Growing up and growing old

A longitudinal study on aging in zebra finches

Michael Briga

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The research in this thesis was carried out at the Groningen Institute for Evolutionary Life Sciences (GELIFES), University of Groningen. All studies were approved by the animal welfare committee of the University of Groningen.

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by

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Part I

Introduction

Chapter 1

Environment, lifespan and aging: a synthesis

Michael Briga

Until now, the longest confirmed human lifespan ever recorded is that of a French woman, Jeanne Calment (1875-1997), who lived up to 122 years and 164 days (Whitney 1997). This exceptional lifespan lies at the very upper end of the human lifespan distribution (Fig. 1A). In humans and various other species, adult lifespan can vary up to tenfold between individuals (Jones et al. 2014, Fig. 1). This variation in human lifespan is only modestly heritable with approximately 25% being attributed to genetic differences[1] (reviewed in Christensen et al. 2006). In the zebra finch *Taeniopygia guttata*, the model organism used in this study, adults also vary up to tenfold in lifespan (Fig. 1B) and I estimated its heritability to range between the 95%CI of 0 and 0.25[2]. This is considerably less that the heritability of, for example, body mass, for which, I estimated its heritability to range between the 95%CI of 0.20 and 0.69[3]. The low heritability of lifespan indicates that the environment is important. Indeed, for example, in the last 160 years, human life expectancy in various western societies has increased with several decades, even among societies' oldest, and there is yet no sign of this trend slowing down (Oeppen and Vaupel 2002; Vaupel 2010). This change likely has an environmental origin, because it occurred too rapidly to be due to changes in DNA sequence. Thus individuals show variation in lifespan, which is to a large extent determined by the environment, a phenomenon that has important consequences for human society.

Genes can also play an important role in determining lifespan. According to the LongevityMap and GenAge, two extensive databases compiling the majority of genetic studies on lifespan and aging, researchers have currently identified over a hundred genes that are associated with lifespan in humans or that can extend lifespan in model organisms such as yeast, *Saccharomyces cerevisiae*, the nematode worm *Caenorhabditis elegans*, the fruitfly *Drosophila melanogaster* and the mouse *Mus musculus* (Budovsky et al. 2013; Tacutu et al. 2013). For example, there are a large number of long-lived mutants in the aforementioned model organisms (reviewed in Kenyon 2005; Kenyon 2010; Gems and Partridge 2013), some which can result in a doubling of the median lifespan, for example through the inhibition of the insulin or insulin-like growth factor signaling pathway (Kenyon et al. 1993; Garsin et al. 2003; Van Voorhies et al. 2005). These studies on model organisms indicate that certain genes can have a major effect on lifespan. However, the vast majority of these studies were carried out in laboratory environments, which can be very distinct from more natural environments.

[1] This refers to the narrow sense heritability, which technically, is the proportion of phenotypic variance among individuals in a trait that can be attributed to the additive effects of alleles that are independent of other alleles or loci (Kruuk et al. 2014).
[2] Bayesian estimate using an 'animal model' approach (Kruuk 2004; Hadfield 2010) based on data from 440 cross-fostered individuals with a pedigree of 3 generations containing 839 half-sib bonds.
[3] Both traits, lifespan and mass were measured with high accuracy: ± 1 day (**Chapter 6**) and 0.01 g respectively (**Chapters 10 and 11**).

