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LETTER

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Caloric restriction extends lifespan in a clonal plant

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Abstract

When subjected to dietary caloric restriction (CR), individual animals often outlive well-fed conspecifics. Here, we address whether CR also extends lifespan in plants. Whereas caloric intake in animals comes from ingestion, in plants it derives from photosynthesis. Thus, factors that reduce photosynthesis, such as reduced light intensity, can induce CR. In two lab experiments investigating the aquatic macrophyte *Lemna minor*, we tracked hundreds of individuals longitudinally, with light intensity—and hence, CR—manipulated using neutral-density filters. In both experiments, CR dramatically increased lifespan through a process of temporal scaling. Moreover, the magnitude of lifespan extension accorded with the assumptions that (a) light intensity positively relates to photosynthesis following Michaelis–Menten kinetics, and (b) photosynthesis negatively relates to lifespan via a power law. Our results emphasize that CR-mediated lifespan extension applies to autotrophs as well as heterotrophs, and suggest that variation in light intensity has quantitatively predictable effects on plant aging trajectories.

KEYWORDS

aging, demography, duckweed, *Lemna*, Lemnaceae, Lemnoideae, light intensity, longevity, senescence, temporal scaling

INTRODUCTION

Lifespan extension via caloric restriction (CR) refers to the phenomenon in which organisms subjected to restricted caloric intake exhibit longer lifespans compared to conspecifics with ad libitum access to food (Hwangbo et al., 2020; McCay et al., 1935). CR-mediated lifespan extension has been well studied in heterotrophic organisms, and occurs in a broad range of animal species (e.g., rhesus monkeys, Colman et al., 2009; nematodes, Klass, 1977; rats, McCay et al., 1935; fruit flies, Partridge et al., 2005; and mice, Weindruch et al., 1986), as well as the non-animal heterotroph, yeast (Jiang et al., 2000). In addition to the positive effects of CR on lifespan, several health benefits have been reported in calorie-restricted animals, including evidence that CR protects against common age-related diseases including cancer (Kritchevsky, 2001; Weindruch & Walford, 1982), neurodegenerative disorders (Castiglioni et al., 1991; van Cauwenberghe et al., 2016), stroke and ischemia (Chiba & Ezaki, 2010; Ciobanu et al., 2017), epilepsy (Greene et al., 2001; Rubio Osornio et al., 2018), and cardiovascular disease (Bales & Kraus, 2013; Walford et al., 1992).

A number of non-exclusive theories contribute to our current understanding of CR-mediated lifespan extension in animals. Among these, the longest standing are the adaptive trade-off theory (Box 1) and the rate of living theory (Box 2). The former suggests that CR-mediated lifespan extension reflects an adaptive trade-off in times of food scarcity, wherein individuals prioritize survival (and thus longevity) at the expense of immediate reproduction (Holliday, 1989; Kirkwood & Shanley, 2005). The latter suggests that CR reduces metabolism, reducing the rate that bodies accrue damage, and thereby extending lifespan (Pearl, 1928; Sohal, 1986). Both of these foundational theories have been subject to critiques (Boxes 1 and 2), with alternatives proposed (Online Appendix A).

BOX 1 Evidence and critiques of the adaptive trade-off theory of CR-mediated lifespan extension.

Evidence

While extended lifespans and reduced incidence of age-related diseases are beneficial from a human perspective, the potential effects of caloric restriction (CR) on organisms in natural environments are more complicated. Most notably, lifespan extension via CR is typically associated with a decrease in reproduction (Brecchia et al., 2006; Chippindale et al., 1993; Holehan & Merry, 1985; Klass, 1977; Speakman & Mitchell, 2011). It has been suggested that this reflects an adaptive trade-off in times of food scarcity, wherein organisms prioritize survival at the cost of immediate reproduction (Holliday, 1989; Kirkwood & Shanley, 2005), drawing from disposable soma theory, a main evolutionary theory of senescence (Kirkwood & Holliday, 1979; Kirkwood & Rose, 1991). This trade-off theory implies that organisms preferentially allocate resources towards somatic maintenance and repair under CR, allowing them to outlast poor environmental conditions. Reproduction and reproductive senescence are delayed, with offspring production deferred until conditions improve, increasing offspring survival (Harrison & Archer, 1989; Holliday, 1989; Kirkwood & Shanley, 2005; Masoro & Austad, 1996). Indeed, studies on animals have observed delayed reproductive senescence under CR, finding that a return to ad libitum feeding allows reproduction at much later ages than conspecifics constantly fed ad libitum (Merry & Holehan, 1979; Nelson et al., 1985; Selesniemi et al., 2008). CR also improves various processes associated with somatic maintenance and repair, including reducing somatic mutations (Aidoo et al., 2003; Dempsey et al., 1993), decreasing damage and increasing repair of DNA (Chung et al., 1992; Haley-Zitlin & Richardson, 1993; Ke et al., 2020), and decreasing oxidative damage (Dubey et al., 1996; Gredilla et al., 2001), and is associated with increased autophagy (Bagherniya et al., 2018; Chung & Chung, 2019; Del Roso et al., 2003; Donati et al., 2001).

The increased somatic maintenance and repair observed under CR are consistent with mechanistic theories of senescence. These often attribute declines with age to an accumulation of damage with age. As such, increased repair processes would result in a slower accumulation of damage, and thus, longer lifespan. One such example is the free radical theory, which proposes that aging results from reactive oxygen species and the oxidative damage that they cause (Harman, 1956, 1992). As new information in this field is discovered, the theory has been updated, taking into account the role of antioxidants and homeostatic mechanisms in the complex process of oxidative damage and defence (Gems & Doonan, 2009; Lin & Beal, 2003; Pomatto & Davies, 2018). Overall, the notion that CR leads to increased lifespans by mediating a trade-off between survival and reproduction has gained significant traction in the literature.

Critiques

More recently, evidence that does not support the trade-off theory has arisen. This evidence includes that the longevity-reproduction trade-off has been uncoupled in *Caenorhabditis elegans*, for which great longevity has been shown to occur without a cost to reproduction (Crawford et al., 2007; Hsin & Kenyon, 1999; Leroi, 2001). In *Drosophila melanogaster*, a study tracking resource uptake and investment found that while diet-restricted flies invested a greater *proportion* of resources towards their soma (rather than reproduction), they invested fewer *total* resources to their soma (and also fewer *total* resources to reproduction). This showed that lifespan extension is not caused by a greater sum of resources to the soma alone (O'Brien et al., 2008).

Additionally, CR has been found to have detrimental effects on organisms in addition to the beneficial ones, calling into question whether responses to CR truly increase survival with food scarcity in natural environments (Adler & Bonduriansky, 2014). These detrimental effects include slowed wound healing during CR (Dirks & Leeuwenburgh, 2006; Hunt et al., 2012; Reed et al., 1996), and bone thinning and osteoporosis (Devlin et al., 2010; Dirks & Leeuwenburgh, 2006; Sun et al., 2001; Talbott et al., 1998). CR also decreases body and fat mass, which may impact strength, stamina, and cold tolerance (Barzilai et al., 1998; Burger et al., 2007; Dirks & Leeuwenburgh, 2006; Johnson et al., 1982; Puerta & Abelenda, 1987; Sun et al., 2001).

