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Smaller adult fish size in warmer water is not explained by elevated metabolism

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Abstract

Fish and other ectotherms living in warmer waters often grow faster as juveniles, mature earlier, but become smaller adults. Known as the temperature-size rule (TSR), this pattern is commonly attributed to higher metabolism in warmer waters, leaving fewer resources for growth. An alternative explanation focuses on growth and reproduction trade-offs across temperatures. We tested these hypotheses by measuring growth, maturation, metabolism and reproductive allocation from zebrafish populations kept at 26 and 30°C across six generations. Zebrafish growth and maturation followed TSR expectations but were not explained by baseline metabolic rate, which converged between temperature treatments after a few generations. Rather, we found that females at 30°C allocated more to reproduction, especially when maturing at the smallest sizes. We show that elevated temperatures do not necessarily increase baseline metabolism if sufficient acclimation is allowed and call for an urgent revision of modelling assumptions used to predict population and ecosystem responses to warming.

KEYWORDS
climate change, fish, life history, metabolism, temperature size rule, trade-off, warming

INTRODUCTION

The temperature-size rule (TSR) postulates that ectotherms living in warmer conditions grow faster as juveniles but attain smaller adult body sizes (Atkinson, 1994). This phenomenon has been observed in organisms ranging from bacteria to vertebrates (Atkinson, 1994) and is particularly pronounced in aquatic environments (Horne et al., 2015, 2017; Rollinson & Rowe, 2018). The 'shrinking of fishes' (Cheung et al., 2013) is expected to have profound implications on population demography (Barneche et al., 2016), intra- (Post et al., 1999) and inter-specific interactions (Audzijonyte et al., 2013), fisheries

productivity (Oke et al., 2020) and community structure (Lindmark et al., 2019). Yet, despite the ubiquity and importance of TSR, the mechanisms underpinning it remain debated (Berrigan & Charnov, 1994; Lefevre et al., 2017; Pauly, 2021; Perrin, 1995), and none of the proposed explanations cover the full range of empirically observed body size responses to temperature (Audzijonyte et al., 2019; Verberk et al., 2020).

Understanding the mechanisms behind TSR is not only of conceptual interest. Our ability to adapt our use of natural resources in a warmer future depends on how well we can predict temperature-driven processes operating at individual, population and community levels

(Verberk et al., 2020). This understanding and prediction relies on the fundamental distinction of whether TSR-type body size changes (larger juveniles, smaller adults) are driven by some form of temperature-imposed limitation that has an overall negative impact on individuals, or whether it represents a potentially adaptive life-history optimisation or trade-off in warmer conditions (Audzijonyte et al., 2019; Verberk et al., 2020).

The 'limitation' perspective is often based on a general two-term equation, known as von Bertalanffy growth function, where body growth is modelled as a difference between 'anabolism' and 'catabolism', that is, $growth = aW^b - cW^d$ (Barneche et al., 2019; Pauly, 2021; Von Bertalanffy, 1938; West et al., 2001) (but see: Audzijonyte et al., 2019; Marshall & White, 2019 for why this model should not be used). The model can only explain asymptotic individual growth, as seen in fishes, if the catabolism term (be it for energy, oxygen or protein degradation rate as in Pauly (2021)) has steeper body size scaling than intake ($d > b$). When this model is used to explain TSR, it is generally assumed that the catabolism term responds at a faster rate to higher temperatures than the anabolism term (Pauly, 2021; Pauly & Cheung, 2018; Perrin, 1995; Schaum et al., 2018). As a result, growth at higher temperatures stops at an earlier age (Figure 1: Hypothesis 1).

The assumption that higher temperatures lead to higher metabolic rates is a central tenet of metabolic theory of ecology (Brown et al., 2004). This assumption is so widely accepted that it is used in most models aiming to predict species and ecosystem responses to warming (DeLong et al., 2017; Sentis et al., 2021; Woodworth-Jefcoats et al., 2019; Zhang et al., 2017), including those that suggest >20% declines in body weight across hundreds of marine fish species (Cheung et al., 2013). Yet, studies of hundreds of species show that metabolic rates can acclimate to temperature changes (Donelson et al., 2012; Scott & Johnston, 2012; Seebacher et al., 2015). The universal temperature dependence of metabolism has also been questioned on evolutionary grounds, as it is unclear why organisms should use precious resources for elevated metabolism without a clear evolutionary gain in fitness (Clarke, 2004; Clarke & Fraser, 2004). According to this contrasting view, the commonly observed higher metabolic rates in warm temperatures reflect an adaptive temperature-dependent selection for a faster pace of life rather than a physiological inevitability (Clarke & Fraser, 2004; Kingsolver & Huey, 2008; Schulte et al., 2011). Elevated metabolism at warmer temperatures then becomes an advantageous response that enables, for example, faster food intake rates (Denderen et al., 2020).