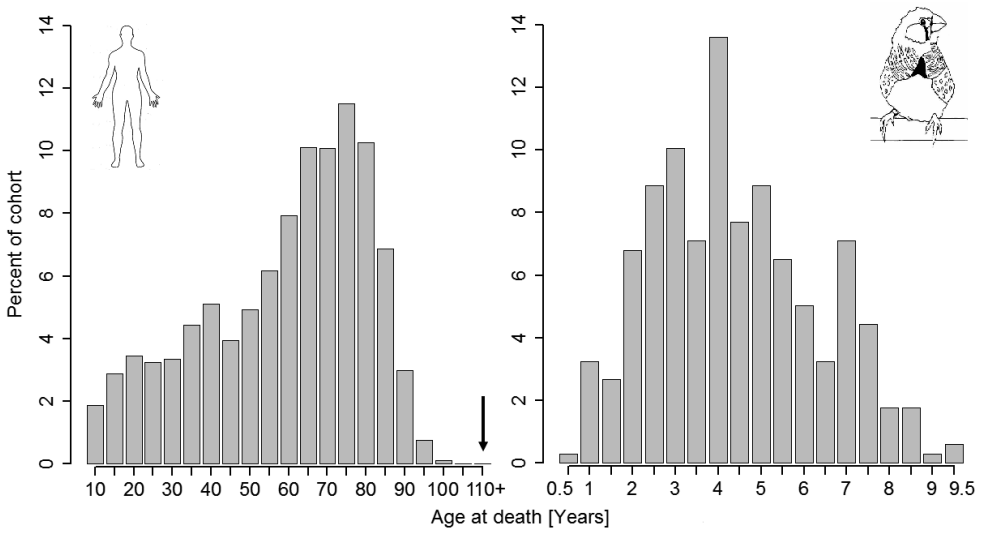

Fig. 1 Variation in lifespan within a birth cohort. (A) Distribution of age at death, from age 10 onwards, for all humans born in France in 1875 (N=708.610). The black arrow indicates the lifespan of Jeanne Calment, currently the person with the longest recorded lifespan. Data are from Human Mortality Database (www.mortality.org/) (B) Distribution of age at death for zebra finches from this study. Shown here are the first three cohorts, i.e. those for which all individuals have died (N=338).

For example, laboratory environments are often characterized by a constant climate, minimal exposure to pathogens, no opportunity to reproduce (depending on the species) and *ad libitum* food that can be obtained with little or no physical effort. Therefore, the lifespan achieved by long-lived mutants in a laboratory environment is only one of the many possible lifespan phenotypes. The question then arises whether long-lived mutants would also display such a lifespan advantage in more natural environments. This issue is important, for example because humans are exposed to a variety of natural environments that are also distinct to the environment encountered in a laboratory setting. In **chapter 2** we investigated the evidence for an environment specific lifespan advantage of long-lived mutants over their wild type controls under a range of laboratory conditions. We showed that in challenging environments (e.g. exposure to cold, pathogens or competition for food) the lifespan advantage of long-lived mutants disappeared or even that the wild type controls outlived their mutant counterparts. These results show that the role of environment in determining lifespan is also important when studying the genetic basis of lifespan. Hence, when studying the mechanistic basis of lifespan, attention should be payed to genotype x environment interactions. More controversially, these results suggest that genetic mechanisms generating lifespan variation in natural populations are different from those studied in the laboratory environments. Therefore, when studying variation in lifespan, careful consideration should be given in choosing

an environment that is relevant to the organism of interest. This obviously opens the question as to what defines a relevant environment. Below here we study in more detail one of several possible variables of interest, foraging costs, which are often encountered by free-living animals.

Long term effects of developmental conditions

The environment can exert effects on lifespan at many ages. However, the development phase is thought of as particularly important for adult lifespan and health (Lindström 1999; Metcalfe and Monaghan 2001; Lummaa and Clutton-Brock 2002; Bateson et al. 2004). For example in humans, Gambians born during the harvest season (i.e. with high food abundance) had a 20% higher chance to reach the age of 45 years relative to individuals born during a season with low food abundance (65 vs. 45% respectively; Fig. 2; Moore et al. 1997). Birth season effects, supposedly via food abundance, on lifespan were also shown in 20th century Austrians, Danes and Australians (Doblhammer and Vaupel 2001), although they were not found in 19th century Finns (Kannisto et al. 1997). More generally, there are several studies in a variety of human populations that have shown that cohorts with high childhood mortality are also characterized by a shorter adult lifespan (Kermack et al. 2001; Finch and Crimmins 2004; Crimmins and Finch 2006; Beltran-Sanchez et al. 2012; reviews in Galobardes et al. 2004; Lumey et al. 2011). Thus various cohort studies in humans have shown that adverse developmental conditions can negatively affect adult lifespan.