With these new observations, critiques of the trade-off hypothesis have been made (Adler & Bonduriansky, 2014; Blagosklonny, 2008; Gems & Partridge, 2013; Lemaître et al., 2024; Mitteldorf, 2001). Alternative hypotheses have been suggested; these are summarised in Online Appendix A.

It is against this backdrop that we turn to CR-mediated lifespan extension in plants. Whereas caloric intake in animals predominantly comes from ingestion (notwithstanding other modes of intake such as endosymbiosis), in plants it predominantly comes from photosynthesis (notwithstanding the existence of heterotrophs

BOX 2 Evidence and critiques of the rate of living theory of CR-mediated lifespan extension.

Evidence

The rate of living theory is an older theory of aging, linking metabolism with lifespan. It proposes that the rate of metabolism determines the rate at which the body wears out, and thus is negatively related to longevity (Pearl, 1928; Sohal, 1986; Stark et al., 2020). The theory was based on observations of similar metabolic energy use per gram of tissue per lifespan among different mammalian species (Rübner, 1908; Speakman, 2005), and observations of the negative relationship of temperature and lifespan (Sohal, 1986). This negative relationship is visible in ectothermic animals (Arnqvist et al., 2017; Bronikowski & Vleck, 2010; Klass, 1977; Liu & Walford, 1966; Munch & Salinas, 2009), which is commonly attributed to the strong influence of temperature on metabolic rate (Flouris & Piantoni, 2015; Gillooly et al., 2001; Loeb & Northrop, 1916; Sacher, 1978; Speakman, 2005), since ectothermic animals are dependent on external conditions for their body temperature.

In endothermic animals, CR has been found to decrease body temperatures (Carrillo & Flouris, 2011; Dirks & Leeuwenburgh, 2006; Duffy et al., 1989; Ferguson et al., 2007; Klass, 1977; Lane et al., 1996; Rikke et al., 2003). It is suggested that the lifespan extension observed may therefore result from changes in metabolism based on body temperature, similarly to ectotherms (Jin & Koizumi, 1994; Koizumi et al., 1996; Turturro & Hart, 1991). Raising ambient temperatures to account for this difference has been found to attenuate the lifespan-extending effects of CR (Koizumi et al., 1996), providing support. Additionally, CR can result in an altered distribution of fat (Barzilai et al., 1998; Bertrand et al., 1980; Redman et al., 2007), and mitochondrial function (Higami et al., 2004; Linford et al., 2007; Rohrbach et al., 2006), which could affect body temperature and metabolism.

Critiques

In contrast to the studies supporting the rate of living theory, some studies find no relationship between metabolism and longevity (Hulbert et al., 2004; Magalhães et al., 2007; Stark et al., 2020). Cross-clade comparisons have found the predictions of rate of living theory are not upheld. For example, compared to mammals, birds have been found to both expend more metabolic energy, and have longer lifespans, opposing predictions of the rate of living theory (Healy et al., 2014; Holmes et al., 2001; Holmes & Austad, 1995; Speakman, 2005). Comparisons across clades may be complicated by confounding factors from the inherent intrinsic differences of the species involved (e.g., different capacities for oxidative defence, or production of reactive oxygen species with metabolism) (Speakman, 2005). Thus, it has been suggested that the rate of living theory may be more suited to intra-species comparisons (Issartel & Coiffard, 2011; Kelemen et al., 2019; Speakman, 2005).

In the context of CR, studies that directly examine the effect of CR on metabolic rate are conflicting. Some report that CR indeed reduces metabolism and improves metabolic efficiency (e.g., Blanc et al., 2003; Dulloo & Girardier, 1993; Greenberg, 1999; Heilbronn et al., 2006; Knight et al., 2011; MacLean et al., 2004). Other studies find that CR does not reduce metabolism (e.g., Dillin et al., 2002; McCarter et al., 1985; McCarter & Palmer, 1992; Selman et al., 2005; Speakman et al., 2004; Yen et al., 2004), leading to ambiguity.

and mixotrophs). Thus, factors that reduce photosynthesis, such as reduced light intensity, can lead to CR in plants. In this context, similarities and differences between plants and animals must be noted. As with ectothermic animals, low temperatures can extend plant lifespan (Körner, 1999; Nobis & Schweingruber, 2013; Paiha, 2021; Rosbakh & Poschlod, 2018; Wangermann & Ashby, 1951), potentially caused by a decreased metabolic rate at these temperatures. While there is conflicting evidence on whether CR affects metabolic rate in animals (see Box 2), the capacity for photosynthesis adds a new layer to investigating the effects of CR on plant metabolism. Photosynthesis directly contributes to metabolism, and is strongly affected by light intensity (Peeters & Eilers, 1978), a relationship often modelled using photosynthesis-irradiance curves based on the Michaelis–Menten equation and its variants (Lin et al., 2015). Thus, CR may have a clearer impact on metabolic rate in plants, and further investigation of relationships between metabolic rate, CR, and longevity in plants is needed.

While the logical first question of whether CR-mediated lifespan extension occurs in plants has been raised (Salguero-Gómez et al., 2013), we are aware of only a single study, Minina et al. (2013), that has investigated it (though Wangermann and Ashby (1951) provides an early, inconclusive antecedent). In that study, Minina et al. (2013) found evidence that CR, manipulated via two light intensities, led to reduced tissue glucose concentrations and subsequent lifespan extension in *Arabidopsis thaliana*, possibly mediated by autophagy.

Our current study also focuses on lifespan extension via CR in plants—specifically in ramets of the short-lived, clonally reproducing macrophyte *Lemna minor*. In our first

of two experiments ('Experiment 1'), we induced CR by reducing the light intensity to which plants were exposed, as in Minina et al. (2013), although we investigated seven light intensities in total. We predicted that mean lifespan would be greatest in the light-restricted treatments, thus indicating that CR-mediated lifespan extension does occur in *L. minor*. We further predicted that the quantitative relationship between light intensity and lifespan would emerge from the straightforward assumptions that (a) light intensity is positively related to photosynthesis (/metabolism) following Michaelis–Menten kinetics (as is *Lemna* growth rate; Docauer, 1983), and (b) photosynthesis (/metabolism) is negatively related to lifespan via a power law (e.g., Issartel & Coiffard, 2011). Finally, in accordance with the view that lifespan extension may come at a cost to reproduction, we predicted that total offspring produced would be greatest under high-light conditions, and would decrease with decreasing light intensity. The first two of these predictions were supported by the data; that is, there was strong evidence of CR-mediated lifespan extension for which our quantitative approach provided a good fit. Contrarily, CR led to slightly greater total reproduction.

