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RESEARCH ARTICLE

Temperature-dependent scaling of fitness traits with body size in hydra

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Abstract

1. Body size strongly depends on developmental temperature. In more than 80% of the ectotherm species investigated, including bacteria, protists, invertebrates and vertebrates, individuals developing at lower temperatures exhibit protracted growth and achieve larger sizes than individuals developing at higher temperatures (referred to as the 'temperature-size rule', TSR). One hypothesis to explain the TSR posits that reproduction and/or survival change more steeply with size in cold environments, resulting in larger optimal body sizes and consequently increased selection for growth. However, clearly ascertaining whether size directly affects fitness traits in a temperature-dependent way is challenging due to the interdependence of size, reproduction and survival.
2. To address this problem, experimental body size manipulation was performed in two male and two female strains of *Hydra oligactis*, a cold-adapted temperate freshwater invertebrate. Experimentally enlarged and reduced individuals were followed at two distinct temperatures (8 and 12°C) in the laboratory to record sexual investment and postreproductive senescence. To gain insight into the underlying physiological processes, phenotypic observations were complemented with a large transcriptomic data set obtained from enlarged and reduced individuals from different temperatures.
3. Within male hydra strains, fecundity increased, while survival decreased more steeply with size in cold, compared with warmer temperature. Females showed similar, though less emphasized, trends. Reduced animals in the cold had slower sexual development and were less able to undergo compensatory growth, suggesting temperature-dependent constraints on physiological performance.
4. Reduced and enlarged males differed dramatically in the expression of reproductive genes at low, but not at higher temperature, while in females, a complex transcriptomic restructuring was seen. In particular, metabolic genes were strongly affected by size manipulation, suggesting resource acquisition and allocation as a central mechanism driving allometric patterns.
5. These results suggest that being large is more beneficial in cold environments, at least in terms of reproduction, while at higher temperature even small individuals

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can maintain reproductive output. Lower reproduction, however, can be compensated by improved survival in small individuals facing colder environments. The reproductive advantage of large size can provide selection for increased growth at low temperature, thereby explaining the TSR in hydra.

KEYWORDS
allocation trade-offs, allometry, compensatory growth, life history evolution, metabolism, reproduction, senescence, temperature-size rule

1 | INTRODUCTION

Body size is a key determinant of energetics, physiological performance, biotic interactions and ultimately Darwinian fitness of organisms (Reiss, 1991). Ecologists have shown great interest in the factors that influence variation in organism size and have made substantial efforts to document and explain body size variation in the natural world. This interest has grown, particularly due to reports of declines in body sizes in terrestrial and aquatic ecosystems as a result of global climate change (Gardner et al., 2011; Sheridan & Bickford, 2011; Verberk et al., 2021).

Temperature is one of the most important drivers of body size variation. In more than 80% of the ectotherm species investigated, including bacteria, protists, invertebrates and vertebrates, individuals developing at lower temperatures have protracted growth periods and achieve a larger size than individuals developing at higher temperatures (Atkinson, 1994; Ray, 1960). This phenomenon is known as the temperature-size rule (TSR). The widespread occurrence of the TSR within the living world is puzzling because it does not readily align with existing theories of growth and energy allocation, which predict growth rates near physiological limits to maximize reproductive advantages (Dmitriew, 2011).

Several hypotheses have been proposed to explain why organisms developing at lower temperatures grow to larger sizes. These hypotheses can be broadly classified as mechanistic or adaptive explanations (see Angilletta et al., 2004; Atkinson, 1994; Audzijonyte et al., 2019; Tabi et al., 2020; Verberk et al., 2021; Walczyńska & Sobczyk, 2021 for comprehensive reviews). Mechanistic hypotheses aim to explain the smaller body size of organisms developing at higher temperature due to physical constraints on the biological processes of growth and development. For example, resources required for metabolism and growth (such as oxygen) might be insufficient at high temperature, especially in an aquatic environment, because oxygen solubility in water is temperature-dependent and low oxygen levels can constrain somatic growth (Forster et al., 2012; Horne et al., 2015; Pauly, 2021; Rubalcaba et al., 2020). Differences in the temperature sensitivity of biochemical processes responsible for growth (protein synthesis) and development (DNA synthesis) could be an alternative or additional factor that influences final body size under different thermal conditions (Forster & Hirst, 2012; Horne et al., 2019; van der Have & de Jong, 1996). The larger size could also be a consequence of increased cell volume enabled by increased oxygen availability at low temperature (Atkinson et al., 2003; Blanckenhorn & Llaurens, 2005; Leiva et al., 2023; Verberk et al., 2021).

In contrast to these mechanistic explanations, adaptive interpretations aim to elucidate temperature-dependent differences in body size from an evolutionary point of view, as a result of individual choices selected to maximize fitness under different thermal conditions (Angilletta et al., 2004; Arendt, 2015; Kozłowski, 1992; Kozłowski et al., 2004; Marshall & White, 2019; Wootton et al., 2022). Specifically, if larger body size increases fecundity and/or survival more strongly at low temperature, then it would be optimal for animals to prioritize growth, attaining larger body size in cold environments (Arendt, 2011, 2015; Thunell et al., 2023; Verberk et al., 2021). Evidence for temperature-dependent size-fecundity or size-mortality relationships has been obtained from correlative life history studies performed on animals under different thermal regimes. For example, in mosquitofish (*Gambusia affinis*) inhabiting geothermal springs along a thermal gradient and in freshwater snails (*Physa*) subjected to experimentally altered temperatures, the fecundity advantage of large size decreased with temperature (Arendt, 2015; Fryxell et al., 2020). Similarly, in *Daphnia*, both reproduction and mortality changed more steeply with body size depending on temperature (Bruijning et al., 2018; Weetman & Atkinson, 2004). In contrast, fertilization increased less steeply with size at low temperature in the water strider (*Aquarius remigis*) (Arendt & Fairbairn, 2012). These contradictory results make it difficult to evaluate the adaptive hypothesis of the TSR. However, even if a clear correlation is established, the intricate causal factors contributing to covariation among life history traits make it challenging to definitively discern whether the temperature-dependent scaling of reproduction and survival directly stems from body size's influence on these traits, or whether it is an indirect outcome of past conditions that impact both. For instance, a less steep size-fecundity relationship could be the result of an increased fecundity cost of rapid growth (Auer et al., 2010; Barneche et al., 2019; Lee et al., 2012), or higher energetic expenditure on growth at high temperature (Clarke et al., 2010; Clarke & Johnston, 1999; Ohlberger et al., 2012).

Here, a recently developed method (Ngo et al., 2021) was used to perform experimental body size manipulation in the freshwater cnidarian *Hydra oligactis* and directly test the adaptive hypothesis of TSR by disentangling direct body size effects from other factors. Hydra polyps possess a simple body organization and have remarkable

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regeneration capacities, allowing reciprocal grafting of tissue pieces between genetically identical clones to increase or decrease their body size (Ngo et al., 2021). Furthermore, they show complex allocation decisions in response to internal and external conditions (Gergely & Tökölyi, 2023; Ngo et al., 2021; Sebestyén et al., 2018). Importantly, hydra follow the TSR (Mortzfeld et al., 2019). To evaluate the adaptive hypothesis of TSR in hydra, polyps with experimentally reduced or enlarged body size were followed at two distinct temperatures (8 or 12°C) to record their sexual investment and postreproductive senescence, expecting that their reproduction and/or survival should increase more steeply with body size at low temperature.