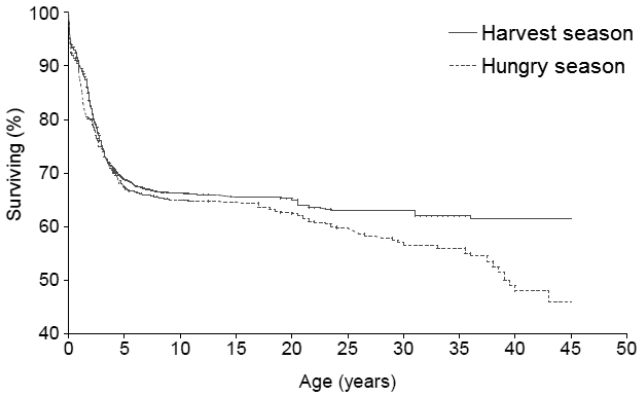

Fig. 2 Harsh developmental conditions can negatively affect adult survival. Shown here as an example are the survival curves of three rural Gambian villages monitored from 1949–1994 (N=3102 births and 1077 deaths). The 'hungry' season refers to the wet season when food reserves are depleted (Moore et al. 1997). At the age of 45, individuals born during the ´harvest season´ (solid line) had a survival 20% higher than those born during ´hungry season´ (dashed line). Data from Moore et al. (1997).

In our model species, we manipulated the developmental conditions by experimentally manipulating brood size. In zebra finches developmental conditions affect survival in adulthood and, and other aspects of the phenotype that can be interpreted as being important for adult health (de Kogel 1997; Alonso-Alvarez et al. 2006; Griffith and Buchanan 2010; Holveck and Riebel 2010). During development, my collaborators and I performed brood size manipulations by cross-fostering chicks to either small or large broods (as in de Kogel 1997). Chicks that grow up in large broods show increased costly begging and diminished food reward relative to those from small broods (Kilner 2001; Neuenschwander et al. 2003; Kim et al. 2011; Redondo et al. in press; also in our study system: **Box A**). Hence, one consequence of growing up in large broods is that chicks are exposed to increased foraging costs. Chicks that grew up in large broods showed impaired growth (**Box A**), a result in concordance with previous studies (Griffith and Buchanan 2010). I therefore interpret large broods as a harsh developmental condition. Despite this, the brood size manipulation did not affect survival until adulthood, nor did we find an effect on adult survival (**Chapter 3**). The harsh developmental conditions generated by the brood size manipulation did thus impinge on development but not on survival in our zebra finches. While this may seem surprising at first, it is certainly not the only negative result of harsh developmental conditions on adult survival. In the blue footed boobies (*Sula nebouxii*), parents sometimes raise two young, and the second young suffers aggressive subordination and food deprivation relative to the first born young. However, both young have similar recruitment and survival rates (Drummond et al. 2011). Similarly, and in contrast to the aforementioned cohort studies in humans, there are various other studies that showed that human infants born in malnourished cohorts have similar juvenile and adult survival as those born just before or after the famine period (Kannisto et al. 1997; Lumey et al. 2011). These studies suggest that harsh developmental environments do not (always) produce the expected long lasting negative consequences on adult survival, including in our captive zebra finches.

Environmental conditions during adulthood

The environment during adulthood can also affect lifespan. For example, hard work during adulthood, which can be manipulated by increasing reproductive effort, can shorten lifespan (Santos and Nakagawa 2012; Boonekamp et al. 2014). Adult survival can thus be affected by the environment during development and in adulthood. At this point, the association between environment and lifespan can become more complex. Does a manipulation during adulthood, for example the increase in reproductive effort above, affect all individuals equally? Or are some individuals more sensitive than others the challenges during adulthood? There are two contrasting predictions on this matter. One scenario is that individuals from benign developmental conditions always perform

at least as well or better during adulthood relative to those from harsh developmental conditions, independent of the adult environment (the 'silver spoon hypothesis'; Fig. 3A; Grafen 1988). However, developmental conditions may not only constrain, but can also lead to differential developmental, potentially adaptive, trajectories (Gilbert 2001; West-Eberhard 2003). In such cases, harsh developmental conditions could prepare individuals to specific challenges during adulthood, a 'predictive adaptive response' (PAR; Fig. 3B; Gluckman and Hanson 2004; Hanson and Gluckman 2014). However, when the developmental and adult environments do not match, these types of developmental adjustments may have negative consequences, for example on adult health and lifespan. Such mismatches have been suggested to be the at the root of health problems such as insulin resistance, type 2 diabetes mellitus and other components of the metabolic syndrome (Gluckman and Hanson 2004; Hanson and Gluckman 2014). Thus, the long-term effects of developmental conditions on adult lifespan and health may depend on the environmental conditions encountered during adulthood.

