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LETTER

# Experimental manipulation of body size alters life history in hydra

**Kha Sach Ngo, Berta R-Almási, Zoltán Barta and Jácint Tökölyi***

**Abstract**

Body size has fundamental impacts on animal ecology and physiology but has been strongly influenced by recent climate change and human activities, such as size-selective harvesting. Understanding the ecological and life history consequences of body size has proved difficult due to the inseparability of direct effects of body size from processes connected to it (such as growth rate and individual condition). Here, we used the cnidarian *Hydra oligactis* to directly manipulate body size and understand its causal effects on reproduction and senescence. We found that experimentally reducing size delayed sexual development and lowered fecundity, while post-reproductive survival increased, implying that smaller individuals can physiologically detect their reduced size and adjust life history decisions to achieve higher survival. Our experiment suggests that ecological or human-induced changes in body size will have immediate effects on life history and population dynamics through a growth-independent link between body size, reproduction and senescence.

**Keywords**

Ageing, allometric scaling, development, growth, life history trade-offs, longevity, phenotypic engineering, physiological regulation, reproductive allocation, sexual maturity.

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## INTRODUCTION

The size of an organism predicts many aspects of its biology from reproductive investment to physiological performance, metabolic rate and senescence (Peters 1983, Reiss, 1989). Understanding how body size affects other biological traits is key to interpret patterns of life history variation in the natural world and explain the ecological, evolutionary and demographic processes that depend on these traits (Brown *et al*. 2004; Killen *et al*. 2010; Malerba & Marshall 2019). Additionally, adult body size is increasingly affected by human activity worldwide. For instance, size-selective fishing and hunting commonly removes the largest individuals and causes size declines in exploited populations (Fenberg & Roy 2008; Allendorf & Hard 2009). Likewise, urbanisation affects animal body sizes through altered food availability and urban heat island effects (Liker *et al*. 2008; Guralnick *et al*. 2020). On a much larger scale, recent climate change impacts organism growth and development, resulting in the shrinking of body size across a range of habitats and taxa (Sheridan & Bickford 2011; Ohlberger 2013). Therefore, it is crucial to understand how size affects key life history traits (such as sexual maturation, fecundity and survival), as these traits will ultimately determine population dynamics and persistence in a changing world.

Decades of research on the scaling of biological traits with body size has uncovered consistent patterns within and across species (Peters 1983; Allaine *et al*. 1987; Reiss 1987; Hendriks & Mulder 2008; Shingleton 2011; Barneche *et al*. 2018). However, while some traits follow well characterised scaling laws, others display more equivocal relationships that prove difficult to understand. Animal longevity in particular shows complex covariation with body size. Across species, size is one of the most important predictors of longevity, with larger-bodied species having longer life spans (Promislow & Harvey 1990; Speakman 2005; Magalhães *et al*. 2007). Increased longevity of large-bodied species is expected based on evolutionary theory because large species tend to have reduced extrinsic mortality (e.g. because they are more buffered against starvation, water loss and temperature fluctuations and experience reduced predation; Austad 2010), which selects for longevity-promoting mechanisms and a shift of resource allocation from reproductive to somatic functions (Williams 1957; Kirkwood & Rose 1991; Cichoń 1997; Reznick *et al*. 2004; Buttemer *et al*. 2010; Vágási *et al*. 2019).

Within species, however, the relationship between size and longevity can be more complex, showing correlations that are positive, non-existent, or even negative, that is, when smaller individuals live longer than larger-bodied conspecifics (Austad 2010). In mammals, for instance, body size correlates negatively with individual life span in rats, mice, dogs and horses (Rollo 2002; Austad 2010; Kraus *et al*. 2013; Bartke 2017). Dietary restriction or genetic manipulations in growth hormone or insulin/insulin-like growth factor-1 signalling (IIS) pathways have produced smaller animals with substantially longer life span than conspecifics in several species (reviewed in Bartke 2017). Even in humans, short stature was shown to predict increased longevity (Samaras *et al*. 2003; Austad 2010; He *et al*. 2014). Conversely, examples in invertebrates suggest that the size–longevity relationship depends on genetic background and environmental conditions (Norry & Loeschcke 2002; McCulloch & Gems 2003; Khazaeli *et al*. 2005). For instance, in a study involving 29 *Drosophila melanogaster*

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strains, statistically significant negative and positive regressions between life span and body size were both observed, depending on the strain (Khazaeli *et al.* 2005).

A possible reason why body size and individual longevity display more complex relationships within species is that size has contrasting effects on survival. On the one hand, large size is presumed to mean better individual condition and a positive relationship between body size and longevity has been described in field populations under resource limitation (e.g. Forsman 1993; Gaillard *et al.* 2000). On the other hand, larger individuals bear the viability costs of longer development and/or accelerated growth (Blanckenhorn 2000; Metcalfe & Monaghan 2003; Austad 2010; Kraus *et al.* 2013). Fast growth can have detrimental effects through the accumulation of metabolically induced damage which impairs subsequent performance and reduces survival (Metcalfe & Monaghan 2003). However, having a large body could negatively affect survival also through mechanisms independent of growth. First, a large structural size implies larger absolute energetic costs for a fixed food intake, since tissue maintenance costs scale positively with tissue size (Kleiber 1947; Sebens 1981). If these maintenance costs drain resources available to the organism, then other life functions, such as costly physiological repair processes might be negatively impacted, impairing survival. Second, size-dependent adjustment in reproductive effort might exist (Karlsson & Wickman 1990; Hendriks & Mulder 2008; Barneche *et al.* 2018). If large individuals achieve increased reproductive success or produce offspring with higher reproductive value, then it might pay for them to invest into current reproduction even at the cost of reducing future survival prospects.

