Calorie restriction brings no benefits to lifespan under stochastic environments

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RSG conceived the idea; JAD, RSG and AA designed the methodology; JAD and PH collected and digitized data; JAD analysed data; JAD led the writing of the manuscript with assistance from RSG.

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DATA AVAILABILITY STATEMENT

The data in this paper are published open access in multiple formats (e.g., .pdf, .R, .cpproj).
Abstract

The impacts of resource availability on senescence—the loss of vitality with age— are formalised under the Calorie Restriction (CR) theory, which predicts that the onset of senescence is delayed and life expectancy prolonged due to the ultimate effects of restricted resource intake without malnutrition. However, CR studies are largely implemented in unrealistic environments that do not consider how interacting, stochastic drivers impact longevity. Indeed, little is known about the impact of stochastic resource availability on senescence, even though environmental stochasticity is the norm rather than an exception in natural populations. Here, we examine whether and how stochasticity in the quantity, quality, and frequency of resources impact lifespan, life history trait trade-offs, and population structure in two long-lived planaria: *Schmidtea mediterranea* and *Dugesia tahitiensis*. For each species, we estimate weekly population size, survival, and a size distribution metric that quantifies population structure and skew. Over the 19-week study, survival in *S. mediterranea* is lower than *D. tahitiensis* across all feeding regimes. However, for both species, CR does not diminish survival. There are also no clear shifts in population structure over time across the different feeding regimes. For *S. mediterranea*, in most treatments, population structure changed to fewer smaller than larger individuals (right-skewed). In the case of *D. tahitiensis*, only treatments where resources are provided frequently cause right-skewed population structures. Population size also varied between species, with that of *D. tahitiensis* never declining across treatments, and always becoming larger than *S. mediterranea*; in the case of *S. mediterranea*, most treatments show a decline in population counts over the study period. As before, no clear pattern emerges in the changes in population counts under CR conditions for both species. As such, we did not find evidence of CR providing benefits in terms of lifespan nor trade-off between population counts, survival, and body size. We call for the careful re-evaluation of decades of CR work in short-lived species, by expanding and testing predictions in more realistic settings and across a wider range of life histories.
Keywords: ageing, diet, life history trait, longevity, planaria, population structure, senescence, trade-off, vitality
INTRODUCTION

Resource availability is a key driver of individual development and population performance (Stearns 1992; Ozgul et al. 2009; Smallegange 2011). Yet, studies on how resource availability impacts the life cycle of an organism have largely focused on specific life transitions (e.g., age at maturity (Stearns 1992; Auer 2010; Smallegange 2011) or features of the life cycle (e.g., reproduction (Williams 1996; Rutstein et al. 2005); survival (Ozgul et al. 2009)). An important trait that has lacked similar attention is senescence; the physiological decline of an organism’s vitality with age that ultimately reduces reproduction and increases mortality risk. Reproduction and survival are key demographic processes of the life cycle of a species with variation in these processes defining life history traits (e.g., age at maturity, net reproductive rate, longevity, etc.) which, in turn, result in life history strategies that impact individual fitness and the dynamics of a species (Roff 1992; Stearns 1992; Capdevila & Salguero-Gómez 2019). Life history theory predicts a trade-off in resource allocation between maintenance (development and survival), and reproductive output of individuals (Roff 1992; Stearns 1992). However, the impact of resource availability on demographic processes, and therefore on life history traits, is not straightforward (Mysterud et al. 2001; Maron et al. 2015). For example, winter climate has been shown to have an indirect effect on foraging conditions for red deer (Cervus elaphus) and domestic sheep (Ovis aries) in Norway (Mysterud et al. 2001). Mysterud et al. (2001) found that high North Atlantic Oscillation (NAO) values had a positive effect on individual body mass, a proxy for increased survival and reproduction in ungulates. Years of high NAO values correlates with increased warm and wet winters, and a potential mechanism explaining the response the authors found was that more snow in the high-elevation summer areas will provide prolonged periods of access to high-quality forage during summer.

Trade-offs in resource allocation between maintenance and reproduction form the predictions of two of the three main theories of senescence (Williams 1957; Kirkwood 1977). Currently the
impacts of resource availability on senescence are formalised under the Calorie Restriction (CR, hereafter) theory. Under CR theory, life expectancy is extended and the onset of senescence delayed due to restricted resource intake without malnutrition (Weindruch & Walford 1988). The benefits of restricted resource intake are hypothesised to be mediated at the molecular and cellular level by lowering molecular oxidative damage (Sohal & Weindruch 1996; Sanchez-Roman & Barja 2013) and reducing free radical-induced cellular damage (Barja 2004; Fontana & Klein 2007). The predicted effects of CR have been observed in several species, from yeast (Lin et al. 2002; Masoro 2005), to invertebrates (Mair et al. 2003; Carey et al. 2005), and mammals (Weindruch and Walford 1988; Masoro 2005; Colman et al. 2014). However, recently the benefits of CR have been brought to question. Several studies have shown no effects on lifespan or senescence (Troen et al. 2007; Smith et al. 2010), with others even showing negative impacts, i.e., shortening lifespan and accelerating rates of senescence (Weithoff 2007; Fisher et al. 2017). Possible reasons for this diversity in results include the lack of standardised protocols of nutritional demands (Cava & Fontana 2013; Deere et al, in prep) and protein:carbohydrate ratio (P:C) of diets (e.g. in Drosophila, Lee et al. 2008). The environment of an organism too plays a role in determining senescence. Recent work by Roper et al. (2021) suggest that key life history traits (e.g., adult body size) and ecology of the organism may be vital in determining senescence outcomes.

The rich diversity of life history strategies is not well represented in CR research (Deere et al. 2020). This is particularly notable given the link between an organism’s life history traits and its ecology (Jansen et al. 2012; Arlettaz et al. 2017). Indeed, most studies exploring the impacts of CR on senescence have focused on short-lived species, while CR studies on longer-lived species remain scarce: one study on the grey mouse lemur (Microcebus murinus; Pifferi et al. 2018) and two studies on the Rhesus monkey (Macaca mulatta; Colman et al. 2009; Mattison et al. 2012)). Other common life history strategies are even less frequently examined in CR, such as the ability to reallocate...
resources internally. For instance, in both the Galápagos marine iguanas (*Amblyrhyncus cristatus*) (Wikelski & Thom 2000) and some Planaria (Child 1914; González-Estévez 2009) individuals can shrink via the digestion of internal organs when resource are scarce and subsequently re-grow when resources become more abundant. In both cases, this ability to shrink enhances survival.