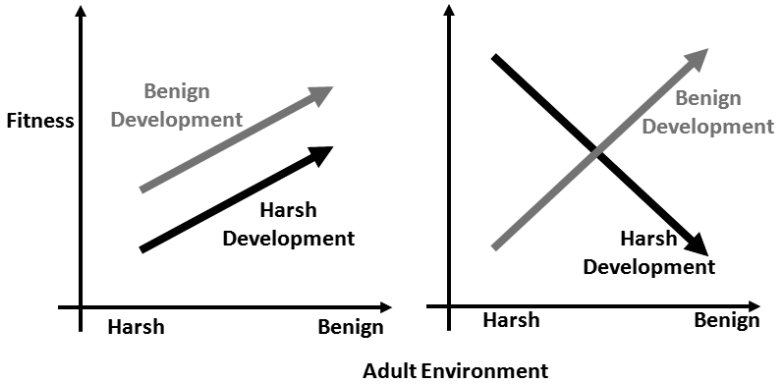

Fig. 3 Schematic illustration of two scenarios showing how the long term effects of developmental conditions on adult fitness may depend on the environment during adulthood. Panel (A) illustrates a silver spoon outcome, i.e. individuals from benign developmental conditions always outperform individuals from harsh developmental conditions (Grafen 1988). Note that here we drew lines in parallel, but that according to the silver spoon hypothesis the advantage of benign over harsh developmental conditions need not be as big over the whole range of adult conditions. Panel (B) illustrates a match-mismatch scenario or predictive adaptive response (Bateson et al. 2004; Gluckman and Hanson 2004; Hanson and Gluckman 2014). In this scenario developmental conditions guide development such as to better prepare individuals to certain challenges during adulthood. These developmental adjustments however result maladaptive when there is a mismatch between the developmental and adult environment.

In the aforementioned cohort examples, harsh developmental conditions yielded low quality phenotypes that had shorter lifespans, suggesting that these were 'silver spoon' responses. However, many of these studies used only one type of environment during adulthood. For the studies in which the environment did vary, in the field for instance,

developmental and adult conditions are still likely to be correlated, for example because individuals that grew up in poor quality territories are more likely to settle in poor quality territories (van de Pol et al. 2006), or because of temporal correlations of environmental variables. Therefore, the ability of the majority of the previous studies (see below for exceptions) to distinguish 'silver spoon' effects from predictive adaptive responses scenarios is limited. The most robust approach to distinguish between these alternatives involves an independent experimental manipulation of the environment during development and in adulthood in a crossover design. To the best of our knowledge, there are only a few such experiments that have tested such effects on lifespan and most of these have failed to find interaction effects (Taborsky 2006; Barrett et al. 2009; Zajitschek et al. 2009; Auer 2010; Dmitriew & Rowe 2011; but see Saastamoinen et al. 2010). Unfortunately, all these studies used species with indeterminate growth and/or with developmental phases of flexible duration (i.e. insects and one study in fishes). Species with such developmental patterns can mitigate effects of harsh developmental conditions in ways that are not available to species with determinate growth, such as birds and humans. Thus to what extent lifespan is subject to match-mismatch versus silver spoon effects is unknown for species with determinate growth.

To test this I expanded upon the usual experimental manipulation of developmental conditions and added a foraging cost manipulation during adulthood in a full factorial (2x2) design. This expansion of the experimental design is interesting because manipulations of developmental conditions like ours are often used (Griffith and Buchanan 2010), but they always involve only standardized adult housing conditions. Furthermore, earlier studies have followed individuals until early adulthood only (e.g. one year de Kogel 1997) or allowed birds to reproduce (Alonso-Alvarez et al. 2006), possibly masking effects of developmental conditions on survival due to trade-offs between lifespan and reproduction (Santos and Nakagawa 2012; Boonekamp et al. 2014). Here, my collaborators and I monitored over 500 individual birds for up to 8 years (**Chapter 3**: Table 1) in conditions where they could never reproduce, and we exposed them to different experimental conditions until their natural death. We chose to manipulate foraging costs, defined as flight costs per food reward, because we believe that free-living animals often experience it (Koetsier and Verhulst 2011) and because it can have major effects on lifespan and/or reproduction. For example, in natural populations, foraging costs are manipulated by food supplementation experiments. It is generally thought that food supplementation increases survival and fecundity (Martin 1987; Boutin 1990) and several studies in birds and mammals have confirmed this (reviewed in: Robb et al. 2008; Prevedello et al. 2013; Ruffino et al. 2014). However, food supplementation interacts with other ecological factors such as population density,

