Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

Exp Gerontol. Author manuscript; available in PMC 2019 January 17.

Published in final edited form as:

Exp Gerontol. 2018 December ; 114: 99–106. doi:10.1016/j.exger.2018.10.023.

Congeneric variability in lifespan extension and onset of senescence suggest active regulation of aging in response to low temperature

Kristin E. Gribble[*], **Benjamin M. Moran**, **Shannon Jones**[1], **Emily L. Corey**, **David B. Mark Welch**

Josephine Bay Paul Center for Comparative Molecular Biology and Evolution, Marine Biological Laboratory, Woods Hole, MA 02543, USA

Abstract

Lifespan extension under low temperature is well conserved across both endothermic and exothermic taxa, but the mechanism underlying this change in aging is poorly understood. Low temperature is thought to decrease metabolic rate, thus slowing the accumulation of cellular damage from reactive oxygen species, although recent evidence suggests involvement of specific cold-sensing biochemical pathways. We tested the effect of low temperature on aging in 11 strains of *Brachionus* rotifers, with the hypothesis that if the mechanism of lifespan extension is purely thermodynamic, all strains should have a similar increase in lifespan. We found differences in change in median lifespan ranging from a 6% decrease to a 100% increase, as well as differences in maximum and relative lifespan extension and in mortality rate. Low temperature delays reproductive senescence in most strains, suggesting an extension of healthspan, even in strains with little to no change in lifespan. The combination of low temperature and caloric restriction in one strain resulted in an additive lifespan increase, indicating these interventions may work via non- or partially-overlapping pathways. The known low temperature sensor TRPA1 is present in the rotifer genome, but chemical TRPA1 agonists did not affect lifespan, suggesting that this gene may be involved in low temperature sensation but not in chemoreception in rotifers. The congeneric variability in response to low temperature suggests that the mechanism of low temperature lifespan extension is an active genetic process rather than a passive thermodynamic one and is dependent upon genotype.

Keywords

Low temperature; Congeneric variability; Reproductive senescence; Transient receptor potential channels; TRPA1

This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/BY-NC-ND/4.0/).

[*]Corresponding author at: Josephine Bay Paul Center for Comparative Molecular Biology and Evolution, Marine Biological Laboratory, 7 MBL St., Woods Hole, MA 02543, USA., kgribble@mbl.edu (K.E. Gribble).

[1]Present Address: University of Notre Dame Environmental Research Center and Department of Biological Sciences, University of Notre Dame, 097 Galvin Life Sciences Center, Notre Dame, IN 46556, USA.

Declarations of interest

None.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

1. Introduction

The association between growth at low temperature and increased lifespan is conserved across a range of taxa, including both exotherms and endotherms (Keil et al., 2015; Liu and Walford, 1972; Rikke and Johnson, 2004). Thermodynamic explanations for lifespan extension under low temperature are common and generally accepted: under normal conditions, the by-products of metabolism, including reactive oxygen species, cause cellular damage that accumulates over time, leading to age-related dysfunction. Lowered ambient temperature has been thought to slow metabolism by decreasing the rate of chemical reactions, slowing the accumulation of damage and thereby increasing lifespan (Conti, 2008; Liu and Walford, 1972; Rikke and Johnson, 2004). These hypotheses are consistent with the rate-of-living or free radical theory of aging (Harman, 1956; Harman, 1972).

Recent studies suggest that specific genetic mechanisms invoked by low temperature can lead to increased lifespan, challenging purely thermodynamic explanations. In *C. elegans*, RNAi knockout of the transient receptor protein TRPA1, a transmembrane Ca+ channel cold and electrophile receptor, negates lifespan extension under low temperature (Xiao et al., 2013). Additionally, mutational inactivation of sod-1 decreases cold-induced longevity (Yen et al., 2009). In *Drosophila* and rotifers, short-term exposures to low temperature lead to a non-proportional, long-term increase in longevity and stress resistance (Johnston and Snell, 2016; Le Bourg, 2016), suggesting that it is not a short-term decrease in metabolism but rather a long-term physiological adaptation that controls lifespan. In the annual fish *Nothobranchius rachovii*, the activities of catalase and Mn-superoxide dismutase, and the levels of ATP, ADP, Sirt1 and FOXO are elevated under low temperature, suggesting active regulation (Hsu and Chiu, 2009). Uncoupling cause and effect of intrinsically or extrinsically lowered body temperature from decreased body temperature due to mutation or antiaging therapies will reveal genetic mechanisms linking temperature and longevity (Conti et al., 2006; Hunter et al., 1999; Keil et al., 2015; Rikke and Johnson, 2004; Van Voorhies and Ward, 1999).

Awareness is growing that the response to aging therapies in model systems can vary greatly, both within and between species, depending upon genetic background (Lucanic et al., 2017). The degree and direction of lifespan change in response to therapies or genetic knockdown may differ among strains due to unknown genetic variability or epistatic relationships. Even caloric restriction, considered to be the most widely conserved lifespan extending intervention, results in a range of lifespan outcomes among different strains of mammalian and invertebrate models (Gribble et al., 2014; Liao et al., 2010; Metaxakis and Partridge, 2013; Sutphin and Kaeberlein, 2008). Most studies of the effect of low temperature on lifespan have extrapolated conservation of lifespan extension from results in a single strain or in genetic mutants of a few model organisms, but it is unknown how the response to low temperature varies among genotypes of a given species. Money, time and health may be lost in pursuing targets or treatments that have not been replicated among multiple model systems. The treatments that are most likely to be robust in humans are those that are conserved among a wide range of taxa and genetic backgrounds.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

To expand the evolutionary breadth of study systems available to investigate the biology of aging, we and others are developing monogonont rotifers as a new model system (Gribble and Snell, 2018; Mark Welch, 2018). Rotifers are microscopic, aquatic, invertebrate animals with approximately 1000 cells, with reproductive, digestive, nervous, and muscle systems, and many advantages as a model in aging research. Their small size (500 μm), two-week lifespan, direct development with no larval stage, and easy culture make rotifers highly tractable for lifespan studies in the laboratory. As lophotrochazoans, rotifers provide diversity to the established invertebrate model systems of *C. elegans* and *D. melanogaster*, both ecdycozoans. Additionally, rotifers have not undergone the genome reduction that characterizes worms and flies, and share hundreds of genes with humans that have been lost in these models, many of which show age-specific differential gene expression (Gribble and Mark Welch, 2017). Resources for rotifer research include draft genomes, transcriptomes, RNAi, and many closely related strains and species in culture and available to the community (Gribble and Mark Welch, 2017; Kim et al., 2018; Snell et al., 2011).

The 11 strains of *Brachionus* spp. used in this study are part of the *Brachionus plicatilis* species complex, a clade of closely related, morphologically similar species primarily defined by DNA sequence divergence, with a gradient of mate recognition and reproductive compatibility between species (Gómez et al., 2002; Gribble and Mark Welch, 2012; Mills et al., 2016; Suatoni et al., 2006). These strains have been in culture from 7 to 45 years. They evolved in diverse environments, ranging in average temperature, salinity, hydration period, and food availability (Table S1). This diversity of selective pressures has resulted in strains that vary in lifespan and responses to environmental change (Gribble et al., 2014; Kim et al., 2017; Serra et al., 1994) allowing them to be used in a comparative fashion as “natural mutants.”

In this study we characterize the degree of conservation of lifespan extension due to exposure to low temperature among strains of the same or closely related species from the *Brachionus* species complex. Additionally, we investigate the evolution of TRPA1, a known temperature sensor and mediator of low temperature lifespan extension, explore the interaction between low temperature and CR facilitated lifespan extension, and elucidate possible trade-offs between lifespan extension and fitness.