Empirical investigations of CR impacts on senescence have been largely focused on constant resource conditions. However, mimicking the environment to which an individual is typically exposed is key to examine senescence outcomes (Koons *et al.* 2014; Roper *et al.* 2021). This consideration is particularly important because environmental stochasticity is known to shape life history traits (Stearns 1992; Pardo *et al.* 2013; Behrman *et al.* 2015), which in turn play a crucial role in population structure and dynamics (Tuljapurkar & Orzack 1980; Tuljapurkar *et al.* 2003; Coulson *et al.* 2006; Simmonds & Coulson 2015). As far as we are aware, there are only two studies that explicitly investigate CR impacts on senescence in stochastic environments (*Drosophila*, Mair *et al.* 2003; medfly, Carey *et al.* 2005). In them, longevity was extended under a stochastic feeding regime. In the case of *Drosophila*, three reduced dietary treatments were initiated; one where flies received reduced diet for the length of adulthood and two where fully fed flies were switched to reduced diet during their adulthood. When fully fed flies were switched to reduced diets at day 14 or day 22 of the experiment there was a reduction in age-specific mortality, equivalent to that in treatments where diets were permanently reduced, when compared to treatments where flies were fully fed. The medfly study was more complex in that 12 stochastic dietary treatment regimes were used. Treatments varied in how sparse or dense the food available was and how persistent each food patch was (e.g., available for one day or 10 days). In this study, nine of the 12 treatments showed an increase in life expectancies compared to the full diet treatment. However, little can be gleaned from two studies alone to determine how stochastic environments impact senescence in the face of CR.

Here, we contribute to fill in the gap of knowledge in CR research by examining the impact of
stochasticity of resources in two long-lived invertebrate species, the planarians *Schmidtea mediterranea* and *Dugesia tahitiensis*. We use these species to examine whether and how stochasticity in three different dimensions of resource availability (quantity, quality, and frequency) impact their lifespan, life history trait trade-offs, as well as population structure and dynamics.

Specifically, we hypothesize that: (H1) In high quantity, low quality environments, population size will be larger, and individual size will be normally distributed. High quantity, low quality environments are richer in protein; these environments typically promote reproduction (Lee 2015) and in turn individuals attain shorter lifespans, which free up more resources for reproduction to improve population viability (Bayliss & Choquenot 2002); (H2) In low quantity, high quality environments, population size will be smaller, dominated by larger individuals skewing the population size structure to the right, thus leading to increased longevity but lower reproduction. Environments with fewer, but higher quality resources, will likely be dominated by larger individuals that outcompete smaller individuals (Szabo 2002; Ward *et al.* 2006). In it, available resources will be allocated to maintenance because, in organisms with indeterminate growth like planarians, energy allocation is typically prioritised for maintenance over reproduction and growth (Jokela & Mutikainen 1995); and (H3) Environments with a higher frequency and quality of resource availability will result in an increase in clonal reproduction and a larger population size. Where resource supply is high and constant, populations support higher numbers, which decrease population extinction risk (Bayliss & Choquenot 2002; Lande *et al.* 2003).

**METHODS**

**Study system and stock culture**
Planaria have unique life history traits. Most notably, the majority of planarians are able of restorative and physiological regeneration due to injury or damaged/sick cells (Elliott & Sánchez Alvarado 2013). The replacement of damaged or sick cells also contributes to the typically long-lived life history of planarians. Indeed, under favourable, constant conditions, planaria are potentially immortal (Elliott & Sánchez Alvarado 2013). Planarians are also able to shrink when faced with unfavourable conditions (Child 1914; González-Estévez 2009). Here, we use Schmidtea mediterranea (Benazzi, Baguna, Ballister, Puccinelli & Del Papa, 1975) and Dugesia tahitiensis (Gourbault, 1977), two closely related, freshwater planarian species from the order Tricladida that are easily reared in the lab (Sousa & Adell 2018). The two species also differ in size with D. tahitiensis larger (=10mm in length) than S. mediterranea (=5mm in length). S. mediterranea can reproduce sexually or asexually whereas D. tahitiensis is an obligate asexual. We used asexual lines of S. mediterranea for our experiments in order to directly compare the two species. Asexual reproduction in planaria occurs via a process called binary fission that results in two genetically identical clones of smaller size from the fissioning individual (Malinowski et al. 2017).

To form cultures, a clonal line of the wild type, asexual strain of S. mediterranea and D. tahitiensis were initiated from long-term cultures maintained by the Aboobaker laboratory in the Department of Zoology at the University of Oxford. The S. mediterranea cultures originate from the main laboratory stock used worldwide, first collected from Monjuïc, Barcelona. D. tahitiensis originate from the cultures in the Egger laboratory at the University of Innsbruck. A total of three stock cultures per species were started with 10 individuals each. To explore the impact of three different dimensions of resource availability, we increased initial numbers of planarians to ensure a large sample size. Once a week, for three weeks, 10 individuals per species were taken from each culture and two transverse amputations per individual were performed (thus forming three individuals) following methods in Sousa and Adell (2018). In doing so all individuals were of a similar chronological age and the potential age variance was reduced to three weeks which is significantly less than that
of individuals within the main laboratory stock. Once fissioned, during regeneration, polarity of their body axes is maintained. This means that each fissioned piece conserves the anterior-posterior, dorsal-ventral and medial-lateral axes and, as such, subsequent organogenesis (Elliott & Sánchez Alvarado 2013). Individuals were reared in a mixture of distilled water and sea salt (Instant Ocean sea salt, Aquarium Systems) at a concentration of 0.5g/L. Cultures were fed on organic calf’s liver twice a week; the liver was left for two hours in the containers before being removed to ensure full satiation of individuals within the population. After removal, the water-salt mix was replaced and the containers housing the cultures were cleaned to avoid potential infection through debris build-up. This feeding regime frequency was sufficient to ensure continued fissioning of individuals and increasing numbers in the cultures. Stock cultures were housed in six 30cm long × 30cm wide × 15cm high plastic containers (3 for *S. mediterranea*, 3 for *D. tahitiensis*) within a climate room set at 20°C and kept in 0:24hr light:dark regime. Populations were only exposed to low levels of light for short periods of time during feeding and data collection.

