Intergenerational plasticity aligns with temperature-dependent selection on offspring metabolic rates

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Abstract

Metabolic rates are linked to key life history traits that are thought to set the pace of life and affect fitness, yet the role that parents may have in shaping the metabolism of their offspring to enhance survival remains unclear. Here, we investigated the effect of temperature (24 °C or 30 °C) and feeding frequency experienced by parent zebrafish (*Danio rerio*) on offspring phenotypes and early survival at different developmental temperatures (24 °C or 30 °C). We found that embryo size was larger, but survival lower, in offspring from the parental low food treatment. Parents exposed to the warmer temperature and lower food treatment also produced offspring with lower standard metabolic rates – aligning with selection on embryo metabolic rates. Lower metabolic rates were correlated with reduced developmental and growth rates, suggesting selection for a slow pace of life. Our results show that intergenerational effects on offspring size and metabolic rate can be adaptive when parent and offspring temperatures are matched: the direction of selection on embryo size and metabolism aligned with intergenerational plasticity towards lower metabolism at higher temperatures, particularly in offspring from low condition parents. These findings highlight the importance of anticipatory parental effects, but only when parental and offspring environments match.

Keywords

Development, energy, maternal effects, metabolism, parental investment, reproductive investment

Introduction

Selection on life history strategies can drive the evolution of metabolic rate, which represents the energetic cost of living [1,2]. Metabolic rates expressed during early life are associated with key life history traits: individuals with faster minimal metabolic rates have faster developmental and growth rates, earlier onset of reproduction, and shorter lifespan, than slow metabolic phenotypes [3,4]. The majority of ectotherms undergo embryonic development in eggs, with a finite amount of energy reserves available to sustain cell division, differentiation, and maintenance costs until post-hatching feeding [5]. Hence, variation in metabolic rates will also determine how quickly energy reserves are depleted for these species, with important consequences for survival [6]. It might be expected therefore, that selection should act to suppress minimal rates of metabolism to conserve energy, yet variation in metabolism is ubiquitous – varying by up to three-fold, even after accounting for embryo size and developmental temperature [7]. Furthermore, selection for a fast pace-of-life may mediate the expression of higher metabolic rates [8]] that can be beneficial in high competition environments [9]. Investigating the interplay between metabolic rates and survival – and the environmental dependence of this relationship – is crucial for understanding the potential adaptive capacity of variation in metabolic rates [10].

Metabolic rates have been studied for over a century [11], yet the adaptive potential of this variation in metabolism remains unclear [12]. Mixed evidence shows that metabolism is sometimes under selection (e.g., [13–15]) and is somewhat heritable [16–18] and repeatable [19,20], suggesting that the fitness consequences of slow and fast metabolic rates are context-dependent [21,22]. It is unresolved whether metabolism has evolved as a driver or simply a by-product of the pace of life. However, metabolic rates (often measured as oxygen consumption or carbon dioxide production) reflect the energy use of an organism, so that measures of metabolic rate are meaningful in linking the physiology of an individual with its life history. Metabolic rates are not fixed across ontogeny however, and within-generation acclimation can act to down-regulate metabolism under low food availability [23]. While this metabolic suppression may slow the pace of life, it can also facilitate survival under stressful conditions [24]. If there is a causal relationship between metabolism and the pace of life, then context-dependent selection may drive a correlated suite of responses [8]. Elucidating the links between metabolic rate, the pace-of-life, and its fitness consequences, is critical for understanding the capacity for organisms to respond to changing environments [25].

The environment a parent experiences can shape the phenotype of their offspring, known generally as parental effects [26]. This form of epigenetic inheritance across two or more generations (termed inter- and trans-generational plasticity respectively) [27] can be

adaptive or maladaptive – acting as either a buffer or conduit to the effects of environmental stress [28]. Adaptive Adaptiveparental effects arise when parents anticipate and respond to environmental cues, to produce shifts in their offspring's phenotype that maximise their fitness in the offspring environment [26].[26]. For example, when exposed to cool temperatures, mothers tend to produce larger offspring [29], leading to enhanced offspring survival in that same environment [30,31]. Alternatively, under a bet hedging strategy, parents in stressful or unpredictable environments increase variance in their offspring phenotypes, with variable consequences for offspring fitness, but overall enhancing parental fitness [32]. If intergenerationalIf intergenerational plasticity is adaptive such that it confers fitness benefits for offspring, then shifts in parental provisioning should be in line with selection on offspring traits. Conversely, increased variance in parental investment that does not enhance offspring fitness consistently may be indicative of a bet-hedging strategy to maximise parental fitness. Overall trends across studies show that intergenerationalintergenerational plasticity on offspring phenotype are generally weak compared with the direct effects of the offspring environment [33,34], and caution needs to be exercised when inferring the adaptive value of trait plasticity [35,36]. Nonetheless, the transmission of the parental environment across generations isis an important source of phenotypic variation. In particular, when environmental conditions are correlated between generations, maternal effects can account for up to half of the phenotypic variation within populations as additive genetic effects [37,38].

Positive anticipatory effects are thought to evolve in changing but predictable environments to enhance offspring fitness [39], however formal selection analyses are lacking. Selection is the phenotypic covariance between fitness and a trait [40], yet most transgenerational studies have reported the effect of parental environment on an aspect of offspring performance that may trade off with actual fitness [41]. Selection analysis uses multiple regression of individual relative fitness on traits of interest to estimate standardised linear and nonlinear selection coefficients [42]. Used in combination with experimental manipulation of environmental predictability across generations, selection analysis can reveal the relative scope for evolutionary change on an offspring trait. If parents can anticipate the environment their offspring will experience, and provision accordingly, then selection on offspring metabolic rates should align with shifts in offspring investment. However, in cases where the offspring environment differs unpredictably from the parental environment, the direction, form, and strength of selection may not align with the mean and variance of offspring phenotypes that parents produce. Selection analysis cannot clarify whether shifts in offspring phenotype in response to parental environment have evolved in response to selection (i.e., whether they are due to genetic or epigenetic causes), however it

104 does provide a meaningful first step to understanding whether transgenerational plasticity is
105 likely to be adaptive in a given environment.
106
107 Food availability and environmental temperature experienced by the parental generation are
108 known to alter parental investment with performance consequences for subsequent
109 generations [30,31]. Poor parental condition may elicit an adaptive response in offspring via
110 transgenerational plasticity, and offspring from parents exposed to low food may suppress
111 their metabolic rate, or alter energy allocation towards maintenance or growth, to
112 compensate for lower energy provisioning from the mother. Alternatively, investment in
113 offspring can be the direct result of parental condition transfer effects, which can be
114 adaptive, but are not contingent on environmental predictability across generations [43].
115 Regardless of the source of offspring trait variation, the implications of intergenerational
116 plasticity are likely to be context dependent. For example, warmer temperatures increase the
117 metabolic rates of ectotherms and may thereby exacerbate the fitness consequences of
118 variation in energy acquisition and allocation in low resource environments [44]. Food
119 availability in the parental generation is likely to alter maternal energy allocation (e.g.,
120 offspring size and composition and/or number) towards offspring as well as mediate the
121 physiology of the offspring; the same is true for environmental temperatures in the case of
122 ectotherms. However, it remains so far unclear as to the direction of these responses,
123 whether they are under selection, and whether they constitute an adaptive parental strategy
124 to maximise offspring fitness.
125
126 Despite evidence that metabolic rates are under selection, it is yet to be established whether
127 parents can modify the metabolic rates of their offspring in adaptive ways. Recent work on
128 ectotherms has shown evidence for both the presence [45,46] and absence [47] of
129 transgenerational responses of metabolic rates to temperature. However, offspring fitness in
130 these studies was measured indirectly as growth [46,47] or aerobic scope [45], which may
131 trade off with actual fitness, and in [45] some treatments showed extensive mortality, hence
132 results may be due to selective mortality. Formal tests of whether transgenerational plasticity
133 aligns with selection on offspring metabolic rates, via measures of offspring metabolism and
134 fitness under different environments, are currently lacking. Further, it is often unclear how
135 selection on metabolic rates may be mediated by its correlation with traits that set the pace
136 of life, such as developmental and growth rates. Here we manipulate parental food
137 availability and temperature in zebrafish to determine whether context-dependent selection
138 on offspring metabolic rates is in line with intergenerational plasticity on metabolic rates and
139 traits that set the pace of life. Here we manipulate parental food availability and temperature
140 in zebrafish to determine whether context-dependent selection on offspring metabolic rates

is in line with intergenerational plasticity on metabolic rates and traits that set the pace of life. We hypothesise that warm offspring temperatures will select for smaller embryo size and lower metabolic rates, while selection at the cool (benign) offspring temperature will be relaxed. Shifts in parental investment should mirror selection on offspring phenotype when their environments match – thus parents in the warm environment should produce smaller offspring with lower metabolic rates compared to parents from the cool temperature (Figure 1A). Further, we predict that parental effects should be exaggerated when parental food availability is low, under which conditions parents should produce offspring with lower metabolic rates than parents from the high food availability environment.

Materials and Methods

Parent maintenance and treatments

All procedures were approved by the University of Sydney Animal Ethics Committee (protocol number: 2021/1932). Adult zebrafish were obtained from a commercial supplier (Livefish, Childers, QLD, Australia), and housed in a controlled temperature room (22 °C with 12L:12D). The supplier maintained zebrafish at 22-24°C in large communal ponds, and so the parental fish were unlikely to have been closely related. The experiment was run in two replicate blocks, one month apart. Within each block, fish were first allocated randomly across four 35 l tanks (35-38 fish per tank) for two weeks to acclimate. Fish were then sexed as per [48], and 60 females and 60 males were allocated evenly across 12 experimental (11 l) tanks; each tank was filled with aged water and contained a sponge filter and a plastic plant. We conducted four parental treatments (with three replicate tanks each) in a fully factorial design (Figure 1B). Parents were held at either 30 °C or 24 °C temperature, referred to hereon as 'high' and 'low' parent temperature, respectively, and either a high feeding frequency (three times per day, five days per week) or low feeding frequency (once per day, four days per week). Previous studies have shown that 30 °C is higher than optimal, and that the low food regime was sufficient to allow growth but at a submaximal level [49,50]. The 24 °C treatment represents a relatively low but benign temperature previously shown to facilitate normal growth [51].