2. Methods

2.1. Culturing

Brachionus spp. strains were kept in serial culture and fed the chlorophyte algae *Tetraselmis suecica*. Algae cultures were maintained in 2 L flasks of bubbled f/2 medium, minus silica (Guillard, 1975), made in 15 ppt Instant Ocean Sea Salt (Instant Ocean, Blacksburg, VA). Both rotifer and algae stock cultures were kept at 21 °C on a 12:12 h light:-dark cycle prior to experiments.

2.2. Lifespan assays

2.2.1. Low temperature—For each of 11 rotifer strains, amictic (asexual) eggs were collected and allowed to hatch overnight to obtain an age-sychronized cohort. For every

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

temperature treatment, two neonates were isolated into 1 ml 15 ppt Instant Ocean with 6×10^5 cells/ml *T. suecica* (ad libitum food) and 20 μM FUDR (5-fluorodeoxyuridine, Sigma-Aldrich, St. Louis, MO) to prevent egg hatching in every well of a 24 well plate (n = 48). For each strain, one plate was incubated at 21 °C and a second plate at 16 °C. For the RUS strain of *Brachionus manjavacas* (BmanRUS), an additional plate was incubated at each temperature with 6×10^4 cells/ ml *T. suecica* (10% of ad libitum, chronic caloric restriction). Survival of individuals was scored daily; death was defined as lack of movement of the cilia, mastax (jaw), muscles, gut, and foot. Rotifers were transferred to new plates with fresh food, water, and FUDR every three days.

2.2.2. TRP agonists—Menthol and allyl isothiocyanate (AITC) were purchased from Acros Organics (Geel, Belgium), ligustilide from Alomone Labs (Jerusalem, Israel), and ASP 7663 from Tocris (Minneapolis, MN). Rotifers were hatched and isolated into 24 well plates with 15 ppt Instant Ocean and 6×10^5 cells/ml *T. suecica*, as above. For the menthol exposure, menthol was added to cultures at a final concentration of 10 μM, 50 μM, or 100 μM, and plates were maintained at 21 °C. In a second experiment, 50 mM AITC in ethanol was added to cultures at a final concentrations of 1 μM, 5 μM or 10 μM; plates with AITC were tested at both 21 °C and 16 °C. To prevent egg hatching, 20 μM FUDR was used in these experiments. For the third experiment, 50 mM ligustilide in ethanol was added to a final concentration of 50 μM, 100 μM, or 200 μM, and 50 mM in ethanol of ASP 7663 to 0.5 μM, 2 μM, or 5 μM, all without FUDR. The concentrations used were based on the EC 50 in cell or mammal studies. The controls for AITC, ligustilide, and ASP 7663 contained 0.4% ethanol. All agonist and control treatments were maintained in a 12:12 h light:dark cycle. Menthol, AITC, food, and FUDR were renewed every 3 days throughout life. For ligustilide and ASP 7663, transfers to fresh food and agonists were performed daily. Survivorship was scored as described above.

2.3. Body size

For each strain, eggs were collected and hatched overnight at 21 °C. Neonates were isolated into 6-well plates with 5 ml *T. suecica* and grown until the age of first reproduction (3–7 d, depending upon the strain and treatment) at 16 °C or 21 °C. Rotifers were fixed with 0.15% Lugol's solution, viewed at 200× on a Zeiss AxioSkop, and photographed using a Zeiss AxioCam. Lorica length and width measurements were made for 30 individuals per treatment using Zeiss Axiovison software. Body volume was calculated assuming body shape approximates a cylinder. Significance of differences in body volume was calculated using heteroscedastic two-tailed t-tests.

2.4. TRPA1 gene sequence and phylogeny

The full-length sequence of TRPA1 was obtained by BLAST search to our draft genome of *Brachionus manjavacas* RUS. TRPA1 homologs for the bdelloid *Adineta vaga* were similarly identified from the published *Adineta vaga* genome (Flot et al., 2013). Rotifer TRPA1 sequences were aligned with homologs from other taxa (Table S2) using Muscle in Mega7. A phylogenetic tree was constructed with MrBayes 3.2, using a mixed amino acid substitution model; posterior probabilities were estimated using a Markov Chain Monte Carlo simulation over 4,000,000 generations.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

2.5. Analysis and statistics

Survivorship, body size, and reproduction data were analyzed using Prism 7 software. Kaplan Meier survivorship curves were compared using a Mantel-Cox test. Differences between median lifespan were determined using a Mann-Whitney test. The instantaneous mortality rate, calculated as $\mu_x = -\ln(1 - q_x)$, where q_x is the age-specific probability of death in the interval Δx, was determined daily. Gompertz curves were constructed from the log of the mortality rate, $\ln(\mu_x)$ and analyzed using segmented linear regression. The significance of differences between correlations or between slopes or intercepts at different temperatures was determined from linear regression using ANCOVA in Prism 7. The latitude of origin was known for 8 of the 11 strains tested. We used linear regression to examine the relationships between lifespan, change in lifespan, body size, post-reproductive period, and latitude of origin for strains at 21 °C or 16 °C. Analysis of the relationship between changes in lifespan under low temperature and under caloric restriction used previously published data on intermittent fasting (Gribble et al., 2014).

3. Results

3.1. Lifespan, reproductive senescence, and body size

To determine the effect of low temperature on health and aging, we measured lifespan and reproductive period of 11 strains of *Brachionus* rotifers at 21 °C and 16 °C. We observed a high degree of variability in the response to low temperature among these congeneric strains. When grown at 21 °C under ad libitum food conditions, median lifespan varied from 10 d to 33.5 d among the 11 strains; minimum lifespan was 2 days, while maximum lifespan ranged from 14.7 to 33.6 d (Fig. 1; Table S1). With continuous growth at 16 °C, median lifespan increased by 6%–100% in nine strains ($p = 0.0001$), decreased by 6% in one strain (JPNAG062; p = 0.0001), and did not change in one strain (BpL1; $p = 0.15$). While both median and maximum lifespan at 21 °C were positively correlated with lifespan at 16 °C, there was no correlation between lifespan at 21 °C and the percent change in lifespan with growth at 16 °C (Fig. 2). There was no correlation between latitude of origin and lifespan at 21 °C, 16 °C, or percent change in lifespan (Fig. S1; linear regression; $R^2 < 0.04$ for all comparisons).

Comparison of the rate of aging at 16 °C and 21 °C, measured as the slope of the Gompertz regression, showed significant differences in the rate of aging for all 11 strains (Sum-of-squares F test, $p < 0.05$; Fig. 3, Table S1). Changes in the slope of the Gompertz regression better reflect a change in aging than comparison of median lifespan, particularly for those strains where 21 °C and 16 °C survivorship curves cross, such as JPNAG062, AUBUS001, and BpL1. The steeper slope at 21 °C indicates a higher mortality rate than at 16 °C for all strains. In four cases mortality rate decreased in late life at 21 °C; this change in slope was usually driven by just a few individuals surviving to late ages. At 16 °C, shallow slopes suggested low mortality rates at young ages; at later ages, Gompertz regressions rapidly steepened for those strains with significantly extended lifespan at low temperature.