2 | METHODS

2.1 | Replication statement

Scale of inference	Scale at which the factor of interest is applied	Number of replicates at the appropriate scale
Species	Two male and two female strains	$N = 463$ individuals for fecundity and survival data; $N = 157$ individuals for compensatory growth measurement; $N = 48$ individuals for RNA-Seq

2.2 | Model species

Hydra oligactis is a temperate freshwater cnidarian occurring in permanent water bodies with low-to-moderate temperature (this species has an attenuated heat shock response and temperatures above 25°C are stressful to them; Bosch et al., 1988). *H. oligactis* polyps reproduce asexually most of the year through budding. The size of adult polyps, just as their budding rate, strongly depends on food availability: When food is abundant, they produce an asexual offspring every few days. When food is scarce, adult polyps stop budding and shrink in size, until food becomes available again and they can regrow and resume budding. In this asexual phase, *H. oligactis* polyps are assumed to lack any forms of senescence (Tomczyk et al., 2015), similar to another hydra species (*H. vulgaris*), which has been shown to survive in the laboratory for years without evidencing declines in reproduction or survivorship (Schaible et al., 2015). More recent evidence, however, indicates that increased mortality and reduced reproduction with age might be observed in asexually reproducing *H. oligactis* (Boutry et al., 2022).

During the autumn, sudden drops in water temperature trigger sexual reproduction in *H. oligactis*. Polyps develop gonads (testes in males and ovaries in females, i.e. this is a gonochoristic species), external fertilization occurs and fertilized eggs transform into a dormant stage (resting egg, or more correctly a resting embryo) that survives freezing. Not all polyps with the same genotype and facing similar environmental conditions initiate sexual development (Miklós et al., 2021): some of them persist asexually throughout the winter and continue budding even in low temperature. Age, size, warm exposure and genotype are factors that are known to influence sexual propensity (Miklós et al., 2022; Ngo et al., 2021; Sebestyén et al., 2020; Tökölyi et al., 2021). Individuals that initiate sex, in contrast to asexual individuals, undergo a clear and marked postreproductive senescence associated with stem cell depletion, loss of regeneration ability and feeding capacity, shortening of tentacles, shrivelling in size, reductions in motility, and, ultimately, increased mortality (Sebestyén et al., 2018, 2020; Tomczyk et al., 2020; Yoshida et al., 2006), similar to some species of salmon undergoing catastrophic senescence after reproduction (Morbey et al., 2005). The probability of surviving postreproductive senescence correlates negatively with sexual investment, such that individuals with more gonads have higher rates of senescence and are more likely to die (Ngo et al., 2021; Sebestyén et al., 2020; Tomczyk et al., 2020). Conversely, sexual individuals with fewer gonads, despite undergoing senescence and shrivelling down, are able to recover after sexual reproduction; they completely regenerate their body and revert to asexual reproduction.

2.3 | Hydra strains and laboratory conditions

Two male and two female strains of *H. oligactis* were used: C2/7 and M83/4 male strains and X11/14 and M26/9/10 female strains. These strains originate from polyps collected in Hungarian lakes at least 1 year before the experiments reported here (Gergely & Tökölyi, 2023) and asexually propagated in the laboratory under standard conditions: 18°C temperature, 12/12 h light/dark cycle, fed four times per week with freshly hatched *Artemia* nauplii and kept in a standard hydra medium consisting of: 1 mM Tris, 1 mM NaCl, 1 mM CaCl2, 0.1 mM KCl, 0.1 mM MgSO4; pH: 7.6 (Gergely & Tökölyi, 2023).

2.4 | Size manipulation

The experimental design is depicted in Figure 1a. Experimental size manipulation was done following (Ngo et al., 2021). Briefly, pairs of animals belonging to the same strain and similar in size were selected to perform size manipulation. The body column of each individual was cut into three pieces with a scalpel by two transverse cuts so that the size of the middle portion differed between members of the pair: it was small in one individual and large in the other. The two middle parts were then reciprocally exchanged between members of the pair and the body parts were reassembled by tightly stringing them on a microcapillary and letting them heal for several hours at room temperature. After healing, the reassembled animals were removed from the microcapillary and allowed to recover overnight at 18°C. Individuals that did not heal normally or developed a secondary body axis later were excluded from the analysis. The final sample size, after excluding failed individuals, was: $N = 125$ for strain C2/7,

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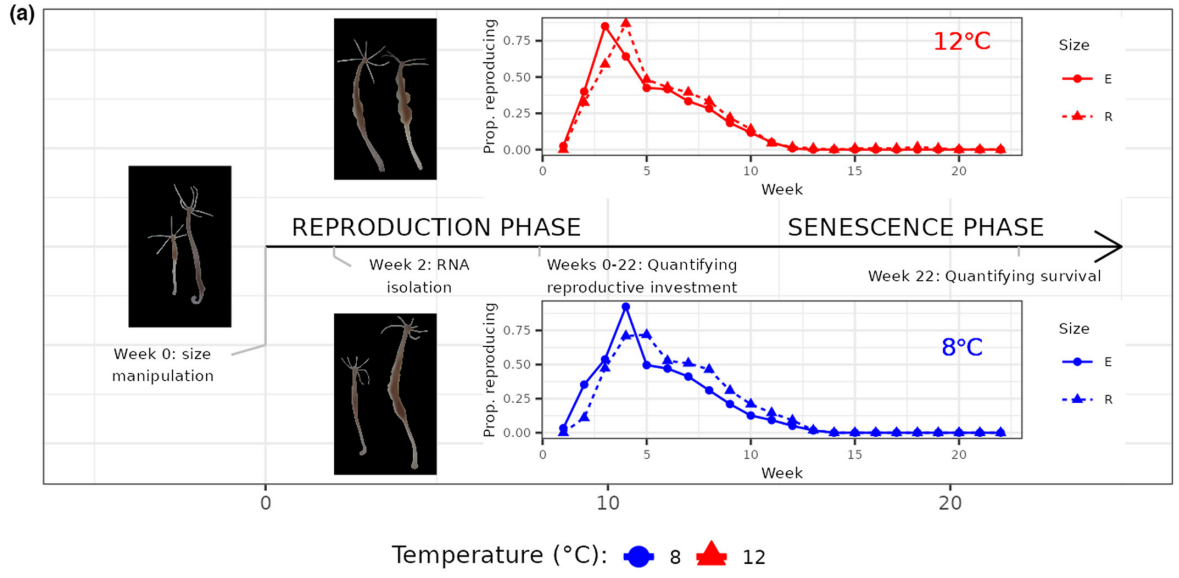