competition, and exposure to predators and pathogens (McNamara and Houston 1987; Krebs et al. 1995; Gilroy and Sutherland 2007; Robb et al. 2008; Prevedello et al. 2013; Forbes et al. 2015). For example, an increase in food availability is likely to reduce starvation risk but also reduces exposure to predators due to, among other things, a reduction in foraging time. Therefore, increased food availability could affect survival primarily through an effect on predation rate, with a negligible contribution of food intake *per se* (McNamara and Houston 1987). Perhaps because of these ecological confounds, a recent meta-analysis on 148 food supplementation experiments in small mammals found on average no effect of food supplementation on survival (Prevedello et al. 2013). Similarly, in birds, the association between food supplementation and adult survival remains debated (Robb et al. 2008). The complexity of the association between food availability and survival is further illustrated by the finding that dietary restriction in laboratory animals generally *increases* lifespan (Nakagawa et al. 2012). Thus, foraging costs are an ecologically relevant variable to manipulate, but the effects *in isolation* on survival in natural populations remain an open question.

In **chapter 3** we thus investigated whether foraging costs shortened lifespan. We indeed found that high foraging costs shortened lifespan, but only for individuals that had grown up in large broods (**Chapter 3**: Fig. 1). The difference in lifespan was considerable: an approximately six months shorter life expectancy relative to an average life expectancy of approximately 3 years, which is a difference of 17%. These results thus show that the effect of high foraging costs on lifespan is conditional and only detectable in individuals that grew up in poor environments. Similarly, these results also show that the effect of developmental conditions on lifespan is conditional on the quality of the adult environment: birds from large broods suffer a shorter lifespan only when they are facing high foraging costs. These results can be put in the context of the contrasting predictions of the silver spoon and PAR scenarios. In our study, we found that birds from benign developmental conditions performed as well as or better than birds from harsh developmental conditions, and therefore our results are more consistent with the predictions of the silver spoon than the PAR scenario (Fig. 3). These results are in contrast with the many food supplementation in free living animals, which did not find an effect on survival (Prevedello et al. 2013) also indicate that, when everything else being equal, food supplementation can increase survival. Interestingly, our results are in contrast with dietary restriction results, which increases lifespan in model organisms in laboratory environments. We should note however that in laboratory rodents, dietary restriction is often applied by decreasing food intake. This is in sharp contrast with foraging costs manipulations, because both type of manipulations can have very different effects on the size and allocation of the energy budget (Carvalho et al. 2005; Wiersma et al. 2005; Schubert et al. 2008).

Population level dynamics in mortality and reproduction

Because individuals die only once, the dynamics of lifespan and mortality are population level phenomena. At this level, the parameters that are typically quantified are median lifespan or life expectancies, which we used when comparing groups that had experienced different environmental conditions (**Chapter 3**: Table 2). Unfortunately, these parameters capture only one time point and this may be insufficient when the differences between groups change with age. A useful mathematical approach to capture changing dynamics with age was developed by Benjamin Gompertz in 1825 (Gompertz 1825). In the Gompertz function ($M_t = Ae^{Bt}$ or, in the notation we use, $log(M_t) = log(A) + Bt$), the force of mortality at time t (M_t) is a function of an age independent parameter A (baseline mortality rate) and increases exponentially with age according to the parameter B (actuarial senescence or aging rate). The Gompertz function shows that differences in lifespan between groups or populations can arise from two mutually non-exclusive reasons (Fig. 4). Populations may differ in the probability of dying at young age, which is reflected in a change in the baseline mortality rate. In addition, populations may differ due to a faster increase in mortality rate with age, which will be reflected in actuarial senescence. This difference is important, for example with regard to interventions that alter lifespan (Partridge et al. 2005). When an intervention changes lifespan through age independent mortality rate (Gompertz A), the effect is immediate. However, when an intervention changes lifespan through actuarial senescence (Gompertz B), the effect is cumulative. In such cases, applying the intervention will not change mortality abruptly. Instead, the intervention needs to be applied over longer time periods in order for differences in mortality to appear clearly (Partridge et al. 2005). Considerable research is devoted to finding via which parameter(s) a treatment affects lifespan. For example, dietary restriction in invertebrates extends lifespan via changes in Gompertz A (e.g. Mair et al. 2003; Nakagawa et al. 2012). In contrast, in laboratory rodents, dietary restriction changes lifespan via decreases in Gompertz B (Simons et al. 2013). These results suggest that in rodents, dietary restriction changes lifespan by slowing a cumulative process, while this seems not to be the case in invertebrates. Thus changes in lifespan may occur via distinct processes, either immediate and/or cumulative, and the Gompertz model is a useful tool in distinguishing these processes.