**Experiment**

To test our hypotheses regarding how CR impacts longevity and its trade-offs with other life history traits in stochastic environments, we randomly assigned populations to a full-factorial design for each of the two species. This design included three resource factors: quality, quantity, and timing (Fig. 1). Quality of resource had two levels, differing in energy content and relative protein-to-carbohydrate ratio: high-quality (high carbohydrate level, HQ hereafter) vs. low-quality (low carbohydrate level, LQ hereafter). The high-quality diet was based on organic calf’s liver and the low-quality diet on bloodworm. The protein-to-carbohydrate ratio indicates resource quality, with low carbohydrate to protein content corresponding to poorer quality (Lee et al. 2008). The two diets differed not only in their energy content (109 kcal/100g in the liver vs. 14 kcal/100g in the bloodworm) but also in their relative protein-to-carbohydrate ratios (1:0.35 in liver vs. 1:0.17 in
bloodworm). Quantity of resources had three levels, which differed in the amount of calories available in each feeding: standard calorie intake (SI: 0.001g/individual, representative of intake to maintain laboratory populations (Jochen 2018)), restricted calorie intake (RI: 0.0005g/ind.), hyper-calorie intake (HI: ad libitum which was at least 10 fold, approx. 0.01g/individual, that of the SI). We always fed treatments the day after population counts and after photographs were taken (see below) to ensure that counts were reflective of the previous feeding event. We based the amount of resource provided a given week on the population counts of the previous day and the relevant treatment (i.e., total number of individuals multiplied by the resource weight for the specific treatment). All resources were weighed to the nearest 0.0001g using an Adventurer Analytical microbalance (Ohaus). After feeding, all the water-salt mix solutions were replaced in the populations of all treatments. This step was essential to ensure an oxygenated solution and to prevent infection from the solution mix that contained excess debris. In the HI treatment, resources were left for 2 h before removal to ensure full satiation. Different combinations of resource quantity and quality were provided in two different frequencies: even (E), once every 7 days, or delayed (D), every 14 days.

We replicated all treatments in the full factorial (n = 12 levels: 2 qualities: HQ vs. LQ; 3 quantities: RI, SI & HI; 2 frequencies: E vs. D) five times for each species. A replicate consisted of a population (25mm deep × 150mm Ø) containing 10 individuals each, henceforth referred to as a population (total n = 600 ind/spp). For each population, individuals were randomly isolated from one of the three stock cultures and placed in the population 7-8 days before the start of the experiment. During this time, individuals were not fed to ensure that individuals across all treatments were in a similar starved state at the start of the experiment. Treatment populations were randomised into three blocks containing 20 populations per species per block. For each species, all experimental blocks lasted 19 weeks (133 days). Blocks were staggered in three different start dates due to the logistical constraint of sampling all 60 populations on the same day, as such staggered blocks allowed a more logistically feasible sampling of 20 populations per day. Block 1 of the experiment started on the 4th February.
2020, and block 2 on the 30th April 2020. In the case of block 3, the start date for *S. mediterranea* and *D. tahitiensis* differed due to low numbers available for *S. mediterranea* from the original source. As such, block 3 for *D. tahitiensis* started on the 1st May 2020 and for *S. mediterranea* on the 19th June 2020. Part way through the experiment, due to the COVID-19 pandemic, the location of the experiment to be changed, while retaining the same environmental conditions. For further details of this change, see the Supporting Information.

Within each population, every week, survival and asexual reproduction (clonal reproduction) were estimated by counting all living and dead individuals. This was done to determine changes in population size, as we expect that, (H1) in high quantity/low quality environments population size will be larger, (H2) in low quantity/high quality environments population size will be smaller, and (H3) increased frequency of resources will result in increased clonal reproduction. Additionally, size (area in mm²) of each individual flatworm within each population was measured to determine growth/shrinkage. Our expectations here are that, (H1) in high quantity/low quality environments individual size will be normally distributed, (H2) low quantity/high quality environments will be dominated by larger individuals, and (H3) increased frequency of resources will result in greater rate of size oscillation.

To estimate individual size, once a week, photos of each population were taken. We placed a population on an A4 size light table and within an opened bottomed box (to allow light through). The top of the box had an 8cm diameter aperture to allow for a camera lens. A Canon EOS 700D digital SLR camera and a Canon 18-135mm EFS lens were attached to a tripod (Vanguard Alta Pro 263AP) so the lens was facing downwards and protruding into the top aperture of the box. The lens was set at a constant height of 29cm above the population. The camera settings were constant and set manually at F5.6, ISO 200 and a shutter speed of 1/100.

**Image digitization**
To obtain size measurements of individuals, images taken during the experiment required digitization and analysis. All images (n = 1,140) were digitized and analysed using the CellProfiler software (Lamprecht et al. 2007). CellProfiler is flexible open-source software that allows processing of batches of images automatically while allowing users to adjust settings to measure the phenotypes of interest. Based on the user settings, the software identifies possible entities (here individuals of planarian) within the image and calculates the relevant data (e.g., area of an individual) (see SI and Fig. S2). Image settings were selected depending on the species in question as there are significant size differences between *S. mediterranea* and *D. tahitiensis*. Within the experiment, because *S. mediterranea* individuals were of a much smaller size than *D. tahitiensis* that the required minimum and maximum values of the parameters used to identify individuals in the image (the ‘object diameter identification’ parameter) had to be set to values that differed from *D. tahitiensis*. The specific user defined settings were then used for the relevant species. All settings were saved as CellProfiler project files and are accessible to use via the online Supplementary Information (Smed.cpproj and Dtah.cpproj). In the case of *D. tahitiensis*, the procedure differed slightly for week 19 for the high quality even feeding treatments for all resource intakes (high, standard and reduced intake). Briefly, due to the high number of individuals present where there were large discrepancies between the number of individuals identified by the program and the count by hand, we randomly chose one quarter of the population to analyse using the user defined settings within the CellProfiler project file (Dtah.cpproj). For the populations showing large discrepancies, before an image was taken, if individuals were clumped together, we allowed individuals time to move around freely and become less aggregated. This ensured that when the photograph was taken individuals were more evenly distributed within the population. Results were then extrapolated by reproducing the data obtained from the analysed quadrant for the three quadrants not analysed, further details can be found in the Supplementary Information. Finally, because the individual area data calculated by CellProfiler is
provided in pixel values, we converted all values into mm² by multiplying pixel values by a conversion factor (see Supplementary Information).