To validate the feeding treatments used, measures of parent body mass and length taken at the end of the experiment were used to assess condition [52]. Parents were weighed (to the nearest 0.001 g) and total body length (to the nearest 0.1 cm) measured, and the exponent for the slope of ln(length) and ln(mass) calculated as 2.79. Measures of body condition were then calculated as mass/length$^{2.79}$. To maintain fish in stable temperature treatments, tanks were held within water baths, containing three submersible heaters (Aqua One 200 W; Techden, Sydney, Australia) and a powerhead water pump (Aqua One maxi). Temperature loggers recording every 15 min were placed into two tanks per temperature treatment. Tanks were maintained within ±1.5 °C of their target temperature for the duration of the treatment. Fish were fed flake food (5mg per fish; Supervit Fish Flakes, Tropical, Chorzów, Poland) [49] at each feeding event according to the regime described above and at randomised times between 8am – 8pm each feeding day. A 50% water change was conducted twice per week. The adult food and temperature treatments were applied for eight weeks, after which adult fish were bred.

The evening before breeding, all fish from each replicate tank were transferred into 10L plastic breeding tanks containing a coarse mesh base, through which fertilised eggs could pass to avoid being eaten by adults. Maintaining males and females in the same tank promotes the release of pheromones that stimulate ovulation and oviposition in females and spawning by males [53]. The next morningbreeding tanks were inspected within 1h post-fertilisation, and eggs were filtered through a sieve onto a petri dish containing buffered E3 medium as per standard procedure for embryo rearing [54]. Unfertilised eggs or dead embryos were immediately removed.

Embryo and yolk size measurements, treatments, and rearing

Within one hour of collection from parental tanks, individual fertilised embryos were sampled by sifting gently through a sieve, and then photographed under a dissecting microscope (x30 magnification; Leica S9D stereomicroscope with FLEXACAM C3 camera). Developmental stages of *D. rerio* are easily identifiable due to the transparency of embryos. The sphere stage shows a flat border between the blastodisc and yolk, and total embryo area and yolk area were measured to the nearest μm^2. The ratio of yolk area to total embryo area was consistent among treatments (Figure S2). Hence, assuming density of embryo tissue did not change with embryo size, we calculated embryo mass (μg) from embryo diameter at the sphere stage using a relationship previously determined for *D. rerio* [50]. Embryos were then placed individually into wells of 24-well culture plates containing E3 medium. For each of the four parental treatment combination, 72 embryos were randomly allocated to each of two offspring temperature treatments (24°C and 30°C), resulting in a total of 576 embryos equally divided across 8 treatment groups: two parental temperatures (24 °C versus 30 °C) x two parental conditions (low versus high) x two offspring temperatures (24 °C versus 30 °C) [Figure 1B]. Offspring were maintained in incubators (Eurotherm Micro Digital Control Model i-80, Steridium, Australia) on a 12L:12D light cycle for the remaining duration of the experiment. Since offspring were removed from the parental treatment, photographed, and placed into their treatment temperatures within three hours of fertilisation (approximately 2-4% of their total development time), we were able to separate the parental and offspring environment.

Offspring metabolic rate measures

The rate of oxygen consumption ($\dot{V}O_2$) was measured as a common proxy for metabolic rate (MR) of the offspring at three developmental stages: 1) 25% through embryonic development (14 and 30 hours post-fertilisation (hpf) for embryos incubated at 30 °C and 24 °C, respectively, 2) 1-4 hours post-hatching (hph) and 3) one week post-hatching (wph),

hereon referred to as MR_{embryo}, MR_{hatch}, and MR_{larva}, respectively. Individual offspring of known identification were photographed to measure diameter (MR_{embryo}) or length (MR_{hatch}, and MR_{larva}) to the nearest μm, then placed into individual 80 μl (MR_{embryo} and MR_{hatch}) or 500 μl (MR_{larva}) glass vials containing Milli-Q water and a nonconsumptive O_2 sensor spot. We used two 24-channel PreSens sensor dish readers (SDR2, PreSens, Germany), each with 24-chamber glass microplates (Loligo Systems Aps, Tjele, Denmark) to measure $\dot{V}O_2$ in 40 offspring and four blank vials simultaneously over a 2-h interval at their respective treatment temperature (24 °C or 30 °C). For a detailed description of methods, see [50]. To calculate the most linear rates of decrease in oxygen concentration within each timeseries dataset (adjusted for background oxygen extraction), we used the *RespR* package, designed for processing closed chamber aquatic respirometry data in R [55]. Slopes were then converted into rate of oxygen consumption, accounting for oxygen solubility of 5.91 ml O_2 at 24 °C and 5.29 ml O_2 at 30 °C (0 ppt salinity, STDP) [56].

Offspring hatching time and survival measures

Eggs were held in their individual wells of the culture plates to allow recording of embryo development time (time in hours from fertilisation until hatching; hpf) and survival; their water was changed daily using a solution of Milli-Q water with 0.5 g l^{-1} of red sea salt at the treatment temperature. Based on hatching time pilot data, we monitored embryos every two hours from 30 hpf at 30 °C and 90 hpf at 24 °C, until all embryos were recorded as either hatched or deceased. Within two hours of hatching, larvae were photographed for measures of larval length (0 hph) and moved into larger 6-well culture plates filled with fresh water and placed back into incubators at their respective treatment temperature. At four days post-hatching (dph), once feeding structures were fully formed, offspring were fed paramecium (4-5 dph), egg yolk (5-14 dph), flake food (5-14 dph), and *Artemia* sp. (from 15 dph) *ad libitum*. Larvae were measured again at one week post hatchling (1wph) to obtain measures of growth rate (mm day^{-1} = (length at 1wph / length at 0hph) / 7). Larvae were monitored for survival daily until two weeks post hatching. Sample sizes for all measures are provided in Table S1.

Analysis of parent and offspring treatment effects on parent and offspring phenotypes

All analyses were conducted in R v4.2.3 [57]. Linear mixed effects models using the “lmer” function within the *lme4* package [58] were used to analyse the effect of parental environment (feeding frequency and temperature) on parent body condition. The effect of feeding frequency (low/high), temperature (24 °C/30 °C), and their interaction on body condition was tested, as well as the random effect of “Tank ID” within block (three per

treatment). We also used linear mixed effects models to analyse the effect of parental condition (low/high feeding frequency), parental temperature (24 °C/30 °C), offspring temperature (24 °C/30 °C), and all interactions, on offspring phenotypes. The significance of parent "Tank ID" as a random effect was tested for all responses. We focussed on four key offspring traits: 1) embryo mass (parental investment), 2) metabolic rates (MR_{embryo}, MR_{hatch}, and MR_{larva}), 3) development time (time from fertilisation until hatching), and 4) growth rate (length at two weeks post hatching divided by length at hatching). All candidate models for offspring responses are provided in Table S2S2. Embryo mass (μg) was included as a covariate in metabolic rate and development time models (m2 and m3; Table S2). We used embryo mass since we only had estimates of length and area for larvae at hatching and one week post-hatching and have previously shown this to be an important indicator for hatch and larval mass [59]. We used Akaike Information Criteria (AIC) for model ranking and averaged models with Δ conditional AIC (AICc) <22 using the R package *MuMin* [60–62] (Table S3), and the estimated marginal means from the best-fitting model were used for all post-hoc comparisons using the *emmeans* package [63]]

Correlations between developmental and growth rates with metabolic rates

To explore within-individual associations among measures of developmental and growth rates with metabolic rates, we ran repeated measures correlations using the package *rmcorr* [64]. Using a repeated measures framework accounts for the non-independence of observations measured on the same individuals.To explore within-individual associations among measures of developmental and growth rates with metabolic rates, we ran repeated measures correlations using the package *rmcorr* [64]. Using a repeated measures framework accounts for the non-independence of observations measured on the same individuals.

Selection analysis

We used a classic multiple regression approach derived from evolutionary theory to characterise temperature-dependent selection acting on embryo metabolic rates, within each parental environment [42]. This framework allows for standardised and comparable estimates of both linear (β) and nonlinear (γ) selection coefficients. For each form, we estimated the direction (sign of coefficients), and strength (magnitude of coefficients) of selection acting on offspring mass and mass-independent metabolic rate (MIMR),), across incubation temperatures, as per [42]. These measures have been used previously to provide a more complete picture of the adaptive landscape for offspring metabolic rates [9].

293 Fitness was measured as survival from fertilisation to two weeks post-hatching. This period
294 of life typically shows greatest mortality rates in egg-laying fish and is considered a
295 bottleneck to reproduction, and therefore fitness [65]. Survival was treated as binary data –
296 offspring that survived to two weeks post hatching were assigned “1”, whereas offspring that
297 died before two weeks were assigned “0”. First, autocorrelation between traits was checked
298 to determine which traits should be included in the analysis. Metabolic rates at each
299 ontogenetic stage were significantly correlated (when embryo mass was included as a
300 covariate; $F_{3,1724} = 3434$, $p < 0.0001$), particularly between MR_{embryo}, and MR_{hatch} ($r^2 = 0.71$).
301 We decided to use mass-independent metabolic rate (MIMR) since recent work has shown
302 that including both mass and metabolic rate in selection analyses can overestimate the
303 strength of selection on metabolic rates (Cameron and Marshall, In Review). Correlations
304 between embryo mass, $MIMR_{embryo}$, and $MIMR_{larva}$ were relatively weak and variance inflation
305 factors were less than 5, hence both $MIMR_{embryo}$, and $MIMR_{larva}$ were included, but $MIMR_{hatch}$
306 was excluded from the analysis. To prepare data for selection analysis, we followed the
307 method of [42]: first, within each combination of parent and offspring treatment, we
308 converted predictor variables of embryo mass, $MIMR_{embryo}$, and $MIMR_{larva}$ into units of
309 standard deviation (mean of 0, standard deviation of 1), and divided each measure of
310 absolute fitness by mean absolute fitness to mean-centre survival.
311
312 Survival data were fitted using logistic regression in a generalised linear model using the
313 “glm” function. We ran a series of nested models to test for differences in linear and
314 nonlinear forms of selection. We first tested whether there were significant differences in
315 selection among parental and offspring environments, via a sequential model fitting method
316 [66,67]. We then tested for significant interactions between selection (linear and nonlinear)
317 and environment (parental condition, parental temperature, and offspring temperature).
318 Since we only found significant interactions between selection and offspring temperature,
319 fitness data were mean-centred (see details above) within offspring temperature, and
320 selection coefficients were estimated for offspring incubated at 24 °C and 30 °C separately.
321 Selection coefficients from the logistic regression were transformed into linear estimates as
322 per [68]. Following [69], we doubled quadratic regression coefficients and their standard
323 errors before reporting selection gradients.
324

Results

Effects of parental environment on parent body condition and offspring size

Parents in the low feed treatment showed significantly lower body condition than individuals within the high feed treatment ($t = -6.44$, $df = 7.34$, $p < 0.001$), however, there were no differences in condition between high and low temperature treatments (Figure S1). Despite low body condition, parents held under the low feed frequency regime produced embryos that were heavier than those from high-condition parents (Table 1). Although there appeared to be a trend for heavier offspring from cool-reared parents (Figure 2A), there was no significant effect of parent temperature on embryo mass (Table 1).Table 1).