Intriguingly, in Experiment 1, the survivorship curves associated with the different light treatments, while very different when age was plotted in absolute units of time, demonstrated conspicuous overlap when age was instead plotted relative to the treatment members' mean lifespans. This suggests the phenomenon of temporal scaling, a promising framework that helps describe and explain environmental dependence of senescence, whereby lifespan distributions respond to environmental variation as if time has been stretched or compressed, which further implies the existence of a unifying process underpinning aging, and evokes intuitive notions of 'resilience' (Stroustrup et al., 2016). Thus, in a follow-up second experiment ('Experiment 2'), we again varied light intensity to induce CR, though this time we used much larger sample sizes for just two light intensity treatments. This allowed us to examine survival curves with greater resolution, and formally compare them between light intensities. Following from the results of Experiment 1, and results indicating temporal scaling due to CR in the nematode *Caenorhabditis elegans* (Stroustrup et al., 2016), we predicted that the empirical distributions of relative lifespan would be similar for Experiment 2 plants grown in bright versus dim light. This was indeed the case, suggesting that CR lengthens *L. minor* lifespan by stretching time.

MATERIALS AND METHODS

Overview

In Experiment 1, we investigated the effect of seven levels of light intensity on lifespan and other plant traits. Experiment 1 was primarily focused on evaluating the presence of CR-mediated lifespan extension, with a secondary aim of providing a preliminary assessment of the quantitative nature of this lifespan-light intensity relationship.

Experiment 1 indeed demonstrated CR-mediated lifespan extension. We subsequently designed Experiment 2 to follow up on Experiment 1, but here we investigated just two levels of light intensity, with a much larger sample size per level. Thus, Experiment 2 was able to test whether decreasing light intensity lengthens lifespan by stretching time, as in the phenomenon of temporal scaling (Stroustrup et al., 2016), or, alternatively, by a more fundamental modification of the shape of the trajectory of aging.

Due to their conceptual similarity, Experiments 1 and 2 had many common features. We describe these in the subsequent five sub-sections before delving into the specifics of each experiment.

Study species

Lemna minor L. (common duckweed) is a small, floating, aquatic macrophyte classified either within the family Lemnaceae or within the family Araceae, subfamily Lemnoideae (Landolt, 1986; Sree et al., 2016). With short-lived ramets and rapid population growth, *Lemna* species are suitable for lab studies in ecology and evolution, among other disciplines (e.g., Laird & Barks, 2018; Wang, 1990; Ziegler et al., 2016), and have been used to study senescence for 75 years (e.g., Ashby et al., 1949; Chmilar & Laird, 2019; Claus, 1972). Reproduction in *L. minor* is mainly clonal, with offspring plants (i.e., ramets) detaching alternatingly from left and right meristematic pockets. Individual plants are composed of a single frond (leaf-stem structure) and a single root (Landolt, 1986; Lemon & Posluszny, 2000). In this study, we used an *L. minor* culture obtained from the Canadian Phycological Culture Centre (CPCC 492 *L. minor*; originally collected from Elk Lake, BC, Canada, 48°31′30″ N, 123°23′18″ W) to investigate lifespan and senescence at the ramet level.

Growth conditions

We grew plants individually in axenic conditions in 35 × 10 mm petri dishes (nominal diameter × height; cat. no. 229638, Celltreat Scientific Products, Pepperell, MA, USA) containing 4 mL of Schenk and Hildebrandt growth medium (cat. no. S6765, Sigma-Aldrich, St. Louis, MO, USA) diluted to one-quarter strength (0.8 g L^{-1}). We replaced the growth medium in each petri dish weekly to ensure a constant environment. While previous studies supplemented the growth medium with sucrose and other substances to facilitate the detection of contaminating microorganisms, this study did not. We made this choice due to the mixotrophic

capabilities of *L. minor*, which can take up and use dissolved sugars as a method of nutrition (Frick, 1994; Vidaković Cifrek et al., 2013). While a study on the effects of heterotrophic caloric restriction would be valuable, we focus solely on autotrophic caloric restriction, as in Minina et al. (2013).

We grew the plants on two growth shelves, each with its own light fixture (cat. no. FLT46, Agrobrite Hydrofarm, Petaluma, CA, USA). Each fixture had six 122 cm, 54 W, T5 HO fluorescent grow lights (6400 K) positioned 25 cm above the plants. The photoperiod was 15:9 (light:dark) for Experiment 1, and 24:0 for Experiment 2. During the light phase, this set-up yielded an average photon flux density at plant height in the wavelength range of photosynthetically active radiation (PAR) of 442 and 446 $\mu mol\, m^{-2}\, s^{-1}$ for Experiments 1 and 2, respectively, as measured with a Hobo Micro Station data logger and PAR sensor (Hoskin Scientific, Edmonton, AB, Canada). The average ambient air temperature at plant height was 24.1 and 23.6°C for Experiments 1 and 2, respectively.

Obtaining focal plants

Focal plants—that is, our experimental subjects—were obtained via a preliminary process designed to ensure recent genealogical homogeneity and thus reduce the potential for parental and grandparental age effects (Barks & Laird, 2015); see Online Appendix B.

Manipulating light intensity

To manipulate light intensity, we placed each focal plant, in its petri dish, into an outer container whose base was an opaque 43 × 13 mm aluminium weigh dish (diameter × height; cat. no. 087-32-105, Fisher Scientific, Waltham, MA, USA) and whose lid was the lid of a 60 × 15 mm petri dish (nominal diameter × height; cat. no. 25384-090, VWR, Radnor, PA, USA). We affixed two discs to the top of this outer container's lid using opaque 6.3 mm aluminium foil tape (Eco-Fused, Sheridan, WY, USA), which also covered the sides of the lid. Our aim was that no light should reach the plants, except that which passed through the discs, if any. The discs were cut to size from either neutral density gel sheets (cat. nos. 130, 209, 210, and, 211, LEE Filters, Burbank, CA, USA), which changed the degree of transmittance of light, but not its colour, or aluminium foil (Alcan, Mississauga, ON, Canada), which was opaque. We used different disc combinations to obtain seven light intensity treatments, the first six of which followed a $\log_2$-series (L_1, $L_{1/2}$, $L_{1/4}$, $L_{1/8}$, $L_{1/16}$, $L_{1/32}$), such that plants in each successive treatment received, putatively, half the light intensity as those in the preceding treatment. Thus, the subscripts in the treatment names refer to those treatments' light intensities relative to the 'full light' treatment, L_1. The seventh light intensity treatment, L_0, was a 'no light' treatment. Disc combinations are detailed in Online Appendix C, along with a schematic diagram of the containers (Figure C1). We selected the light intensity of the full light treatment as the intensity around which *Lemna* population growth rates plateau (i.e., brighter light intensities provide diminishing returns on growth) (Docauer, 1983), indicating saturation of photosynthetic capacity.