The post-reproductive period decreased significantly for 8 of 11 strains grown at 16 °C (Fig. 4). Actual days post-reproductive was significantly lower at 16 °C for five of the 11 strains.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

While there was a trend toward increasing lifespan being associated with a smaller decrease in the post-reproductive period, the correlation was not significant ($R^2 = 0.120$). The lack of correlation was largely due to a single outlying point for JPNAG023. This strain had a small change in median lifespan but a large increase in the post-reproductive period and a large increase in maximum lifespan. With this single outlier removed from the analysis, $R^2 = 0.750$ (Fig. 4).

Body size at the time of first reproduction was significantly larger for all strains grown at 21 °C except CGAL6 and BmanL5, for which there was no significant difference in size between 21 °C and 16 °C (Fig. S2). Neither lifespan and body size nor change in body size and change in lifespan at lower temperature were significantly correlated (Fig. S2).

3.2. Relationship between low temperature and caloric restriction (CR) response

Previously, we found significant variation between many of these strains in their response to chronic caloric restriction (CCR, constant low food) and to intermittent fasting (IF; ad libitum feeding and starvation on alternate days) (Gribble et al., 2014). Some strains, including BmanRUS and AUYEN020, had significantly increased lifespan under both caloric restriction and decreased temperature, while others, particularly BpL1, had no change in lifespan under either intervention. However, there was no correlation between lifespan response to intermittent fasting and to decreased temperature across the eight strains tested with the two interventions (Fig. 5).

To determine if lifespan effects of caloric restriction and low temperature were additive, we tested BmanRUS under a combination of low temperature and CCR. Median lifespan increased by 60% under low temperature alone, 13% under CCR alone, and by 80% under the combination of low temperature and CR (Table S1; Fig. 1). Maximum lifespan was significantly increased by low temperature, but not by CCR.

3.3. Phylogeny of TRPA1 and effect of transient receptor protein agonists

We identified a single potential TRPA1 ortholog in our draft genome of *Brachionus manjavacas* RUS but found no recognizable ortholog to TRPM8, both known cold-temperature sensors. To confirm our TRPA1 candidate as a true ortholog, we collected TRPA1 sequences from diverse metazoans, including two divergent copies in several lophotrochozoan species (the bdelloid rotifer *Adineta vaga*, the brachiopod *Lingula anatine*, and the mollusks *Aplysia californica* and *Crassostrea gigas*, Table S2). Our phylogenetic analysis revealed two major clades of TRPA1: one clade that includes TRPA1 from vertebrates, insects, and the lophotrochozoans, including BmanRUS; and another clade that includes the second copy from the same lophotrocozoan taxa, not including BmanRUS, as well as single copies from other invertebrates, including the chordate *Ciona intestinalis*. The single copy of TRPA1 from *Caenorhabditis* nematodes falls within this second clade (Fig. S3).

To assess the role of temperature sensing transient receptor proteins in regulating the lifespan response to lower temperature, we exposed the low-temperature responder, BmanRUS, and the non-responder, BpL1, to three concentrations of AITC or menthol, agonists for the known cold-sensing channels TRPA1 and TRPM8, respectively

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

(Macpherson et al., 2007; Peier et al., 2002). AITC did not change lifespan in either strain at any concentration; menthol caused no change in lifespan in BmanRUS and decreased lifespan in BpL1 (Fig. S4). We also exposed BmanRUS to three concentrations of each of the specific TRPA1 agonists ASP 7663 and ligustilide (Zhong et al., 2011); these had no significant effect on aging, except that at high concentrations ligustilide decreased median and maximum lifespan (Fig. S4).

4. Discussion

We examined the effect of low temperature on aging in 11 strains from the *Brachionus* species complex. Differences in lifespan, mortality rate, post-reproductive period, and body size reflect genetic differences among strains.

4.1. Low temperature increases lifespan differently in varied genetic backgrounds

Low temperature increased total median lifespan in some but not all strains. The change in median lifespan varied considerably between strains, from a small but significant decrease to an increase of 100%. Maximum lifespan increased in all strains but was highly variable, with extension ranging from 26% to 110%. Unlike the response of these same strains to caloric restriction (Gribble et al., 2014), there was no correlation between lifespan under control conditions (21 °C) and the percent change in median lifespan under the intervention condition (16 °C).

4.2. Low temperature decreases mortality rates

Segmented Gompertz regressions revealed a change in the mortality rate with age under low temperature conditions for all strains. Lower temperature both slowed mortality rate in early life and delayed the onset of rapid mortality late in life, as has been seen in the blowfly *Calliphora stygia* (Kelly et al., 2013). Previous studies in *B. manjavacas* have shown that short-term cold temperature exposure early in life results in a greater increase in lifespan than does cold exposure in either mid-life or late-life (Johnston and Snell, 2016). The reason for the greater sensitivity of lifespan to low temperature in early life is unknown, but may be due to late-age associated dysfunction in other pathways (Gribble and Mark Welch, 2017) or an inability to overcome accrued cellular damage with a late-age intervention.

The inflection points for low temperature Gompertz regressions were at or near the maximum age under high temperature for most strains, but were not correlated with the onset of reproductive senescence. The sharp inflection point of the mortality curves implies a “tipping point,” at late age. These results suggest that the low temperature response may slow but not abrogate the accumulation of cellular damage throughout life. Alternatively, low temperature may delay but not prevent the induction of another deleterious age-related genetic mechanism. Identifying the mechanistic cause of the sudden increase in mortality rate in late life under low temperature will be an interesting area for future investigation.

4.3. Low temperature decreases post-reproductive senescence

Low temperature prolonged the reproductive period and shortened the post-reproductive period, suggesting an extension of healthspan in most strains. As with the overall lifespan

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

response to low temperature, the change in the length of the post-reproductive period, measured in relative or absolute terms, varied among strains. In 10 of the 11 strains, low temperature decreased the relative post-reproductive period, in 8 strains significantly. For 5 of these 8 strains the absolute time of the post-reproductive period also decreased. Both BpL1, which did not have a significant change in total lifespan, and JPNAG062, which had a decrease in median but an increase in maximum lifespan, experienced significant decreases in the post-reproductive period. An increase in the reproductive period and decrease in the length of reproductive senescence is generally considered an improvement in health at late ages. Some other interventions and mutations that increase lifespan are associated with decreased reproduction, lowered stress resistance, and declines in motility, suggesting that improved health does not always accompany increased longevity (Avanesian et al., 2010; Bansal et al., 2015; Jenkins et al., 2004; Partridge et al., 2005).

4.4. Caloric restriction and low-temperature lifespan extension

The additive increase in lifespan with both CR and low temperature compared to either treatment alone suggests these interventions work via independent or only partially overlapping pathways. The variability among strains in interaction between the two interventions will be an interesting avenue for future research. Previous studies have suggested that lower body temperature and CR use alternative pathways to decrease mortality, as evidenced by the different shape of Gompertz curves (Keil et al., 2015). In *Drosophila*, for example, while CR generally delays mortality in younger animals, the slope of the mortality rate is the same as in non-CR flies (Mair et al., 2003). Lower temperature, on the other hand, both delays mortality and reduces the rate of the mortality trajectory. We saw no correlation between the percent change in lifespan under IF conditions and under low temperature among strains, suggesting that genotypes that respond to one intervention will not necessarily respond positively to a different intervention.

Interestingly, the strain with the smallest lifespan response to low temperature, BpL1, also had no response to CR in a previous study (Gribble et al., 2014), suggesting a difference between this strain and others in the genes at the intersection of low-temperature and CR pathways. Both SGK-1, a serum and glucocorticoid kinase of FOXO (DAF-16), and the FOXO transcription factor, are involved in low temperature and CR mediated longevity (Xiao et al., 2013). Future work will determine how these and other aging-related genes vary among *Brachionus* strains.