Effects of parental and offspring environments on offspring metabolic rates

Offspring reared at the high temperature treatment showed significantly higher metabolic rates than those reared at the low temperature treatment (Table 1, Figure 2B). We also found significant parental environment effects on offspring metabolic rates (Table 1, Figure 2B). Parents exposed to the high temperature or low food treatments produced offspring with lower metabolic rates at embryo, hatch, and larval stages. We also found a significant interaction between embryo mass and parent feed frequency, where the slope between embryo metabolic rate and mass was steeper in offspring from low-feed frequency parents (Table 1, Figure 3).

Effects of parental and offspring environments on offspring developmental and growth rates

Offspring incubated at the low temperature took almost twice as long to develop than those incubated at the warm temperature (Table 1, Figure 2C). More interestingly, development time at a given offspring temperature was affected by the parental temperature as well as the parental feed frequency, being extended in offspring from low-feed frequency or high-temperature parents; thus hatching was delayed by 9h on average when offspring reared at the cool temperature came from low feed compared with high feed parents (Table S1, Figure 2C). We also found significant interactive effects between offspring temperature and parent feed treatment, and between offspring temperature and parent temperature on development time. High food treatment parents produced offspring that developed faster when reared at the low offspring temperature, but not high offspring temperature treatment (Table 1, Figure 2C, $t = -2.34$, $p = 0.088$). Embryos developing at the cool treatment developed faster when their parents were also from the cool temperature, relative to parents from the warm temperature (Table 1, Figure 2C, $t = -0.90$, $p = 0.807$).

Larval growth rates during the second week post-hatching were faster in offspring from high-feed parents (Table 1, Figure 2D). We also found a significant three-way interaction between

362 parental temperature, parent condition, and offspring temperature for larval growth rate:
363 growth was slowest in offspring from the low offspring temperature treatment and when
364 parents were from both low feeding frequency and from the low temperature (Table 1).
365
366 *Correlations between offspring traits*
367 We found significant positive correlations between all metabolic rates (embryo, hatch, larval),
368 and between larval growth rates and these three metabolic rates (Figure 4). In contrast,
369 embryo development time was significantly negatively correlated with metabolic rates. We
370 found no significant correlation between embryo development time and larval growth rate
371 overall, however they were significantly negatively correlated within offspring temperature
372 treatments (Figure S3).
373
374 *Effects of parental environment on offspring survival*
375 Overall, we found that survival was lowest in offspring from parents in the low feed frequency
376 regime, but offspring and parent temperatures showed no effect on offspring survival to two
377 weeks post hatching (Table 1). Although parents in the low feed treatment produced larger
378 offspring, embryo mass did not itself predict survival.
379
380 *Selection on offspring metabolic rates*
381 Offspring from low-food parents showed greater survival when they had relatively low
382 embryo metabolic rates, as shown by significant negative directional selection (Table 2,
383 Figure 5E-H). Across all offspring high-temperature treatments, we found evidence for
384 negative directional selection on embryo metabolic rates (Table 2, Figure 5B,D,F,H). We
385 also found positive directional selection on offspring embryo mass when they were reared at
386 the low temperature from high-feed parents (P24HO24 and P30HO24; Figure 5A and 5C) or
387 they were reared at the high temperature but from high-feed and low temperature parents
388 (P24HO30; Figure 5B). Conversely, we found negative directional selection on embryo mass
389 when offspring originated from parents reared at the high temperature and low food
390 treatments (P30LO24 and P30LO30; Figure 5G-H). There was also evidence for stabilising
391 selection on embryo metabolic rate in P30LO24 (Figure 5G), as shown by a significant
392 negative quadratic coefficient (Table 2). We found no significant directional selection on
393 larval metabolic rates, however there was significant positive correlational selection for
394 embryo and larva metabolic rates in P24LO30 (Table 2, Figure 5F), suggesting that
395 consistently lower metabolic rates were favoured in this environment..

Discussion

Intergenerational effects can be an important source of offspring phenotypic variation – here we provide evidence of adaptive intergenerational plasticity for offspring metabolic rates. We found that low parental food availability negatively impacted offspring survival, but also altered offspring metabolic phenotypes in a direction that aligned with selection on offspring traits. The low feeding frequency treatment in our study produced low condition parents that invested in larger offspring, compared with parents from the high feeding frequency treatment. We also found that when parents were reared under either the warm (30 ºC) temperature, low feeding frequency treatment, or both, they produced offspring with lower metabolic rates. Warm developmental temperatures generally increase the metabolic rates of offspring; however, we show that at these temperatures selection acts to decrease offspring metabolism, and that parents modify their offspring accordingly.

Parental condition and offspring temperature increased selection on offspring metabolism

Overall, we found that low parental food levels increased the presence and strength of negative selection acting on embryo metabolic rate (MR_{embryo}), such that offspring with lower MR_{embryo} were more likely to survive a critical period of early development (compare Fig. 4 E-H with A-D). Previous work has clearly demonstrated the direct effects that environmental temperature and food availability produce on metabolic rates [21,24,70,71]. Acute effects of warming generally increase metabolic rates in ectotherms, yet acclimatisation or adaptation can act to suppress energy expenditure [72,73]. Similarly, low food availability often selects for reduced metabolic rates [74], presumably to conserve energy reserves. Further, temperature and food availability can interact to affect metabolism in complex ways, with evidence for temperature mediating both an increase and decrease in metabolism with increases in food availability [75–77].

Intergenerational plasticity is adaptive when environments are consistent across generations

We found similar patterns between intergenerational plasticity and selection on offspring metabolic rates when parent and offspring temperatures matched. Parents reared under the warm temperature treatment produced offspring with lower metabolic rates, which were more likely to survive than warm-reared offspring from cool-reared parents. Consequently, offspring with slower metabolic rates showed greater survival in warm developmental temperatures, particularly when they originated from parents from the low food treatment. The downregulation of offspring metabolism is likely to be particularly crucial when food availability is low, where offspring are more likely to be reliant on internal energy reserves to fuel early life growth, maintenance, and development. The alignment of intergenerational plasticity and selection provides evidence that shifts in offspring metabolic phenotypes can

be adaptive when the environment in the parent generation matches that of the offspring generation. This has often been assumed in studies measuring performance metrics, such as growth or aerobic capacity, which may trade off with actual fitness [31,39,78]. Through use of a selection analysis, our study provides standardised, comparable estimates of selection, showing that parents can program their offspring with metabolic phenotypes that enhance early life survival. Our findings, however, have worrying implications for environmental mismatches between generations. We acknowledge that ourour study was conducted on zebrafish reared under stable lab conditions, and thatthat wild-caught fish or other taxa may respond differently [79]. However, underHowever, under increasingly warmer and more variable climates, parents may not be able to keep pace with provisioning their offspring to enhance survival during a vulnerable life stage, and there may be increasing reliance on thermal acclimation to buffer populations to environmental change.

Potential proximal mechanisms underlying intergenerational effects on offspring metabolism

Metabolic suppression as a means to conserve energy has been well documented, yet intergenerational mechanisms are less well explored [24,72,73]. Across generations, epigenetic mechanisms such as changes in DNA methylation can facilitate developmental thermal plasticity to buffer offspring from stressful temperatures [80–82]. One clear mechanism by which parents may alter the transgenerational thermal sensitivity of offspring metabolic and life-history traits is through changes in the density and efficiency of mitochondria [46]. Fasting and warm temperature regimes can enhance mitochondrial efficiency, such that a greater amount of ATP is produced per amount of oxygen consumed [49]. For species that provision their offspring with finite energy reserves in eggs, energy-demanding warm temperatures may elicit an adaptive response in parents to produce energy-efficient offspring. It may be that parents can program their offspring with more efficient mitochondria to compensate for a predicted energetically costly environment as reflected by lower metabolic rates [46]. We found that metabolism until two weeks post-hatching was unrelated to growth rates, supporting previous work that these two rates can be decoupled and that low metabolic rates do not necessitate slow growth rates because it is mitochondrial efficiency rather than metabolic rate per se that determines availability of ATP for growth [83]. While fitness benefits of reduced metabolism were observed within this study, trade-offs with such as oxidative stress may manifest later in life, affecting fitness-enhancing processes [84]. While our study did not detect any negative consequences of metabolic suppression for early life survival in zebrafish, previous work has shown that slow metabolic phenotypes possess lower competitive ability, compared with fast metabolic phenotypes [9]. What is needed now is to go beyond measures of oxygen consumption to

investigate the capacity for parents to alter the efficiency of ATP production in their offspring and mediate fitness under warmer and more nutrient poor environments.

The presence and form of selection on metabolism varied across ontogeny

Despite clear evidence for selection on metabolic rate during embryonic development, we found that, across all environments, directional selection on larval metabolic rate (MR_{larva}) was absent. A recent meta-analysis showed limited evidence for selection on metabolic rate, where the majority of selection coefficients were measured during the adult life stage [85]. Variation or flexibility in metabolic rate may confer a fitness advantage, particularly under selection regimes that change across time and space [24]. Metabolic rate is not a single trait, hence metabolic rates expressed at particular life stages may also affect fitness [12,22]. Metabolic rates may be repeatable, such that they are correlated across the life history, yet we found differences in selection on metabolic rates measured one week apart. In our study, we fed hatched larvae ad libitum, which may have relaxed selection on larval traits. Alternatively, it may be that there are fitness consequences for a low or high larval metabolic rate that were not measured in this study. Survival is a key component, but not an absolute measure of fitness, and further measures are needed of both metabolism across ontogeny and lifetime reproductive output. We did, however observe negative correlational selection on embryo mass and $MR_{larva,}$ in offspring reared at the cool (24 ºC) temperature, from high condition parents also reared at 24 ºC. Offspring mortality was greatest in smaller embryos with relatively high MR_{larva}, possibly because the reduced endogenous energy reserves often attributed to smaller offspring were insufficient to sustain higher metabolism in the larval stage. Variation in the strength, form, and direction of selection on combinations of early life traits across environments reveals the diversity of adaptive landscapes that organisms may enter, and the challenges that parents face when matching offspring phenotype to enhance performance within a given environment.