**Survival analysis**

To determine whether high quantity/low quality environments (H1) and increased frequency of higher quality resources (H3) results in decreased lifespan, and low quantity/high quality environments (H2) results in increased lifespan, we calculated weekly survival in each population for each species using a Bayesian framework. We modelled survival as a function of covariates using a Binomial multilevel model with a logit link function with adaptive priors (Eq. 1). Fixed covariates were population (POP; categorical with 60 levels corresponding to each of the populations in the experiment; 5 replicates per treatment), treatment (T; categorical with 12 levels) and block (B; categorical with three levels).

\[
\text{Survival}_i \sim \text{Binomial}(n_{i-1}, p_i)
\]

\[
\logit(p_i) = \alpha_{POP[i]} + \beta_{T[i]} + \gamma_{B[i]}
\]

\[
\alpha_{POP} \sim \text{Normal}(\alpha, \sigma_{POP})
\]

\[
\beta_T \sim \text{Normal}(\alpha, \sigma_T)
\]

\[
\gamma_B \sim \text{Normal}(\alpha, \sigma_B)
\]

\[
\alpha \sim \text{Normal}(0, x)
\]

\[
\sigma_{POP} \sim \text{Exponential}(1)
\]

\[
\sigma_T \sim \text{Exponential}(1)
\]

\[
\sigma_B \sim \text{Exponential}(1)
\]  

(1)
To fit the model in Eq. (1), we used the package “rethinking” (McElreath 2020) in the software R version 4.0.3 (R Core Team 2020). We then applied the model to each week of the experiment separately to determine weekly survival (as opposed to survival at the end of the experiment after 19 weeks), with the exception of week 1, as this week was the start of the experiment and so there was 100% survival in all populations. In this way, we could identify if at a specific point (i.e., week) a significant reduction in survival occurred or if a constant decline in survival occurred over the 19 weeks. The model calculates the mean log-odds of survival between two given consecutive weeks where $Survival_i$ represents the number of individuals surviving in week $i$ out of an initial count the week prior, $n_{i-1}$. Models for each week only differed in the standard deviation ($\sigma$) of the $\alpha$ prior. The expectation was that, across weeks, populations (replicates of all treatments) would show variation in the standard deviation in the prior for each population’s intercept ($\alpha$). As such, not all weeks had the same $\sigma$ value, for each week $i$ the value of $\sigma$ of the $\alpha$ prior was varied and a model comparison was done to ensure best model fit (models with the lowest WAIC values and highest weight value were taken as best fit). Final $\alpha$ priors for each model are provided in the supplementary information (final models used are in bold, Deere et al_SI_Model_comparisons.pdf). To calculate expected survival probability, predicted log-odds values were transformed using the logistic transformation.

To provide insight into how weekly changes in size distribution may impact survival probability at any given week, we calculated a change in survival index ($f$), which assesses how survival in a given week compares to that of the previous week across treatments. To do so, we subtracted survival probability in week $i + 1$ from survival probability in week $i$. Any value above zero would indicate an increase in survival probability from one week to the next, any value below zero would indicate a reduction in survival probability from one week to the next. As we expect population size to be higher
in high quantity/low quality environments (H1) and low quantity/high quality environments (H3),
values in these environments should be below zero.

**Size distribution analysis**

To test whether individual body size is normally distributed (H1) in high quantity/low quality
environments, (H2) dominated by larger individuals in low quantity/high quality environments, we
used the size spectrum of populations as a method of quantifying the distribution of body size
(Edwards et al. 2017). To do so, we calculated the size spectra slope values ($b$) for each population,
of both species, across treatments following Carvalho et al. (2021). Briefly, here $b$ describes the
relative abundance of a population in relation to body sizes, thus providing a good proxy to quantify
the distribution of body size within a population. A steeper (more negative) $b$ indicates fewer large-bodied and/or more small-bodied individuals, which is an indication of a right-skewed size population
structure. Values of $b$ can vary substantially with estimates ranging from -6.75 to -0.40 been reported
(Arranz et al. 2021; Carvalho et al. 2021). To estimate $b$, for each species separately, we used the
abundance density function (Carvalho et al. 2021):

$$N(x) = nx^b \left( \frac{b+1}{x_{max}^b - x_{min}^b} \right)$$

where $n$ is the number of individuals, $x$ is the area of each individual (mm$^2$), $x_{max}$ and $x_{min}$ are the
maximum and minimum area of individuals that were digitized. To calculate the maximum and
minimum values, all populations for each treatment were pooled and maximum and minimum values
were calculated separately for each treatment for each week.