4.5. TRPA1 phylogeny and role of TRPs in temperature-mediated lifespan extension

The TRP superfamily is divided into seven subfamilies: TRPC, TRPA, TRPV, TRPN, TRPM, TRPP, and TRPML, based on conserved domains. These genes have a wide array of functions, and the gene family has expanded and contracted in different animal lineages. Our analysis indicates that TRPA1 underwent duplication early in metazoan evolution, with subsequent loss of one or the other copy in some lineages but retention of both copies in other lineages. While the monogonont rotifer *B. manjavacas* has only one copy, the bdelloid rotifer *Adineta vaga* maintains both. Insects and vertebrates have the same copy as *B. manjavacas*, while *Caenorhabditis* spp. have the other.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Chemical agonists of TRPA1 did not induce lifespan changes in BmanRUS (a low temperature responder) or BpL1 (a low temperature non-responder), suggesting that either chemical induction of this TRP channel does not induce the same life-extending pathways as does low temperature (Xiao et al., 2013) or that the rotifer TRPA1 is insensitive to the chemical agonists tested (Snell et al., 2016). The covalent binding of AITC to TRPA1 and subsequent Ca+ channel activation is not well understood but appears to require three of the many cysteines in the cytoplasmic N terminus (Hinman et al., 2006; Macpherson et al., 2007). *Drosophila* TRPA1 has two of these three specific cysteines and binds to AITC (Kang et al., 2010) while *C. elegans* TRPA1 is missing all three and does not bind AITC (Chaudhuri et al., 2016; Xiao and Xu, 2009; Xiao et al., 2013). The *B. manjavacas* TRPA1 gene has one of these cysteines. Thus, it is likely that TRPA1 is involved in temperature sensing but not nociception in *Brachionus*.

The mechanism by which TRPA1 mediates temperature sensation is also not well understood and appears to be highly varied among taxa. Cold activates rodent TRPA1 but not primate TRPA1; these activities are controlled by the difference of a single amino acid (G878 in rodent and V875 in primates) within the S5 transmembrane domain (Chen et al., 2013). In *D. melanogaster* and other arthropods, TRPA1 has been shown to act as a high temperature sensor (Kim et al., 2010; Peng et al., 2016). Directly testing the role of TRPA1 low temperature sensing in rotifers may provide insights into the congeneric variability in low-temperature induced lifespan extension seen in this study.

Previous work implicated TRP7 in lifespan increase in rotifers under low temperature (Johnston and Snell, 2016). Little is known about the role of this gene in temperature, chemical, or mechanical reception. In rotifers, RNAi of TRP7 at 28 °C increased lifespan, suggesting that it may play a role in high or noxious temperature sensation or that decreased Ca+ influx is adaptive at this higher temperature. With RNAi of TRP7 at 16 °C, lifespan decreased below that at 21 °C, suggesting that the receptor is involved in maintenance of lifespan beyond temperature-mediated changes. In mice, the TRPC7 homolog is activated by diacylglycerol or intracellular calcium store depletion, and is not known as a temperature sensor. However, TRPC7 is one of the least studied TRP channels, and thus its full range of functions is not known.

4.6. Conclusions

Differences in the response to low temperature among *Brachionus* strains challenge the common idea that lifespan extension at low temperature is simply a byproduct of decreased metabolic rate. If purely thermodynamic, all strains, having essentially the same physiology, would be expected to demonstrate the same change in lifespan, either absolute (number of days) or relative (percent change relative to life-span at higher temperature). Instead, the high degree of variability in lifespan extension indicates that lifespan response to low temperature depends upon genetic background and may be driven by specific genetic pathways.

Low temperature decreases the mortality rate for all strains, and inflection points in mortality curves suggest that low temperature delays the onset of aging. This is supported by our finding that low temperature increases lifespan while decreasing the relative,

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

and in many cases absolute, time in post-reproductive senescence. Taken together, these observations suggest low temperature confers a genetic response that delays aging and extends healthspan. The varied responses to low temperature among the *Brachionus* strains, coupled with their power as a tractable aging model, makes a study of the comparative genetics of this group in response to low temperature an attractive strategy for understanding the biology of aging.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.exger.2018.10.023.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding

This work was financed by grants from the National Institute on Aging to DMW (R01 AG037960-01) and to KEG (K01 AG049049-01) and by funding from the Vetlesen Foundation to the Josephine Bay Paul Center for Comparative Molecular Biology and Evolution at the Marine Biological Laboratory.

References

Avanesian A, Khodayari B, Felgner JS, Jafari M, 2010. Lamotrigine extends lifespan but compromises health span in Drosophila melanogaster. Biogerontology 11, 45–52. [PubMed: 19430925]

Bansal A, Zhu LJ, Yen K, Tissenbaum HA, 2015. Uncoupling lifespan and health-span in Caenorhabditis elegans longevity mutants. Proc. Natl. Acad. Sci. U. S. A 112, E277–E286. [PubMed: 25561524]

Chaudhuri J, Bose N, Gong J, Hall D, Rifkind A, Bhaumik D, Peiris TH, Chamoli M, Le CH, Liu J, Lithgow GJ, Ramanathan A, Xu XZS, Kapahi P, 2016. A Caenorhabditis elegans model elucidates a conserved role for TRPA1-Nrf signaling in reactive alpha-dicarbonyl detoxification. Curr. Biol 26, 3014–3025. [PubMed: 27773573]

Chen J, Kang D, Xu J, Lake M, Hogan JO, Sun C, Walter K, Yao B, Kim D, 2013. Species differences and molecular determinant of TRPA1 cold sensitivity. Nat. Commun 4, 2501. [PubMed: 24071625]

Conti B, 2008. Considerations on temperature, longevity and aging. Cell. Mol. Life Sci 65, 1626–1630. [PubMed: 18425417]

Conti B, Sanchez-Alverez M, Winsky-Sommerer R, Morale MC, Lucero J, Brownell S, Fabre V, Huitron-Resendiz S, Henriksen S, Zorrilla EP, de Lecea L, Bartfai T, 2006. Transgenic mice with a reduced core body temperature have an increased life span. Science 314, 825–828. [PubMed: 17082459]

Flot J-F, Hespeels B, Li X, Noel B, Arkhipova I, Danchin EGJ, Hejnol A, Henrissat B, Koszul R, Aury J-M, Barbe V, Barthelemy R-M, Bast J, Bazykin GA, Chabrol O, Couloux A, Da Rocha M, Da Silva C, Gladyshev E, Gouret P, Hallatschek O, Hecox-Lea B, Labadie K, Lejeune B, Piskurek O, Poulain J, Rodriguez F, Ryan JF, Vakhrusheva OA, Wajnberg E, Wirth B, Yushenova I, Kellis M, Kondrashov AS, Mark Welch D.B., Pontarotti P, Weissenbach J, Wincker P, Jaillon O, Van Doninck K, 2013. Genomic evidence for ameiotic evolution in the bdelloid rotifer Adineta vaga. Nature 500, 453–457. [PubMed: 23873043]

Gómez A, Serra M, Carvalho GR, Lunt DH, 2002. Speciation in ancient cryptic species complexes: evidence from the molecular phylogeny of Brachionus plicatilis (Rotifera). Evolution 56, 1431–1444. [PubMed: 12206243]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Gribble KE, Mark Welch D.B., 2012. The mate recognition protein gene mediates reproductive isolation and speciation in the Brachionus plicatilis cryptic species complex. BMC Evol. Biol 12, 134. [PubMed: 22852831]

Gribble KE, Mark Welch D.B., 2017. Genome-wide transcriptomics of aging in the rotifer Brachionus manjavacas, an emerging model system. BMC Genomics 18, 217. [PubMed: 28249563]

Gribble KE, Snell TW, 2018. Rotifers as a model for the biology of aging. In: Conn's Handbook of Models for Human Aging Academic Press, London, U.K.