Potential indirect selection on developmental and growth rates

We found that metabolic rates measured from the embryo stage through to one week post hatching were consistently negatively correlated with development time and positively correlated with growth rate, but that developmental and growth rates were onlyonly correlated within offspring temperature treatments. Pace-of-life theory proposes that natural selection should favour the integration of a suite of life-history and metabolic traits that together enhance fitness [86]. In our study, warm and low condition parents produced offspring with lowerPace-of-life theory proposes that natural selection should favour the integration of a suite of life-history and metabolic traits that together enhance fitness [86]. In our study, warm and low condition parents produced offspring with lower metabolic rates,

with evidence for a slower pace-of-life, including extended development time and reduced growth rates. Potential mechanisms underlying this response from parents include epigenetic modification such as DNA methylation in gametes or early developmental stages, or genetic constraints. Our finding that selection acts to reduce embryo metabolic rate in the warm offspring treatment may inadvertently also act to reduce the pace of life if these traits are both phenotypically and genetically, correlated. There is evidence however, that pace-of-life traits can be decoupled, whereby growth and developmental rates, for example, can evolve independently [87]. Further measures of multivariate selection will help to disentangle the underlying drivers of correlated traits related to the pace of life [88]

Conclusions

Our study shows the importance of intergenerational plasticity as a source of variation in metabolic rates during early life stages. When parent and offspring environments match, parents can program offspring to express metabolic phenotypes that align with selection on embryonic metabolic rate. Offspring with lower metabolic rates showed greater survival when reared under warm temperatures, and this response was particularly evident when offspring originated from low condition parents. Our findings support previous evidence that the unpredictability of offspring environment may in part explain why anticipatory parental effects are not always, or only weakly, observed. However, identifying the mechanistic basis of parental effects on variation in metabolic rate is an important next step.

Table 1. Output from best fitting linear mixed effects models. Estimates provided for fixed effects of Parent ('P') temperature (24 °C or 30 °C), parent feeding frequency (High; H or Low; L) and offspring ('O') temperature (24 °C or 30 °C) on offspring phenotypes: 1) Embryo mass, 2) Metabolic rates (a. MR_{embryo}, b. MR_{hatch}, c. MR_{larva}), 3) Development time, 4) Growth rate, and 5) Survival to two weeks post hatching. For survival, logistic generalised linear mixed effect regression was used and individuals were assigned either "1" for alive at two weeks post hatching or "0" for dead. Parental Tank ID was included as a random effect in all models. All candidate models are provided in Table S2 and ranked in Table S3). All comparisons are made in relation to 'L' parent feed frequency and 30 °C parent and offspring temperature. Significance level set at p <0.05.

Predictors	Estimate	SE	df	t-value	p-value
1. Embryo mass					
Intercept	56.70	1.49	15.03	38.12	<0.0001***
P feed (L)	8.45	1.06	298.95	7.98	<0.0001***
P temperature (30)	0.18	1.26	233.14	0.14	0.89
P feed (L) × P temperature (30)	0.37	1.16	367.29	0.25	0.81
2a. Log_{10} MR_{embryo}					
Intercept	-4.27	0.08	184.05	-56.04	<0.0001***
Log_{10} Embryo mass	0.69	0.04	174.21	16.02	<0.0001***
P feed (L)	-1.01	0.11	340.08	-9.30	<0.0001***
O temperature (30)	0.06	0.00	505.68	25.81	<0.0001***
P temperature (30)	-0.03	0.00	13.88	-6.02	<0.0001***
Log_{10} Embryo mass × P feed (L)	0.51	0.06	333.03	8.27	<0.0001***
2b. Log_{10} MR_{hatch}					
Intercept	-4.00	0.13	105.98	-30.44	<0.0001***
Log_{10} Embryo mass	0.72	0.07	103.55	9.79	<0.0001***
O temperature (30)	0.07	0.00	442.76	17.79	<0.0001***
P feed (L)	-0.78	0.20	181.16	-3.93	<0.001**
P temperature (30)	-0.01	0.01	12.80	-2.48	0.03
Log_{10} Embryo mass x P feed (L)	0.38	0.11	179.19	3.43	<0.001***
2c. Log_{10} MR_{larva}					
Intercept	-3.19	0.14	198.41	-22.60	<0.0001***
Log_{10} Embryo mass	0.35	0.08	187.11	4.38	<0.0001***
O temperature (30)	0.03	0.01	370.10	6.09	<0.0001***
P feed (L)	-0.06	0.01	14.94	-8.31	<0.0001***
P temperature (30)	-0.02	0.01	9.29	-2.75	0.02*
3. Development time					
Intercept	110.66	0.96	24.89	115.63	<0.0001***
O temperature (30)	-48.77	0.82	564.05	-59.61	<0.0001***
P feed (L)	6.79	1.17	76.00	5.79	<0.0001***
P temperature (30)	6.24	1.24	55.30	5.03	<0.0001***
O temperature (30) × P feed (L)	-5.72	1.19	548.31	-4.80	<0.0001***
O temperature (30) × P temperature (30)	-6.49	1.16	565.52	-5.59	<0.0001***
P feed (L) × P temperature (30)	0.65	1.54	124.97	0.42	0.68
O temperature (30) × P feed (L) × P temperature (30)	2.40	1.81	486.64	1.32	0.19
4. Growth rate					
Intercept	89.78	0.65	460	138.73	<0.0001***
O temperature (30)	-0.10	0.93	460	-0.10	0.92
P feed (L)	-3.04	1.01	460	-3.02	0.003**
P temperature (30)	0.57	0.95	460	0.60	0.55
O temperature (30) × P feed (L)	2.32	1.41	460	1.64	0.10
O temperature (30) × P temperature (30)	-0.12	1.37	460	-0.09	0.93

P feed (L) × P temperature (30)	1.37	1.43	460	0.96	0.34
O temperature (30) × P feed (L) × P temperature (30)	-4.50	2.03	460	-2.22	0.02*
5. Survival					
Intercept	1.08	0.20		5.39	<0.0001***
O temperature (30)	-0.104	0.21		-0.49	0.63
P feed (L)	-0.72	0.21		-3.42	<0.001***
P temperature (30)	-0.08	0.22		-0.37	0.71
O temperature (30) × P temperature (30)	0.16	0.35		0.48	0.66
P feed (L) × P temperature (30)	-0.14	0.36		-0.40	0.69
O temperature (30) × P feed (L	0.11	0.36		0.32	0.75

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Table 2. Selection coefficients (mean and standard error). Direction and strength of linear (β) and nonlinear (γ) selection on embryo mass and mass-independent metabolic rates across two life stages ($MIMR_{embryo}$ and $MIMR_{larva}$; μlO_2h^{-1}) in *Danio rerio*. Fitness was measured as survival to two weeks post hatching. Results shown for each combination of Parent ('P') temperature (24 °C or 30 °C), parent feeding frequency (High; H or Low; L) and offspring ('O') temperature (24 °C or 30 °C). Significant selection gradients ($p < 0.05$) shown in bold.

Parent environment	Offspring environment		β	γ		
				Embryo mass	$MIMR_{Embryo}$	$MIMR_{Larva}$
		Embryo mass	**0.134 (0.041)**	-0.053 (0.114)	0.010 (0.055)	-0.020 (0.043)
	O24	$MIMR_{Embryo}$	-0.061 (0.041)		-0.054 (0.092)	0.082 (0.048)
		$MIMR_{Larva}$	-0.013 (0.033)			0.011 (0.075)
P24H				Embryo mass	MR_{Embryo}	MR_{Larva}
		Embryo mass	**0.039 (0.036)**	-0.117 (0.105)	0.052 (0.057)	-0.030 (0.060)
	O30	$MIMR_{Embryo}$	**-0.157 (0.040)**		0.051 (0.113)	-0.084 (0.065)
		$MIMR_{Larva}$	-0.055 (0.035)			0.181 (0.095)
				Embryo mass	MR_{Embryo}	MR_{Larva}
		Embryo mass	**0.180 (0.056)**	0.371 (0.224)	0.040 (0.125)	0.004 (0.129)
	O24	$MIMR_{Embryo}$	0.052 (0.033)		0.002 (0.068)	0.056 (0.079)
		$MIMR_{Larva}$	-0.002 (0.041)			-0.179 (0.119)
				Embryo mass	MR_{Embryo}	MR_{Larva}
P30H		Embryo mass	-0.109 (0.089)	0.210 (0.215)	-0.165 (0.181)	0.181 (0.258)
	O30	$MIMR_{Embryo}$	**-0.167 (0.074)**		-0.091 (0.074)	0.320 (0.218)
		$MIMR_{Larva}$	-0.021 (0.045)			0.100 (0.073)
				Embryo mass	MR_{Embryo}	MR_{Larva}
		Embryo mass	-0.092 (0.070)	-0.106 (0.166)	-0.083 (0.124)	-0.160 (0.095)
	O24	MR_{Embryo}	**-0.122 (0.047)**		0.346 (0.281)	0.157 (0.138)
		MR_{Larva}	0.046 (0.059)			0.090 (0.159)
P24L				Embryo mass	MR_{Embryo}	MR_{Larva}
		Embryo mass	-0.200 (0.057)	-0.343 (0.270)	-0.329 (0.222)	0.043 (0.099)
	O30	MR_{Embryo}	**-0.070 (0.035)**		0.329 (0.320)	**0.297 (0.149)**
		MR_{Larva}	-0.050 (0.037)			-0.060 (0.093)
				Embryo mass	MR_{Embryo}	MR_{Larva}
		Embryo mass	**-0.122 (0.056)**	-0.039 (0.113)	0.038 (0.095)	0.077 (0.086)
	O24	MR_{Embryo}	**-0.161 (0.054)**		**-0.353 (0.138)**	-0.038 (0.093)
		MR_{Larva}	-0.017 (0.038)			-0.051 (0.081)
P30L				Embryo mass	MR_{Embryo}	MR_{Larva}
		Embryo mass	**-0.166 (0.056)**	0.218 (0.233)	0.171 (0.111)	-0.173 (0.125)
	O30	MR_{Embryo}	**-0.120 (0.042)**		-0.168 (0.104)	-0.008 (0.065)
		MR_{Larva}	0.022 (0.030)			0.144 (0.120)