For each week of the experiment, $b$ values were calculated using the area measurements of each
individual within each population. We then took the mean $b$ for the five populations of each
treatment to represent the weekly $b$ value. As such, $b$ indicates how the populations responds to the
varying quality, quantity, and frequency of resources provided and here is also used to examine potential trade-offs between weekly changes in size and survival. As with the survival analysis, we applied a Bayesian framework to determine changes in the mean $b$ values using the R package “rethinking”. To model $b$ as a function of covariates, a Gaussian model with an identity link function was used (Eq. 3), with treatment ($T$, categorical with 12 levels) and week ($W$, 19 weeks) as fixed variables.

$$\text{Size}_i \sim \text{Normal}(\mu_i, \sigma)$$

$$\mu_i = \alpha_{T[i]} + \beta_{W[i]}$$

$$\alpha_T \sim \text{Normal}(\alpha, \sigma_T)$$

$$\beta_W \sim \text{Normal}(\alpha, \sigma_W)$$

$$\alpha \sim \text{Normal}(0, 0.5)$$

$$\sigma_T \sim \text{Exponential}(1)$$

$$\sigma_W \sim \text{Exponential}(1)$$

$$\sigma \sim \text{Exponential}(1)$$  \hspace{1cm} (3)

The model in Eq. (3) predicts the posterior mean size spectra slope value ($b$). We applied the model to the estimated mean $b$ values, as described above, and within the model we varied standard deviation in the $\alpha$ prior as the expectation is shrinkage and regrowth of individuals across treatments. The standard deviation values of the $\alpha$ prior that were used for comparison were 0.05 and 1. We then conducted a model comparison to ensure best model fit; models with the lowest WAIC values and highest weight values were taken as best fit (see SI Table S1).
Population size analysis

To determine whether (H1) high quantity/low quality environments and (H2) low quantity/high quality environments result in a smaller population size, and whether (H3) increased frequency of higher quality resources result in a larger population size, we calculated changes in weekly population counts for each species. We applied a Bayesian framework to determine changes in population counts using the same “rethinking” R package as before. Population counts were modelled as a function of covariates using a Poisson multilevel model with a log link function using adaptive priors (Eq. 4), with treatment ($T$, categorical with 12 levels), week ($W$, 19 weeks) and block ($B$, 3 blocks) as fixed variables.

$$\text{Counts}_i \sim \text{Poisson} (\mu_i)$$

$$\mu_i = \alpha_T[i] + \beta_T[i] * w_T[i] + \gamma_B[i]$$

$$\alpha_T \sim \text{Normal}(2,1)$$

$$\beta_T \sim \text{Normal}(0,0.2)$$

$$\gamma_B \sim \text{Normal}(2,0.5)$$

(4)

The model in Eq. (4) predicts the posterior mean population count. We applied the model to the population counts for all blocks and varied the mean and standard deviation in the $\alpha_T$ prior. Following the model simulations based on the various mean and standard deviation values, we conducted a model comparison to ensure best model fit (models with the lowest WAIC values were taken as best fit, see SI Table S2). The model with the best fit had a mean value of 2 and standard deviation value of 1 for the $\alpha_T$ prior.
RESULTS

Survival analysis

We predicted that (H1) in high quantity/low quality environments, and (H3) in environments with increased frequency of resources lifespan would decrease, while (H2) in low quantity/high quality environments lifespan would increase. Survival of *S. mediterranea* was most impacted by week of the experiment rather than CR treatment; in it, for all treatments, weekly mean proportion survival was generally high (0.65 – 0.92) but it did decline over the course of 19 weeks across all treatments (Fig. 2). There was little difference in survival between treatments across all weeks, with weekly mean log-odds of survival similar for all treatments (Fig. S3 - S5). Where few exceptions did occur, and differences between treatments were found, these were largely in the high-quality resource (HQ) treatments (Fig. S3 - S5). In the case of *D. thaitiensis*, survival was also mostly impacted by week rather than CR treatment. The weekly mean proportion survival was generally high (0.54 – 0.91), although there was less of an overall decline in mean proportion survival over the 19 weeks of the experiment compared to *S. mediterranea*. However, proportion survival did fluctuate more between weeks than *S. mediterranea* (Fig. 3). As with *S. mediterranea*, there was little difference in survival between treatments across all weeks and treatments had similar weekly mean log-odds of survival (Fig. S6 – S8). The one exception was week 13, where the log-odds of survival for the high-quality treatments with delayed feeding having lower log-odds of survival than those of the high-quality treatments that had even feeding (Fig. S7). However, for several of the weeks, the variation in log-odds of survival across experimental blocks was greater than across treatments (Fig. S6- S8). A closer examination shows that, for both species, block 1 had a larger impact on planarian survival than treatment for several weeks, although the weeks where this impact was seen differed between species (*S. mediterranea*, Fig. S3 - S5; *D. thaitiensis*, Fig. S6 – S8).
For both species no treatment showed a consistent pattern in survival index ($f$) (Fig. 4). This index assessed how survival in a given week compared to that of the previous week across treatments. However, *D. tahitiensis* had higher variation in the change in survival index than *S. mediterranea*. In general, even feeding regimes showed less variation in $f$, and values were closer to zero, across weeks, than delayed feeding regimes. Similarly, in HQ treatments, $f$ was less variable, and values were closer to zero, compared to low quality resource (LQ) treatments.

**Body size distributions**

We predicted that body size will be (H1) normally distributed in high quantity/low quality environments, (H2) dominated by larger individuals in low quantity/high quality environments, and (H3) that increased frequency of resources will result in a greater rate of size oscillation. Over time, size spectra slope values ($b$) for *S. mediterranea* became more negative for most treatments (Fig. 2), implying a more right-skewed population. From week 1 to week 13, there was a consistent decline in $b$ values, indicating a change in the size structure of the populations with an increase in smaller individuals and fewer larger individuals. From week 14, however, size structure remained similar until week 19. Overall, there was greater variation in size structure over time (Std. deviation: Week ($b$) = 0.24, 89% compatibility intervals (5.5% - 94.5%) (CPI) = 0.10 – 0.24) than between treatments (Std. deviation: Treatment ($b$) = 0.16, CPI = 0.17 – 0.32) (Fig. S9). In the high quality treatments only the even feeding standard intake and even feeding reduced intake treatments did not show a change in size structure (i.e., no decline in $b$ values) through time, while all other high quality treatments had more right skewed populations at the end of the experiment compared to the start. This outcome suggests that in high quality treatments, populations changed in size structure with an increase in the number of small individuals and a decrease in the number of larger individuals over time (Fig. 2). However, in the case of the low quality treatments, only the even feeding high intake and even...
feeding standard intake treatments did not show a change in size structure at the end of the experiment compared to the start. The remaining low quality treatments showed a change in size structure with more right skewed populations at the end of the experiment compared to the start (Fig. 2).