The alternative, life-history based, explanation for TSR emphasises how the trade-off in resource allocation to growth and reproduction is shaped to maximise individual fitness (Kozlowski, 1992; Roff, 2002) (Figure 1: Hypothesis 2). If higher temperatures enable
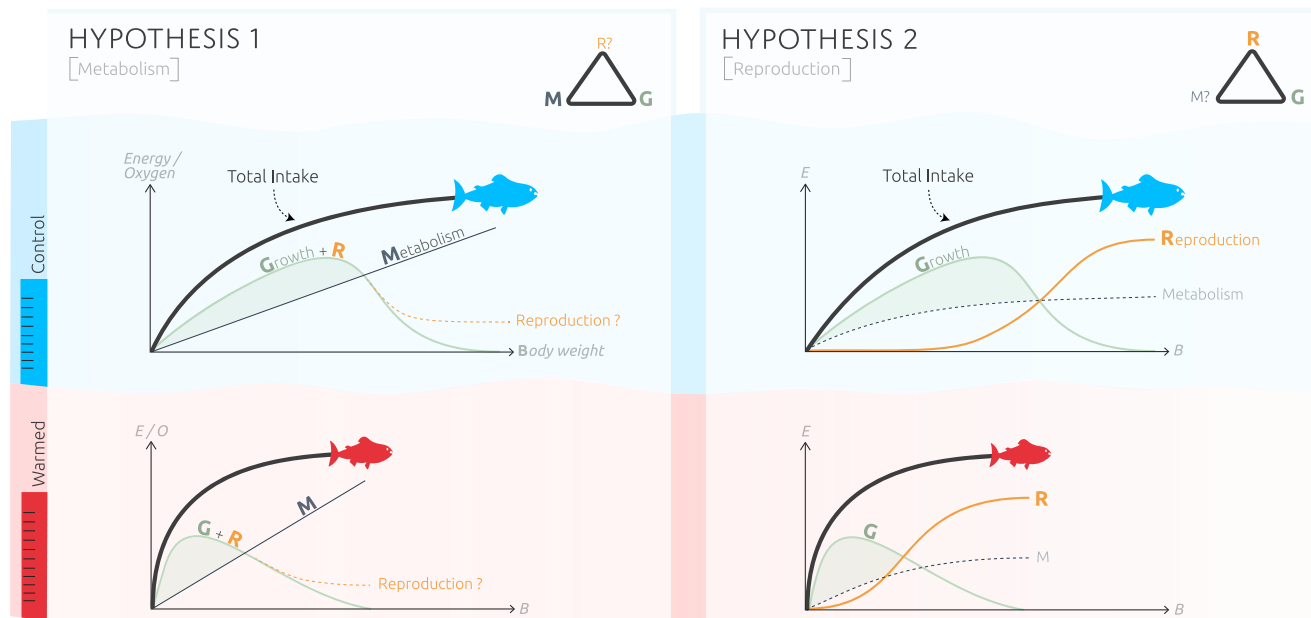

FIGURE 1 Alternate hypotheses for the temperature-size rule (TSR). Hypothesis 1 explains the TSR via a temperature-driven increase in metabolic rate, where energy or oxygen intake cannot match increases in metabolism as ectotherms grow larger, resulting in a smaller maximum size. Reproductive allocation is not explicitly discussed in Hypothesis 1. Hypothesis 2 focusses on faster early growth and increased development rate to explain the TSR, rather than metabolism. Temperature-induced juvenile growth acceleration leads to earlier maturation and consequently a diversion of energy towards reproduction. Smaller adult size is, thus, seen as the outcome of a potentially adaptive life-history strategy. Metabolism is not explicitly discussed in Hypothesis 2

faster developmental rates (Kingsolver & Huey, 2008), this would allow earlier maturation and energy investment in reproduction at younger ages (Forster & Hirst, 2012). The resulting smaller adult body sizes would then not be driven by temperature-induced metabolic limitations, but rather adaptive energy allocation (Kingsolver & Huey, 2008; Kozlowski, 1992). Such shifts to earlier and potentially higher investment in reproduction can have important implications for population growth rates in warming environments (Engen & Saether, 2016; Oli et al., 2002).

The adaptive life-history perspective for TSR generally does not deal with metabolic rates or maintenance costs and is also rarely used in models predicting population or species responses to climate change. This is not only because of the immense popularity of the metabolic theory of ecology (Brown et al., 2004), but also because most existing data on the temperature sensitivity of growth is inadequate to fully address the two competing explanations for TSR. In particular: (i) most studies are based on spatial comparisons across distinct species or populations (Brown et al., 2004; Cheung et al., 2013; Clarke & Johnston, 1999; Dillon et al., 2010), which cannot separate the cause and effect of temperature impacts; alternatively, (ii) they rely on short term experiments (Lefevre, 2016; Seebacher et al., 2015) and cannot be extrapolated to inter-generational processes in wild populations and iii) most studies do not assess the full range of energy intake, growth, metabolism and reproductive allocations (but see Boratynski & Koteja, 2010), and by design cannot separate the alternative mechanisms (Audzijonyte et al., 2019).

To better predict individual species' responses to climate change, and understand the underlying mechanisms driving observed patterns, we need to study body size, metabolism and life-history responses to warming over inter-generational timescales. Here, we took population-level body size, growth, metabolism and reproductive allocation measurements repeatedly from zebrafish (*Danio rerio*, Hamilton 1822) across six generations, with three independent populations kept at each of two temperatures (controls: 26°C and warmed: 30°C). We were specifically interested in assessing intergenerational population-level trait sensitivities to temperature across different life-history stages, and thus understand how long-term acclimation to elevated temperatures affects growth, metabolism and reproduction.

MATERIALS AND METHODS

We followed six populations of zebrafish through six generations of selection, as part of a broader experiment involving 18 populations, explained in detail in Wootton et al. (2021). A founding population of 2000 individual wild-type zebrafish was held at 28°C for a parental generation (PG1) whilst acclimating to laboratory conditions, and then allowed to spawn. Offspring from PG1 were split into the experimental populations, and temperature was adjusted by 1°C day^{-1} until they reached treatment levels (control: 26°C and warmed: 30°C). Populations were then held at their experimental temperature for another six treatment or 'F' generations. Populations were kept as independent lines throughout the experiment in one of six 200L tanks, alongside two additional populations in separate compartments relating to a broader experiment as described in Wootton et al. (2021). The tanks had independent heating and filtration, and populations were fed ad libitum twice daily (at approx. 9 am and 5 pm) for 10 min, after which excess food was removed. Random size selection, which imposed 80% mortality evenly across sizes, was applied shortly after maturation in all six F generations. This was done as the six experimental populations were fishing controls in our broader experiment (Wootton et al., 2021).