Evaluating whether body size affects reproduction and survival through growth-independent mechanisms is, however, difficult because of the tight linkage between body size, growth and individual condition. Most previous studies relied, unsurprisingly, on correlations between body size and other life history traits (Norry & Loeschcke 2002; Rollo 2002; McCulloch & Gems 2003; Samaras *et al.* 2003; Khazaeli *et al.* 2005; Kraus *et al.* 2013; He *et al.* 2014; Bartke 2017). Attempts to experimentally manipulate body size, such as dietary restriction or manipulating growth hormone levels have not provided sufficient evidence whether it is body size itself that impacts longevity, as all these methods influence body size through modifying growth rate. Engineering transgenic strains, for example, in IIS pathway, can introduce unintentional side effects as IIS plays key roles in controlling many physiological processes other than size and aging, including protein synthesis and glucose metabolism (McCulloch & Gems 2003). Therefore, it is necessary to examine the effect of direct experimental manipulation of body size, isolated from growth and genetic and environmental factors, to determine if body size itself influences fecundity and ageing.

Here, we present results obtained from direct experimental manipulation of body size in an emerging model system in ageing research, the freshwater cnidarian *Hydra oligactis* (Pallas 1766). *Hydra* species are renowned for their regenerative capability, which permits surgical manipulations to change body size with negligible risk of debilitating injuries or death (Bosch 2007). *H. oligactis*, in particular, exhibits cold-induced sexual reproduction that is followed by accelerated senescence and high mortality (Martínez 1998; Yoshida *et al.* 2006; Schaible *et al.* 2015; Tökölyi *et al.* 2017; Sebestyén *et al.* 2018; Tomczyk *et al.* 2020; Sun *et al.* 2020). To assess the role of body size variation in determining post-reproductive survival in hydra, we first performed a correlative study involving multiple clonal lineages established from a natural population to show that individuals with smaller natural size have delayed sexual development, lower fecundity and higher post-reproductive survival. Next, by reciprocally exchanging different-sized pieces of the body column between hydra individuals we conducted experimental size manipulation standardised for temperature, age, diet and genetic background to show that patterns observed in the correlative study are causal effects of body size variation *per se*.

## MATERIALS AND METHODS

### Study species

*Hydra oligactis* is a small freshwater cnidarian inhabiting Northern temperate environments. It has a simple body organisation consisting of a tube-like body column with an oral region surrounded by tentacles and a foot peduncle with adhesive basal disk. Reproduction can be both sexual (production of ovaries and testes on different individuals, i.e. this species is gonochoristic) and asexual (budding, which results in the production of physiologically independent polyps). Sexual reproduction is limited to autumn, when cooling inhibits budding and promotes sexual development, although in natural populations only a subset of individuals reproduce sexually at the same time (Sebestyén *et al.* 2018). Fertilisation results in production of resting eggs that can survive harsh conditions (e.g. freezing). Sexual reproduction is followed by post-reproductive senescence during which movement and feeding ability declines, polyps become unresponsive to touch, their body becomes discolored and shrinks, ultimately resulting in an amorphic, necrotic mass. In the lab strains originally characterised in detail by Yoshida *et al.* (2006), all individuals show these symptoms within three months after sexual reproduction. In another lab strain (Ho_CR) characterised by Tomczyk *et al.* (2020), polyps develop sexually but do not show morphological signs of senescence. The hydra strains derived from our study population (see below) show an intermediate pattern, wherein most polyps undergo post-reproductive senescence (interstitial stem cell depletion and diminishing regeneration ability), but some survive, regain the ability to feed and resume asexual reproduction (Sebestyén *et al.* 2020).

### Population sample

*Hydra* strains (population sample hereafter) were collected from an oxbow lake near Tiszadorogma, Hungary (47.67 N, 20.86 E). Sampling was performed four times in two consecutive years (31st May 2018, 1st Oct. 2018, 16th May 2019, 24th Sept. 2019). On each collection date, animals were collected from multiple locations within the lake (at least 2 m distance from each other, to reduce the chances of sampling clones), brought to the laboratory and propagated asexually for

10 weeks to obtain clonal lineages (strains), while keeping them individually under standard conditions (constant 18 °C, 12/12 h light/dark cycle, artificial hydra medium, fed and cleaned twice per week; Tökölyi *et al.* 2020). After 10 weeks, temperature was lowered to 8 °C and photoperiod changed to 8/16 h light/dark cycle to stimulate autumn conditions and induce gametogenesis. The presence and number of gonads and survival was recorded twice weekly for five months.

We examined altogether $N = 1584$ specimens. A subset of $N = 330$ individuals died during asexual propagation or after cooling, without producing either buds or gonads and hence were not considered during data analysis (most of these individuals ($N = 198$, 60%) died during the first phase of the experiment, prior to cooling, probably reflecting stress associated with accommodation to the laboratory environment; further $N = 81$ polyps (25%) died within three weeks after cooling, likely due to cold stress). We further excluded $N = 18$ individuals belonging to two strains with sex-changed animals. Final sample size was $N = 1236$ polyps belonging to $N = 181$ clonal lineages.

### *H. oligactis* strains used for size manipulation

We used one strain of male (C2/7) and one strain of female (X11/14) *H. oligactis* for experimental manipulations. These strains were established from two polyps collected from Tiszadorogma in Sept. 2016 and maintained asexually under standard conditions in the lab since then. Both strains are well characterised in terms of their post-reproductive senescence (Sebestyén *et al.* 2020). Polyps were housed individually in 6-well tissue culture plates in a climate chamber at 18 °C and 12/12 hours light/dark cycle. Maintenance was carried out 4 times per week, during which adult polyps were fed and cleaned. We maintained strains in an age-standardised manner: polyps reaching 3 weeks of age were removed from culturing and exchanged with one of their detached buds, which remained in the strain. This regular replacement was maintained for 8 months, hence experimental animals derive from an asexual lineage where the asexual parent was approximately of the same age (3 weeks) for multiple asexual generations. After 8 months, adult polyps removed from rearing were retained for experimental treatment.

### Size manipulation

Experimental manipulation of body size was done by reciprocal exchange of intercalary parts of the body column differing in size between individuals (Fig. 1). Polyps that were 3–4 weeks old and derived from age-standardised strains were randomised into three treatment groups: enlarged, reduced, and control body size. Polyps were photographed before and after manipulation using Euromex StereoBlue microscope, Euromex camera with standard 1 mm grid sheet beneath the polyp. Pre- and post-treatment body surface area of each polyp was measured as proxy for body size, using ImageJ (Schneider *et al.* 2012).