For *D. tahitiensis*, size spectra slope values (*b*) reflected populations that were less right skewed in size structure throughout the experiment, with the even feeding treatments having more negative values (*i.e.*, more right skewed) than all other treatments (Fig. 3). Compared to *S. mediterranea*, *D. tahitiensis* populations were less right skewed but did fluctuate more between weeks. Overall, variation in size structure over time (Std. deviation: Week (*b*) = 0.16, CPI = 0.11 – 0.22) and between treatments (Std. deviation: Treatment (*b*) = 0.16, CPI = 0.10 – 0.24) were similar (Fig. S10). In the high quality resource treatments only the even feeding treatments showed a change in size structure with more right skewed populations at the end of the experiment compared to the start. In contrast, the delayed feeding treatments showed little to no change in size structure of the populations (Fig. 3).

For the low quality treatments, changes to the size structure of populations to more right skewed populations by the end of the experiment only occurred in the even feeding high intake and even feeding standard intake treatments (Fig. 3).

**Population trends**

We predicted that in (H1) high quantity/low quality environments (H1.1) and low quantity/high quality environments population will decline, and that in (H3) an increased frequency of resources clonal reproduction will increase, resulting in increasing populations. Overall, *D. tahitiensis* populations had higher numbers than populations of *S. mediterranea* at the end of the experiment (Fig. 5). Population counts of *S. mediterranea* varied between treatments that differed in resource
quantity (HI: high intake, SI: standard intake, RI: reduced intake) and frequency (D: delayed, E: even)
but not resource quality (HQ: high quality, LQ: low quality) (Fig. 5A & B). The exception being the high
intake resource quantity treatments with even feeding (HI-E). In this case, the HQ-HI-E treatment, where the resource was of a high quality, the population initially increased but then declined
compared to the LQ-HI-E treatment, where the population increased linearly over time (Fig. 5A & B,
Table 1). All populations where resource was delayed showed a decrease in population counts over
time compared to even feeding populations, where counts either stayed constant (HQ-RI-E, LQ-RI-E)
or increased over time (HQ-SI-E, LQ-HI-E, LQ-SI-E) (Fig. 5A & B, Table 1). Again, the exception being
the HQ-HI-E treatment where population counts declined over time.

In *D. tahitiensis*, populations either increased or remained stable (Fig.5C & D). When comparing
HQ and LQ treatments, HQ treatments with even feeding, regardless of resource quantity, had higher
population counts than LQ treatments with even feeding. When resource was delayed there was no
clear trend; the HQ-HI-D treatment had lower counts than the LQ-HI-D treatment, HQ-SI-D had higher
counts than LQ-SI-D and HQ-RI-D had similar counts to LQ-RI-D (Fig.5C & D, Table 2). For both species,
experimental block 1 had population growth rates lower than block 2 and block 3 (*S. mediterranea*:
block 1 - estimate -0.2, std dev. 0.13; block 2 - estimate 0.49, std dev. 0.13; block 3 - estimate 0.45,
std dev. 0.13. *D. tahitiensis*: block 1 - estimate 0.98, std dev. 0.2; block 2 - estimate 1.66, std dev. 0.2;
block 3 - estimate 1.55, std dev. 0.2). However, for all treatments, the population response
(increasing, decreasing or stable counts) of block 1 did not differ from that of block 2 or block 3 (Fig.
5, Table 1 & 2).

**DISCUSSION**
A comprehensive understanding of the impact of calorie restriction (CR) on senescence and longevity has been hampered by the lack of realism in empirical manipulations (Deere et al. 2020) and narrow focus on short-lived species (but see Colman et al. 2009; Mattison et al. 2012; Pifferi et al. 2018). Here, we begin to address this shortcoming by incorporating stochasticity in resource availability in a long-lived species. Our findings suggest that the fundamental predictions of CR do not hold when multiple factors interact with reduced calorie intake. CR theory predicts extended organismal longevity due to reduced calorie intake (Weindruch & Walford 1988). However, our results show that for both of our study species survival is not affected by any moment of resource availability: frequency, amount, or quality. Our findings suggest that interaction effects of these three moments plays a significant part in the extent to which CR can be beneficial.

The exact way these moments in resource availability impacts survival is context dependent. A possible reason for the variable responses we report could be the choice of quality of diet, with our treatments lacking a more graduated component, in terms of variation in macronutrients, to the quality of the resource provided. Indeed, in studies where the ratio of macronutrients have been varied, in addition to calorie manipulation, the macronutrient ratios have a greater impact on longevity than when manipulating calories (Lee et al. 2008; Solon-Biet et al. 2014). The response we report here is also likely driven by the specific life history of planarians. Planarians have the unique ability to restore damaged cells (Elliott & Sánchez Alvarado 2013). However, restoring damaged cells is costly and requires resources and, if most resources are used for maintenance, less resources will be available for reproduction.

The reduced reproduction and high survival within populations are indicative of the fundamental trade-off between survival and reproduction under variable environmental conditions (Stearns 1992). In general, for *S. mediterranea* a trade-off between survival and reproduction can be seen across the delayed feeding regimes but not for even feeding regimes. For *D. tahitiensis*, only
populations of low quantity and delayed feeding show a trade-off between survival and reproduction. This begs the question, for populations of both species, where there is a lack of trade-off between survival and reproduction do we see a trade-off with body size, another key life history trait?

When considering body size, our findings showed that the size structure of populations varied across treatments. For *S. mediterranea*, populations become more right skewed across all treatments (with two exceptions when feeding is even). The right skewed populations signify an increase in the number of smaller individuals and fewer larger individuals in the populations (Carvalho *et al.* 2021). Such changes in the population structure can occur when there is increased reproduction, as more recruits of a smaller size enter the population. *S. mediterranea* reproduce asexually through fission where larger individuals fission into two, with the anterior product (new recruits) generally smaller than the posterior fission product (now a smaller asexual individual) (Peter *et al.* 2001). Thus, the fissioning process results in fewer larger individuals and an increase the number of smaller individuals. However, the right skewed size structure in populations as a consequence as a result of reproductive events, will only be reflected in populations that are increasing in number. Where *S. mediterranea* populations show a decline, the change in structure is not driven by reproduction and in turn the new recruits in the population. Here, the life history of planaria is driving the response. A key trait in the life history of planaria is the ability to shrink when food resources become scarce (Mangel *et al.* 2016), with individuals experiencing up to a 50-fold change in size (Rink 2013).