We measured fish size (total weight and length), reproductive investment (gonad weight) and size- and age-at-maturation in each population across all six F generations. We additionally measured standard metabolic rate (SMR) and maximum metabolic rate (MMR) from four populations (two at 26°C, two at 30°C). Biological measurements were taken at juvenile, recently matured and post-spawn adult life stages to correspond with key predictions of TSR and the two competing explanatory hypotheses.

Size-at-age measurements

At each of the juvenile and recently matured life stages across all generations, 20 fish were randomly selected from each population once a week for 3 weeks, weighed (0.001 g precision), measured for length (total length: 1mm precision) and returned to their home tank. After maturation (see below) and fishery-related random size selection, we sampled 10 individuals per tank for each of the following three weeks to assess post-maturation (adult) growth. In addition, 20 recently matured fish were measured for length in each generation during the imposition of random size selection.

Metabolic rate measurements

Measurements of SMR and MMR were taken using an intermittent-flow respirometry system (Figure S1), following the methodology outlined in Clark et al. (2013). Respirometry techniques estimate SMR and MMR by measuring oxygen saturation within respirometer chambers over time, from which a consumptive rate (M_{O_2}) is calculated (Clark et al., 2013). SMR is estimated as equivalent to 'standard M_{O_2}', which is the minimum M_{O_2} to sustain life (Clark et al., 2013). MMR

describes an organism's maximum capacity to take up oxygen from the environment, i.e. its maximum aerobic capacity (via the oxygen consumption rate as a proxy: Norin & Clark, 2016). We also estimated the absolute aerobic scope (AAS), defined as the difference between MMR and SMR. AAS can be considered a fitness parameter linked to the consumption of larger meals, the expression of faster digestive rate or higher burst activity (Clark et al., 2013). Reductions in AAS are often described as a cause (Atkinson et al., 2006) or consequence (Portner et al., 2017) of TSR-type responses. See the Supplement for further details of metabolic rate measurements.

Maturity estimation

We monitored the maturity status of populations from when fish were eight weeks old until 80% of all populations had reached ≥80% maturity in each generation (~12 weeks old). Each week, five individuals were randomly selected from each population using a dip net and humanely euthanised (overdose of anaesthetic). Individuals were weighed, their gonads dissected, and maturity status determined based on visual inspection (mature/not mature, after Chen and Ge (2013)). We used logistic regression to estimate maturation schedules as a function of age and weight. Here, the logit of the probability p of individual b being mature M (pM_b) was modelled as a function of age or weight (a)

$$logit\ (pM_b)\ =\ \beta_0\ +\ \beta_1 a_b$$

where β_0 and β_1 are estimated regression coefficients. We used these models to generate estimates of the age (A50) and weight (W50) at which each population in each generation reached 50% maturity.

Gonad size measurements

We measured female gonad weight for each population and generation when fish were recently matured (170 individuals in total at time of maturity estimation, see above) and as older adults after they had spawned the following generation (approx. 21 weeks old, 136 individuals in total). Post-spawn adult female gonad size measurements were taken from 5–8 individuals from each population per generation and were processed fresh or after being stored in 95% ethanol (no significant difference in weights derived from the two methods was detected, see Figure S3). Fish were euthanised, dry blotted and weighed, then one gonad lobe was dissected, weighed, and then doubled to estimate an individual's total gonad weight. Note that we did not measure post-spawn adult gonad size in the first generation of the experiment due to logistical constraints associated with initiating the experiment.

Feed rate

We recorded the approximate weight of daily food consumption for each population in the first and sixth treatment generations (logistical constraints prevented data collection in intervening generations). Fish were fed ad libitum for 10 min using a small spoon. This daily data was averaged to a population's weekly feed amount, and then divided by its weekly abundance to generate a feed rate (g fish^{-1} week^{-1}). We did not take individual feed rate measurements; hence the population level data is used only as a general indication of relative food consumption.

Statistical analyses

All data analyses, calculations and graphical representations were performed using R 3.5.1 (R Development Core Team, 2013) in the RStudio (RStudio Team, 2015) interface (version 1.4.1106), with the additional packages 'lme4' (Bates et al., 2015) and 'effects' (Fox & Weisberg, 2018, 2019). We used linear mixed effects models to assess how temperature, individual size and generation affect population- and generation-specific body size, metabolic rate, and reproductive investment. Separate model suites were developed and tested for each of the 11 response variables –total weight (g), total length (mm), SMR (M_{O2min}), MMR (M_{O2max}), AAS, recently matured and post-spawn adult female gonad weight (g), W50 (g), A50 (days), and feed rate (g fish^{-1} week^{-1}). Fixed effects included all three factors of interest in our experiments - temperature (T, two levels), generation (G, continuous), and stage (S, three levels: juvenile, recently matured and post-spawn adult). We replaced stage with individual weight (W, continuous) in metabolic models to compare responses across body sizes because metabolic rate is usually related to body weight and not life stage. Fixed effects for the feed rate analysis were similar and included a generation term (Gc, categorical) and an average population weight term (Wp, continuous). In all models, random effects for population (Pop) and generation (Gen) accounted for variation across populations and deviations from generational trends (Wootton et al., 2021). AAS models were fit with an additional random effect of chamber 'position' within the water bath (Pos) to account for any potential differences in observed oxygen consumption caused by being closer to air stones or flush pumps (see Figure S1). Fitting the chamber position term to SMR and MMR models explained no additional variation and so was excluded from final analyses. The most complex models for each response included all interactions among fixed effects:

$$(\text{Total weight, Total length}) = T * G * S + (1|Pop) + (1|Gen) + \text{error}$$

$$(W50, A50) = T * G + (1|Pop) + (1|Gen) + \text{error}$$

$$\left\{ \begin{array}{l} SMR, MMR, \\ Recently\ mature\ female\ gonad\ weight, \\ Post\ spawn\ adult\ female\ gonad\ weight \end{array} \right\} = T * G * W + (1|Pop) + (1|Gen) + \text{error}$$

$$(AAS) = T * G * W + (1|Pop) + (1|Gen) + (1|Pos) + \text{error}$$

$$(\text{Feed rate}) = T * Gc * Wp + (1|Pop) + (1|Gen) + \text{error}$$

Competing models for each response were fit with maximum likelihood (ML) (Zuur et al., 2009) and comparisons performed among nested models with and without key interaction terms of interest using backwards sequential model selection (Zuur et al., 2009). Specifically, these competing models directly assessed whether the temperature sensitivity of traits varied between life stages (T*S (or W)), across generations (T*G), or both (T*S*G). If we failed to find evidence supporting an interaction term of interest (based on Chi-squared tests), the term was dropped. We considered a model including just additive fixed effects integral to our study design and set this as our null. The final model (either including interactions of interest or the null) for each response was re-fitted using restricted maximum likelihood to produce unbiased parameter estimates for interpretation (Zuur et al., 2009: see Figure S6 for comparison of parameter estimates from the full three-way interactive model and the supported/null models, as appropriate). All data was visually inspected for errors and outliers and responses were square-root or log transformed as necessary, to satisfy assumptions of statistical tests.

RESULTS

Smaller adult body sizes at warmer temperatures were observed across all six generations

Our observations of body size supported TSR expectations: Body size showed a clear temperature sensitivity, and this differed across life stages and generations (test

TABLE 1 Alternative models tested to assess the impacts of temperature (T), stage (S), weight (W) and generation (G) and their interactions on zebrafish growth, maturation, metabolism and reproductive allocation responses. Supported models are shown in bold

| Test number and explanation	Full model	Alternative model	Chi^2	df	p
1:Ttotal weight	**T*G*S**	T*G + T*S + G*S	14.01	2	<0.001
2: Total length	**T*G*S**	T*G + T*S + G*S	32.46	2	<0.001
3: Standard metabolic rate	**T*G*W**	T*G + T*W + G*W	7.63	1	<0.01
4: Juvenile feeding rate	**T*G+T*W+G*W**	T*W+G*W	28.51	1	<0.001
5: Maximum metabolic rate (MMR)	T*G*W	**T*G + T*W + G*W**	0.26	1	0.61
6: MMR simpler model	T*G+T*W+G*W	**T*G+G*W**	0.06	1	0.81
7: MMR trend through generations	T*G+T*W+G*W	**T*W+G*W**	0.80	1	0.37
8: Absolute aerobic scope (AAS)	T*G*W	**T*G+T*W+G*W**	0.45	1	0.50
9: AAS simpler model	T*G+T*W+G*W	**T*G+G*W**	0.00	1	0.96
10: AAS trend through generations	**T*G+T*W+G*W**	T*W+G*W	1.30	1	0.25
11: Maturation size trend across generations	T*G	**T+G**	0.57	1	0.45
12: Maturation age trend across generations	**T*G**	T+G	7.27	1	<0.01
13: Gonad weight in recently matured females	**T*G*W**	T*G+T*W+G*W	4.08	1	<0.05
14: Gonad weight in later, post-spawn females	T*G*W	**T*G+T*W+G*W**	0.09	1	0.76
15: Gonad weight in later, post-spawn females, simpler model	T*G+T*W+G*W	**T*W+G*W**	0.66	1	0.41
16: Gonad weight in later, post-spawn females, alternative simpler model	T*G+T*W+G*W	**T*G+G*W**	0.54	1	0.46
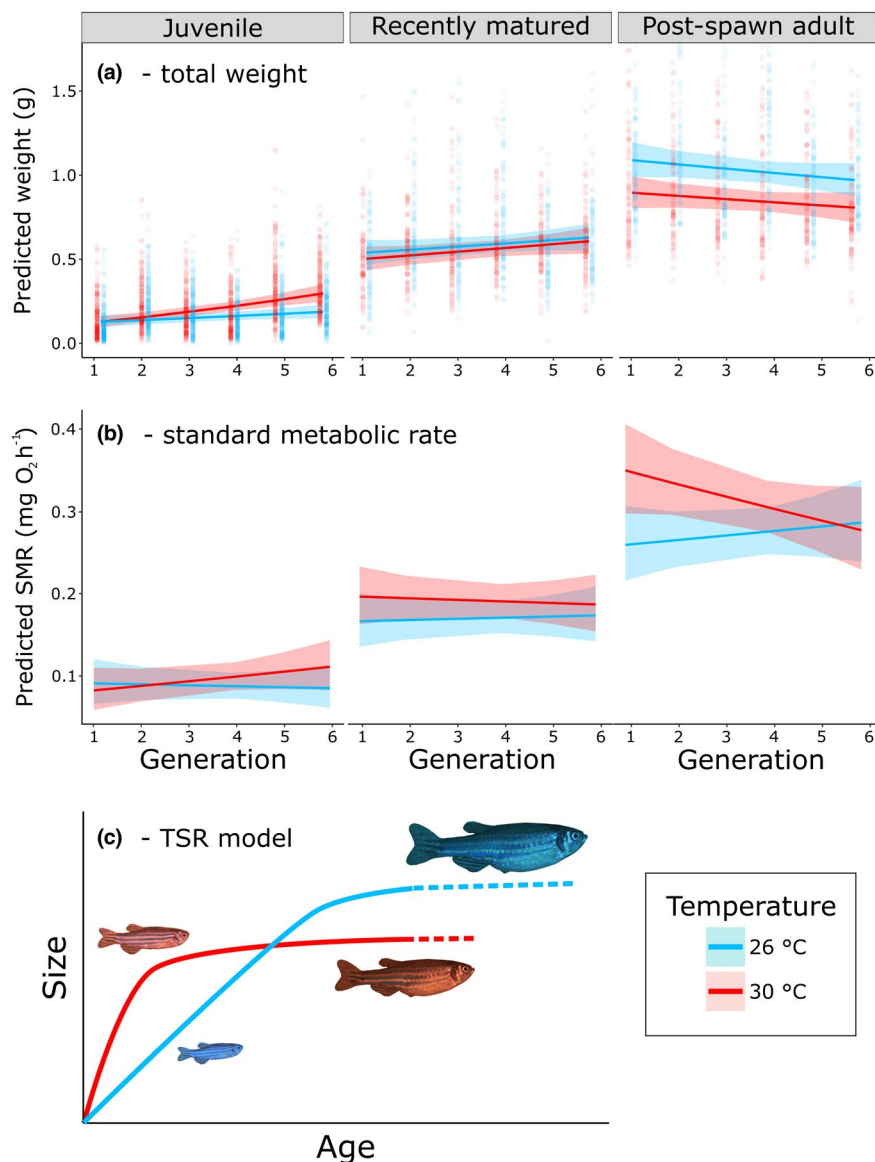
FIGURE 2 Predicted effects (±95% CIs) of warmed (30°C) and control (26°C) temperature treatments across six generations at three life-history stages (juvenile, recently matured and post-spawn adult): (a) somatic weight (g), and (b) standard metabolic rate (SMR). Points in (a) show raw data. SMR is shown at the average weight measured at each life-history stage in a (juvenile = 0.2 g, matured = 0.6 g and adult = 1 g). Some raw data from the largest fish in (a) (mostly control temperature) is truncated for clarity (full detail in Figure S7). Full illustration of raw SMR data across generations is shown in Figure S9. (c) The tested temperature-size rule model is shown with solid lines, where dashed lines represent possible growth and longevity past the limit of our experiment