Gribble KE, Kaido O, Jarvis G, Mark Welch D.B., 2014. Patterns of intraspecific variability in the response to caloric restriction. Exp. Gerontol 51, 28–37. [PubMed: 24384399]

Guillard RRL, 1975. Culture of phytoplankton for feeding marine invertebrates. In: Smith WL, Chanley MH (Eds.), Culture of Marine Invertebrates Plenum Publishing Corporation, New York, New York.

Harman D, 1956. Aging: a theory based on free radical and radiation chemistry. J. Gerontol 11, 298–300. [PubMed: 13332224]

Harman D, 1972. The biologic clock: the mitochondria? J. Am. Geriatr. Soc 20, 145–147. [PubMed: 5016631]

Hinman A, Chuang HH, Bautista DM, Julius D, 2006. TRP channel activation by reversible covalent modification. Proc. Natl. Acad. Sci. U. S. A 103, 19564–19568. [PubMed: 17164327]

Hsu CY, Chiu YC, 2009. Ambient temperature influences aging in an annual fish (Nothobranchius rachovii). Aging Cell 8, 726–737. [PubMed: 19780720]

Hunter WS, Croson WB, Barktke A, Gentry MV, Meliska CJ, 1999. Low body temperature in long-lived Ames dwarf mice at rest and during stress. Physiol. Behav 67, 433–437. [PubMed: 10497963]

Jenkins NL, McColl G, Lithgow GJ, 2004. Fitness cost of extended lifespan in Caenorhabditis elegans. Proc. Biol. Sci 271, 2523–2526. [PubMed: 15590605]

Johnston RK, Snell TW, 2016. Moderately lower temperatures greatly extend the lifespan of Brachionus manjavacas (Rotifera): thermodynamics or gene regulation? Exp. Gerontol 78, 12–22. [PubMed: 26939542]

Kang K, Pulver SR, Panzano VC, Chang EC, Griffith LC, Theobald DL, Garrity PA, 2010. Analysis of Drosophila TRPA1 reveals an ancient origin for human chemical nociception. Nature 464, 597–600. [PubMed: 20237474]

Keil G, Cummings E, de Magalhaes JP, 2015. Being cool: how body temperature influences ageing and longevity. Biogerontology 16, 383–397. [PubMed: 25832892]

Kelly MA, Zieba AP, Buttemer WA, Hulbert AJ, 2013. Effect of temperature on the rate of ageing: an experimental study of the blowfly Calliphora stygia. PLoS One 8, e73781. [PubMed: 24019937]

Kim SH, Lee Y, Akitake B, Woodward OM, Guggino WB, Montell C, 2010. Drosophila TRPA1 channel mediates chemical avoidance in gustatory receptor neurons. Proc. Natl. Acad. Sci. U. S. A 107, 8440–8445. [PubMed: 20404155]

Kim H-J, Iwabuchi M, Sakakura Y, Hagiwara A, 2017. Comparison of low temperature adaptation ability in three native and two hybrid strains of the rotifer Brachionus plicatilis species complex. Fish. Sci 83, 65–72.

Kim HS, Lee BY, Han J, Jeong CB, Hwang DS, Lee MC, Kang HM, Kim DH, Kim HJ, Papakostas S, Declerck SAJ, Choi IY, Hagiwara A, Park HG, Lee JS, 2018. The genome of the freshwater monogonont rotifer Brachionus calyciflorus. Mol. Ecol. Resour 18, 646–655. [PubMed: 29451365]

Le Bourg E, 2016. Life-time protection against severe heat stress by exposing young Drosophila melanogaster flies to a mild cold stress. Biogerontology 17, 409–415. [PubMed: 26704618]

Liao C-Y, Rikke BA, Johnson TE, Diaz V, Nelson JF, 2010. Genetic variation in the murine lifespan response to dietary restriction: from life extension to life shortening. Aging Cell 9, 92–95. [PubMed: 19878144]

Liu RK, Walford RL, 1972. The effect of lowered body temperature on lifespan and immune and non-immune processes. Gerontologia 18, 363–388. [PubMed: 4618218]

Lucanic M, Plummer WT, Chen E, Harke J, Foulger AC, Onken B, Coleman-Hulbert AL, Dumas KJ, Guo S, Johnson E, Bhaumik D, Xue J, Crist AB, Presley MP, Harinath G, Sedore CA, Chamoli

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

M, Kamat S, Chen MK, Angeli S, Chang C, Willis JH, Edgar D, Royal MA, Chao EA, Patel S, Garrett T, Ibanez-Ventoso C, Hope J, Kish JL, Guo M, Lithgow GJ, Driscoll M, Phillips PC, 2017. Impact of genetic background and experimental reproducibility on identifying chemical compounds with robust longevity effects. Nat. Commun 8, 14256. [PubMed: 28220799]

Macpherson LJ, Dubin AE, Evans MJ, Marr F, Schultz PG, Cravatt BF, Patapoutian A, 2007. Noxious compounds activate TRPA1 ion channels through covalent modification of cysteines. Nature 445, 541–545. [PubMed: 17237762]

Mair WB, Goymer P, Pletcher SD, Partridge L, 2003. Demography of dietary restriction and death in Drosophila. Science 301, 1731–1733. [PubMed: 14500985]

Mark Welch D.B., 2018. The potential of comparative biology to reveal mechanisms of aging in rotifers. In: Conn's Handbook of Models for Human Aging Academic Press, London, United Kingdom.

Matsuura H, Sokabe T, Kohno K, Tominaga M, Kadowaki T, 2009. Evolutionary conservation and changes in insect TRP channels. BMC Evol. Biol 9, 228. [PubMed: 19740447]

Metaxakis A, Partridge L, 2013. Dietary restriction extends lifespan in wild-derived populations of Drosophila melanogaster. PLoS One 8, e74681. [PubMed: 24040317]

Mills S, Alcántara-Rodríguez A, Ciros-Pérez J, Gómez A, Hagiwara A, Galindo KH, Jersabek CD, Malekzadeh-Viayeh R, Leasi F, Lee J-S, Mark Welch D.B., Papakostas S, Riss S, Segers H, Serra M, Shiel RJ, Smolak R, Snell TW, Stelzer C-P, Tang CQ, Wallace RL, Fontaneto D, Walsh EJ, 2016. Fifteen species in one: deciphering the Brachionus plicatilis species complex (Rotifera, Monogononta) through DNA taxonomy. Hydrobiologia 39–58.

Partridge L, Gems D, Withers DJ, 2005. Sex and death: what is the connection? Cell 120, 461–472. [PubMed: 15734679]

Peier AM, Monqrich A, Hergarden AC, Reeve AJ, Andersson DA, Story GM, Earley TJ, Dragoni I, McIntyre P, Bevan S, Patapoutian A, 2002. A TRP channel that senses cold stimuli and menthol. Cell 108, 705–715. [PubMed: 11893340]

Peng G, Kashio M, Li T, Dong X, Tominaga M, Kadowaki T, 2016. TRPA1 channels in Drosophila and honey bee ectoparasitic mites share heat sensitivity and temperature-related physiological functions. Front. Physiol 7, 447. [PubMed: 27761115]

Rikke BA, Johnson TE, 2004. Lower body temperature as a potential mechanism of life extension in homeotherms. Exp. Gerontol 39, 927–930. [PubMed: 15217694]

Serra M, Carmona MJ, Miracle MR, 1994. Survival analysis of three clones of Brachionus plicatilis (Rotifera). Hydrobiologia 277, 97–105.