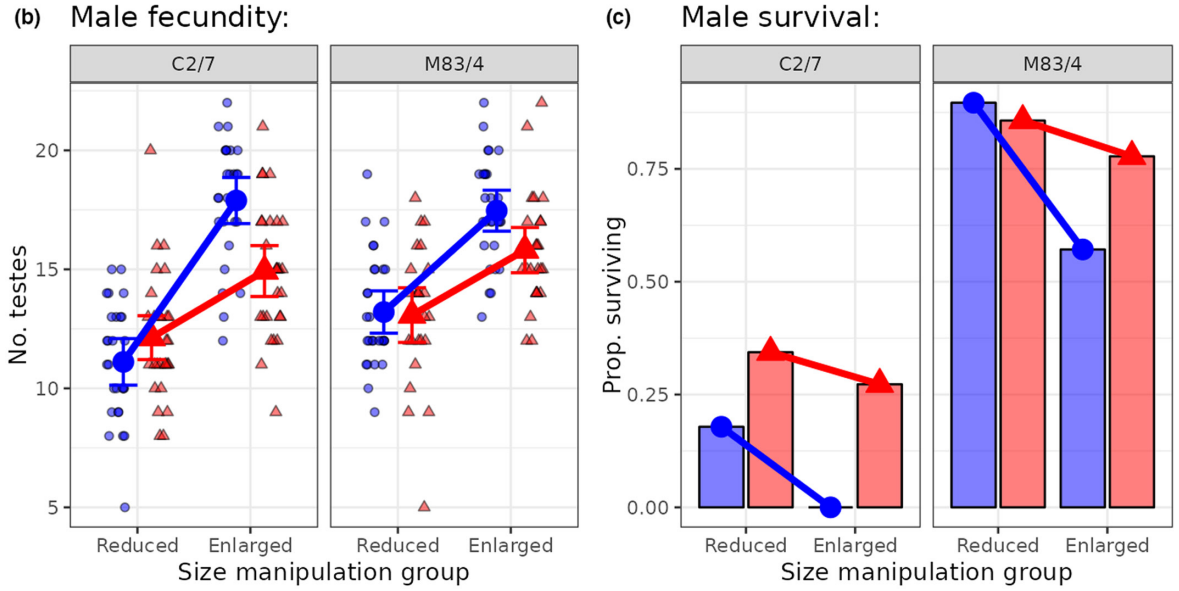

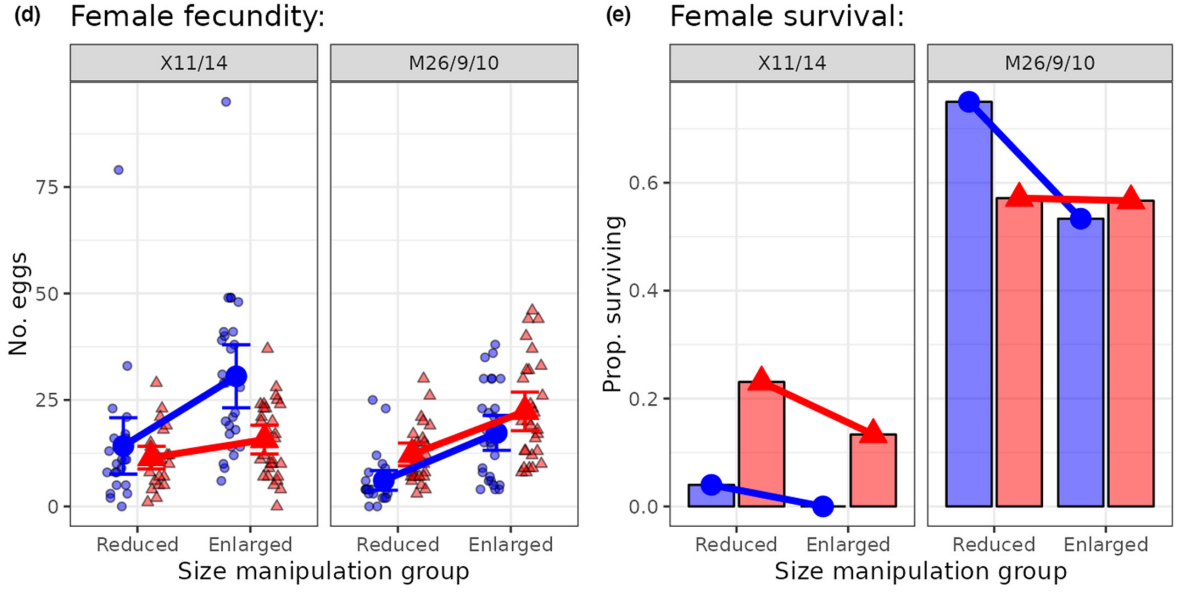

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FIGURE 1 Temperature-dependent scaling of reproduction and survival with body size in hydra. (a) experimental body size manipulation was done on pairs of clonally descended hydra polyps at time 0. After size manipulation, polyps were moved to 8 or 12°C and their reproduction and survival recorded for 22 weeks. A subset of animals were measured for size change 2 weeks after size manipulation. A second subset was used for RNA extraction 2 weeks after size manipulation. Inset graphs show the temporal distribution of reproduction (all four strains combined). Inset photographs depict reduced (left) and enlarged (right) individuals after size manipulation and 2 weeks later. Reproductive output (testes or egg number, mean ± 95% confidence interval) in males (b) and females (d) increased significantly more steeply with body size at low temperature. Conversely, survival rate decreased significantly more steeply with body size in males (c), but was not significantly affected in females (e).

$N = 112$ for strain M83/4, $N = 110$ for strain X11/14 and $N = 116$ for strain M26/9/10.

2.5 | Sex induction and recording of phenotype data

The day after size manipulation, animals were randomly assigned to one of the 12°C or 8°C temperature treatment groups (temperatures below 12°C are required in this species for sex induction; Tökölyi, 2023). The animals were housed individually in six-well plates, fed two times a week with freshly hatched *Artemia* and checked four times a week for the presence and number of gonads. They were kept at their respective temperatures for 22 weeks and their survival was recorded. Animals were scored dead if they degenerated following sexual reproduction and became a mass of necrotic tissue, or disappeared completely. On the contrary, they were scored as survived if they recovered after sexual reproduction, had intact tentacles, and were able to feed. *H. oligactis* polyps become necrotic and shrink in size following sexual reproduction, but some of them retain sufficient stem cells to regenerate to a large, healthy animal. This postreproductive degeneration means that it is not possible to tell whether they are alive or dead through visual inspection during the senescent phase. However, animals that regenerate from this necrotic condition can be scored survived a few weeks after sexual reproduction. A period of 22 weeks was chosen because very few regenerations are seen after 20 weeks in the cold in these strains (results not shown); hence, most surviving animals can be detected within this time frame.