In **chapter 3** we used this approach to distinguish whether the effect of our manipulations on lifespan arose via age-independent or age-dependent changes in mortality. We showed that our environmental manipulations increased mortality immediately and thus shorten lifespan via an increased age independent effect '*A*' and despite diminished actuarial senescence '*B*' (**Chapter 3**: Fig. 2). We also found that zebra finch females live shorter lives than males. This effect, however, arose cumulatively via actuarial

senescence *'B'*. Thus our environmental manipulations shortened lifespan via an age-independent effect on mortality, but the sex difference in lifespan arose because females aged faster (demographically, see below for explanation) than males.

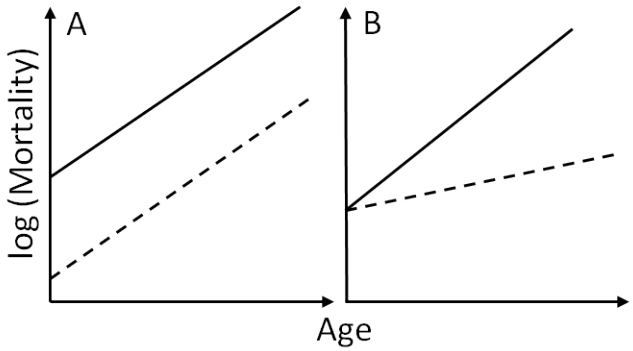

Fig. 4 Schematic diagram showing how differences in mortality between groups or populations (full vs. dashed line) can arise following the Gompertz equation. (A) Differences may be age independent, (Gompertz A), which means they are immediate. (B) In contrast, differences may accumulate with time and be captured in the age dependent parameter (Gompertz B).

Natural selection acts on individual contributions to future generations. Therefore, reproduction is an essential aspect of an individual's life history. In the above studies, we have focused on lifespan, and the birds involved were not allowed to reproduce. This has the advantage that environmental effects on lifespan can be tested with less interference due to other life history traits such as reproduction. However, this may limit the ecological suitability of our foraging cost manipulation. In order to investigate whether birds facing our environmental manipulations were at all able to reproduce, we thus carried out two short-term breeding experiments. One study was carried out in autumn and winter, and we found that in the harsh treatment birds did not lay any eggs, while their benign counterparts readily did (Simons et al. 2014). We then carried out a follow-up study during spring **(Chapter 4)**. We found that birds from both treatments readily reproduced, but that in the harsh treatment brood size was reduced and the young experienced a higher mortality. Thus birds in the high foraging cost manipulation could reproduce, but there were effects of seasonality on reproductive behavior and high foraging costs impaired chick development and survival.

The Gompertz model was developed a long time ago (published in 1825). Since then, many other equations have been developed to capture the dynamics of mortality (for an overview, see Colchero et al. 2012). Nevertheless, the Gompertz equation captures demographic patterns well and in many instances outperforms other demographic