We conducted an ancillary experiment to investigate the effect of the treatments on light intensity (Online Appendix D). This showed that actual PAR values for each treatment were of the expected light intensity relative to the 'full light' treatment (Figure D1).

We conducted a second ancillary experiment to determine whether light intensity treatments altered the growth medium temperature (Online Appendix E). We found no significant effect of light intensity treatment on growth medium temperature for treatments L_1 to $L_{1/32}$; however, the growth medium temperature in treatment L_0 was significantly cooler than in the other treatments (Figure E1). Thus, while we continued to include the L_0 treatment in Experiment 1, we cannot disentangle the effects of temperature and light for that treatment.

Both ancillary experiments detected either a significant main effect of growth shelf or a significant light treatment-by-shelf interaction. This influenced the subsequent design of the main experiments with respect to randomisation.

Daily observations and data collection

We positioned focal plants randomly across eight wire racks (i.e., four racks per shelf), with up to 28 plants per rack. We re-randomised rack positions daily to homogenize environmental conditions, following the results of the ancillary experiments described above. (In fact, this re-randomisation process started with the focal plants' recent ancestors, which had occupied the same spatial positions during the process of obtaining the focal plants.)

We tracked focal plants throughout their entire lives (with exceptions noted for Experiment 1, described below), and recorded reproduction daily, discarding offspring plants as they detached. We defined a focal plant's date of birth as the date it detached from its parent. Likewise, we defined a focal plant's date of death as the date it released its last offspring. We calculated lifespan as the number of days between birth and death, inclusive, so 'lifespan' is tantamount to 'reproductive lifespan'. This is necessary because of a paucity of reliable cues of physiological death in *Lemna* (Barks & Laird, 2015). Moreover, reproductive

lifespan is more relevant to the evolution of senescence compared to physiological lifespan in this plant species that exhibits no post-reproductive parental care. To ensure accurate determination of the full lifespan, we observed focal plants for 2 weeks following the detachment of all visible offspring and only considered focal plants dead, retroactive to their last reproduction, if no visible, attached offspring appeared during this time.

Experiment 1

Experimental approach

In Experiment 1, we deployed all seven light intensity treatments (L_1, $L_{1/2}$, $L_{1/4}$, $L_{1/8}$, $L_{1/16}$, $L_{1/32}$, and L_0). Each treatment began with 32 replicates (initial $n=224$). One plant from $L_{1/2}$, one from $L_{1/32}$, and four from L_0 were excluded due to growth medium contamination (final $n=218$). We observed the plants daily until all replicates from the L_1, $L_{1/2}$, $L_{1/4}$, and $L_{1/8}$ treatments, and all but two, 11, and 18 plants from the $L_{1/16}$, $L_{1/32}$, and L_0 treatments, respectively, were dead (with the 31 remaining plants yet to be classified as alive or dead).

As plants in the low-light treatments were very long-lived, Experiment 1 was terminated following a 2-week interval during which no offspring detached from either of the two remaining plants in the $L_{1/16}$ treatment. At this point, the 31 plants still under observation in the three lowest-light treatments entered a post-experiment phase. We removed the lids of the outer containers, exposing the plants to full light for 2 weeks. If plants did not produce any new offspring in this time, we considered them dead, retroactive to their date of last reproduction. If plants did produce new offspring, we considered them alive at experiment termination, with lifespan calculated to the experiment's termination date. Following this process, we determined that all plants in the $L_{1/16}$ treatment were dead prior to the termination date (i.e., in addition to all of those in the L_1, $L_{1/2}$, $L_{1/4}$, and $L_{1/8}$ treatments). We determined that three (of 11) plants in $L_{1/32}$ treatment and 16 (of 18) plants in the L_0 treatment were alive at the termination date. Thus, lifespan was underestimated for 19 individuals.

Frond surface area measurements were collected after death (/experiment termination); light intensity did not affect frond surface area in Experiment 1 (Online Appendix F).

Data analysis

We conducted all analyses in R version 4.3.2 (R Core Team, 2024). Code and data are available on Zenodo (https://zenodo.org/records/10267550) and Dryad (https://doi.org/10.5061/dryad.4qrfj6qgz), respectively.

We used the daily reproduction data to calculate focal plants' lifespan and total number of offspring. To connect our reproduction results with the capacity for population growth, we also calculated plants' intrinsic rate of increase (r) at the level of the individual (McGraw & Caswell, 1996). This quantity, hereafter called simply 'intrinsic rate of increase', is the natural logarithm of the dominant eigenvalue of a Leslie matrix populated by the survival and reproduction data of a single focal individual (McGraw & Caswell, 1996), and can be construed as the projected intrinsic growth rate of a population composed of identical copies of that focal individual in a given environment. The three measures were underestimated, likely subtly in the case of total offspring and intrinsic rate of increase, for the 19 individuals in treatments $L_{1/32}$ and L_0 that were still alive at Experiment 1's termination.

We tested the effects of light intensity on lifespan, total number of offspring, and intrinsic rate of increase using one-way ANOVAs (R function *aov*). In these ANOVAs, only treatments L_1 to $L_{1/16}$ were tested formally, as these were the treatments for which every individual's lifespan was known (due to the termination of the experiment, described above).

In each case, we assessed ANOVA assumptions with residual-by-predicted plots and normal quantile-quantile plots. Following this, lifespan, total number of offspring, and intrinsic rate of increase were log-transformed to better meet the assumptions (i.e., normally distributed residuals and homoscedasticity), and re-tested. In the case of significant ANOVAs, pairwise comparisons among light intensity treatments were performed using Tukey–Kramer post hoc tests (R function *TukeyHSD*).

To conduct a preliminary investigation of the quantitative nature of the lifespan-versus-light intensity relationship, we fit the following three-parameter model that combines Michaelis–Menten kinetics (for the photosynthesis-vs-light intensity relationship) and a power law (for the lifespan-vs-photosynthesis relationship):

$$\log[\text{lifespan}] = w - v\log\left(\frac{[\text{light intensity}]}{q + [\text{light intensity}]}\right) \quad (1)$$

where q is the Michaelis constant, v is the power law's exponent, and w is the asymptotic log lifespan at high light intensity (Online Appendix G). The fit was executed by non-linear regression using the R function *nls* in the package *nlme* (Pinheiro et al., 2022). This was done twice, once for light intensity treatments L_1 to $L_{1/16}$ and again for L_1 to $L_{1/32}$. In the latter fit, three individuals in $L_{1/32}$ were included despite having underestimated lifespans, as visual inspection of the data suggested that the trend in lifespan between L_1 and $L_{1/16}$ continued to $L_{1/32}$.

To compare the time-invariant shape of survivorship curves across light intensity treatments, we first

time-standardised lifespan data (Baudisch, 2011). Thus, the 'relative age' of each individual was calculated by dividing its absolute age (in days) by the mean absolute lifespan of the members of its treatment. Relative age is measured in number of mean lifespans, and allows the comparison of survivorship curves that occur on different timescales. Specifically, if the different treatments' survivorship-versus-relative lifespan curves coincide, this implies the presence of temporal scaling (Online Appendix H).