Snell TW, Shearer TL, Smith HA, 2011. Exposure to dsRNA produces RNA interference in Brachionus manjavacas (Rotifera). Mar. Biotechnol 13, 264–274.

Snell TW, Johnston RK, Srinivasan B, Zhou H, Gao M, Skolnick J, 2016. Repurposing FDA-approved drugs for anti-aging therapies. Biogerontology 17, 907–920. [PubMed: 27484416]

Suatoni E, Vicario S, Rice S, Snell T, Acaccone A, 2006. An analysis of species boundaries and biogeographic patterns in a cryptic species complex: the rotifer—Brachionus plicatilis. Mol. Phylogenet. Evol 41, 86–98. [PubMed: 16815046]

Sutphin GL, Kaeberlein M, 2008. Dietary restriction by bacterial deprivation increases life span in wild-derived nematodes. Exp. Gerontol 43, 130–135. [PubMed: 18083317]

Van Voorhies WA, Ward S, 1999. Genetic and environmental conditions that increase longevity in Caenorhabditis elegans decrease metabolic rate. Proc. Natl. Acad. Sci. U. S. A 96, 11399–11403. [PubMed: 10500188]

Xiao R, Xu XZ, 2009. Function and regulation of TRP family channels in C. elegans. Pflugers Arch 458, 851–860. [PubMed: 19421772]

Xiao R, Zhang B, Dong Y, Gong J, Xu T, Liu J, Xu XZ, 2013. A genetic program promotes *C. elegans* longevity at cold temperatures via a thermosensitive TRP channel. Cell 152, 806–817. [PubMed: 23415228]

Yen K, Patel HB, Lublin AL, Mobbs CV, 2009. SOD isoforms play no role in lifespan in ad lib or dietary restricted conditions, but mutational inactivation of SOD-1 reduces life extension by cold. Mech. Ageing Dev 130, 173–178. [PubMed: 19059428]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Zhong J, Pollastro F, Prenen J, Zhu Z, Appendino G, Nilius B, 2011. Ligustilide: a novel TRPA1 modulator. Pflugers Arch 462, 841–849. [PubMed: 21894528]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

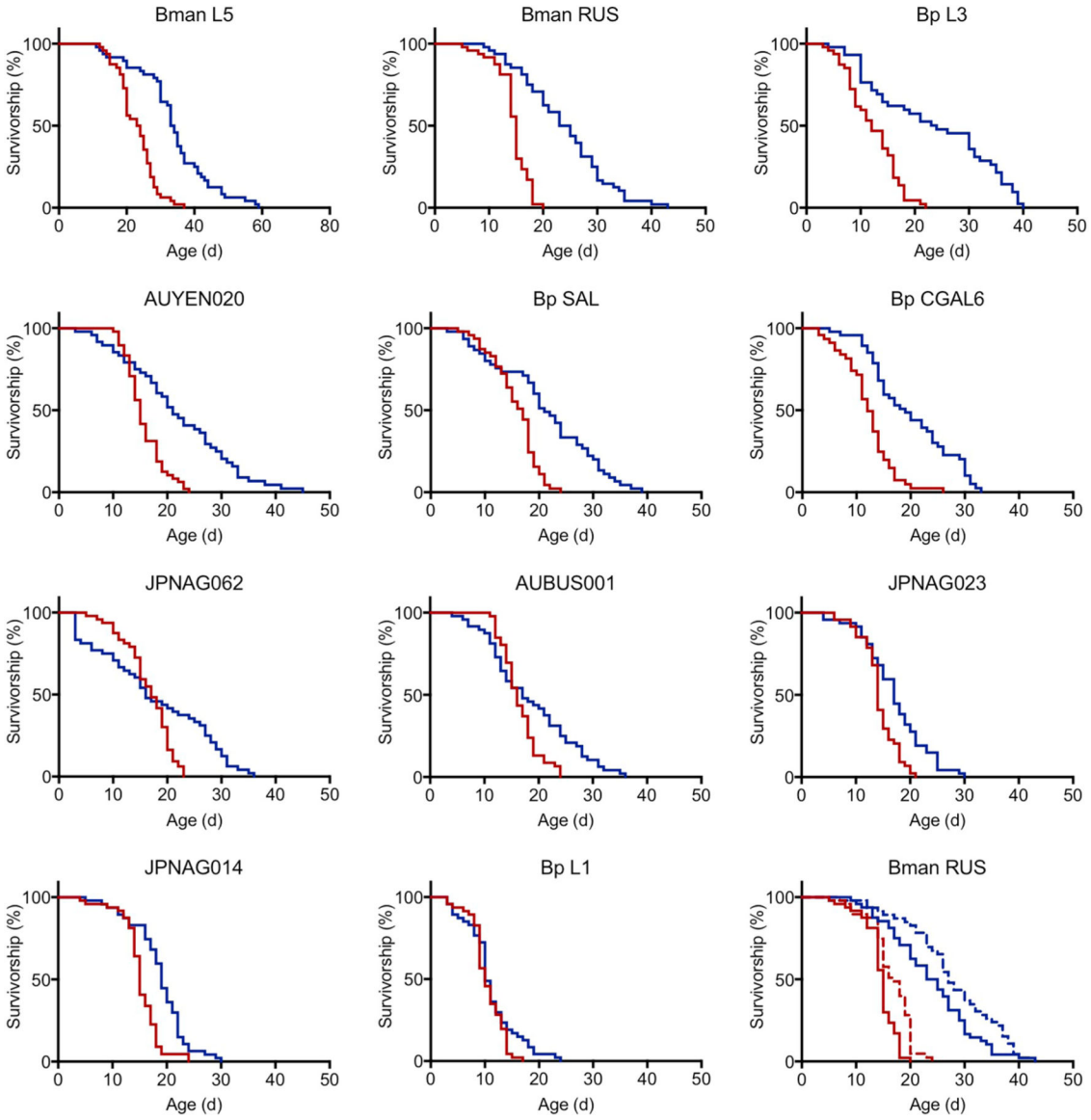

Fig. 1.