1 in Table 1 for total weight and test 2 for total length). Adult fish (measured post spawning) from the warmed 30°C treatment were on average 3% shorter and 17% lighter than control 26°C fish (Figure 2a,d, Figures S7, S8, Table S2). This difference in adult body size persisted across all six generations. Observed adult body size differences could not be explained by a warming-induced shift to a male biased sex-ratio (males are smaller than females) as sex-ratios remained constant through the experiment (data presented in Wootton et al. (2021)). In

contrast, juvenile body size patterns did not initially conform to TSR expectations as control and warmed juveniles grew at similar rates over the first two generations. Juvenile growth rates of 30°C fish began to increase in the third generation, and then by the sixth-generation 30°C juveniles were 15% longer and 33% heavier than 26°C controls (Figure 2a, Figures S7, S8, Tables S2). There was an overall cross-generational trend towards smaller adult size at both temperatures which most likely reflected a common multi-generational experimental

artefact related to artificially shortened generation length (Hoffmann & Ross, 2018).

By the sixth generation, there was no difference in SMR between the two temperatures

The metabolism-centred 'limitation' hypothesis (Hypothesis 1) predicts that smaller adult body size in warmed populations is driven by higher SMR. Whilst SMR did initially differ between temperatures, this effect was conditional on life stage (test 3, Table 1), and importantly displayed a different pattern to that observed in body size. In the first generation, SMR was ~20% higher in warmed populations for the recently matured and post-spawn adult fish (model predicted SMR (±SE) at 26°C: 0.166 ± 0.034 mg O_2 h^{-1} and 30°C: 0.196 ± 0.037 in recently matured individuals and 26°C: 0.259 ± 0.047 and 30°C: 0.350 ± 0.056 in post-spawn adults: Figure 2b, Figure S9, Table S2). This difference progressively weakened, such that by the sixth generation, SMR of zebrafish kept at 26 and 30°C were not detectably different (model predicted SMR (±SE) in recently matured individuals at 26°C: 0.173 ± 0.035 mg O_2 h^{-1} and at 30°C: 0.187 ± 0.035, and in post-spawn adults at 26°C: 0.286 ± 0.052 and at 30°C: 0.277 ± 0.053). Equally, SMR could not explain the increasingly faster juvenile growth rates in warmed fish as the experiment progressed: juvenile SMR remained similar between temperatures yet by the sixth-generation juveniles were ~30% larger (in weight). Instead, the observed faster juvenile growth at 30°C in the last generations correlated with an increased juvenile feeding rate relative to controls (test 4 in Table 1, Figure S10, Table S2). We therefore conclude that there was no correlation, at least at a population level, between temperature-driven differences in juvenile and adult body size and SMR.