A separate subset of individuals was used to quantify compensatory growth. These individuals received the same treatment as other individuals but were photographed right after size manipulation and 2 weeks later on a millimetre grid to record their size. From the photographs, body size was estimated as the area of the body column, excluding any buds, gonads and tentacles, using ImageJ (Schneider et al., 2012). The sample size for the size measurements was $N = 48$ for strain C2/7, $N = 36$ for strain M83/4, $N = 37$ for strain X11/14 and $N = 36$ for strain M26/9/10.

From the recorded phenotype data, the following variables were extracted: (1) fecundity (maximum number of testes observed on a male individual or total number of detached eggs in the case of a female animal); (2) survival (survived or not); (3) size change (size at 2 weeks after the initiation of the experiment divided by postmanipulation body size); and (4) sexual development speed (the number of days that elapsed after initiation of the experiment and the appearance of the first gonads).

2.6 | Statistical analysis of phenotype data

Phenotype data were analysed with generalized linear mixed-effects models (GLMM) implemented in the glmmTMB package (*v1.1.7.9000*; Brooks et al., 2017) in R *v4.3.1* (R Core Team, 2023). As dependent variables, the models included either fecundity, survival, sexual development time or size change. Temperature treatment (8 vs. 12°C), size manipulation group (reduced vs. enlarged) and their interaction were included as fixed effects. Strain ID was included as an additional fixed effect. Although differences between strains were not the focus of this study, the low number of levels in this variable (two per sex) suggested a better treatment of strain ID as fixed rather than a random effect. Strain effects were modelled additively but models containing strain ID in interaction with other terms are included in the Appendix (Tables S1 and S2). The ID of the size manipulation pair was included as a random effect to take into account the possibility that individuals who exchanged tissue pieces between them might be more similar to each other. Models were built separately for males and females.

Phenotype data that approximately followed a normal distribution (male sexual development time, male and female compensatory growth after log transformation) were analysed with GLMM with a Gaussian distribution. Model diagnostics were then performed with the DHARMa R package *v0.4.6* (Hartig, 2022) to ensure that the assumptions of the models with Gaussian distribution were met (DHARMa residual plots are shown in Supplementary Material S1). Compensatory growth after size manipulation was analysed by taking log(week 2. size/postmanipulation size) as dependent variable, to ensure that assumptions of a Gaussian model (nonbounded, normal distribution) are met. For phenotype data that showed substantial deviations from a normal distribution (male fecundity, female fecundity and female sexual development time), models were fitted with different distributions (Poisson, Negative Binomial with linear parametrization and Negative Binomial with quadratic parametrization) and ranked using AICc. The highest-ranking model (with the lowest AICc) was then used to test the effect of the size × temperature interaction. Finally, survival data were analysed with GLMM with a binomial distribution. Model diagnostics were evaluated with DHARMa for non-Gaussian models as well (see Supplementary Material S1). In each case, a full model containing the size × temperature interaction

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was built, along with a reduced model that only included the additive effects of size manipulation group and temperature treatment. These two models were compared using likelihood ratio tests implemented in the *anova* function in R.

2.7 | RNA isolation and sequencing

A separate set of experimental animals was produced for RNA extraction. These animals were handled exactly the same as those used for the measurement of the phenotype. 2 weeks after size manipulation and cooling, they were isolated and used for RNA extraction. One polyp was used for each RNA-Seq sample and three biological replicates were used for each experimental group within each strain (a total of $N = 48$ samples).

RNA extraction was performed with TRIzol™ Reagent (Life Technologies, USA), using the following protocol. Polyps were first gently moved to individually labelled Eppendorf tubes. Hydra medium was removed, 100 μL TRIzol™ Reagent was added and polyps were homogenized with a pellet pestle. After homogenization, 400 μL additional TRIzol™ Reagent was added to each sample, followed by careful vortexing and incubation at room temperature for 10 min. Next, 100 μL of chloroform was added; tubes were inverted 15 times and incubated for 5 min at room temperature, after which they were centrifuged for 15 min at 4°C at 12,000 RCF. The supernatant was removed, mixed with 100 μL chloroform, inverted 15 times, incubated for 5 min at room temperature and centrifuged again (15 min at 4°C at 12,000 RCF). After this step, the supernatant was removed, mixed in an approximately 1:1 volume ratio with isopropanol, inverted 10 times then incubated for 10 min at room temperature. This was followed by centrifuging for 10 min 15,000 rpm 4°C to produce pellets. The pellets were then washed three times with 1 mL of 70% ethanol with a short centrifuging step between washes (3 min, 14,000 rpm, 4°C). Finally, the pellets were dried for approximately 5 min, dissolved in 25 μL MilliQ water and incubated for 10 min at 65°C in a dry block thermostat. Immediately after this step, samples were moved to −80°C and subsequently shipped to Novogene, Cambridge, UK, for 2 × 150 paired-end sequencing on an Illumina NovaSeq platform.

2.8 | De novo transcriptome assembly

Reads were first processed with *fastp v0.23.4* (Chen et al., 2018) to detect adaptor contamination and remove low-quality reads, with the following parameters: quantified quality score of 30, minimum required length of 50 bp, sliding window from front to tail and tail to front with a window size of 4 bp, and required mean quality of 30, adaptor detection and base correction enabled. The reads were then processed with *SortMeRNA v4.3.6* (Kopylova et al., 2012) to detect and remove ribosomal RNA contamination, using the smr_v4.3_sensitive RNA reference database. To remove contamination from other organisms that might be found along the hydra or introduced into the samples before sequencing, a two-step decontamination process was performed. First, reads were mapped to a recently published high-quality draft genome of *H. oligactis* (Cazet et al., 2022) using *HISAT2 v2.2.1* (Kim et al., 2019) and concordantly aligned reads were retained. Second, reads that did not align with the draft genome of *H. oligactis* were mapped with *Kraken2 v2.1.3* (Wood et al., 2019) to the prebuilt Kraken2 *nt* database (5/2/2023) to identify hydra sequences that could be missing in the draft genome. After the Kraken2 taxonomy assignments, reads that mapped to cnidarian sequences were retained, and all other reads were eventually discarded. The remaining reads (i.e. those mapping to the *H. oligactis* genome and those mapping to cnidarian sequences) were individually concatenated for each sample.

De novo transcriptome assembly was performed with *Trinity v2.15.1* (Haas et al., 2013), using the concatenated set of forward and reverse reads as input. Following assembly, the transcriptome quality was assessed by remapping reads from individual samples to the assembly using *Bowtie2 v2.5.1* (Langmead & Salzberg, 2012) and *BUSCO v.5.4.7* (Manni et al., 2021) completeness analysis, with the *Metazoa_odb10* database as a reference.

2.9 | Transcript annotation and differential expression analysis

Transcripts were annotated with the *Trinotate* pipeline *v4.0.1* (Bryant et al., 2017). First, coding regions within the transcripts were identified with *TransDecoder v5.7.0* (https://github.com/TransDecoder/TransDecoder), followed by homology searches against the Swissprot database with *blastx* and *blastp* (*ncbi blast v1.14.0*, Altschul et al., 1990) and against the Pfam database with hmmscan involving HMMER v3.3.2 (Eddy, 2011). Further annotation was done with *eggnog-mapper v2.1.11* (Cantalapiedra et al., 2021).