Experiment 2

Experimental approach

To further investigate the shape of survivorship curves in high- versus low-light conditions, we conducted a second main experiment. In Experiment 2, replicates were spread across only two light intensity treatments, L_1 and $L_{1/4}$, chosen based on significant results from Experiment 1, with 112 replicates per treatment (initial $n=224$). Having a larger sample size per treatment allowed a finer-resolution of the survivorship curves, and a formal assessment of temporal scaling. Ten plants in L_1 and eight in $L_{1/4}$ were excluded due to growth medium contamination (final $n=206$). We observed the plants daily until all replicates were dead (unlike in Experiment 1, it was unnecessary to terminate Experiment 2 before that point).

Frond surface area measurements were collected after death; light intensity did not affect frond surface area in Experiment 2 (Online Appendix F).

Data analysis

As in Experiment 1, in Experiment 2 we conducted analyses in R version 4.3.2 (R Core Team, 2024). Code and data are available on Zenodo (https://zenodo.org/records/10267550) and Dryad (https://doi.org/10.5061/dryad.4qrfj6qgz), respectively.

We again used the daily reproduction data to calculate lifespan, total number of offspring, and intrinsic rate of increase. We compared each of the response variables between the two light intensity treatments using *t*-tests (R function *t.test*), after having log-transformed those variables to better meet the test's assumptions.

To assess temporal scaling in Experiment 2, we determined the relative age-at-death for plants in both treatments, as in Experiment 1. We then compared the relative lifespan distributions using a Kolmogorov–Smirnov (KS) test (R function *ks.test*); this accords with the approach of Stroustrup et al. (2016) (Online Appendix H). Because a *non*-significant KS test implies that the relative lifespans in the two treatments have the same distribution (and therefore exhibit temporal scaling), before conducting this KS test we first estimated the critical value of KS's D, $D_{crit}=0.1339$, by conducting a bootstrap procedure on Experiment 1's data, with the aim that our subsequent test on Experiment 2's data would have high power (Online Appendix H).

RESULTS

Experiment 1

Lifespan, total number of offspring, and intrinsic rate of increase as functions of light intensity treatment

Lifespan (log-transformed) differed significantly across the analysed light intensity treatments (L_1 to $L_{1/16}$) (Figure 1a; one-way ANOVA, $F_{4,154}=61.51$, $p<2.2\times10^{-16}$). Plants in dimmer treatments lived longer, on average, than those in brighter treatments, a trend that was significant for all pairs except $L_{1/2}$ versus $L_{1/4}$ (Figure 1a; Tukey–Kramer post hoc tests). This trend appeared to continue in the light intensity treatments that were not analysed formally ($L_{1/32}$ and L_0). The assumption of Michaelis–Menten kinetics coupled with the assumption that lifespan and metabolic rate follow a power law, gave a good fit for the relationship between lifespan and light intensity treatment (Figure 1a), even up to $L_{1/32}$.

Total number of offspring (log-transformed) differed subtly but significantly across the analysed light intensity treatments (L_1 to $L_{1/16}$) (Figure 1b; one-way ANOVA, $F_{4,154}=7.58$, $p=1.34\times10^{-5}$). Plants in dimmer light intensity treatments had more offspring than plants in full light, but this plateaued in treatments $L_{1/2}$ and dimmer (Figure 1b; Tukey–Kramer post hoc tests). However, while plants in treatment $L_{1/32}$ had similar total numbers of offspring compared to plants in brighter treatments, plants in treatment L_0 had far fewer offspring, on average (Figure 1b).

Intrinsic rate of increase (log-transformed) differed significantly across the analysed light intensity treatments (L_1 to $L_{1/16}$) (Figure 1c; one-way ANOVA, $F_{4,154}=39.41$, $p<2.2\times10^{-16}$). Plants in dimmer light intensity treatments had lower intrinsic rates of increase, on average, than those in brighter treatments, a trend that was significant for all pairs except L_1 versus $L_{1/2}$, and $L_{1/2}$ versus $L_{1/4}$ (Figure 1c; Tukey–Kramer post hoc tests). This trend appeared to continue in the treatments that were not analysed formally ($L_{1/32}$ and L_0). These results were unsurprising, given that while plants in dimmer treatments had slightly more offspring (excepting treatment L_0; Figure 1b), they were spread out over a considerably longer time (Figure 1a).