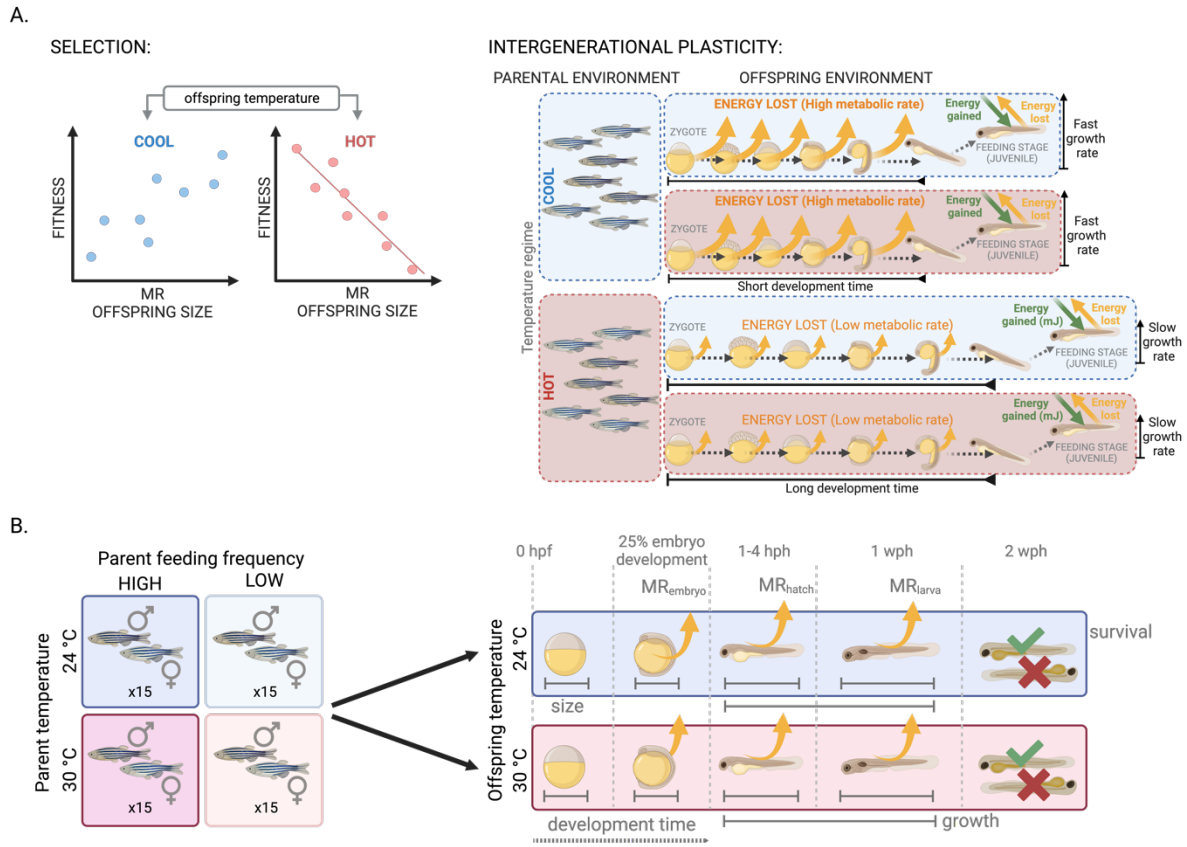


Figure 1. A. Conceptual diagram: predicted responses of temperature-dependent selection on and intergenerational plasticity of embryo metabolic rates at cool and hot offspring temperatures. We hypothesise that hot offspring temperatures will select for lower metabolic rates, while selection at the cool offspring temperature will be relatively relaxed (positive but not significant correlation between fitness and metabolic rate shown). If intergenerational plasticity aligns with selection when environments across generations match, then similar trends in the direction and strength of selection should be observed. We therefore predict that parents in the warm environment (pink) will produce offspring with lower metabolic rates (smaller curved arrows) compared to parents from the cool temperature (blue), and that this will be correlated with development time and growth rates, with fitness benefits for offspring. **B. Experimental design**: parents were held under one of four treatment combinations: 24 °C or 30 °C and low or high feeding frequency then bred to produce offspring that were reared at either 24 °C or 30 °C. Embryo size (diameter, area, mass) and yolk area were measured at 1-4 hours post fertilisation (hpf), and metabolic rates (measured as rate of oxygen consumption) measured at three stages: 25% of embryonic development (MR_{embryo}), 1-4 hours post hatching (hph; MR_{hatch}), and 1 week post-hatching (1wph; MR_{larva}). Offspring were then monitored for survival up to two weeks post hatching.

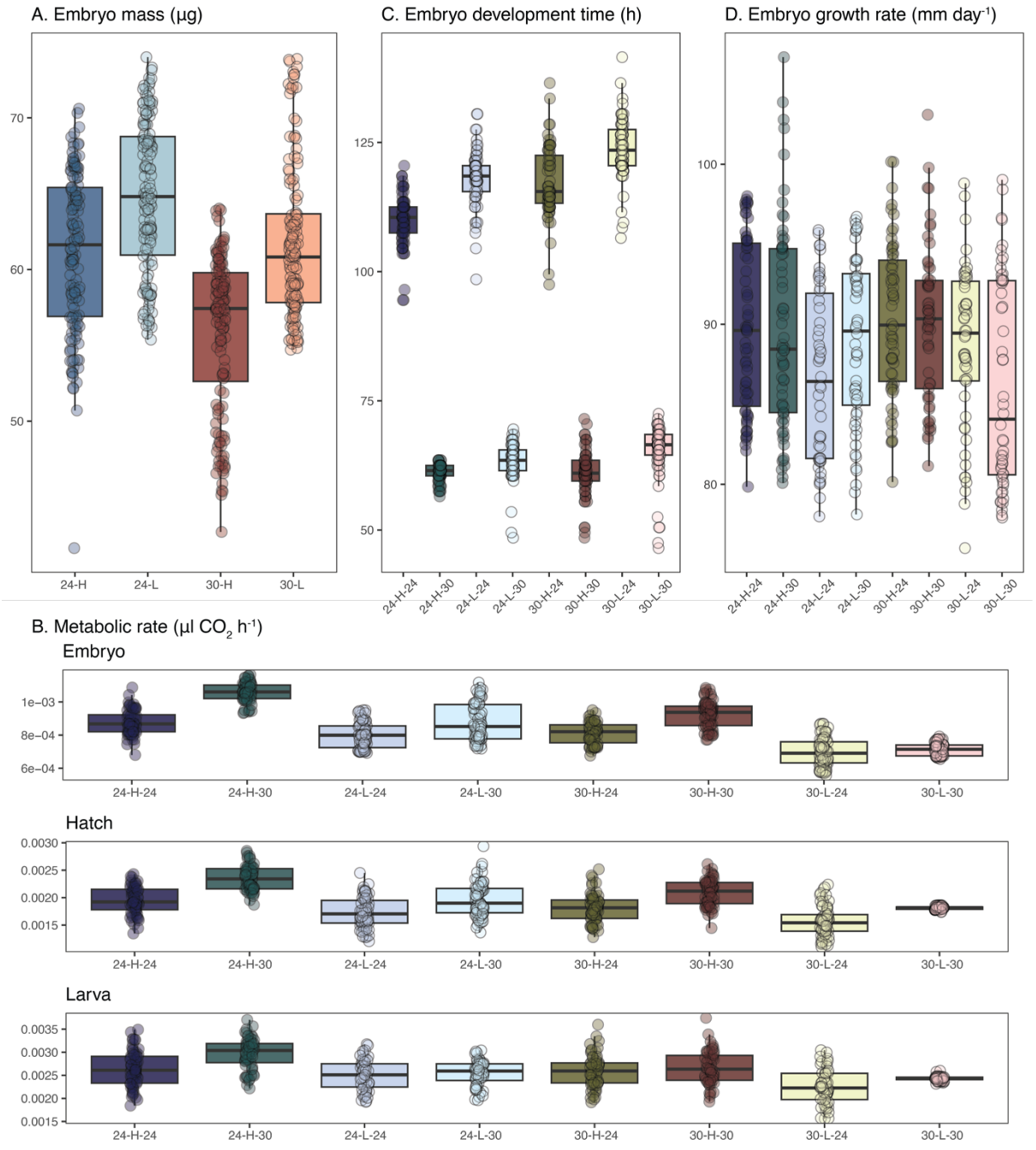


Figure 2. Offspring phenotypes in response to parent and offspring treatments. Responses of A. Embryo mass, B. Metabolic rate (MR_{embryo}, MR_{hatch}, MR_{larva}), C. Development time, and D. Growth rate, measured across combinations of parent temperature (24 °C or 30 °C), feeding frequency (Low; 'L' or High; 'H'), and offspring temperature (24 °C or 30 °C). First number in treatment description refers to parent temperature and the second refers to offspring temperature. Note that metabolic rates shown in panel B do not account for the significant effect of embryo mass.