Differential expression was performed with the *DESeq2 v.1.40.2* package (Love et al., 2014) in *R v.4.3.1* (R Core Team, 2023) at the transcript level for all strains separately. First, transcripts with low and sporadic expression (<10 counts in at least 9 out of 12 samples) were removed. Next, *DESeq2* analysis was performed with the experimental group (8°C reduced, 8°C enlarged, 12°C reduced, 12°C enlarged) as predictor. From the resulting model object, size manipulation contrasts (reduced vs. enlarged) were specified for both temperatures to identify transcripts that were up- or downregulated at 8 or 12°C in reduced animals compared to enlarged animals. Log_2 Fold Change values were adjusted with the *apeglm* shrinkage estimator (Zhu et al., 2019) to reduce overestimation of effect sizes due to small sample size and high variability in transcriptomic data. Transcripts with adjusted p-value <0.05 and $|\text{log}_2$ Fold Change$| > 1$ were selected. Subsequently, these transcripts were subjected to Gene Ontology (GO) enrichment analysis with length bias correction using the *GOseq v1.52.0* R package (Young et al., 2010). Finally, the enriched GO terms were clustered based on semantic similarity with the binary cut method and plotted with the *simplifyEnrichment v1.10.0* R package (Gu & Hübschmann, 2023). A second set of

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enrichment analysis was performed on genes from the metabolic and digestive pathway alone, as encoded by the Kyoto Encyclopedia of Genes and Genomes (KEGG, path numbers KO00010-KO01500 and KO04970-KO04979).

3 | RESULTS

3.1 | Phenotype data

Testes number in males depended on the interaction between temperature and size manipulation group (Poisson GLMM, $\lambda^2 = 5.913$, $p = 0.015$, $N = 223$) so that it increased more steeply with body size at low temperature (an effect mostly driven by strain C2/7, although apparent as a trend in M83/4 as well; Figure 1b). A significant temperature × size interaction was found in females as well (Negative binomial GLMM with quadratic parametrization, $\lambda^2 = 7.607$, $p = 0.006$, $N = 218$), with the number of eggs increasing more steeply with body size at low versus higher temperature, although this effect was mainly driven by one strain (X11/14; Figure 1d). These results suggest that on higher temperature fecundity does not depend strongly on body size and even small individuals can produce a large number of gametes, while they are unable to achieve high reproductive output on low temperature.

To gain insight into potential explanations driving temperature-dependent scaling of reproduction, patterns of compensatory growth and reproductive development were analysed. Enlarged animals changed only slightly in body size, but reduced animals doubled their size within 2 weeks after size manipulation, providing evidence for compensatory growth (Figure S1). Reduced animals appeared to undergo compensatory growth to a higher degree at 12°C, although this effect was only statistically significant in females, but not in males, where strong variation was observed in compensatory growth among reduced individuals (Gaussian GLMM, males: $\lambda^2 = 0.823$, $p = 0.364$, $N = 73$; females: $\lambda^2 = 6.950$, $p = 0.008$, $N = 65$; Figure S1). Sexual development time was significantly affected by the interaction between body size manipulation group and temperature in both males and females (Gaussian GLMM, males: $\lambda^2 = 13.874$, $p < 0.001$, $N = 223$; females: $\lambda^2 = 14.965$, $p < 0.001$, $N = 218$) so that individuals with reduced body size needed more time to mature at low versus high temperatures (Figure S2). In general, these observations suggest that higher temperatures allow faster compensatory growth and reproductive development, thereby being more permissive to reproductive investment in small individuals.

Survival rate was significantly affected by the temperature-size manipulation interaction in males (binomial GLMM, $\lambda^2 = 4.210$, $p = 0.040$, $N = 218$), but not females (binomial GLMM, $\lambda^2 = 1.157$, $p = 0.282$, $N = 210$), where one of the strains (X11/14) had a very low survival rate, which could have prevented the detection of a statistically significant effect. Within males, reduced individuals showed a higher survival than enlarged ones, a difference that was much more emphasized at low temperature (Figure 1c).

3.2 | Transcriptomic data

De novo transcriptome assembly with *Trinity v2.5.1* from 1024.6 million 150 bp paired-end reads (after removal of low-quality reads, rRNA sequences, and contamination from other organisms; all strains combined) resulted in 506,839 individual transcripts (see Figure S3 and Table S3 for read statistics and transcriptome evaluation). After filtering out transcripts with low and sporadic expression (<10 reads in ≥9 out of 12 samples/strain), there were 55,102 transcripts in strain M26/9/10, 54,142 in strain X11/14, 62,035 in strain M83/4 and 62,753 in strain C2/7. These transcripts were subjected to differential expression analysis (DE) and transcripts significantly up- or downregulated (with an adjusted p-value <0.05 and a $|\log_2$ Fold Change$| > 1$) were extracted.

Both male strains showed a much higher number of DE transcripts in reduced vs. enlarged individuals at 8°C compared to 12°C (C2/7: 4648 DE transcripts at 8°C, 422 DE transcripts at 12°C; M83/4: 1362 DE transcripts at 8°C, 39 DE transcripts at 12°C; Figure 2a,b). Patterns of differential expression were more complex in females: while the number of DE genes was higher at 8°C, a substantial number of transcripts were upregulated on 12°C (X11/14: 2663 DE transcripts at 8°C, 1894 DE transcripts at 12°C; M26/9/10: 2983 DE transcripts at 8°C, 1195 DE transcripts at 12°C; Figure 2c,d). Furthermore, a substantial number of transcripts were upregulated at both temperatures (Figure 2c).

Next, to identify which functional categories of genes were enriched in size-manipulated animals, functional enrichment analysis was performed with Gene Ontology (GO) terms. Clustering of GO terms significantly enriched with an adjusted p-value <0.05 revealed that in males the largest category of DE transcripts belongs to genes involved in meiosis, spermatogenesis and assembly of sperm cells (Figure 3a). A large number of male reproduction-related transcripts were downregulated in reduced individuals on 8°C, but not at 12°C, aligning well with phenotype-level data that showed reduced reproductive investment of males at low (but not high) temperature. A second functionally different cluster of enriched GO terms involved in metabolism was also more strongly impacted by size manipulation at low versus high temperatures (Figure 3a). In females, by contrast, the largest class of differentially regulated transcripts was associated with metabolism (Figure 3b). However, these GO terms showed complex restructuring with respect to body size and temperature: while metabolism-associated GO terms were enriched within transcripts up- or downregulated at low temperature, a large number of metabolism-associated GO terms were also found within the upregulated transcripts at 12°C (Figure 3b).