Survivorship curves for 11 strains of *Brachionus* grown under high (red, 21 °C) and low (blue, 16 °C) temperature, and for *B. manjavacas* RUS (BmanRUS) grown under ad libitum (solid line), or chronic caloric restriction (dashed line) food conditions. For each strain, survivorship at 16 °C was significantly different than at 21 °C (Mantel-Cox test, $p < 0.0003$) except for AUBUS001 ($p = 0.012$), JPNAG062 ($p = 0.026$) and BpL1 ($p = 0.149$).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

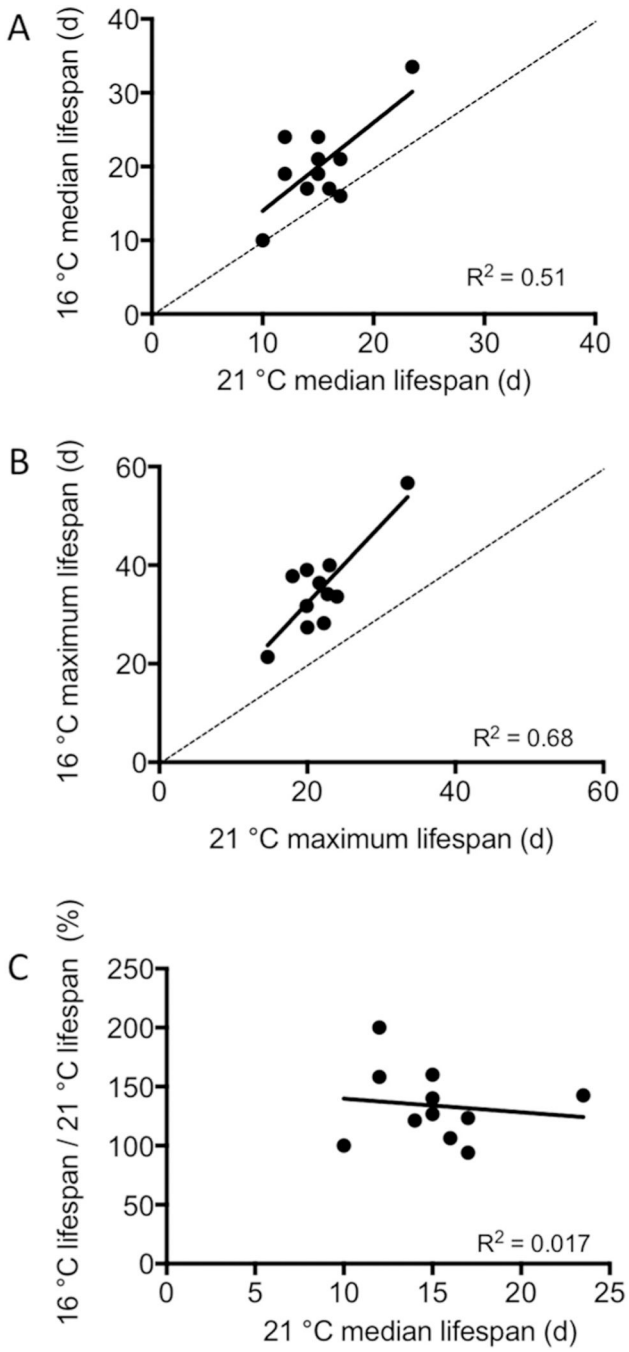

Fig. 2.

Relationship between (A) median lifespan, (B) maximum lifespan, and (C) change in lifespan under 21 °C versus 16 °C for 11 strains of *Brachionus*.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

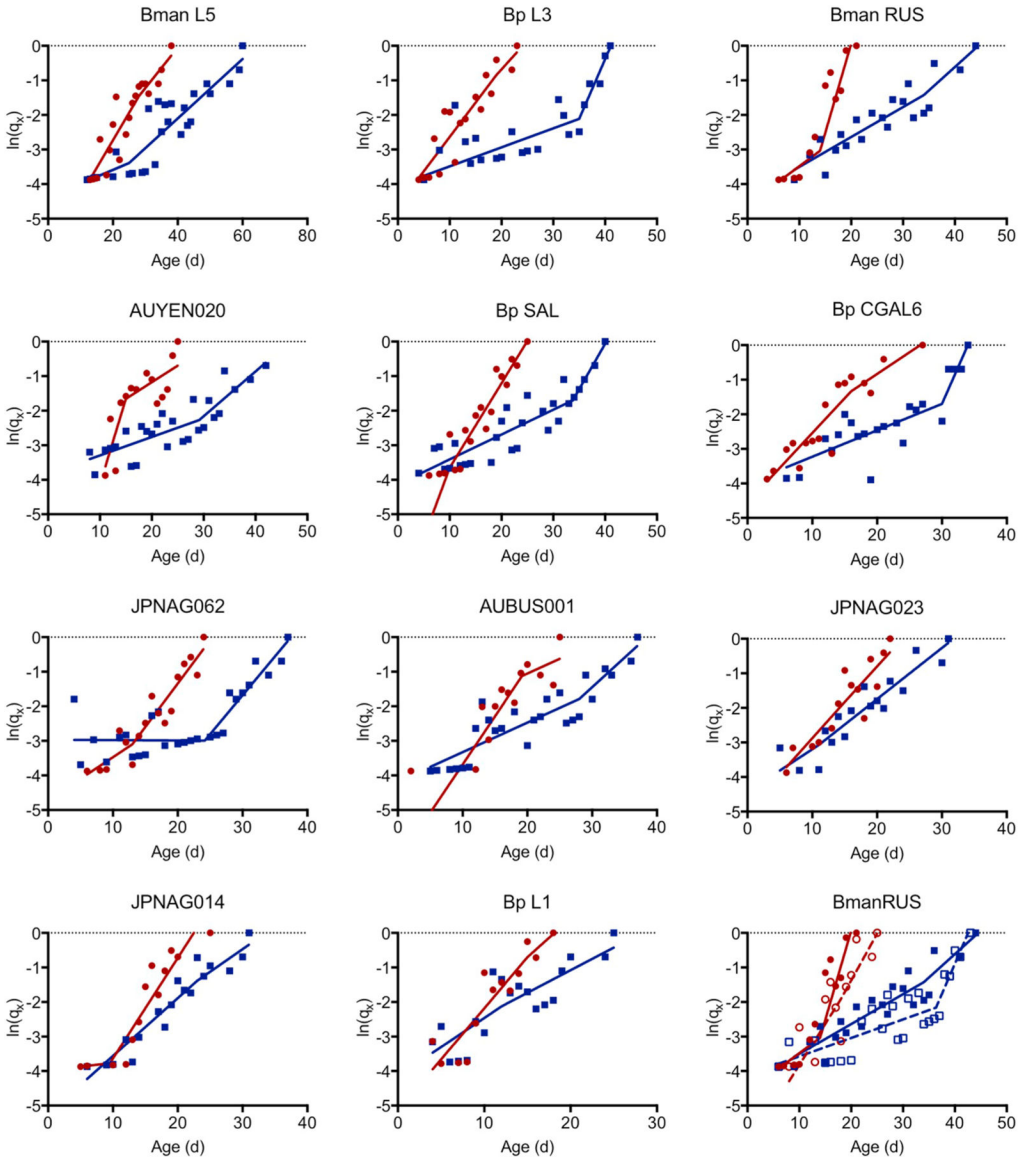

Fig. 3.

Gompertz relationships for 11 strains of *Brachionus*, grown at 21 °C (red) or 16 °C (blue). BmanRUS was grown under ad libitum (solid line), or chronic caloric restriction (dashed line) food conditions. Linear regressions were significantly different between 21 °C and 16 °C and between ad libitum and chronic caloric restriction for all strains (Sum-of-squares F test, $p < 0.05$).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