Maximum metabolic rate always remained higher at warmer temperatures

MMR showed a different temperature sensitivity compared to SMR across generations. Maximum rates of oxygen consumption did not differ across body size and generation at the two temperatures (test 5 in Table 1), nor was there an interaction of temperature and body size alone (test 6 in Table 1). MMR was always higher in fish from warmed populations, with this difference in adults appearing to increase as generations progressed, although the trend was not significant (test 7 in Table 1, Figure S11, Table S2). The temperature sensitivity of MMR shaped the observed AAS of populations (tests 8 and 9 in Table 1, Figure S12, Table S2). AAS of warmed fish was always higher than controls and also seemed to trend upwards across generations, although not significantly (test 10 in Table 1) due to MMR remaining consistently higher in warmed populations while SMR decreased and fully acclimated.

Warmed fish matured smaller, earlier and with relatively larger gonads

Since metabolic rates could not explain the temperature-driven body size changes observed in our experiment, we next explored whether such changes could be related to shifting developmental time or reproductive allocation. Such evidence would provide support for TSR emerging from the growth and reproduction trade-off (Figure 1: Hypothesis 2). Our warmed populations persistently matured at a smaller (17% lighter) size (test 11 in Table 1, with no trend across generations), and progressively at a younger age across generations (test 12 in Table 1, Figure 3a,b, Table S2). By the final generation, warmed populations matured on average 17 days or 23% earlier than populations at 26°C (Figure 3b).

In the first generation, gonads from recently matured females (i.e. excluding later, post-spawn females) in warmed populations were 55% larger than those held at 26°C. However, this difference weakened through generations, especially in larger (>0.7 g) recently matured females such that by the end of the experiment the largest gonads were in larger 26°C females (test 13 in Table 1, Figure 3c, Table S2). A comparative inter-generational shift in gonad weight between temperatures did not occur in smaller (<0.6 g) recently matured females, where 30°C fish always had larger gonads (Figure 3c). For older, post-spawn adult females we detected no difference in size-specific gonad weight across temperatures or generations (tests 14–16 in Table 1; Figure S13, Table S2). However, we did find that delayed maturation in 26°C females (Figure 3b) likely allowed these fish to attain overall larger body sizes (as post-spawn adults) and eventually produce gonads that were absolutely 32% larger than females in warmed conditions.

DISCUSSION

Baseline metabolism, adult body size and oxygen limitation

In this study we confirmed, through a multi-generational experiment, that fish mature at smaller sizes and become smaller adults when exposed to elevated temperatures. The observed rate of decline in adult body weight at warmer temperatures (4.25% per 1°C warming) was similar to other experimental findings (Loisel et al., 2019), and in agreement with field-based observations of wild aquatic ectotherms (Baudron et al., 2014; Daufresne et al., 2009; Horne et al., 2015; Ikpewe et al., 2021). Yet, our study also demonstrates that temperature-induced changes to metabolic rates do not explain these TSR-consistent body size differences in zebrafish reared at 26 and 30°C.

After six generations, SMR in control and warmed populations were indistinguishable, unlike the difference
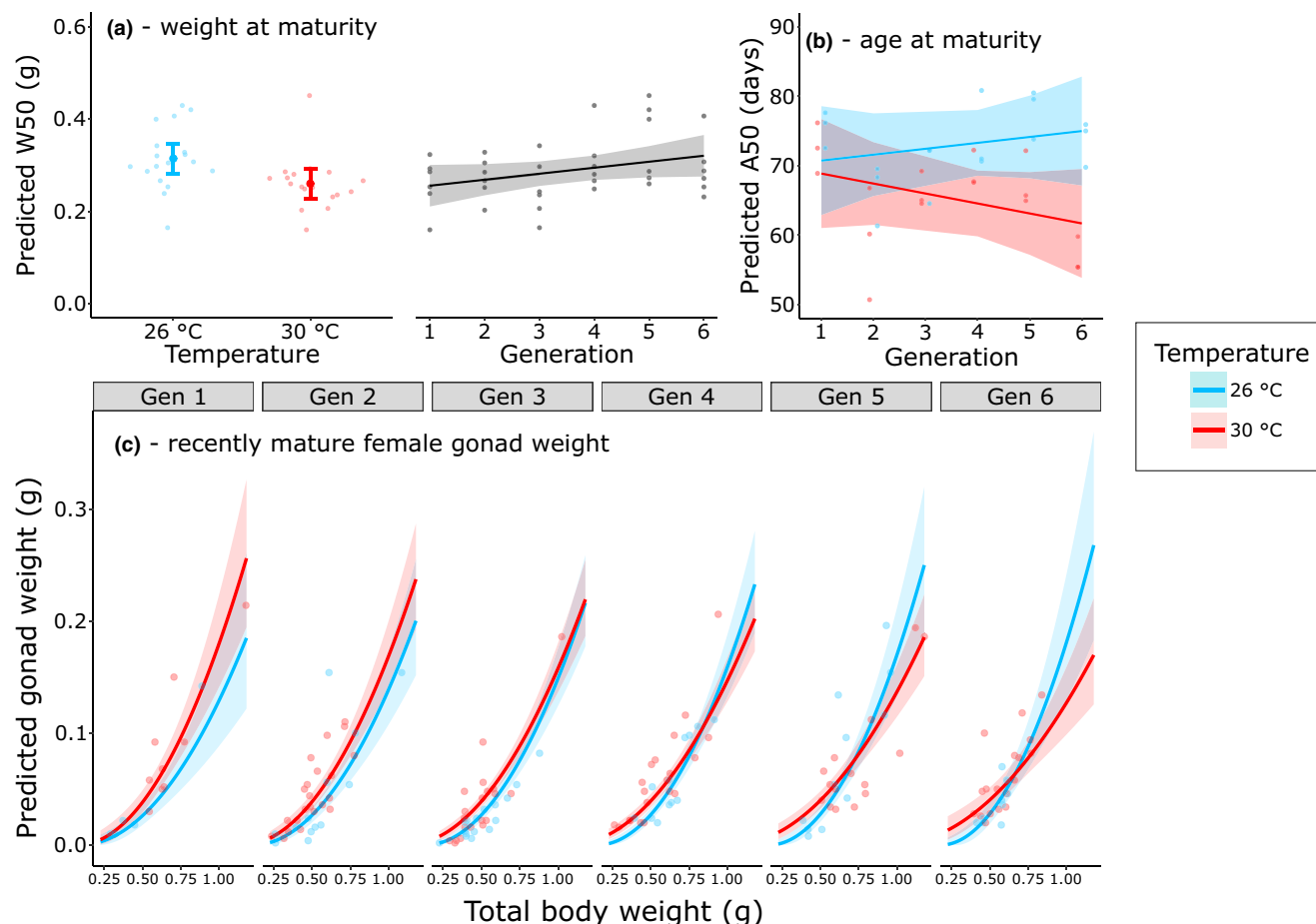
FIGURE 3 Predicted effects (±95% CIs) of warmed (30°C) and control temperature (26°C) treatments on zebrafish maturity and gonad weight: (a) weight at maturity (W50 in g) at each temperature and across generations, (b) age at maturity (A50 in days) where temperature interacts with generation, (c) recently matured female gonad weight as a function of somatic body weight (g) through generations. Age and weight at maturity were estimated using logistic regression at the time at which 50% of the population is mature. Points represent raw data, where spread along the x-axis in the left panel of (a) only indicates data density (all points are exactly at either 26 or 30°C)