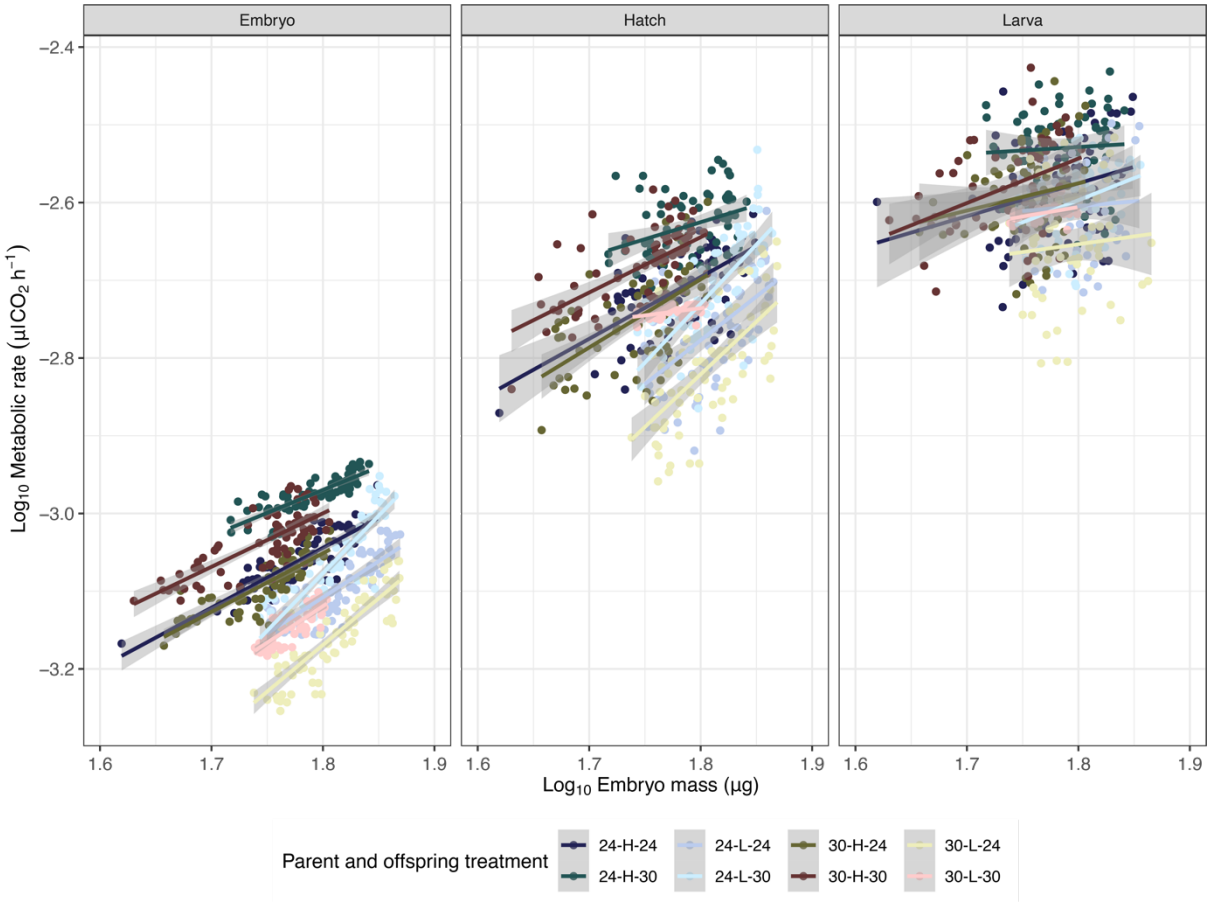
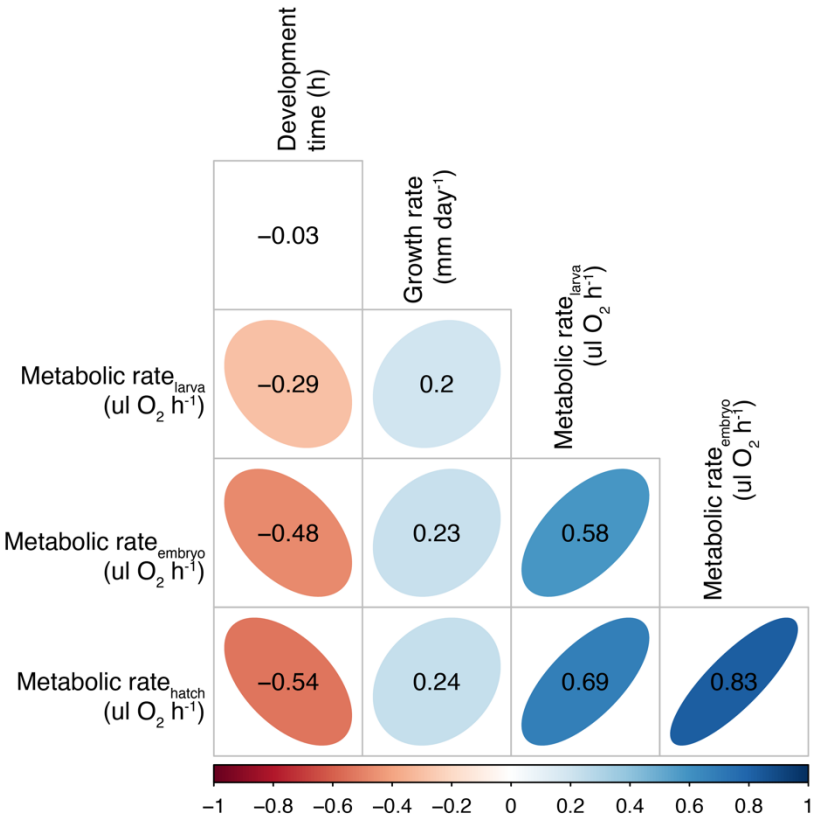


Figure 3. The relationship between offspring metabolic rates (Log_{10} MR_{embryo}, Log_{10} MR_{hatch}, Log_{10} MR_{larva}) and Log_{10} Embryo mass in response to parent and offspring treatments. Coloured data points and lines reflect parent temperature (24 °C or 30 °C), feeding frequency (Low; 'L' or High; 'H'), and offspring temperature (24 °C or 30 °C). Grey bars are standard error. First number in treatment description refers to parent temperature and the second refers to offspring temperature.



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584 **Figure 4. Correlation plots for offspring phenotypes**. Pairwise correlations between
585 offspring traits: metabolic rates (MR_{embryo}, MR_{hatch}, MR_{larva}), embryo development time, and
586 larval growth rate, across combinations of parent and offspring treatments. Coloured plots
587 represent significant correlations between traits.

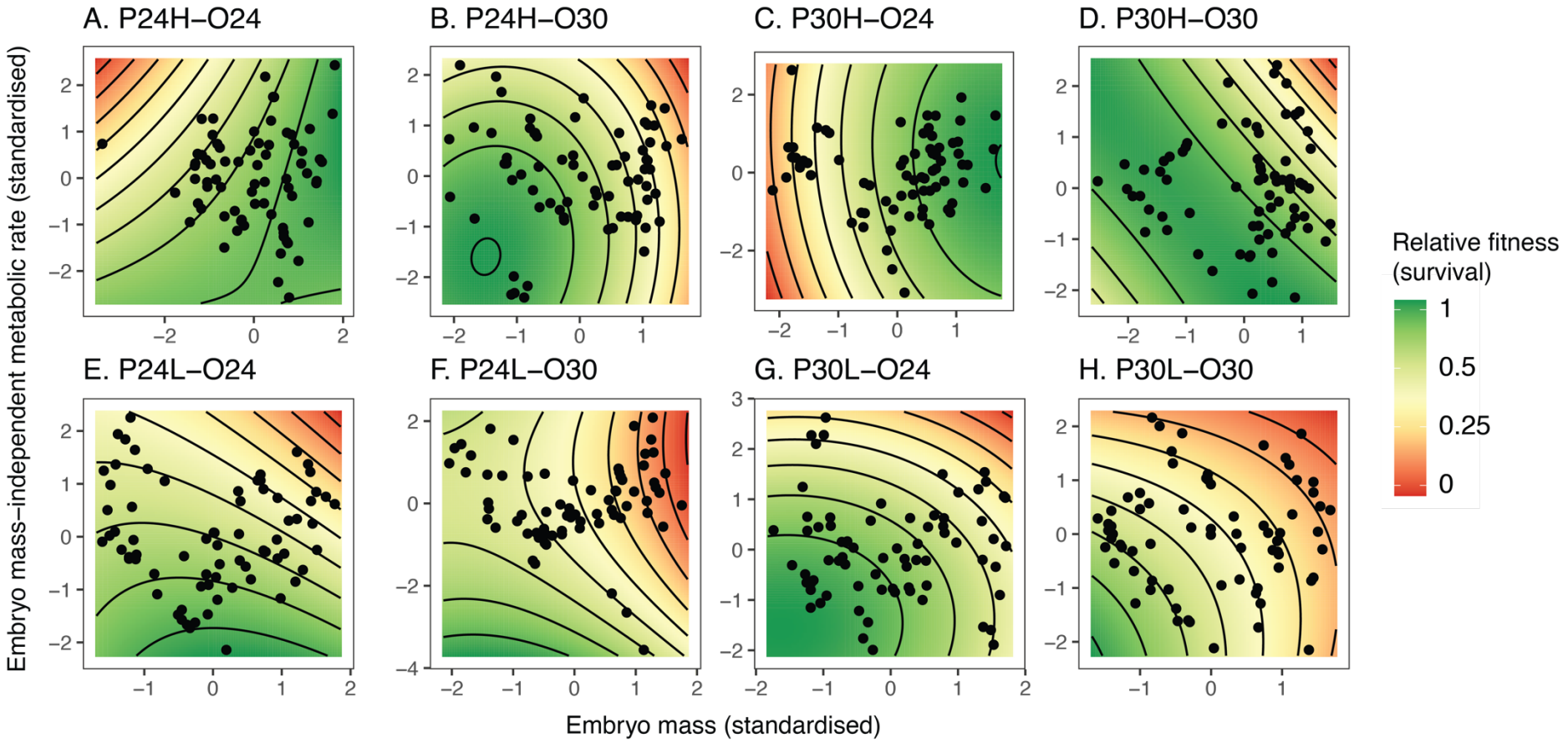

Figure 5. Selection surface plots. Selection on embryo mass (μg) and metabolic rate (MR_{embryo}; μlO_2h^{-1}) across combinations of parent (P) temperature (24 °C or 30 °C) and feeding frequency (high; H or low; L) and offspring (O) temperature (24 °C or 30 °C) environments.

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Competing interests

The authors have no competing interests to declare.

Data accessibility statement

All data and code have been made publicly available for peer review on the Open Science Framework: https://osf.io/6357s/?view_only=9c6e1ac841fb4e6188fd297aeeaa2733.

References

1. Auer SK, Dick CA, Metcalfe NB, Reznick DN. 2018 Metabolic rate evolves rapidly and in parallel with the pace of life history. *Nature Communications* **9**, 14. (doi:10.1038/s41467-017-02514-z)

2. White CR, Alton LA, Bywater CL, Lombardi EJ, Marshall DJ. 2022 Metabolic scaling is the product of life-history optimization. *Science* **377**, 834–839. (doi:10.1126/science.abm7649)

3. Diaz-Cuadros M *et al.* 2023 Metabolic regulation of species-specific developmental rates. *Nature* **613**, 550–557. (doi:10.1038/s41586-022-05574-4)

4. Pettersen AK, White CR, Marshall DJ. 2016 Metabolic rate covaries with fitness and the pace of the life history in the field. *Proceedings of the Royal Society B: Biological Sciences* **283**, 20160323. (doi:10.1098/rspb.2016.0323)

5. Deeming DC, Ferguson MWJ, editors. 1991 *Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles*. Cambridge: Cambridge University Press. (doi:10.1017/CBO9780511585739)

6. Pettersen AK, Nord A, While G, Uller T. In Press. How do fluctuating temperatures alter the cost of development? *Functional Ecology*.