The ability to shrink allows individuals to survive resource scarce environments until conditions improve and resources increase. In planaria, when shrinkage occurs, cell numbers reduce through autophagy, during which energy resources are supplied to neoblasts (stem/regenerative cells) (Romero & Baguñà 1991; González-Estévez 2009). This process ensures maintenance levels of individuals can be met. In our study, *S. mediterranea* populations which are more right skewed in size structure have relatively high survival at the end of the experiment. The proportion survival of these
right skewed populations is above 0.6 and the change in survival index is consistently close to zero, indicating survival from one week to the next is consistent. Shrinkage is a strategy found in several taxa and mitigates the impact of fluctuating or stochastic environments on individual survival (Ebert 1967; Levitan 1988; Marinovic & Mangel 1999; Salguero-Gómez & Casper 2010, 2011). Indeed, shrinkage has been shown to maintain survival during periods of resource scarcity. In the Galápagos marine iguanas (*Amblyrhynchus cristatus*), individuals shrink up to 20% of their body length when food resources are low during El Niño events (Wikelski & Thom 2000); those individuals that shrink more have a higher rate of survival, pointing to the adaptive value of shrinkage. When food resources improve during La Niña events, body length increases, as is the case with planarians when they receive increased food resources after periods of low food (Child 1914; González-Estévez 2009; Thommen et al. 2019).

The shrinkage seen in *S. mediterranea* populations is unlikely occurring in populations of *D. tahitiensis*. Indeed, fewer *D. tahitiensis* treatments showed populations becoming more right skewed over the course of the experiment. Where populations become more right skewed, the populations do not show a decline in population size and, as is the case with *S. mediterranea*, changes in population structure are likely due to fissioning. The populations of *D. tahitiensis* that have a less right skewed size structure all increased in size over the course of the experiment and individuals are likely experiencing increased competition for resource. The increased competition among individuals for available resources results in contest competition, where an individual interferes with other individuals to obtain resources (Nicholson 1954).

In competitive environments where contest competition occurs, smaller individuals can be outcompeted by other larger individuals within the population as larger individuals are dominant (Petersson & Järvi 2000; Stewart & Tabak 2011). In a study by Petersson & Järvi (2000) on brown trout (*Salmo trutta*), the authors found that sea-ranch trout had higher dominance than wild-type
trout and that individuals with higher dominance had larger body sizes. In our study, contest competition results in differences in average resource intake among individuals, with larger individuals able to consume more resources per feeding event. As such, competition between two individuals will result in the larger of the two increasing in size thereby increasing the number of larger individuals in the population, resulting in less right-skewed populations.

The differences found in survival between *D. tahitiensis* and *S. mediterranea* can be attributed to differences in a unique life history strategy of planaria; the ability to regenerate. Regeneration is a life history strategy that can mitigate the impact of stochastic environments on longevity more than restricting calories. The generally higher survival in *D. tahitiensis*, compared to *S. mediterranea*, is likely due to *D. tahitiensis* being able to repair damaged cells more efficiently. *Dugesia tahitiensis* is considered to have one of the highest regenerative and fissioning capacities, and the most stem/regenerative cells (neoblasts) within the Tricladida taxon (Baguñá & Romero 1981; Peter *et al.* 2001). High neoblast density in the parenchyma (the cellular tissue between the body wall and organs) is a prerequisite for efficient regeneration after fission and, when compared to *S. mediterranea*, *D. tahitiensis* have high cell counts (Peter *et al.* 2001). Moreover, neoblast self-renewal activity can be impacted by food availability with reduced food levels resulting in lowered self-renewal activity (Mangel *et al.* 2016). Reduced neoblast self-renewal activity will then enhance the negative impacts experienced by *S. mediterranea*.

An additional, unintentional, contributing factor of the response of *S. mediterranea* to stochastic environments may be due to the popularity of *S. mediterranea* as a model organism. *Schmidtea mediterranea* is a well-known laboratory system (Sousa & Adell 2018) and the populations used in our study have been maintained in the lab for >10 years (Aboobaker, pers. comm.). In contrast, the populations of *D. tahitiensis* used in our study have not been maintained in the laboratory for such an extensive period (< 10 years) (Aboobaker, pers. comm.). Given that our *S. mediterranea*
populations have been lab bred for such an extensive period, and in stable conditions, they may be
more vulnerable to unstable conditions than *D. tahitiensis*. The impact of generational lab breeding
is not uncommon and can have an impact on the response of individuals to experimental treatments
(Hoffmann & Ross 2018). A review, of the rates and patterns of laboratory adaptation of insects and
other invertebrates, found that laboratory-adapted lines tend to be more sensitive to stress,
indicating that stress-related traits experience relaxed selection (Hoffmann & Ross 2018). In the case
of *S. mediterranea*, the regeneration capabilities under stressful resource conditions have likely
become less effective due to the extensive period of lab breeding, a response not seen in *D. tahitiensis*.

Life-histories differences between short- and long-lived species will ultimately have
consequences for CR predictions. There are few CR studies on long-lived species (*i.e.*, species that live
> 5 years), with studies showing benefits to longevity of CR (*e.g.*, Colman *et al.* 2009; Pifferi *et al.*
2018) and others not (*e.g.*, Mattison *et al.* 2012). Here we show that two long-lived species do not
conform to the predictions of CR. Indeed, we go further by highlighting that, in our study, both long-
lived species under stochastic environments do not conform to CR predictions. Our findings are
especially important in the face of CR research, largely as much of the fundamental work have been
on short-lived species in constant environments (*e.g.*, McCay *et al.* 1935; Sanchez-Roman & Barja
2013). An exciting opportunity now arises. Moving forward CR research needs to extend the
important CR work done in short-lived species, that identified mechanisms and pathways impacting
aging, to include long-lived species in stochastic environments. Such a move is key, as the
mechanisms and pathways previously identified may not be the same as those present in species that
are long-lived.