Polyps of each treatment group received 2 complete transverse cuts on their body column using a medical scalpel to create 3 distinct parts: head plus upper body, mid-body ring, and lower body plus foot peduncle (Fig. 1). Control polyps were cut so that their mid-body rings were of similar sizes, and rings were then exchanged between members of the pair. Enlarged and reduced polyps underwent similar procedures, except that body parts were cut at different lengths so that the enlarged polyp would exchange its small mid-body ring for the much larger ring from the reduced polyp (and vice versa). After exchange of rings, the 3 parts of each polyp were secured together with a small glass capillary needle to let heal the cuts for 2–3 hours at room temperature. Using this grafting method each experimental individual underwent the same experimental procedures (they received two cuts), while they retained their original feeding apparatus (head and tentacles). After 24 hours of healing, polyps were photographed for post-treatment size measurements and moved to a climate chamber with 8 °C and 8/16 hours light/dark cycle to induce sex. They were fed and cleaned twice per week during the cold phase.

Out of 59 sets of 4 polyps ($N = 236$ individuals in total), we excluded pairs that contained polyps of failed experimental

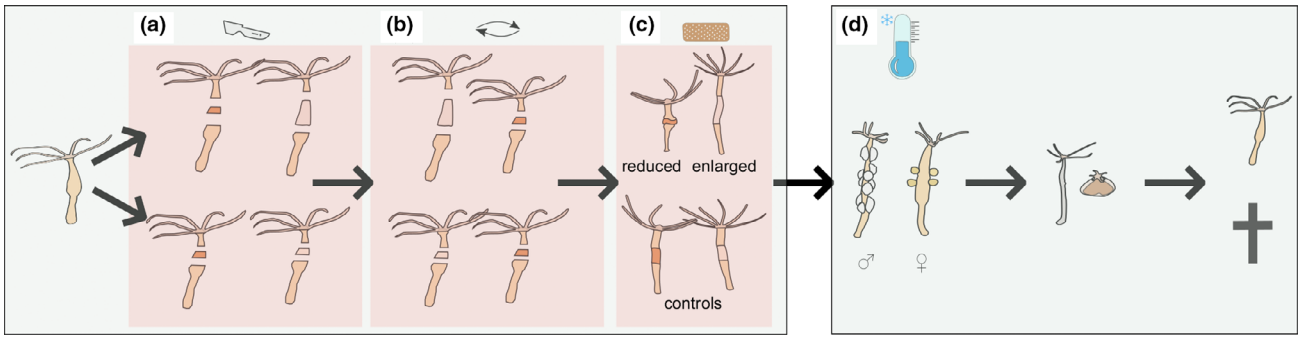


**Figure 1** Experimental design and study system. Experimental manipulation was performed on polyps cultured on 18 °C and 12/12 hours light/dark cycle by (a) excising pieces of body column differing in size in pairs of clonally descended individuals, (b) exchanging the pieces between individuals of a pair and (c) letting the novel mid-body ring heal with the upper and lower body column of the original individual. Size-manipulated individuals had tissue rings differing in size exchanged between them, while in control pairs the pieces were of similar size. Finally (d), 24 hours after grafting, experimental animals were moved to a climate chamber with 8 °C temperature and 8/16 hours light/dark cycle where they were followed for 6 months. Lowering the temperature induces sexual reproduction in this species, which is followed by post-reproductive senescence, whereby a substantial proportion of individuals die, but some of them survive, regenerate and revert to asexual reproduction.

attempts and those that changed sex or remained asexual. Final sample size was $N = 90$ male polyps ($N = 24$ reduced, $N = 42$ control and $N = 24$ enlarged) and $N = 92$ female polyps ($N = 22$ reduced, $N = 50$ control and $N = 20$ enlarged).

### Monitoring sexual development

Animals were monitored for the appearance of gonads during routine maintenance. In males, testes develop simultaneously around the body column; we counted mature testes visible from one side, without rotating the polyp, to avoid counting gonads twice. For each individual we used the maximum number of testes observed during the whole reproductive period as a proxy for male fecundity. In females, eggs develop sequentially, and unfertilised eggs detach from the parent. We counted and removed detached eggs during routine maintenance and used the sum of detached eggs as a proxy for female fecundity.

To measure size-specific reproductive allocation, we also calculated relative gonad number by dividing the number of gonads with body size for both males and females.

### Quantifying survival

To avoid scoring animals dead when they retain the ability to survive, we maintained experimental animals even if they shrank to a very small size and looked a mass of amorphic necrotic tissue, up to five months in the population sample and six months in the size manipulation experiment. Animals were scored 'survived' if they regenerated and produced asexual buds following sexual reproduction in each experiment. Furthermore, in the population sample we also scored animals 'survived' if they did not produce buds but had intact tentacles, looked healthy and were able to feed at the end of the five months cooling period. No such individual was observed in the size–manipulation experiment (i.e. they either produced buds, totally disintegrated and disappeared, or remained senescent by the end of the experiment). Animals that disappeared or became an amorphic mass of necrotic tissue were scored as 'not survived'. Because in post-reproductive individuals it is not possible to clearly tell apart dead and living individuals, we did not produce mortality curves over time, but used the end state instead (i.e. survived or not) as dependent variable in subsequent analyses.

### Statistical analyses

For the population sample (i.e. non-manipulated individuals) we used Linear Mixed Effects Models (LMM) or Generalized Linear Mixed Models (GLMM) to analyse the effect of body size on: 1) probability of sexual reproduction (vs. remaining asexual during the whole cooling phase; binomial GLMM), 2) start of sexual reproduction (days after cooling; LMM), 3) maximum number of testes per male and total number of eggs per female (Poisson GLMM), 4) size-standardised gonad number (LMM) and 5) probability of survival (combined for sexual and asexual individuals and separately for males and females; binomial GLMM). Start of sexual reproduction was log-transformed to improve normality. Strain ID was included as random effect and we controlled for polyp age and collection date. Body size and age were scaled to zero mean and unit variance to improve model convergence. An observation-level random effect was added to the model analysing egg number in females to handle overdispersion.