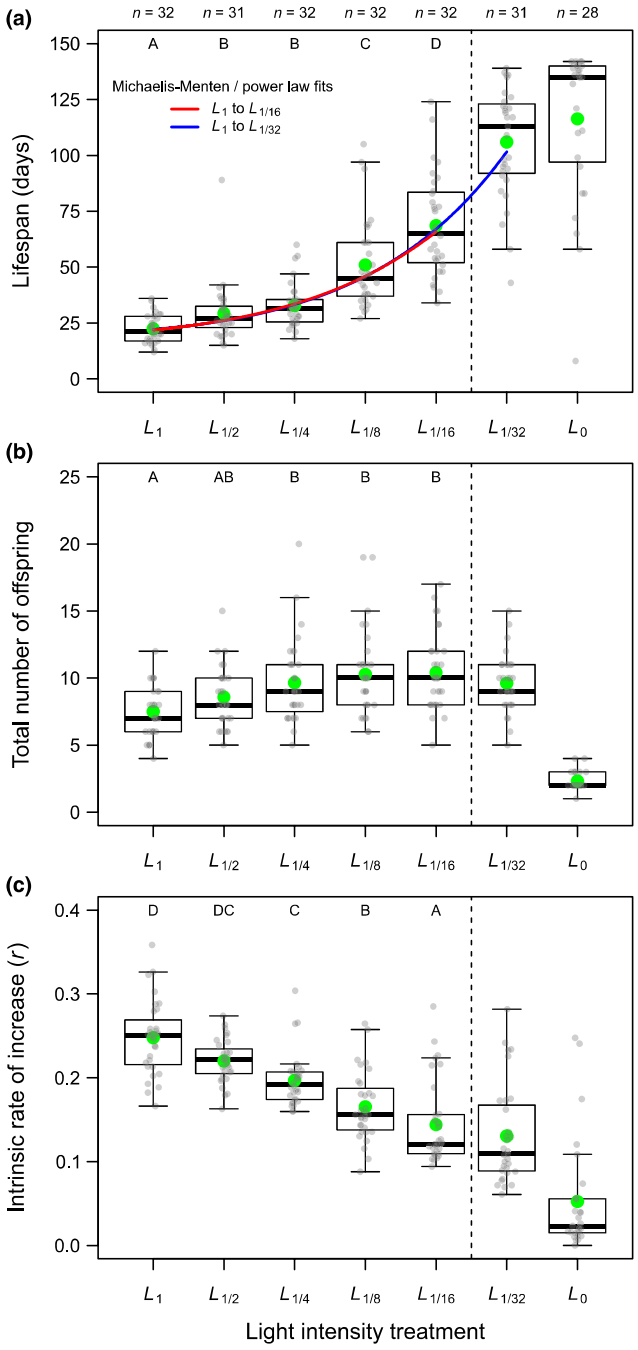

FIGURE 1 Box-and-whisker plots indicating (a) lifespan in days, (b) total number of offspring, and (c) intrinsic rate of increase (r) for the seven light intensity treatments in Experiment 1 (brighter to the left, dimmer to the right). Sample sizes (n) are shown above (a); total $n=218$. The thick horizontal line and the green point in each box represent the median and mean, respectively. The bottom and top of each box represent the first quartile (Q_1) and third quartile (Q_3), respectively. The lower whisker associated with each box represents the smallest value that is no less than $Q_1 - 1.5 \times \text{IQR}$, where $\text{IQR} = Q_3 - Q_1$ (i.e., the interquartile range). The upper whisker associated with each box represents the largest value that is no greater than $Q_3 + 1.5 \times \text{IQR}$. All data points are shown in grey with a slight horizontal jitter to make them easier to distinguish. Within each panel, light treatments that were observed until all individuals were dead (i.e., those left of the dashed line) were included in statistical analyses: Treatments with no letters in common were significantly different; treatments with at least one letter in common were not significantly different. In (a), the red and blue lines represent Michaelis–Menten/power law fits relating lifespan and light intensity. The red line is fit for light intensity treatments $L_{1/16}$ and brighter, while the blue line is fit for light intensity treatments $L_{1/32}$ and brighter.

Survival as a function of age and relative age

When survival curves were plotted against absolute age (in days), they separated out according to light intensity treatment (Figure 2a; consistent with the differences in mean lifespan depicted in Figure 1a). On the other hand, when survival curves were plotted against relative age (in mean lifespans), they appeared very similar (Figure 2b; this was only possible for light intensity treatments L_1 to $L_{1/16}$, where lifespan was known accurately for every individual). This raises the possibility that CR-mediated lifespan extension was related to temporal scaling, an idea that we test formally for the larger sample-size treatments in Experiment 2, below.

Experiment 2

Lifespan, total number of offspring, and intrinsic rate of increase as functions of light intensity treatment

In Experiment 2, plants in the $L_{1/4}$ treatment exhibited a significantly longer lifespan (log-transformed; Figure 3a; t-test, $t_{204} = -4.62$, $p = 6.66 \times 10^{-6}$), a greater total number of offspring (log-transformed; Figure 3b; t-test, $t_{204} = -2.92$, $p = 3.87 \times 10^{-3}$), and a lower intrinsic rate of increase (log-transformed; Figure 3c; t-test, $t_{204} = 2.73$, $p = 6.80 \times 10^{-3}$) compared to those in the L_1 treatment. The directionality of these results is consistent with Experiment 1.

Survival as a function of age and relative age

Similar to Experiment 1, in Experiment 2, when the L_1 and $L_{1/4}$ survival curves were plotted against absolute age (in days), they separated out according to light intensity treatment (Figure 4a; consistent with Figure 3a). When survival curves were instead plotted against relative age (in mean lifespans), they appeared very similar (Figure 4b). There was no significant difference in the distribution of relative lifespan in the L_1 and $L_{1/4}$ light intensity treatments (Kolmogorov–Smirnov test, $D = 0.12 < D_{\text{crit}}$, $n = 206$, $p = 0.41$), consistent with temporal scaling in CR-mediated lifespan extension.

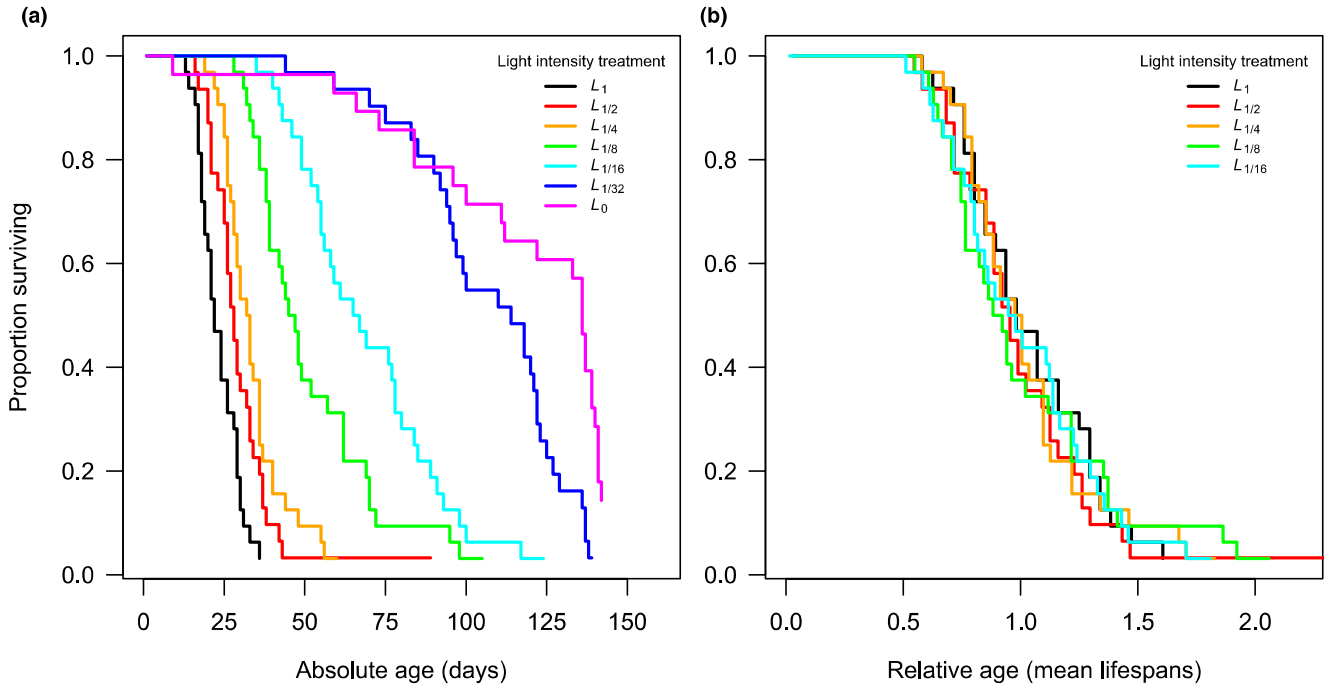

FIGURE 2 Proportion of plants surviving versus (a) absolute age in days, and (b) relative age in mean lifespans in Experiment 1. Sample sizes are as in Figure 1a. Colours represent the seven light intensity treatments (black = L_1; red = $L_{1/2}$; orange = $L_{1/4}$; green = $L_{1/8}$; cyan = $L_{1/16}$; blue = $L_{1/32}$; magenta = L_0). $L_{1/32}$ and L_0 are not given in (b); for those treatments, the mean lifespan could not be calculated because the experiment was terminated before all the plants had died.