Since metabolism seemed to play a central role in transcriptomic changes in reduced vs. enlarged polyps, a second enrichment analysis was performed, this time limited to metabolic pathways as encoded by the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database. Among the metabolic pathways most strongly affected, genes involved in glycosphingolipid biosynthesis were more likely to be found among transcripts upregulated in reduced versus

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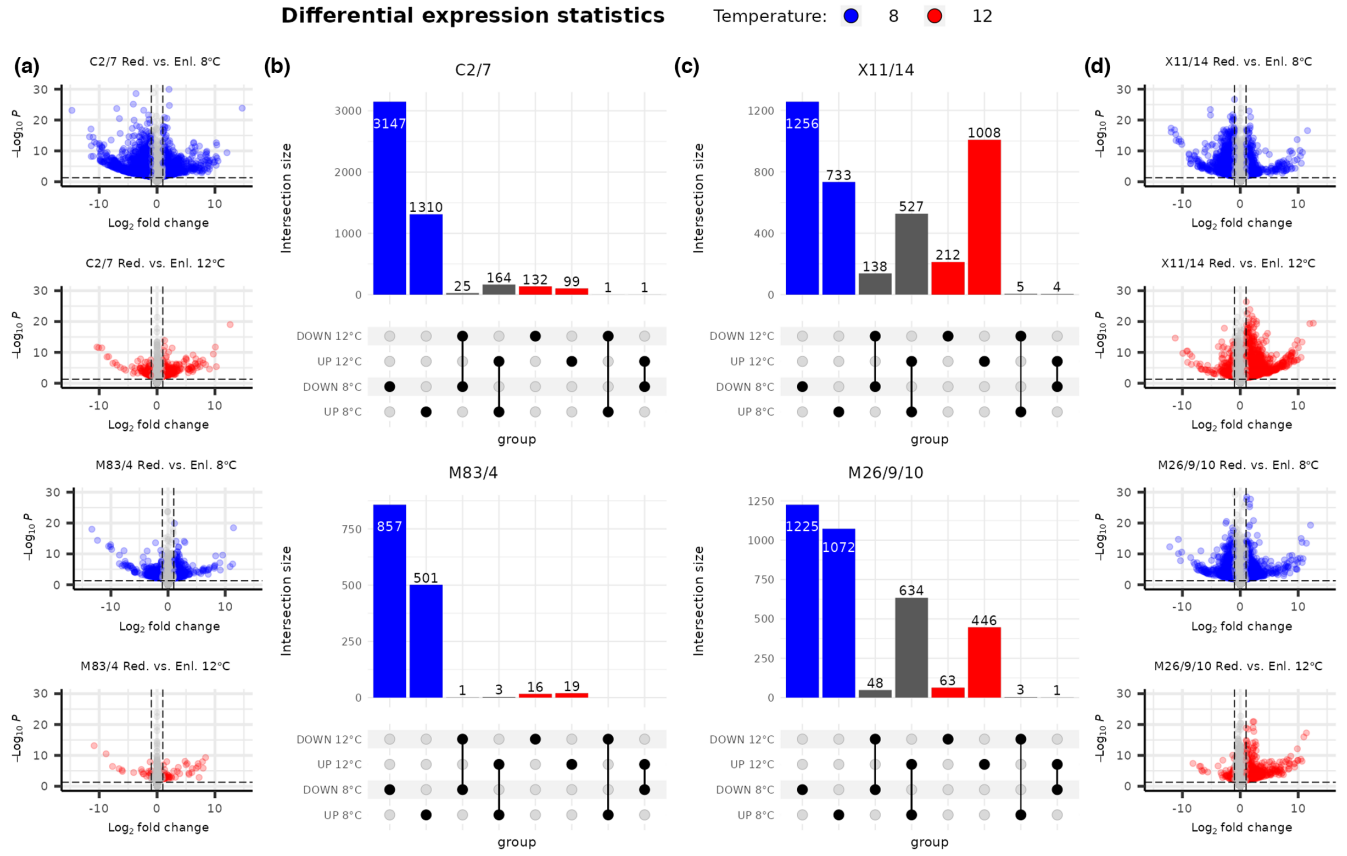

FIGURE 2 Differential expression of transcripts in reduced vs. enlarged individuals. Increased gene expression differences are observed on 8°C compared to 12°C. Volcano plots show the distribution of differentially expressed transcripts with an adjusted *p*-value cutoff of 0.05 and $|\log_2$ Fold Change$| > 1$ in males (a) and females (d). Upset plots show the overall number of differentially expressed transcripts with adjusted $p < 0.05$ and a |log2 Fold Change$| > 1$ in males (b) and females (c).

enlarged individuals. Glycosphingolipid biosynthesis possibly reflects increased reserve accumulation and cell proliferation in these individuals, since glycosphingolipids are major constituents of cell membranes (Schnaar et al., 2022). In addition, protein digestion and absorption pathway genes, as well as various types of glycan degradation genes were significantly enriched in three out of four strains (Figure 4). Finally, among the downregulated metabolic pathways in males with reduced body size, nucleotide metabolism pathways were present, reflecting lower reproductive investment and spermatogenesis in these individuals.

4 | DISCUSSION

Overall, the results presented here suggest that fecundity scales more steeply with body size at low temperature in hydra. Previous research has noted temperature-dependent correlations between body size and reproduction and/or mortality (e.g. Arendt, 2015; Bruijning et al., 2018; Fryxell et al., 2020; Weetman & Atkinson, 2004). However, these earlier studies were unable to distinguish the direct impact of body size on reproduction from other potential confounding factors. By manipulating body size directly, we can clearly discern that body size has direct effects on reproductive output in a temperature-dependent way. The advantage of larger size in cold environments could potentially drive a selection pressure for increased growth at low temperature, thus offering an explanation for the TSR phenomenon in hydra.

Why do small individuals in cold environments have a lower reproductive output? One possible explanation is that these individuals alter their allocation across various life history components. This hypothesis is increasingly proposed to account for allometric patterns and the TSR in general (Arendt, 2011; Audzijonyte et al., 2022; Marshall & White, 2019; Potter & Felmy, 2022; Wootton et al., 2022; Zuo et al., 2011). Altered allocation decisions were likely involved here as well, because variation in reproductive investment in hydra was associated with differences in survival rate, such that individuals with high reproductive output were more likely to die. This suggests that resources invested in reproduction are drawn away from self-maintenance in a life history trade-off, and the optimal resolution of this trade-off depends on the interaction between body size and temperature such that high reproduction is favoured under some conditions and high survival under other conditions. Alternatively (or additionally), low temperature might impose constraints on growth and/or development, thereby altering the relative fitness benefits of reproduction compared to other life history components (Angilletta et al., 2004;