We used LMMs or GLMMs to analyse the effect of experimental treatment on: 1) post-treatment polyp size (LMM), 2) start of sexual reproduction (LMM), 3) maximum testis number per male and total egg number per female (Poisson GLMM), 4) size-standardised gonad number (LMM) and 5) probability of survival (binomial GLMM). We included body ring exchange pair ID as random effect. All analyses were done in R (v 3.6.3; R Core Team 2020), using the *nlme* package for LMM (v. 3.1-144; Pinheiro *et al.* 2020) and *lme4* package for GLMM (v. 1.1-21; Bates *et al.* 2015). We first tested whether experimental groups are significantly different from each other via Likelihood Ratio (LR) tests. When LR tests indicated significant differences, we used the *multcomp* package in R (v. 1.4-13; Hothorn *et al.* 2008) to perform *post hoc* comparisons with Benjamini–Hochberg correction among the three experimental groups.

To gain insight into sex differences, we repeated all analyses with male and female data pooled and the interaction between sex and body size (population sample) or sex and experimental group (size manipulation experiment) included into the models. To make gonad numbers comparable between males and females, we first log-transformed these variables then scaled them to zero mean and unit variance separately for the sexes.

## RESULTS

### Population sample

Out of the $N = 1236$ individuals in the population sample 18.1% ($N = 224$) remained asexual throughout the 5 months of the cold phase and produced only buds. $N = 393$ polyps (31.8%) produced eggs, while $N = 619$ individuals (50.1%) produced testes. Larger individual were more likely to reproduce sexually (binomial GLMM, beta = 2.10, SE = 0.20, $P < 0.001$, $N = 1220$), showed faster sexual development (LMM, males: beta = −0.10, SE = 0.10, $P < 0.001$, $N = 610$; females: beta = −0.08, SE = 0.10, $P < 0.001$, $N = 390$; Fig. 2a,b) and had higher fecundity (Poisson GLMM, males: beta = 0.18, SE = 0.02, $P < 0.001$, $N = 610$; females: beta = 0.21, SE = 0.04, $P < 0.001$, $N = 390$; Fig. 2c and d). However, size-standardised gonad number decreased with body size (LMM, males: beta = −0.17, SE = 0.02, $P < 0.001$; females: beta = −0.10, SE = 0.04, $P = 0.019$).

Overall, including both sexual and asexual individuals there was a significant negative correlation between survival probability and body size (binomial GLMM, beta = −0.68, SE = 0.09, $P < 0.001$, $N = 1213$). The relationship was significant separately for males (binomial GLMM, beta = −0.59, SE = 0.13, $P < 0.001$, $N = 604$) and females (binomial GLMM, beta = −0.28, SE = 0.13, $P = 0.030$, $N = 389$; Fig. 2e and f).

We found a significant sex × body size interaction for the absolute and relative number of gonads: absolute gonad number