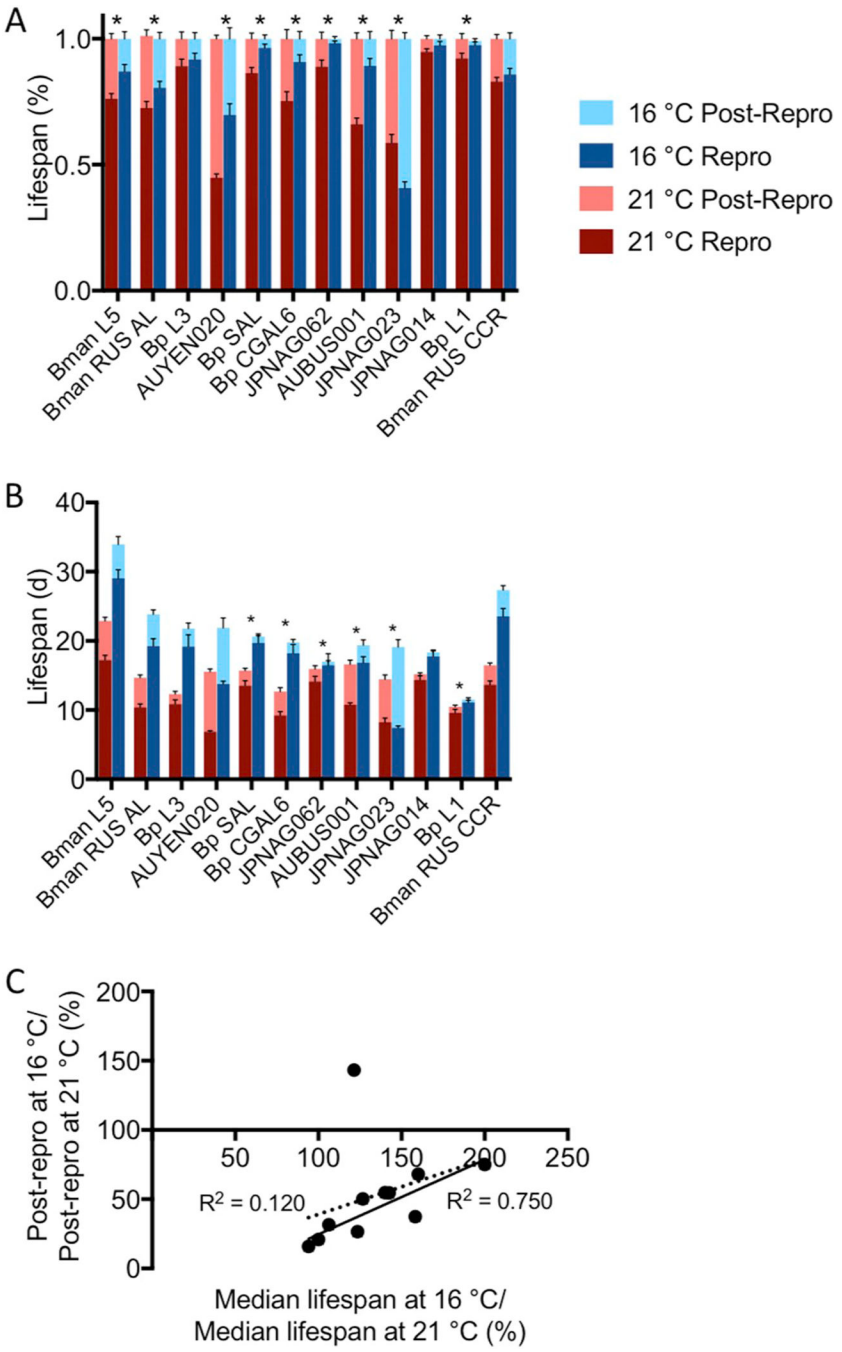

Fig. 4.

The onset of reproductive senescence and the length of the post-reproductive period for 11 strains of *Brachionus*, grown at 21 °C (red) or 16 °C (blue). (A) Reproductive and post-reproductive periods as a portion of lifespan. (B) Reproductive and post-reproductive periods in actual days. * denotes significant difference between percent or length of post-reproductive period between 21 °C and 16 °C (*t*-test, $p < 0.05$). (C) Relationship between percent change in median lifespan and percent change in post-reproductive period between

21 °C and 16 °C, showing R^2 for all the data (dashed line) and also with the single outlier removed (solid line).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

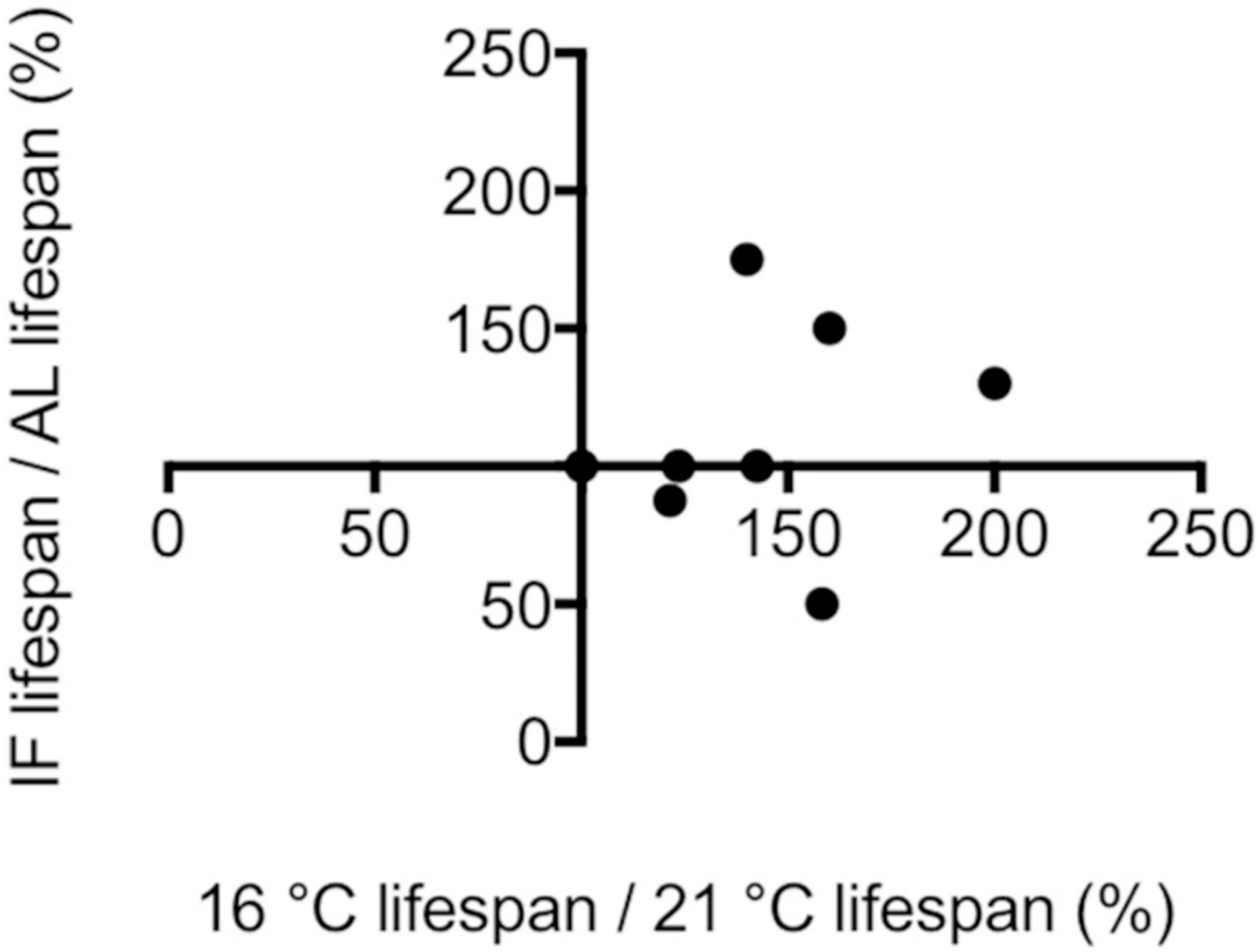

Fig. 5.

Relationship between percent change in median lifespan at 16 °C relative to 21 °C and under intermittent fasting relative to ad libitum feeding for eight strains of *Brachionus*. $R^2 = 0.048$.