**Acknowledgements**
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References


Table 1. Model estimates of log population counts for Schmidtea mediterranea. Mean estimate, standard deviation and compatibility interval (5.5% and 94.5%) values are given for treatments and all blocks. Model diagnostic criteria are given by \( n_{\text{eff}} \) (estimate of number of independent samples) and Rhat4 (Gelman-Rubin convergence diagnostic of the Markov chains, values of Rhat4 that are above 1.00 indicate chains have not converged). HQ indicates high quality diet; HI indicates high food intake; SI indicates standard food intake; RI indicates reduced food intake; D indicates delayed feeding (every 14 days) and E indicates even feeding (every seven days).

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### Table 2. Model estimates of log population counts for *Dugesia tahitiensis*. Mean estimate, standard deviation and compatibility interval (5.5% and 94.5%) values are given for treatments and all blocks.

Model diagnostic criteria are given by $n_{\text{eff}}$ (estimate of number of independent samples) and $\text{Rhat}^4$ (Gelman-Rubin convergence diagnostic of the Markov chains, values of $\text{Rhat}^4$ that are above 1.00 indicate chains have not converged). HQ indicates high quality diet; LQ indicates low quality diet; HI indicates high food intake; SI indicates standard food intake; RI indicates reduced food intake; D indicates delayed feeding (every 14 days) and E indicates even feeding (every seven days).

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**Figure legends**

**Figure 1.** Full factorial experimental design. Design has three resource factors: quality, quantity and timing. Resource quality has two levels (top right panel): high quality (HQ; orange dots) and low quality (LQ; purple dots). Resource quantity has three levels (middle right panel): restricted intake (RI; single grey dot), standard intake (SI; three grey dots) and hyper caloric intake (HI; six grey dots). Resource timing has two levels (bottom right panel): even feeding (E; every seven days – black line), delayed feeding (D; every 14 days – grey line). Each population was initiated with 10 individuals and replicated five times resulting in a total of 600 individuals and 60 populations per species. Populations were counted and photographed weekly.

**Figure 2.** Survival in *Schmidtea mediterranea* populations is high and consistent across treatments however population structure changes across time in most treatments. Weekly proportion survival and size spectra slope values ($b$) for *S. mediterranea*. A) Median estimated size spectra slope ($b$) values (black points) with 95% confidence intervals (black bars), and median estimated proportion survival (red lines) with 89% compatibility intervals (dashed red line) for high quality (HQ, ⬤) treatments over 19 weeks. B) Estimated size spectra slope ($b$) values (black points) with 95% confidence intervals (black bars), and median estimated proportion survival (red lines) with 89% compatibility intervals (dashed red line) for low quality (LQ, ⬤) treatments over 19 weeks. Left panels indicate delayed feeding treatments and right panels even feeding treatments. Top panel represent high intake (HI, ⬤), middles panel standard intake (SI, ⬤) and bottom panel reduced intake (RI, ⬤) treatments.
Figure 3. Survival in *Dugesia tahitiensis* populations is high and consistent across treatments with little change in structure to populations. Weekly proportion survival and size spectra slope values \((b)\) for *D. tahitiensis*. A) Median estimated size spectra slope \((b)\) values (black points) with 95% confidence intervals (black bars), and median estimated proportion survival (red lines) with 89% compatibility intervals (dashed red line) for high quality \((HQ)\) treatments over 19 weeks. B) Estimated size spectra slope \((b)\) values (black points) with 95% confidence intervals (black bars), and median estimated proportion survival (red lines) with 89% compatibility intervals (dashed red line) for low quality \((LQ)\) treatments over 19 weeks. Left panels indicate delayed feeding treatments and right panels even feeding treatments. Top panel represent high intake \((HI)\), middles panel standard intake \((SI)\) and bottom panel reduced intake \((RI)\) treatments.

Figure 4. Week to week survival is largely consistent across treatments but varies between species. Change in survival index values of *S. mediterranea* over the 19 weeks for A) high quality \((HQ)\) treatments and B) low quality \((LQ)\) treatments. Change in survival index values of *D. tahitiensis* over the 19 weeks for C) high quality \((HQ)\) treatments and D) low quality \((LQ)\) treatments. Values (proportion survival at \(t+1\) minus proportion survival at \(t\)) are shown for high intake \((HI)\) treatments (top panel), standard intake \((SI)\) treatments (middle panel), and reduced intake \((RI)\) treatments (bottom panel). Red points indicate estimated proportion survival from the model and black points indicate data points. Dashed line indicates the zero line, values above the line indicate an increase in survival probability from one week to the next, any values below the line indicate a reduction in survival probability from one week to the next.
**Figure 5.** Population size differs between species but with no clear patterns across treatments.

Weekly population size of *S. mediterranea* over the 19 weeks for A) high quality (●) treatments and B) low quality (●) treatments. Weekly population size of *D. tahitiensis* over the 19 weeks for C) high quality treatments and D) low quality treatments. Solid black line indicates mean population counts for all five replicates (across all blocks) of the raw data for a given week. Dashed line indicates the mean estimated population counts for block 1, dotted line the mean estimated population counts for block 2 and the two-dash line the mean estimated population counts for block 3. Shaded grey regions indicate 89% compatibility intervals (5.5% - 94.5%). Left panels indicate delayed feeding treatments and right panels even feeding treatments. Top panel represent high intake (*HI*, ○), middles panel standard intake (*SI*, △) and bottom panel reduced intake (*RI*, □) treatments.
Figure 1

Total individuals = 600

- × 5 (n = 10)

- × 5 (n = 10)

- × 5 (n = 10)
Figure 2

A

Delayed feeding

Even feeding

Size spectra slope ($b$)

Proportion survival

Time (Weeks)

B

Delayed feeding

Even feeding

Size spectra slope ($b$)

Proportion survival

Time (Weeks)
Figure 3

A

Delayed feeding

Even feeding

Size spectra slope (b)

Proportion survival

Time (Weeks)

B

Delayed feeding

Even feeding

Size spectra slope (b)

Proportion survival

Time (Weeks)
Figure 4
Figure 5