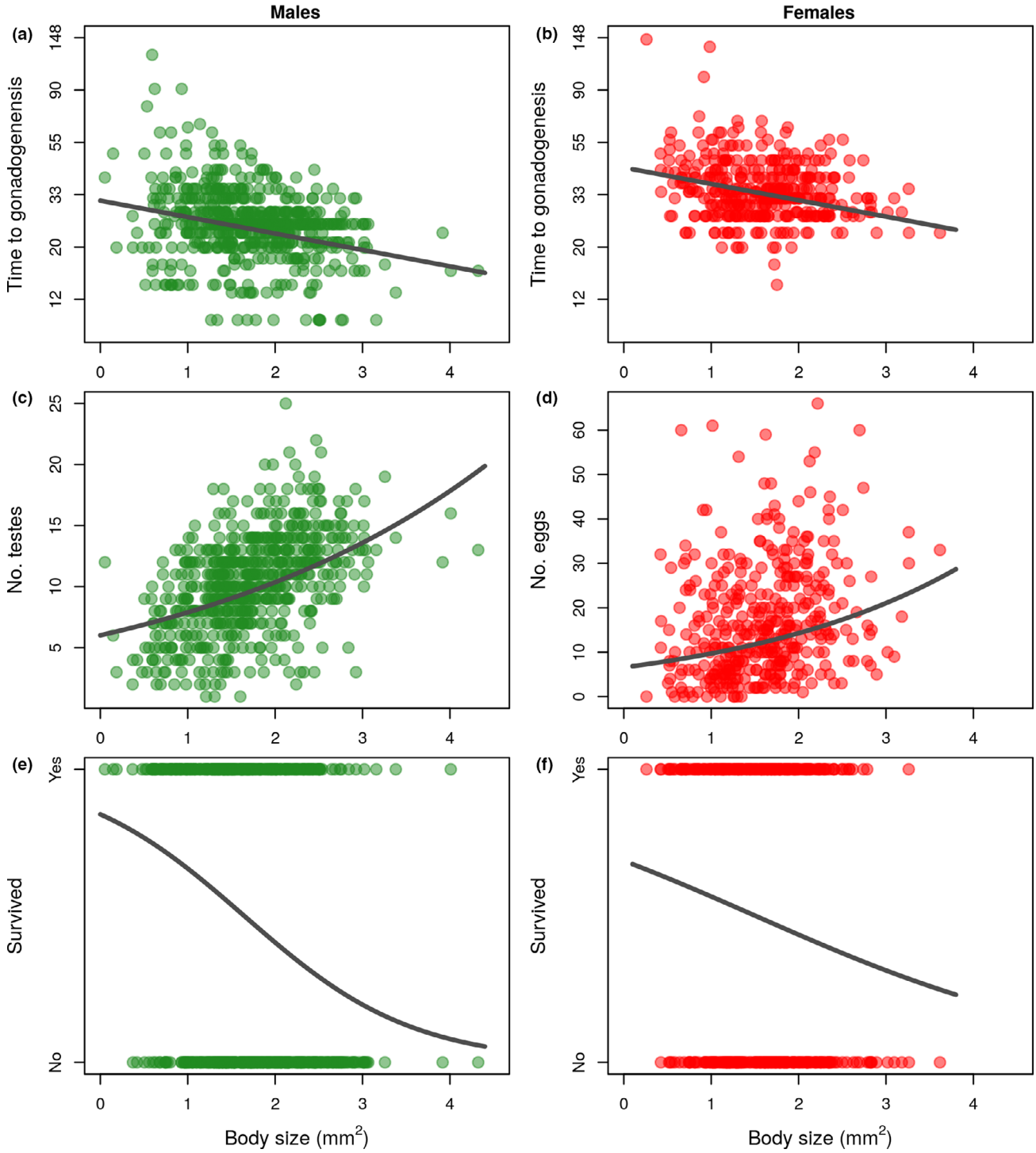


**Figure 2** Relationship between body size and (a) start of gonadogenesis after cooling in males (days, note the logarithmic scale), (b) start of gonadogenesis after cooling in females (days, note the logarithmic scale), (c) number of testes, (d) number of eggs, (e) male survival rate and (f) female survival rate. Data points are from strains derived from populations samples of *H. oligactis* kept under common garden conditions in the laboratory. Prediction lines derive from Linear Mixed-Effects Models (a and b), Generalized Linear Mixed-Effects Models with Poisson distribution (c and d) or Generalized Linear Mixed-Effects Models with binomial distribution (e, f). All models contain strain ID as random factor. Sample sizes are $N = 610$ and $N = 390$ for male and female reproductive traits, respectively and $N = 604$ and $N = 389$ for male and female survival, respectively.

increased with body size more steeply in males, while relative gonad number decreased more steeply in males compared to females (Table S1). The sex × body size interaction did not significantly affect time to gonadogenesis or survival (Table S1).

### Size manipulation

Pre-treatment body size measurements showed that polyps did not differ significantly in size among treatment groups prior

to experimental manipulation (LMM, male strain, LR = 0.69, $P = 0.71$; female strain, LR = 1.83, $P = 0.40$). In contrast, post-treatment size differed in accordance with intended size manipulations (LMM, male strain, LR = 140.71, $P < 0.001$; female strain, LR = 132.17, $P < 0.001$): enlarged and reduced polyps showed significantly larger and smaller body sizes, respectively, relative to control polyps (Table 1; Fig. 3a and b; Fig. 4). The range of post-manipulation size (0.55–3.54 $mm^2$) was within the range of the unmanipulated body sizes in the population sample (0.05–4.32 $mm^2$).

In males, size manipulation affected the timing of sexual development, with enlarged polyps producing testes first (LMM, LR = 14.19, $P < 0.001$; Table 1; Fig. 3c). The number of testes was also highest in enlarged polyps (Poisson GLMM, $\chi^2 = 61.05$, $P < 0.001$; Table 1; Fig. 3e). In females, experimental treatment likewise affected the length of time required to produce the first egg, with enlarged females developing eggs earliest (LMM, LR = 60.42, $P < 0.001$; Table 1; Fig. 3d). The number of eggs produced by females differed significantly between groups, being highest in the enlarged group (Poisson GLMM, $\chi^2 = 9.44$, $P = 0.009$; Table 1; Fig. 3f).

Experimental treatment impacted relative gonad numbers in both sexes (LMM, males, LR = 36.48, $P < 0.001$; females, LR = 15.85, $P < 0.001$). Pairwise comparisons revealed that larger individuals had smaller size-specific gonad numbers. With one exception (enlarged vs. control in females), these differences were statistically significant (Table 1).

Survival rates were significantly impacted by size manipulation in both males and females (binomial GLMM, males, $\chi^2 = 13.58$, $P = 0.001$; females, $\chi^2 = 10.17$, $P = 0.006$). In males, reduced polyps were more likely to survive compared to control or enlarged polyps, but the difference between controls and enlarged polyps was not significant (Table 1; Fig. 3g). In females, reduced polyps had significantly higher survival rate than controls and the difference between reduced and enlarged polyps was marginally significant (Table 1; Fig. 3h). Control and enlarged female polyps did not differ in survival (Table 1; Fig. 3h).

We found a significant sex × experimental treatment interaction for time to gonadogenesis and absolute number of gonads, but not the other variables (Table S2). Gonad number increased more steeply, while time to gonadogenesis decreased less steeply with size in males (Table S2).

## DISCUSSION

Correlations between body size and other life history traits are frequently observed, but the underlying mechanisms are poorly understood due to multiple competing explanations behind size-scaling relationships. Using experimental manipulation, we here unequivocally showed that body size *per se,* rather than the processes associated with it (such as growth or individual condition) has a causal effect in determining sexual development and longevity in a cnidarian model system. Notably, hydra polyps with experimentally reduced body size showed delayed sexual development and reduced fecundity but experienced higher post-reproductive survival. These observations demonstrate an immediate, growth-independent effect of adult body size on key life history traits determining population dynamics.

Previous research on size-scaling relationships has identified several distinct hypotheses to explain the effect of body size on life history traits. Size could influence reproduction and survival through its effect on resource balance: in a favourable environment a large body implies more resources that can be invested in both reproduction and physiological repair, potentially contributing to increased fecundity and survival likelihood (e.g. Forsman 1993; Gaillard *et al.* 2000; Hendriks & Mulder 2008). However, while the increased resource availability of large individuals can explain the increased fecundity we observed, it cannot account for their reduced survival, suggesting that additional mechanisms must be involved.

A frequently cited explanation for the size-dependence of life history traits is centred on the negative effects of growth (Blanckenhorn 2000; Metcalfe & Monaghan 2003), which were hypothesised to explain, for example, the negative correlation between body size and longevity in domesticated species (Rollo 2002; Austad 2010). Our findings, however, clearly show that a small size itself can lead to improved survival without reduced growth, because the size differences in our experiment were achieved in a growth-independent manner.