DISCUSSION

CR-mediated lifespan extension

Decreasing light intensity led to increased lifespan in *L. minor* (Figures 1a and 3a), suggesting the occurrence of CR-mediated lifespan extension. Along with Minina et al. (2013), our study provides some of the first evidence that CR leads to lifespan extension in plant species, not just in animals. Plant senescence has received far less attention than animal senescence (Salguero-Gómez et al., 2013), and this general trend certainly applies in the case of CR-mediated lifespan extension. In light of our results, we amplify calls to redress the imbalance.

Although both plants and animals exhibit CR-mediated lifespan extension, their specific responses to CR are different. In particular, we note that Experiment 1 showed continually increasing lifespan with decreasing light intensity (Figure 1a). In fact, in our most light-restricted treatment (L_0), with plants grown in near-complete darkness, 18 of the initial 28 plants were still alive after over four times the mean lifespan of plants grown in full light conditions (L_1). Contrarily, in animals, extreme calorie-restricted diets typically result in shorter lifespans due to starvation, and maximal mean lifespan occurs at a moderate level of CR (e.g., Bishop & Guarente, 2007; Clancy et al., 2002; Houthoofd et al., 2003; Mair & Dillin, 2008).

Temporal scaling of lifespan distributions

Lifespan extension in our experiments occurred due to the stretching of time (Figures 2b and 4b), wherein the pace of senescence was different but the time-invariant shape was constant (Barks et al., 2018; Baudisch, 2011), in line with the concept of temporal scaling (Stroustrup et al., 2016). Temporal scaling is a framework that has the potential to unify our understanding of how environmental or intrinsic factors influence senescence (Stroustrup et al., 2016). Thus, the fact that temporal scaling occurs in plant as well as animal senescence strongly bolsters the evidence in favour of this burgeoning framework.

Accordingly, our results emphasize the need for further study of temporal scaling in plant senescence. In particular, the effects of factors other than light intensity should be investigated, with the aim of seeing whether variation in these factors leads to a similar stretching of senescence trajectories. With specific reference to *Lemna* senescence, temporal scaling should be further studied with respect to known or suspected sources of lifespan or growth variation including temperature (Paiha, 2021; Wangermann & Ashby, 1951), parental age effects (Barks & Laird, 2015; Dutt, 2023), nutrient availability (Hayden, 2018), the presence and concentration of chemical stressors (Chmilar et al., 2023; Chmilar & Laird, 2023), carbon dioxide concentration (Zenir et al., 2023), and variation in the microbiome (Kose et al., 2023; Zenir et al., 2023).

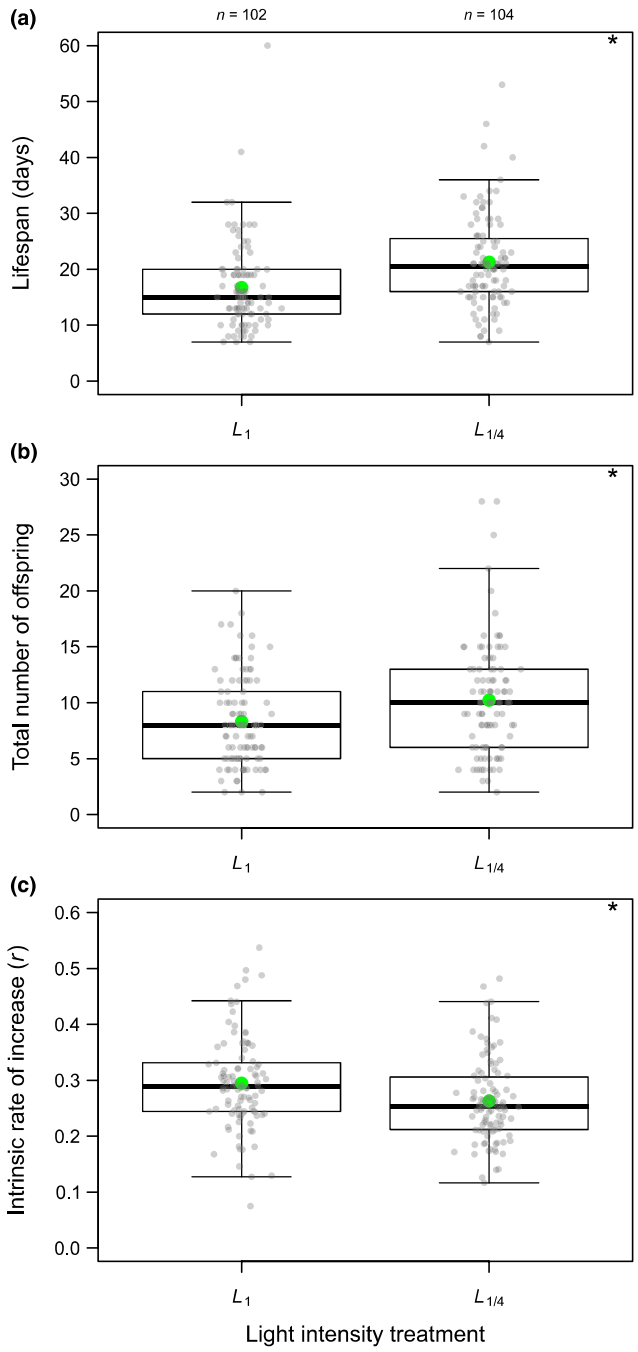

FIGURE 3 Box-and-whisker plots indicating (a) lifespan in days, (b) total number of offspring, and (c) intrinsic rate of increase (r) for the two light intensity treatments in Experiment 2 (brighter = L_1, dimmer = $L_{1/4}$). Sample sizes (n) are shown above (a); total $n = 206$. The thick horizontal line and the green point in each box represent the median and mean, respectively. The bottom and top of each box represent the first quartile (Q_1) and third quartile (Q_3), respectively. The lower whisker associated with each box represents the smallest value that is no less than $Q_1 - 1.5 \times \text{IQR}$, where $\text{IQR} = Q_3 - Q_1$ (i.e., the interquartile range). The upper whisker associated with each box represents the largest value that is no greater than $Q_3 + 1.5 \times \text{IQR}$. All data points are shown in grey with a slight horizontal jitter to make them easier to distinguish. Asterisks indicate significant differences between the treatments.

Furthermore, interactive effects of multiple factors should also be examined. Finally, additional strains and plant species should be tested for temporal scaling (Barks et al., 2018; Paiha & Laird, 2022), including those outside the realm of common model systems like *Arabidopsis* and *Lemna* (see Online Appendix A). In general, we anticipate that environmental or intrinsic factors that slow photosynthesis or metabolism will tend to stretch time in a manner that extends plant lifespan.