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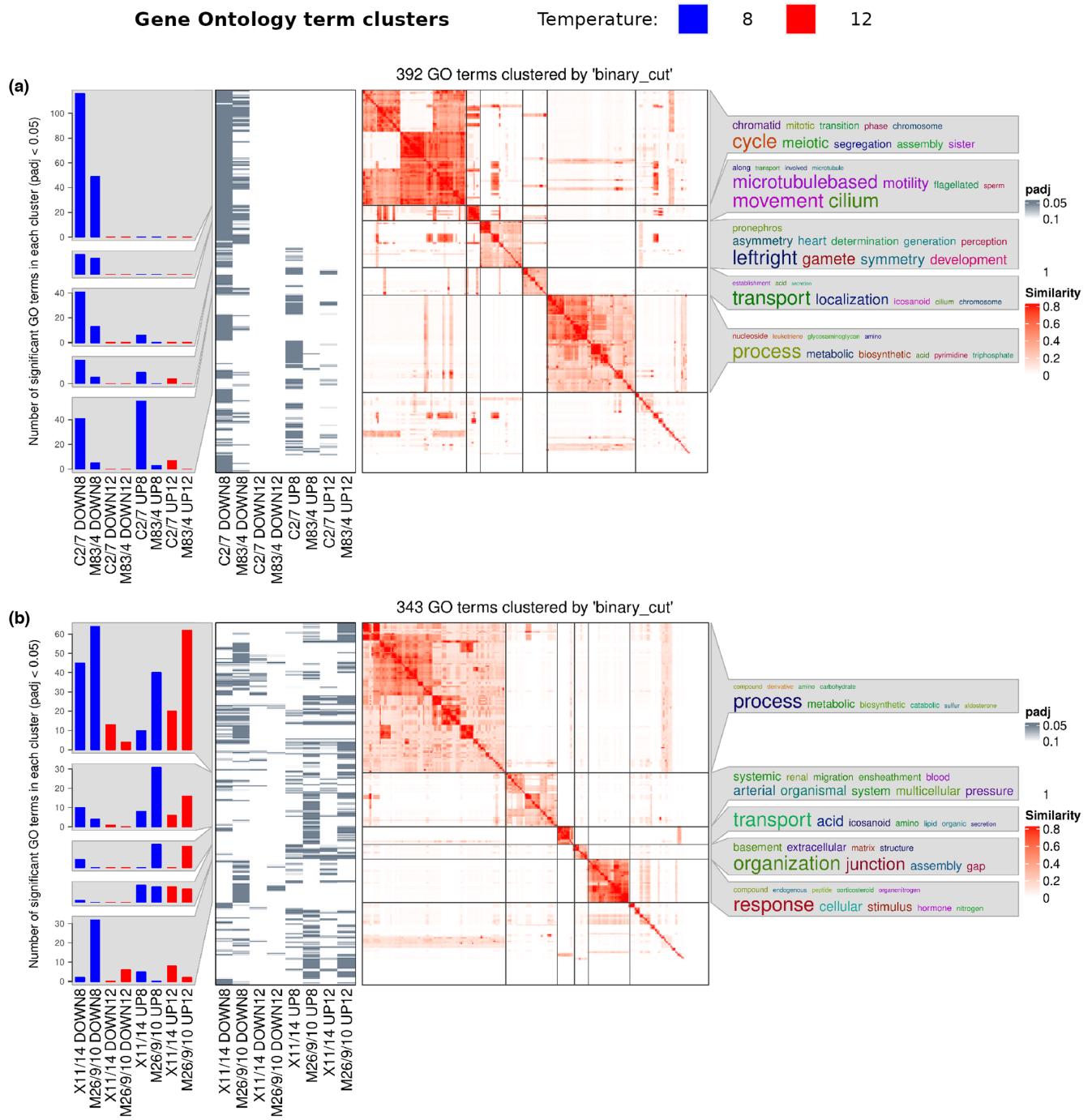

FIGURE 3 Clusters of gene ontology (GO) terms associated with differentially expressed transcripts in males (a) and females (b). Gene ontology terms were compared based on semantic similarity (shown as 'Similarity' on the heatmap) and clustered with binary cut. Only clusters with at least 10 terms are shown. The leftmost heatmap annotation shows the number of up- and downregulated transcripts in reduced vs. enlarged polyps from the two strains on 8 and 12°C. The second heatmap annotation shows the presence of individual GO terms from each cluster in these groups. Word clouds depict the top terms within each cluster. There were more GO clusters differentially regulated on 8°C compared to 12°C, especially in males, where almost no difference is seen between reduced and enlarged males at 12°C.

Forster & Hirst, 2012). For example, reduced oxygen availability at higher temperatures might hinder growth or gamete maturation, prompting individuals to adjust their allocation decisions to maximize fitness according to the prevailing environmental conditions (Arendt, 2015; Verberk et al., 2021). This explanation highlights the fact that adaptive and mechanistic explanations are not mutually exclusive; rather, they can complement each other. The results presented here support this latter explanation: Both compensatory growth and reproductive development were slower at low temperature in hydra. Since small individuals could neither quickly catch up in size nor develop gametes fast, they redirected allocation away from reproduction.

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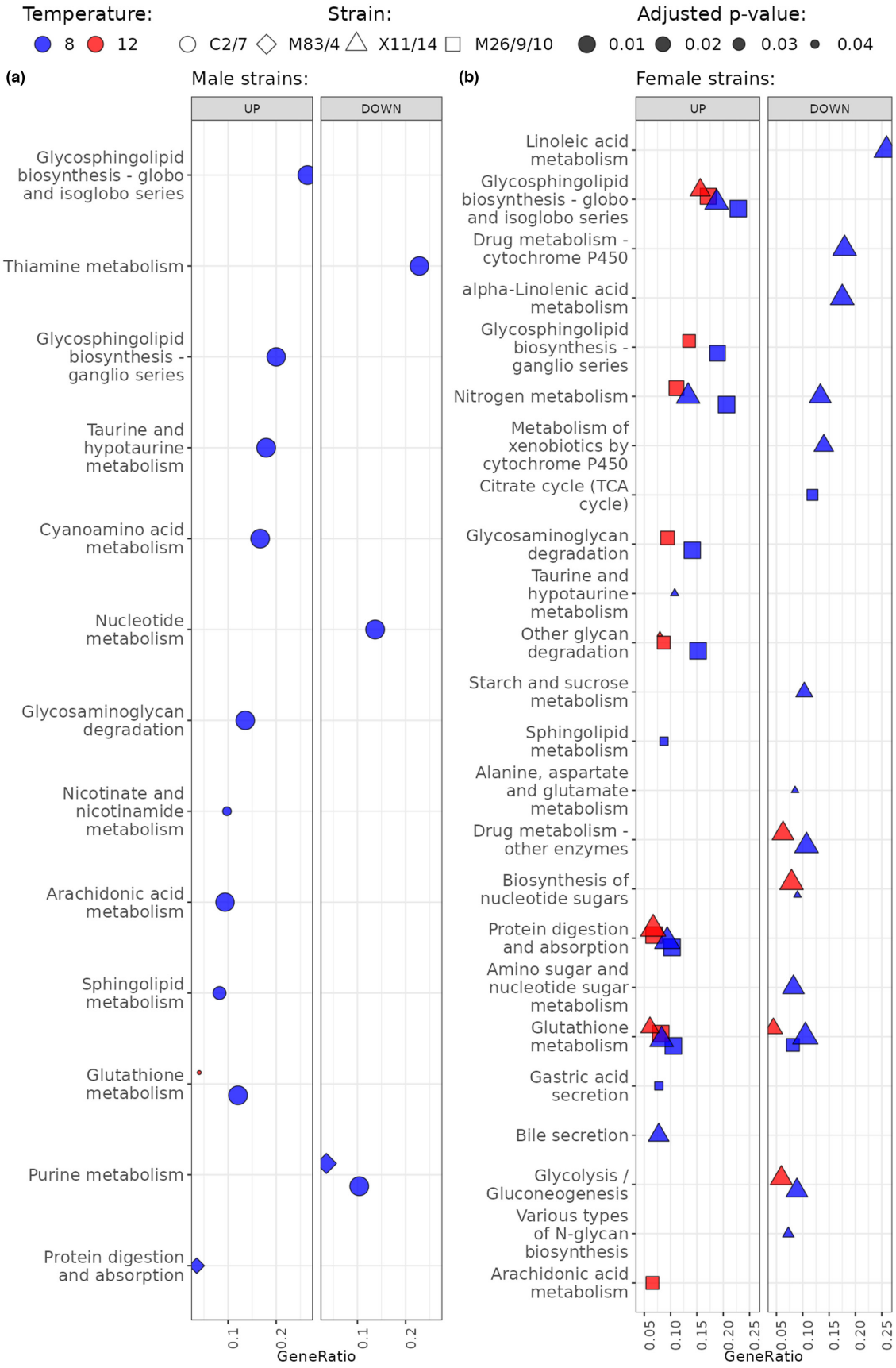