**Table 1** Pairwise comparisons between size manipulation experimental groups, obtained from Linear Mixed-effects Models (LMMs) or Generalized Linear Mixed-effects Models (GLMMs) containing tissue ring exchange pair ID as random effects. Significant differences ($P < 0.05$) are highlighted in bold

| | | Control vs. Reduced | | Enlarged vs. Control | | Enlarged vs. Reduced | |
|---|---|---|---|---|---|---|---|
| | Variable | β (SE) | *P* value* | β (SE) | *P* value* | β (SE) | *P* value* |
| Male strain | Post-manipulation size (LMM) | **0.89 (0.09)** | **< 0.001** | **0.88 (0.09)** | **< 0.001** | **1.76 (0.10)** | **< 0.001** |
| | Time to gonadogenesis (LMM) | −1.41 (1.18) | 0.444 | −0.38 (1.18) | 0.742 | **−1.79 (0.45)** | **< 0.001** |
| | No. testes (Poisson GLMM) | **0.38 (0.08)** | **< 0.001** | **0.24 (0.06)** | **< 0.001** | **0.62 (0.08)** | **< 0.001** |
| | Relative no. testes (LMM) | **−0.30 (0.06)** | **< 0.001** | **−0.16 (0.06)** | **0.011** | **−0.46 (0.07)** | **< 0.001** |
| | Post-reproductive survival (Binomial GLMM) | **−2.01 (0.80)** | **0.024** | −0.53 (0.53) | 0.315 | **−2.56 (0.84)** | **0.007** |
| Female strain | Post-manipulation size (LMM) | **0.81 (0.08)** | **< 0.001** | **0.90 (0.09)** | **< 0.001** | **1.71 (0.10)** | **< 0.001** |
| | Time to gonadogenesis (LMM) | **−4.13 (0.77)** | **< 0.001** | **−1.58 (0.79)** | **0.039** | **−5.72 (0.55)** | **< 0.001** |
| | No. eggs (Poisson GLMM) | 0.17 (0.12) | 0.166 | 0.21 (0.11) | 0.132 | **0.37 (0.12)** | **0.007** |
| | Relative no. eggs (LMM) | **−0.50 (0.14)** | **< 0.001** | −0.15 (0.15) | 0.293 | **−0.65 (0.17)** | **< 0.001** |
| | Post-reproductive survival (Binomial GLMM) | **−1.70 (0.56)** | **0.008** | 0.13 (0.67) | 0.846 | −1.57 (0.70) | 0.052 |

*After Benjamini-Hochberg correction for three comparisons.