The magnitude of temporal scaling

In Experiment 1, which had multiple light intensity treatments, the magnitude of temporal scaling in CR-mediated lifespan extension was quantitatively predictable, under the assumptions that photosynthesis (/ metabolism) is positively related to light intensity following Michaelis–Menten kinetics (c.f. Docauer, 1983), and negatively related to lifespan following a power law (Lin et al., 2015). Thus, this approach led to a good fit between lifespan and light intensity (Figure 1a).

Although other types of curves could also potentially fit our data, Experiment 1 provides intriguing preliminary support for the link between metabolism and senescence, as suggested by the rate of living theory (Box 2). While this theory is now unpopular with regards to animal species, it may be more relevant in plant species (Issartel & Coiffard, 2011). Indeed, metabolism-mediated lifespan extension is also supported by previous findings that lower temperatures extend lifespan in *L. minor* (Paiha, 2021; Wangermann & Ashby, 1951), along with the well-known negative relationship between lifespan and temperature in ectotherm animals (Box 2; e.g., Flouris & Piantoni, 2015; Klass, 1977; Liu & Walford, 1966; Rosbakh & Poschlod, 2018). We emphasize, however, that the hypothesis that differences in metabolic rate contribute to differences in plant lifespan under CR requires further mechanistic study of the processes involved.

The effects of CR on reproduction

Contrary to our prediction, we did not find evidence of a reduction in total offspring with reduction in light intensity, as expected following animal studies on CR (Holehan & Merry, 1985; Speakman & Mitchell, 2011). Rather, in both our experiments, plants in lifespan-extending treatments produced the same ($L_{1/2}$), or significantly more offspring ($L_{1/4}$ to $L_{1/16}$) than those in the L_1 treatment, with L_0 the only exception (Figures 1b and 3b).

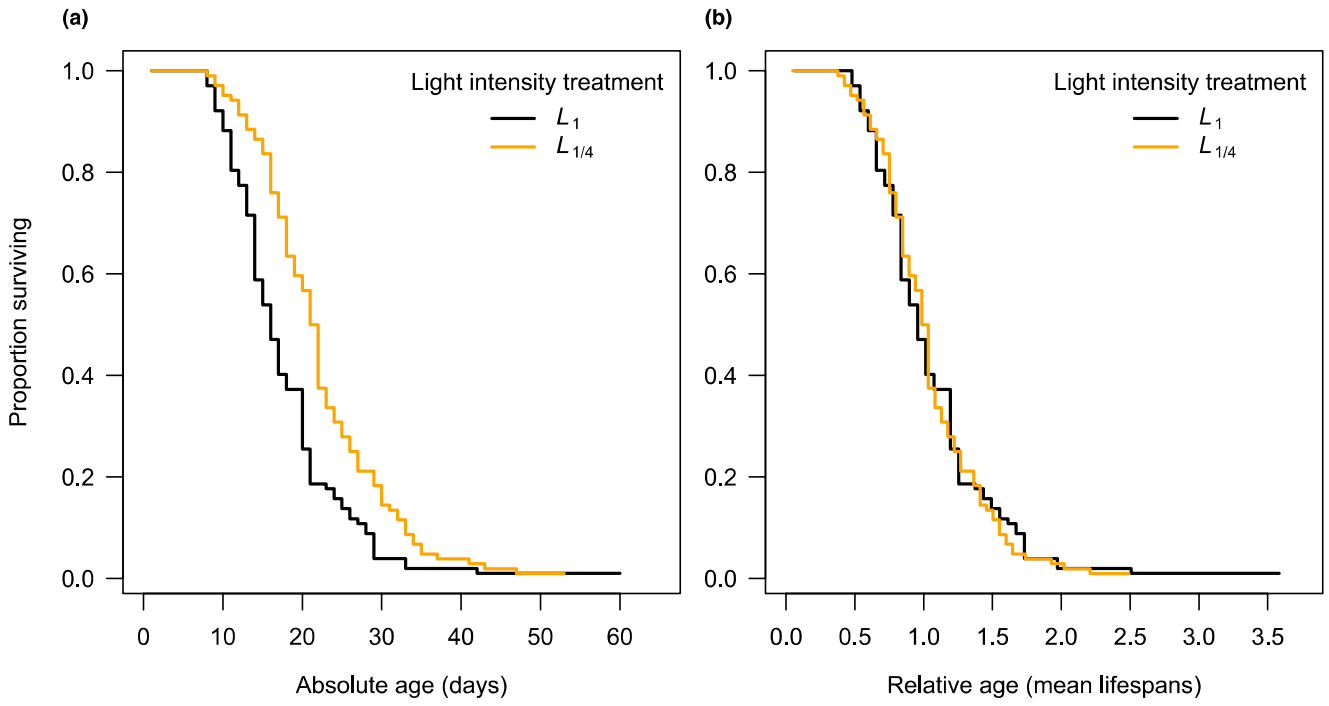

FIGURE 4 Proportion of plants surviving versus (a) absolute age in days, and (b) relative age in mean lifespans in Experiment 2. Sample sizes are as in Figure 3a. Colours represent the two light intensity treatments (black = L_1; orange = $L_{1/4}$).

However, this does not refute the long-standing trade-off theory of CR-mediated lifespan extension (Box 1), as extended lifespan may trade off with aspects of reproduction besides number of offspring—that is, offspring may be produced slower or later in life—as evidenced by strong decreases in intrinsic rates of increase found in treatments with extended lifespans (Figures 1c and 3c) and supplementary analyses suggesting temporal scaling in terms of reproduction (Online Appendix I). These data and additional analyses also reconcile how it is possible that brighter light leads to (a) shorter-lived individuals that produce fewer offspring, and at the same time (b) populations that have much greater growth rates (Docauer, 1983; Paolacci et al., 2018).

Conclusions

Our experiments show that (a) caloric restriction shortens *L. minor* lifespan, (b) it does so by stretching time via temporal scaling in a manner that slows the pace of senescence but leaves its time-invariant shape intact, and (c) the magnitude of the stretch can be related to specific relationships between light intensity, photosynthesis, and aging. Further investigation should focus on the effects of CR in other plant species and the physiological mechanisms underpinning environment-induced lifespan extension. Meanwhile, our results provide promising support to the goal of developing models of senescence that produce quantitative predictions of aging trajectories.

AUTHOR CONTRIBUTIONS

SLC and RAL designed the experiments, with contributions from the other authors. SLC, ACL, PD, AP, and VCT collected the data. SLC and RAL analysed the data. SLC and RAL were the lead writers of the initial draft of the manuscript. All authors edited subsequent drafts.

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DATA AVAILABILITY STATEMENT

The data and R code that support these findings are publicly archived on Dryad (https://doi.org/10.5061/

dryad.4qrfj6qgz) and Zenodo (https://zenodo.org/records/10267550), respectively.

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SUPPORTING INFORMATION

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

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