7. Pettersen AK, Schuster L, Metcalfe NB. 2022 The Evolution of Offspring Size: A Metabolic Scaling Perspective. *Integrative and Comparative Biology* , icac076. (doi:10.1093/icb/icac076)

8. Tüzün N, Stoks R. 2022 A fast pace-of-life is traded off against a high thermal performance. *Proceedings of the Royal Society B: Biological Sciences* **289**, 20212414. (doi:10.1098/rspb.2021.2414)

9. Pettersen AK, Hall MD, White CR, Marshall DJ. 2020 Metabolic rate, context-dependent selection, and the competition-colonization trade-off. *Evolution Letters* **4**, 333–344. (doi:10.1002/evl3.174)

10. Pettersen AK, Metcalfe NB. In Review Consequences of the cost of living: is variation in metabolic rate evolutionary significant? *Philosophical Transactions of the Royal Society B*

11. Lighton JRB. 2008 A Brief History of Metabolic Measurement. In *Measuring Metabolic Rates: A Manual for Scientists* (ed JRB Lighton), p. 0. Oxford University Press. (doi:10.1093/acprof:oso/9780195310610.003.0001)

12. Metcalfe NB *et al.* 2023 Solving the conundrum of intra-specific variation in metabolic rate: A multidisciplinary conceptual and methodological toolkit. *BioEssays* **45**, 2300026. (doi:10.1002/bies.202300026)

13. Artacho P, Nespolo RF. 2009 Natural Selection Reduces Energy Metabolism in the Garden Snail, Helix Aspersa (cornu Aspersum). *Evolution* **63**, 1044–1050. (doi:10.1111/j.1558-5646.2008.00603.x)

14. Boratyński Z, Koteja P. 2010 Sexual and natural selection on body mass and metabolic rates in free-living bank voles. *Functional Ecology* **24**, 1252–1261. (doi:10.1111/j.1365-2435.2010.01764.x)

15. Careau V, Bergeron P, Garant D, Réale D, Speakman JR, Humphries MM. 2013 The energetic and survival costs of growth in free-ranging chipmunks. *Oecologia* **171**, 11–23. (doi:10.1007/s00442-012-2385-x)

16. White CR, Kearney MR. 2013 Determinants of inter-specific variation in basal metabolic rate. *Journal of Comparative Physiology B* **183**, 1–26. (doi:10.1007/s00360-012-0676-5)

17. Mattila ALK, Hanski I. 2014 Heritability of flight and resting metabolic rates in the Glanville fritillary butterfly. *Journal of Evolutionary Biology* **27**, 1733–1743. (doi:10.1111/jeb.12426)

18. McFarlane SE, Ålund M, Sirkiä PM, Qvarnström A. 2021 Low Heritability but Significant Early Environmental Effects on Resting Metabolic Rate in a Wild Passerine. *The American Naturalist* **198**, 551–560. (doi:10.1086/715842)

19. Nespolo RF, Franco M. 2007 Whole-animal metabolic rate is a repeatable trait: a meta-analysis. *Journal of Experimental Biology* **210**, 2000–2005. (doi:10.1242/jeb.02780)

20. Auer SK, Bassar RD, Salin K, Metcalfe NB. 2016 Repeatability of metabolic rate is lower for animals living under field versus laboratory conditions. *Journal of Experimental Biology* **219**, 631–634. (doi:10.1242/jeb.133678)

21. Burton T, Killen SS, Armstrong JD, Metcalfe NB. 2011 What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? *Proceedings of the Royal Society B: Biological Sciences* **278**, 3465–3473.

22. Pettersen AK, Marshall DJ, White CR. 2018 Understanding variation in metabolic rate. *Journal of Experimental Biology* **221**, jeb166876. (doi:10.1242/jeb.166876)

23. Seebacher F, White CR, Franklin CE. 2015 Physiological plasticity increases resilience of ectothermic animals to climate change. *Nature Climate Change* **5**, 61–66. (doi:10.1038/nclimate2457)

24. Auer SK, Salin K, Rudolf AM, Anderson GJ, Metcalfe NB. 2015 Flexibility in metabolic rate confers a growth advantage under changing food availability. *Journal of Animal Ecology* **84**, 1405–1411. (doi:10.1111/1365-2656.12384)

25. Norin T, Metcalfe NB. 2019 Ecological and evolutionary consequences of metabolic rate plasticity in response to environmental change. *Philosophical Transactions of the Royal Society B: Biological Sciences* **374**, 20180180. (doi:10.1098/rstb.2018.0180)

26. Marshall DustinJ, Uller T. 2007 When is a maternal effect adaptive? *Oikos* **116**, 1957–1963. (doi:10.1111/j.2007.0030-1299.16203.x)

687 27. O'Dea RE, Noble DWA, Johnson SL, Hesselson D, Nakagawa S. 2016 The
688 role of non-genetic inheritance in evolutionary rescue: epigenetic buffering,
689 heritable bet hedging and epigenetic traps. *Environmental Epigenetics* **2**, dvv014.
690 (doi:10.1093/eep/dvv014)

691 28. Guillaume AS, Monro K, Marshall DJ. 2016 Transgenerational plasticity and
692 environmental stress: do paternal effects act as a conduit or a buffer? *Functional*
693 *Ecology* **30**, 1175–1184.

694 29. Pettersen AK, White CR, Bryson-Richardson RJ, Marshall DJ. 2019 Linking
695 life-history theory and metabolic theory explains the offspring size-temperature
696 relationship. *Ecology Letters* **22**, 518–526. (doi:10.1111/ele.13213)

697 30. Bownds C, Wilson R, Marshall DJ. 2010 Why do colder mothers produce
698 larger eggs? An optimality approach. *Journal of Experimental Biology* **213**, 3796–
699 3801. (doi:10.1242/jeb.043356)

700 31. Burgess SC, Marshall DJ. 2011 Temperature-induced maternal effects and
701 environmental predictability. *Journal of Experimental Biology* **214**, 2329–2336.
702 (doi:10.1242/jeb.054718)

703 32. Simons AM. 2011 Modes of response to environmental change and the
704 elusive empirical evidence for bet hedging. *Proceedings of the Royal Society B:*
705 *Biological Sciences* **278**, 1601–1609. (doi:10.1098/rspb.2011.0176)

706 33. Uller T, Nakagawa S, English S. 2013 Weak evidence for anticipatory parental
707 effects in plants and animals. *Journal of Evolutionary Biology*. **26**, 2161–2170.
708 (doi:10.1111/jeb.12212)

709 34. Sánchez-Tójar A, Lagisz M, Moran NP, Nakagawa S, Noble DWA, Reinhold
710 K. 2020 The jury is still out regarding the generality of adaptive 'transgenerational'
711 effects. *Ecology Letters* **23**, 1715–1718. (doi:10.1111/ele.13479)

712 35. McGuigan K, Hoffmann AA, Sgrò CM. 2021 How is epigenetics predicted to
713 contribute to climate change adaptation? What evidence do we need?
714 *Philosophical Transactions of the Royal Society B: Biological Sciences* **376**,
715 20200119. (doi:10.1098/rstb.2020.0119)

716 36. Acasuso-Rivero C, Murren CJ, Schlichting CD, Steiner UK. 2019 Adaptive
717 phenotypic plasticity for life-history and less fitness-related traits. *Proceedings of*
718 *the Royal Society B: Biological Sciences* **286**, 20190653.
719 (doi:10.1098/rspb.2019.0653)

720 37. Moore MP, Whiteman HH, Martin RA. 2019 A mother's legacy: the strength of
721 maternal effects in animal populations. *Ecology Letters* **22**, 1620–1628.
722 (doi:10.1111/ele.13351)

723 38. Burgess SC, Marshall DJ. 2014 Adaptive parental effects: the importance of
724 estimating environmental predictability and offspring fitness appropriately. *Oikos*
725 **123**, 769–776. (doi:10.1111/oik.01235)

726 39. Lind MI, Zwoinska MK, Andersson J, Carlsson H, Krieg T, Larva T, Maklakov
727 AA. 2020 Environmental variation mediates the evolution of anticipatory parental
728 effects. *Evolution Letters* **4**, 371–381. (doi:10.1002/evl3.177)

729 40. Falconer DS, Mackay TFC. 1996 *Introduction to Quantitative Genetics*.
730 Harlow: Pearson.

731 41. Baker BH, Sultan SE, Lopez-Ichikawa M, Waterman R. 2019
732 Transgenerational effects of parental light environment on progeny competitive
733 performance and lifetime fitness. *Philosophical Transactions of the Royal Society*
734 *London: Biological Sciences* **374**, 20180182. (doi:10.1098/rstb.2018.0182)

735 42. Lande R, Arnold SJ. 1983 The Measurement of Selection on Correlated
736 Characters. *Evolution* **37**, 1210–1226. (doi:10.2307/2408842)

737 43. Bonduriansky R, Crean AJ. 2018 What are parental condition-transfer effects
738 and how can they be detected? *Methods in Ecology and Evolution* **9**, 450–456.
739 (doi:10.1111/2041-210X.12848)

740 44. Yampolsky LY, Scheiner SM. 1996 Why Larger Offspring at Lower
741 Temperatures? A Demographic Approach. *The American Naturalist* **147**, 86–100.