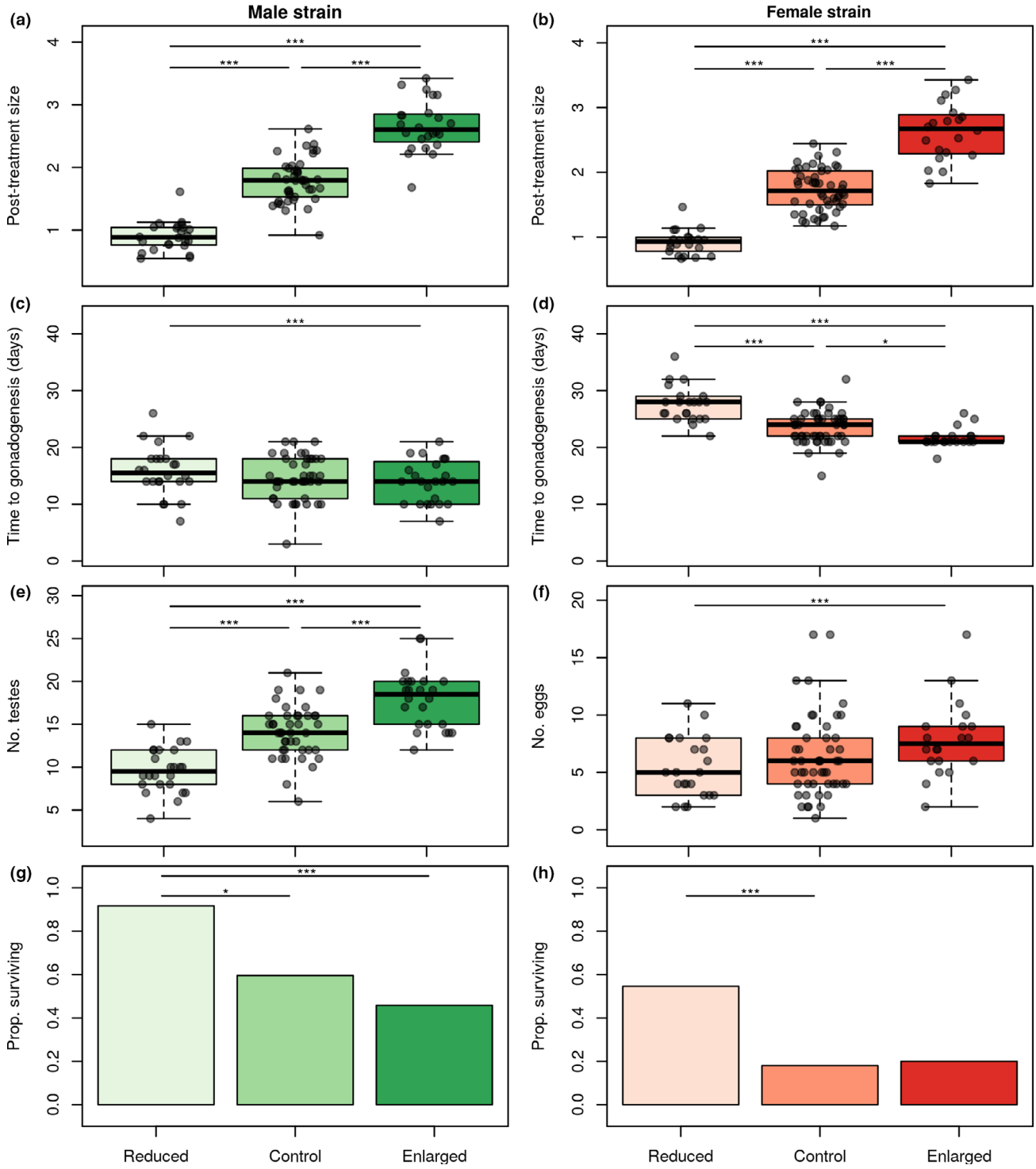


**Figure 3** Comparison of enlarged, control and reduced individuals in post-manipulation size (body surface area measured after experimental treatment and healing; $mm^2$) in the male (a) and female strain (b), start of gonadogenesis after cooling (days) in the male (c) and female strain (d), number of testes in the male strain (e), number of detached eggs in the female strain (f) survival rate (proportion of animals scored as survived after post-reproductive senescence) in the male (g) and female strain (h). Comparisons between groups were done through *post hoc* tests based on Linear Mixed-Effects Models (a–d), Generalized Linear Mixed-Effects Models with Poisson distribution (e and f) or Generalized Linear Mixed-Effects Models with Binomial distribution. Bars above pairs denote significant differences (*$P < 0.05$, ***$P < 0.001$) after Benjamini–Hochberg correction. The box-and-whiskers plot shows median values, lower and upper quantiles and minimum–maximum values. Dots denote individual data points. Sample sizes are $N = 90$ male polyps ($N = 24$ reduced, $N = 42$ control and $N = 24$ enlarged) and $N = 92$ female polyps ($N = 22$ reduced, $N = 50$ control and $N = 20$ enlarged)

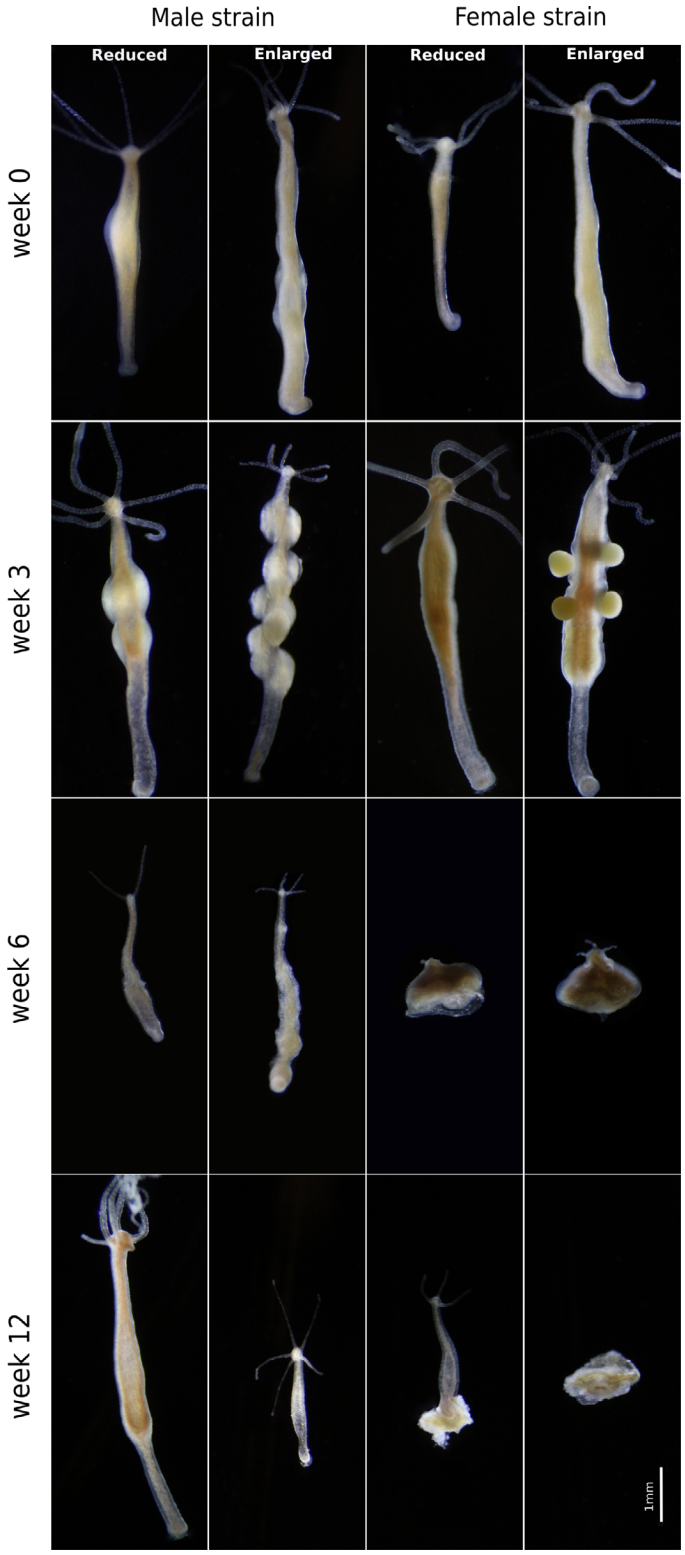


**Figure 4** Photographs illustrating sexual development and post-reproductive senescence of reduced and enlarged males and females. Gonads are visible by week 3 except in females with reduced body size that initiate gonadogenesis latest. By week 6 all polyps show signs of post-reproductive senescence (reduced body size and shortened tentacles). At week 12, some individuals show signs of recovery. Reduced individuals are in a more advanced stage of regeneration and males are more advanced than females.

Growth-independent effects of body size on survival could be explained, in turn, by at least two mechanisms. First, a larger size implies a larger tissue mass with proportionally larger maintenance costs, all else being equal. While animals enlarged in our experiment had larger digestive cavity, their head region and tentacles (which contain most stinging cells and are critical for capturing food) were left intact during the grafting procedure, therefore it is unlikely that their food capture rate increased to meet the higher energetic demands of larger size. As a result, enlarged animals had to divide their ingested food among a larger tissue mass, reducing resources left for physiological maintenance and repair. The fact, however, that we found the same relationships between body size and longevity in the population sample where body size was not manipulated and hence the feeding apparatus should be proportional to body size makes this explanation unlikely.