in adult body weights. Furthermore, MMR was higher in warmed fish across all sizes and generations and thus could also not explain the increasingly larger warmed juvenile body sizes in the last, but not initial generations. The AAS was always higher in warmed treatments, suggesting that a 'maintenance' of aerobic scope was not the cause of observed TSR (Atkinson et al., 2006). Our multi-generational experimental results are therefore incompatible with the 'limitation' hypothesis or the narrow application of metabolic theory of ecology, where metabolic rates are seen as an inevitable consequence of environmental temperature, and smaller adult body sizes are a negative consequence of insufficient energy or oxygen available for growth. For example, if we applied a commonly used activation energy for metabolic rates (0.6l: Brown et al., 2004) we would expect ~40% higher SMR in 30°C fish compared with the 26°C controls. This was somewhat close to the observed difference in adult (but not juvenile) fish of the first generation (model predicted 26% difference for 1g fish; Figure 2a), yet this effect disappeared through generations. Our findings, therefore, better align with the argument that

an individual's baseline metabolism, and by extension its oxygen use, is determined by its needs (Bigman et al., 2021; Kingsolver & Huey, 2008) and not an inescapable effect of temperature. Still, the elevated SMR observed over the first few generations of our experiment suggest that acclimation to a 4°C higher temperature required time, even in a eurythermal species like zebrafish.

Despite increasing evidence to the contrary (Audzijonyte et al., 2019; Verberk et al., 2020), warming-induced increases in metabolism (baseline, or through digestion related energetic costs (Jutfelt et al., 2021)), and hence some form of 'limitation' are still considered the main explanation of TSR-type body size responses. Limitation mechanisms are explained either directly (Pauly, 1981, 2021; Portner et al., 2004) or indirectly through 'ghosts of evolutionary past', where organisms grow to smaller sizes in response to historic selection (e.g. via past oxygen limitation (Verberk et al., 2020)). One possible explanation for the ubiquitous focus on metabolism is the generally short duration of most experiments or observational time series. Even inter-generational studies often only consider two or three generations, yet

the full range of responses in our study was observed only after three generations. If our experiment stopped sooner, it would be logical to conclude that smaller adult body sizes were driven by higher baseline adult metabolic rates. Yet, after six generations our results suggest the opposite – higher temperatures, if anything, actually increased size-specific aerobic scope and thus likely increased metabolic performance in zebrafish. In all, our study supports the view that the generally higher metabolic rates of organisms living in warmer conditions (Brown et al., 2004) are not directly caused by high temperatures. Rather, they more likely reflect an adaptive metabolic response to temperature-induced changes in community structure due to shifting predation rates or faster developmental rates (Clarke & Fraser, 2004).

Do our findings suggest that the gill-oxygen limitation (GOLT) hypothesis cannot be used to explain smaller adult body sizes in warmer water (Pauly, 2021; Pauly & Cheung, 2018)? Strictly speaking, our findings only question GOLT's reliance on an increase in maintenance metabolism as an explanation for TSR (Figure 4a, b in (Pauly, 2021)). In reality, baseline oxygen usage, referred to here as SMR, includes multiple processes, such as cellular repair, protein synthesis and the overheads of growth. It could be argued that even if the total SMR at higher temperatures remains the same, the proportion of oxygen used for repair increases, leaving less oxygen for growth. Teasing apart the relative demand of cellular repair and growth would require measurements of the proportion of SMR spent by separate physiological processes. We are not aware of such analyses, suggesting that an experimental test of GOL might be virtually impossible. Finally, our study shows that SMR responses to warming varied across different life-stages in zebrafish. Adult fish had higher SMR sensitivity to temperature in the first generations, whereas for juveniles, elevated temperatures had virtually no effect on SMR. Our findings strongly support the body size dependency of metabolic rates, at least on acute, short time scales (Lindmark et al., 2018; Ohlberger et al., 2012).