742 45. Donelson JM, Munday PL, McCormick MI, Pitcher CR. 2012 Rapid
743 transgenerational acclimation of a tropical reef fish to climate change. *Nature*
744 *Climate Change* **2**, 30–32. (doi:10.1038/nclimate1323)

745 46. Shama LNS, Strobel A, Mark FC, Wegner KM. 2014 Transgenerational
746 plasticity in marine sticklebacks: maternal effects mediate impacts of a warming
747 ocean. *Functional Ecology* **28**, 1482–1493. (doi:10.1111/1365-2435.12280)

748 47. Kielland ØN, Bech C, Einum S. 2017 No evidence for thermal
749 transgenerational plasticity in metabolism when minimizing the potential for
750 confounding effects. *Proceedings of the Royal Society B: Biological Sciences* **284**,
751 20162494. (doi:10.1098/rspb.2016.2494)

752 48. Kossack ME, Draper BW. 2019 Genetic regulation of sex determination and
753 maintenance in zebrafish (Danio rerio). *Current Topics in Developmental Biology*
754 **134**, 119–149. (doi:10.1016/bs.ctdb.2019.02.004)

755 49. Le Roy A, Mazué GPF, Metcalfe NB, Seebacher F. 2021 Diet and
756 temperature modify the relationship between energy use and ATP production to
757 influence behavior in zebrafish (*Danio rerio*). *Ecology and Evolution* **11**, 9791–
758 9803. (doi:10.1002/ece3.7806)

759 50. Pettersen AK, White CR, Bryson-Richardson RJ, Marshall DJ. 2018 Does the
760 cost of development scale allometrically with offspring size? *Functional Ecology*
761 **32**, 762–772. (doi:10.1111/1365-2435.13015)

762 51. Jahn M, Seebacher F. 2019 Cost of transport is a repeatable trait but is not
763 determined by mitochondrial efficiency in zebrafish (*Danio rerio*). *J Exp Biol* **222**,
764 jeb201400. (doi:10.1242/jeb.201400)

765 52. Clark TS, Pandolfo LM, Marshall CM, Mitra AK, Schech JM. 2018 Body
766 Condition Scoring for Adult Zebrafish (Danio rerio). *Journal American Association*
767 *for Laboratory Animal Science* **57**, 698–702. (doi:10.30802/AALAS-JAALAS-18-
768 000045)

769 53. Hurk R van den, Lambert JGD. 1983 Ovarian steroid glucuronides function as
770 sex pheromones for male zebrafish, Brachydanio rerio. *Canadian Journal of*
771 *Zoology.* **61**, 2381–2387. (doi:10.1139/z83-317)

772 54. Kawakami K, Patton EE, Orger M, editors. 2016 *Zebrafish: Methods and*
773 *Protocols*. New York, NY: Springer New York. (doi:10.1007/978-1-4939-3771-4)

774 55. Harianto J, Carey N, Byrne M. 2019 respR—An R package for the
775 manipulation and analysis of respirometry data. *Methods in Ecology and Evolution*
776 **10**, 912–920. (doi:10.1111/2041-210X.13162)

777 56. Cameron JN. 1986 The solubility of carbon dioxide as a function of
778 temperature and salinity (appendix table). In *Principles of physiological*
779 *measurement*, pp. 254–259. London, UK: Academic Press.

780 57. R Core Team. 2023 R: A language and environment for statistical computing.
781 R Foundation for Statistical Computing, Vienna, Austria.

782 58. Bates D, Mächler M, Bolker B, Walker S. 2015 Fitting Linear Mixed-Effects
783 Models Using lme4. *Journal of Statistical Software* **67**, 1–48.
784 (doi:10.18637/jss.v067.i01)

785 59. Pettersen AK, White CR, Marshall DJ. 2015 Why does offspring size affect
786 performance? Integrating metabolic scaling with life-history theory. *Proceedings of*
787 *the Royal Society B: Biological Sciences* **282**, 20151946.
788 (doi:10.1098/rspb.2015.1946)

789 60. Barton K. 2009 MuMIn : multi-model inference. *http://r-forge.r-*
790 *project.org/projects/mumin/*

791 61. Symonds MRE, Moussalli A. 2011 A brief guide to model selection,
792 multimodel inference and model averaging in behavioural ecology using Akaike's
793 information criterion. *Behavioural Ecological Sociobiology* **65**, 13–21.
794 (doi:10.1007/s00265-010-1037-6)

795 62. Sutherland C, Hare D, Johnson PJ, Linden DW, Montgomery RA, Droge E.
796 2023 Practical advice on variable selection and reporting using Akaike information
797 criterion. *Proceedings of the Royal Society B: Biological Sciences* **290**, 20231261.
798 (doi:10.1098/rspb.2023.1261)

799 63. Lenth RV. 2022 Estimated Marginal Means, aka Least-Squares Means.

800 64. Bakdash JZ, Marusich LR. 2017 Repeated Measures Correlation. *Frontiers of*
801 *Psychology* **8**, 456. (doi:10.3389/fpsyg.2017.00456)

802 65. Kamler E. 1992 *Early Life History of Fish: An Energetics Approach*. 1st edn.
803 Dordrecht: Springer. See https://books.google.se/books?id=rZAWAQAAIAAJ.

804 66. Draper NR, John JA. 1988 Response-Surface Designs for Quantitative and
805 Qualitative Variables. *Technometrics* **30**, 423–428. (doi:10.2307/1269805)

806 67. Chenoweth SF, Blows MW. 2005 Contrasting Mutual Sexual Selection on
807 Homologous Signal Traits in Drosophila serrata. *The American Naturalist* **165**,
808 281–289. (doi:10.1086/427271)

809 68. Janzen FJ, Stern HS. 1998 Logistic Regression for Empirical Studies of
810 Multivariate Selection. *Evolution* **52**, 1564–1571. (doi:10.1111/j.1558-
811 5646.1998.tb02237.x)

812 69. Stinchcombe JR, Agrawal AF, Hohenlohe PA, Arnold SJ, Blows MW. 2008
813 Estimating nonlinear selection gradients using quadratic regression coefficients:
814 double or nothing? *Evolution* **62**, 2435–2440. (doi:10.1111/j.1558-
815 5646.2008.00449.x)

816 70. Auer SK, Salin K, Anderson GJ, Metcalfe NB. 2018 Individuals exhibit
817 consistent differences in their metabolic rates across changing thermal conditions.
818 *Comparative Biochemistry and Physiology Part A: Molecular & Integrative*
819 *Physiology* **217**, 1–6. (doi:10.1016/j.cbpa.2017.11.021)

820 71. Jong GD, Scharloo W, Moed GHD. 1997 The Energetics of Growth in
821 Drosophila Melanogaster: Effect of Temperature and Food Conditions.
822 *Netherlands Journal of Zoology* **48**, 169–188. (doi:10.1163/156854298X00165)

823 72. Clarke A, Fraser KPP. 2004 Why does metabolism scale with temperature?
824 *Functional Ecology* **18**, 243–251. (doi:10.1111/j.0269-8463.2004.00841.x)

825 73. Moffett ER, Fryxell DC, Palkovacs EP, Kinnison MT, Simon KS. 2018 Local
826 adaptation reduces the metabolic cost of environmental warming. *Ecology* **99**,
827 2318–2326. (doi:10.1002/ecy.2463)

828 74. Bochdansky AB, Grønkjær P, Herra TP, Leggett WC. 2005 Experimental
829 evidence for selection against fish larvae with high metabolic rates in a food
830 limited environment. *Marine Biology* **147**, 1413–1417. (doi:10.1007/s00227-005-
831 0036-z)

832 75. Thoral E *et al.* 2023 Temperature-dependent metabolic consequences of food
833 deprivation in the European sardine. *Journal of Experimental Biology* **226**,
834 jeb244984. (doi:10.1242/jeb.244984)

835 76. Andersson MN, Nilsson J, Nilsson J-Å, Isaksson C. 2018 Diet and ambient
836 temperature interact to shape plasma fatty acid composition, basal metabolic rate
837 and oxidative stress in great tits. *Journal of Experimental Biology* **221**, jeb186759.
838 (doi:10.1242/jeb.186759)

839 77. Alton LA, Kutz TC, Bywater CL, Beaman JE, Arnold PA, Mirth CK, Sgrò CM,
840 White CR. 2020 Developmental nutrition modulates metabolic responses to
841 projected climate change. *Functional Ecology* **34**, 2488–2502. (doi:10.1111/1365-
842 2435.13663)

78. Chirgwin E, Marshall DJ, Sgrò CM, Monro K. 2018 How does parental environment influence the potential for adaptation to global change? *Proceedings of the Royal Society B: Biological Sciences* **285**, 20181374. (doi:10.1098/rspb.2018.1374)

79. Morgan R, Andreassen AH, Åsheim ER, Finnøen MH, Dresler G, Brembu T, Loh A, Miest JJ, Jutfelt F. 2022 Reduced physiological plasticity in a fish adapted to stable temperatures. *Proceedings of the National Academy of Sciences* **119**, e2201919119. (doi:10.1073/pnas.2201919119)

80. Beaman JE, White CR, Seebacher F. 2016 Evolution of Plasticity: Mechanistic Link between Development and Reversible Acclimation. *Trends in Ecology & Evolution* **31**, 237–249. (doi:10.1016/j.tree.2016.01.004)

81. Metzger DCH, Schulte PM. 2017 Persistent and plastic effects of temperature on DNA methylation across the genome of threespine stickleback (Gasterosteus aculeatus). *Proceedings of the Royal Society B: Biological Sciences* **284**, 20171667. (doi:10.1098/rspb.2017.1667)

82. Loughland I, Little A, Seebacher F. 2021 DNA methyltransferase 3a mediates developmental thermal plasticity. *BMC Biology* **19**, 11. (doi:10.1186/s12915-020-00942-w)

83. Williams CM, Szejner-Sigal A, Morgan TJ, Edison AS, Allison DB, Hahn DA. 2016 Adaptation to Low Temperature Exposure Increases Metabolic Rates Independently of Growth Rates. *Integrative and Comparative Biology* **56**, 62–72. (doi:10.1093/icb/icw009)

84. Salin K, Villasevil EM, Anderson GJ, Auer SK, Selman C, Hartley RC, Mullen W, Chinopoulos C, Metcalfe NB. 2018 Decreased mitochondrial metabolic requirements in fasting animals carry an oxidative cost. *Functional Ecology* **32**, 2149–2157. (doi:10.1111/1365-2435.13125)

85. Arnold PA, Delean S, Cassey P, White CR. 2021 Meta-analysis reveals that resting metabolic rate is not consistently related to fitness and performance in animals. *Journal of Comparative Physiology B* (doi:10.1007/s00360-021-01358-w)

86. Dammhahn M, Dingemanse NJ, Niemelä PT, Réale D. 2018 Pace-of-life syndromes: a framework for the adaptive integration of behaviour, physiology and life history. *Behavioural Ecological Sociobiology* **72**, 62. (doi:10.1007/s00265-018-2473-y)

87. Czarnołęski M, Kozłowski J, Dumiot G, Bonnet J-C, Mallard J, Dupont-Nivet M. 2008 Scaling of metabolism in Helix aspersa snails: changes through ontogeny and response to selection for increased size. *Journal of Experimental Biology* **211**, 391–400. (doi:10.1242/jeb.013169)

88. Réale D, Garant D, Humphries MM, Bergeron P, Careau V, Montiglio P-O. 2010 Personality and the emergence of the pace-of-life syndrome concept at the population level. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365**, 4051–4063. (doi:10.1098/rstb.2010.0208)