Second, differences in post-reproductive survival of large and small individuals could be the consequence of size-dependent reproductive decisions. If large individuals invest proportionally more of their resources into reproduction, that could drain resources available for survival. Such a hyperallometric investment in reproduction has been recently demonstrated in fish (Barneche *et al.* 2018) and might be common in the natural world (Marshall & White 2019). This hypothesis predicts that relative (size-standardised) gonad number in hydra increases with size. Contrarily, we found a negative relationship between relative gonad number and body size. Hence, reproductive investment in *H. oligactis* appears to be hypoallometric, instead of hyperallometric. However, while we did not observe a disproportionately higher sexual investment in larger individuals, there was clear evidence for a size-dependent adjustment in timing of gonadogenesis. In both the population sample and size-manipulated polyps, smaller individuals took more time to start gonadogenesis than larger individuals. Since in the laboratory animals were kept on constant food, this could have enabled small individuals to accumulate more resources, ultimately increasing survival. Therefore, we think that the most likely explanation for the size-dependent life history patterns observed by us is that small individuals delay sexual reproduction to improve their post-reproductive survival.

Why do small individuals but not large ones delay reproduction? In males, this could be explained by intrasexual competition: in their natural habitat, *H. oligactis* males appear earlier in the season (Sebestyén *et al.* 2018) and likely compete for priority in fertilising females. A small male is expected to lose out in competition for fertilisation due to its lower number of reproductive organs. Small individuals might have a greater fitness payoff by investing into physiological repair to increase the chances of overwinter survival. In females, the situation is much less clear, because we do not expect intrasexual competition in their case. However, delayed sexual development and increased investment into survival in small females could be caused, for instance, by sexually antagonistic selection (selection on males causing correlated changes in females; Bonduriansky *et al.* 2008). This hypothesis is highly plausible since sex-change is known to occur in this species (Miklós *et al.* 2021), therefore the same genotype might experience selection both as female and male. In support of the stronger expected size-dependence of reproductive investment

in males compared to females we found that fecundity increased more steeply, while time to gonadogenesis decreased less steeply in experimentally enlarged males. Although our experimental manipulations were done only on a single male and female strain, the effect of the sex × body size interaction on fecundity (though not on time to gonadogenesis) was detected in the population sample as well, suggesting that these sex differences might be general.

Although multiple mechanisms might be involved, our data clearly show that body size itself influences reproductive decisions, potentially explaining the differential survival rate of small and large individuals. Since the relationship between body size and reproductive decisions is well established in the animal world (Reiss 1987) and reproductive decisions have carry-over effects on survival in animals ranging from nematodes and fruit flies to humans (Hsin & Kenyon 1999; Flatt 2011; Min *et al.* 2012), the link between body size, reproduction and senescence might be a general and major nexus in the natural world. Nonetheless, while the growth-independent effect of body size on life history traits might be present in other species as well, the exact relationship is likely to differ. In particular, Reiss (1987) predicted that the scaling of reproductive investment with body weight would depend on post-reproductive survival, with a steeper relationship in species with poor post-reproductive survival (such as our model species). Hence, the effect of body size on reproductive investment and survival might be different in animals that reproduce more frequently during their lifetime. This prediction could be tested in the future by applying the tissue grafting method presented here on species with different life history patterns, for example, other cnidarian polyps or animals where transplantation of whole body pieces is possible (e.g. flatworms; Saló & Baguñà 1985).

On a proximate level, genetic regulation of body size and senescence is likely to be governed by common mechanisms across the animal kingdom. In several organisms reproductive investment and somatic maintenance are regulated by the IIS pathway which increases reproduction at the expense of survival (e.g. Hsin & Kenyon 1999; Flatt *et al.* 2008). Recent evidence indicates that these effects are observed even if IIS is modulated during adulthood (Lind *et al.* 2019), suggesting a growth-independent effect of this pathway. In hydra, the size of polyps is also assessed and controlled through the IIS pathway, in crosstalk with the Wnt and TGF–β pathways (Mortzfeld *et al.* 2019). Together, these regulate cell proliferation and differentiation in the body column in a size-dependent manner (Hobmayer *et al.* 2000; Mortzfeld *et al.* 2019). Because gametes in hydra derive from the same stock of interstitial stem cell populations that also give rise to somatic derivatives (Nishimiya–Fujisawa & Kobayashi 2018), any shift in the differentiation of stem cells to gametes is likely to reduce cell populations involved in somatic maintenance, thereby impacting survival (Sebestyén *et al.* 2018).

In conclusion, by exploiting the ability of hydra polyps to incorporate tissue from other polyps, we were able to experimentally alter body size in *Hydra* and evaluate the effect of size manipulation on key life history traits. With human activity causing substantial reduction in the body size of harvested species and global climate change affecting animal size worldwide, understanding how life history decisions depend on body size becomes increasingly important. Crucially, our finding that small individuals delay sexual maturation, have lower fecundity and higher post-reproductive survival implies that ecological changes in body size will have immediate effects on population dynamics: even though survival of small individuals increases, the population might be negatively affected due to delayed sexual maturation and reduced fecundity. Given these concerns, understanding the scaling of demographic traits with body size is more important today than ever.

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## AUTHORSHIP

JT and ZB conceived research; KSN performed size manipulation experiment, collected data and wrote first draft of the manuscript; BA and JT collected data for the population sample and analysed data; all authors contributed to the final version of the manuscript.

## PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/ele.13698.

## OPEN RESEARCH BADGES

This article has earned Open Data badge. Data is available at (https://doi.org/10.6084/m9.figshare.12727673.v1 or https://doi.org/10.6084/m9.figshare.12731909.v1), https://github.com/jtokolyi/SizeDependence

## DATA AVAILABILITY STATEMENT

Data used in this study are available on FigShare (https://doi.org/10.6084/m9.figshare.12727673.v1 and https://doi.org/10.6084/m9.figshare.12731909.v1). R code used to analyse data is available on GitHub (https://github.com/jtokolyi/SizeDependence).

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