FIGURE 4 Kegg metabolic pathways emerging from gene set enrichment analysis in males (a) and females (b). The number of significant DE genes, relative to the total number of genes within the pathway (GeneRatio) is shown on the *x*-axis. Symbol size is proportional to statistical significance (*p*-value adjusted with Benjamini–Holm correction).

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While patterns of reproduction could explain the TSR in hydra, the same does not apply to survival, which showed a steeper decrease with body size at lower temperatures. From a survival perspective, it clearly does not pay to be large in the cold, as individuals facing these conditions will have higher mortality, especially in males. Although increased reproduction by these individuals might theoretically compensate for reduced survival, it is difficult to weigh these fitness components in a laboratory setting. Patterns of survival among size-manipulated individuals at different temperatures instead reflect disparities in reproductive allocation: those with high reproductive investment have reduced survival, whereas individuals with reduced reproductive investment (e.g. small individuals in cold environments) invest resources in biosynthesis and buildup of resources, which could later enable them higher postreproductive survival. In fact, three mechanistic hypotheses have been presented so far for postreproductive senescence in *H. oligactis*: depletion of stem cell reserves that differentiate into reproductive, rather than somatic tissue types (Sebestyén et al., 2018), deficient autophagy of epithelial cells resulting in an inefficient response to starvation (Tomczyk et al., 2020) and downregulation of genome maintenance pathways in senescing individuals (Sun et al., 2020). However, these explanations are not mutually exclusive. The transcriptomic data collected here pertains to animals in early stages of sexual development, likely before the onset of senescence. The upregulation of digestive and degrading genes in reduced animals at this stage implies heightened food intake and digestion, leading to enhanced somatic functions. This could subsequently result in greater stem cell availability, improved autophagy, and upregulated genome maintenance pathways. Thus, the accumulation of energy reserves in individuals investing less in reproduction might constitute a mechanism linking disparate mechanistic explanations of senescence in *H. oligactis*. Ultimately, higher energy reserves and improved maintenance could allow small hydra polyps facing cold environments to enhance survival prospects and revert to asexual reproduction as a means of compensating for the fitness loss resulting from reduced reproductive rates.

Throughout the study, sex-related disparities in allometric patterns were pervasive. In males, a clear temperature-dependent scaling of testes number emerged, a finding that received substantial support by the transcriptomic data: while numerous genes linked to spermatogenesis were downregulated in reduced versus enlarged individuals at 8°C, no such downregulation was observed at 12°C. Conversely, females, despite showing a significantly steeper scaling of reproductive output with body size at low temperature, did not exhibit evidence of temperature-dependent scaling at the transcriptome level. Furthermore, survival was not affected by the interaction of temperature and body size manipulation in females. These observations point to a fundamentally different physiological mechanism underpinning phenotypic differences among females compared with males. Although certain differences might stem from methodological factors (e.g. low number of female reproductive cells precluding detection of differentially regulated reproductive genes), biological differences are also likely involved. *H. oligactis* females in general have more flexible reproductive timing than males (Gergely & Tökölyi, 2023), which start gamete production immediately after reproductive cues (in this case, cooling) not to miss fertilization chances. In contrast, females delay reproduction in response to adverse conditions (such as starvation or unfavourable temperature), ultimately allowing decoupling of size-dependent reproduction from current environmental conditions. Extrapolating to other species, this would mean that a temperature-dependent scaling of reproduction might be expected when reproduction is time-constrained, but such a relationship could be lacking otherwise. Taking into account sex differences in reproduction might be required in future studies to obtain a fuller understanding of the TSR and better predict body size responses to climate warming.

AUTHOR CONTRIBUTIONS

Jácint Tökölyi designed research, collected and analysed phenotype data, performed the transcriptomics study and wrote the manuscript.

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CONFLICT OF INTEREST STATEMENT

The author declares no conflict of interest.

DATA AVAILABILITY STATEMENT

Phenotype data generated for this study are available on FigShare (https://doi.org/10.6084/m9.figshare.23857713). Transcriptomic data are available on NCBI as BioProject 1000260 (https://www.ncbi.nlm.nih.gov/bioproject/1000260). Code to analyse data is available on GitHub (https://github.com/jtokolyi/Hydra-TSR).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Data S1. DHARMa residual plots.

Figure S1: Size change (size after two weeks/size after body size manipulation; mean ± 95% confidence intervals) in male (A) and female (B) hydra, as a function of temperature (8 or 12°C) and size manipulation group (reduced or enlarged).

Figure S2: Sexual development time (number of days elapsed until appearance of first gonads; mean ± 95% confidence intervals) in male (A) and female (B) hydra, as a function of temperature (8 or 12°C) and size manipulation group (reduced or enlarged).

Figure S3: Evaluation of de novo transcriptome assembly.

Table S1: Strain-specific effects of the interaction between temperature and body size manipulation group on fecundity, survival, sexual development time and compensatory growth in male strains.

Table S2: Strain-specific effects of the interaction between temperature and body size manipulation group on fecundity, survival, sexual development time and compensatory growth in female strains.

Table S3: Read statistics: the number of paired-end, 150 nt raw reads; the proportion of these reads that survived after filtering for low quality reads and rRNA contamination; the proportion of raw reads that mapped to the *Hydra oligactis* genome using *hisat2*; the proportion of reads that mapped to cnidarian sequences in the *nt* database using Kraken2; final read number (sum of reads mapping to *H. oligactis* genome and cnidarian sequences in the *nt* database); proportion of these final reads mapping back to the assembled transcriptome using *bowtie2*.

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