Can temperature-sensitive reproductive allocations explain smaller adult body sizes?

Our experiments show that zebrafish living at 30°C matured on average eight days earlier and at least initially had significantly larger gonads at maturation when compared to 26°C fish. These observations appear to support the life-history-based 'optimisation' explanation for TSR with a reproduction-growth trade-off at its core (Audzijonyte et al., 2019; Berrigan & Charnov, 1994; Forster & Hirst, 2012). Higher temperatures lead to faster developmental rates and earlier maturation in many ectotherms (Berrigan & Charnov, 1994; Forster & Hirst, 2012). Naturally, an earlier onset of reproductive allocation is expected to divert energy away from growth

(see reviews: Angilletta & Dunham, 2003; Audzijonyte et al., 2019; Verberk et al., 2020), although see Pauly (2021) for a list of arguments of why allocation to reproduction cannot explain TSR type growth trajectories in fish. We note that this reproductive allocation is not only a direct expenditure on gonads but also includes other indirect costs of reproduction such as migration, reproductive behaviours and competition (Watson et al., 1998). If these indirect reproductive costs scale non-linearly with size with an exponent <1 (Audzijonyte & Richards, 2018), then the overall relative energy expenditure of recently matured and smaller fish in warmer conditions could be even higher.

After several generations, only smaller females in warmed populations had larger gonad weights. Thus, overall gonad weight depended on the interaction between size, generation and temperature, as was seen for baseline metabolic rate (Figures 2b and 3c). However, it is not clear to us why, as generations progressed, full SMR acclimation in warmed adult fish correlated to relatively smaller gonad size in the largest (>0.7 g) recently matured females. Perhaps the similarity in SMR and gonad size responses to warming in the largest females is coincidental rather than causative. Our reproductive data were inevitably limited as only a few females per generation could be assessed without compromising the integrity of the experiment. To draw stronger inference, more empirical data is needed from specifically designed multi-generational experiments that, for example, assess large numbers of fish in first and last generations.

Zebrafish living at the warmer temperature always matured earlier, despite any ambiguity in observations of gonad weights across temperatures and adult sizes. Is this earlier maturation at warmer temperature an adaptive response, or some form of limitation (e.g. oxygen supply: Pauly, 2021)? All else being equal, an earlier onset of reproduction is advantageous because it increases an individual's chance to reproduce (Cole, 1954). However, earlier reproduction and a resultant smaller adult body size typically means lower fecundity later in life. One explanation for TSR-type growth could be that size-specific fecundity increases later in life at colder temperatures and hence the risks of delayed maturation are compensated for by higher overall reproductive output in these conditions (Arendt, 2011). Although we did not test for such an increase in size-specific fecundity, females living at the control temperature grew to larger sizes and, therefore, had larger overall gonads which would suggest greater absolute reproductive potential. Another adaptive pathway could lie in evolved responses to shorter lifespans in warmer waters. Reductions to longevity at higher temperature can occur through direct (e.g. oxidative stress: Monaghan et al., 2009) and indirect (e.g. increased predation: Walker et al., 2020) processes, and involve the expression of early maturation or increased reproductive allocation in response to reduced lifetime reproductive opportunity (Kozlowski, 1992). Studies on insects suggest

that TSR-type growth patterns are actively maintained by selection (Kingsolver & Huey, 2008), indicating their adaptive significance, but the generality of these findings should be tested more broadly.

Conclusion and call for better growth models

Evolutionary and ecological theories suggest three general and contradicting rules – 'bigger is better', 'hotter is smaller' and 'hotter is better' (Kingsolver & Huey, 2008). We still do not know how to resolve them. Despite earlier reproduction, warmed populations did not necessarily have higher reproductive output. In fact, after four generations of heating we observed a rapid decline in the recruitment (number of fish that reached 6 weeks of age) of our warmed experimental populations (see: Wootton et al., 2021). It is important to note that the observed decrease in recruitment was relative to the control populations and does not necessarily suggest that earlier maturation itself was disadvantageous. Without earlier maturation, recruitment impacts in the warmed zebrafish populations may have been even more severe. Nevertheless, our findings challenge the prevailing view that smaller body sizes at warmer temperatures are driven by physiological limitations and are necessarily maladaptive. Instead, they highlight that the adaptive significance of a body size response to warming is complex. Our findings also strongly suggest that the TSR puzzle will remain unresolved if we keep attempting to explain the complexity of growth simply as a difference between anabolism and catabolism. Time is ripe for better growth models, which at least specifically include reproductive allocation and its costs (Audzijonyte & Richards, 2018; Marshall & White, 2019). There is also a critical need for further research and long-term experiments into temperature-related body size and reproduction changes. This research must acknowledge that intraspecific responses to temperature through time cannot be automatically attributed to changes in metabolism but require careful assessment of changing development rates, reproductive output and the resulting fitness consequences.

AUTHORSHIP STATEMENT
HFW, TS, AA and JRM conceived the study. HFW and TS carried out the experiment. All authors contributed to data analyses, manuscript construction and editing. All authors approved the manuscript for submission.

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DATA AVAILABILITY STATEMENT
The data supporting these findings and analysis code have been deposited on Figshare (https://doi.org/10.26188/6201f08988bdd).

OPEN RESEARCH BADGES

This article has earned Open Data and Open Materials badges. Data and materials are available at https://github.com/Haychi-86/zebrafishTSR.

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