models in terms of its fit with the data[4], and this was also for our population (**Chapter 3**). However, the Gompertz and many other demographic models are limited in a biological sense: they are descriptive and therefore fail to include the underlying biology. This hampers connecting demography with the study of aging at the mechanistic or organismal level. A first step towards bridging this gap was developed by Gavrilov and Gravilova in 2001. They developed a demographic model using a bottom up approach: utilizing the concept of redundancy of elements and how these elements fail with age, demographic patterns emerge from their model (Gavrilov and Gavrilova 2001). Redundancy remains an abstract concept, but one way to think of it in biological terms is as an organ with redundancy being (the number or the functioning of) the cells in that organ. Redundancy decreases following a certain failure rate until it is depleted and the organ or organism dies. The redundancy model of aging has rarely been fitted to actual data (for exceptions see Boonekamp et al. 2013; Vural et al. 2014) and its fit has rarely been compared with that of other demographic models. In **chapter 5** we make a first attempt to fit a mechanism-based demographic model on mortality data. We show that the redundancy model can fit demographic patterns well and in some cases even better that the traditional Gompertz model. Furthermore, we show that some common interventions that extend lifespan (dietary restriction and lowering ambient temperature) can be interpreted in terms of parameters of the redundancy model. For example, lowering ambient temperature increased lifespan in the fruitfly *Drosophila* through reductions in actuarial senescence (Mair et al. 2003). Following a simplified version of the redundancy model, increases in lifespan can be achieved by reducing failure rate, by increasing redundancy, or both. Fitting the redundancy model to the data indicated the role of failure rate while redundancy remained unchanged. Physiologically, this points towards a decrease in the production of physiological damage rather than a change in organismal strength or resilience. While using demographic changes to identify certain physiological processes remains a challenge, we hope that this study will motivate others to including mechanistic processes into demographic models.

Mortality not only changes with age, but also in response to extrinsic variables such as climate (Coulson et al. 2001). In general, when studying the biological consequences of climatic variables, the considered timescales are long, typically weeks, months or years (Stenseth et al. 2002; Parmesan 2006; Grosbois et al. 2008; Lawson et al. 2015). However, global warming is also associated with changes in climatic variability over much shorter time scales of typically days (Vose et al. 2005; Wang and Dillon 2014). For

[4] Note however that the Gompertz model cannot explain late-life mortality plateaus, i.e. that among the oldest individuals, mortality rate remains constant with age (Carey et al. 1992). This demographic phenomenon can, however, be captured by the redundancy model of aging (**Chapter 5**: Fig. 2).

example, the diurnal temperature range (DTR), i.e. the difference between minimum and maximum temperature within one day, has increased with more than 2 °C since the 1960's in Mexico, Bolivia, Patagonia, Madagascar, Indonesia, central Russia and the Western Himalaya (Yadav et al. 2004; Englehart and Douglas 2005; Jhajharia and Singh 2011; Wang and Dillon 2014). The demographic consequences of such changes for ectotherms are currently under investigation (Paaijmans et al. 2010; Raffel et al. 2012; Paaijmans et al. 2013; Vasseur et al. 2014; Zeh et al. 2014), but the consequences for endotherms are not yet known. In **chapter 6** we address this by investigating whether DTR affects mortality in zebra finches. We find that an increase of 1°C in DTR can cause up to a twofold increase in mortality in zebra finches. This shows that temperature variability on short time scales can have a major impact on endotherm populations. This is to our best knowledge the first report of such an effect in endotherms, and we therefore believe that changes in short-term variability of climatic variables should be taken into account when estimating the possible consequences of climate change.

In our experimental set-up, however, the effect of DTR on mortality depended upon environmental quality. DTR increased mortality on days with low minimum temperature when foraging costs were low, but on days with high minimum temperature when foraging costs were high (**Chapter 6**: Fig. 4). This difference is important for two reasons. First, low foraging costs typically reflect a laboratory type of environment, while high foraging costs are typically encountered in more natural environments. Therefore, these results show that the effects of climatic variables can differ between a laboratory and a (semi-) natural environment, and thus highlight that testing the ecological consequences of climatic factors should be done in environments as natural as possible. Secondly, in the semi-natural environment, DTR decreased mortality on days with high minimum temperature. Global warming is associated with increases in minimum temperatures (Vose et al. 2005), and thus DTR effects will become increasingly important in a warming world.

Individual aging

In the population-level section, we focused on changes in lifespan and the dynamics of death. There we encountered a demographic quantification of aging or senescence, i.e. actuarial senescence or the increase in mortality rate with age. Aging, however, is more often referred to functionally as a decline in organismal functioning with age associated with decreases in fecundity and survival probability. Aging thus becomes a characteristic of individual functioning and we here further consider aging in this sense. Aging is a ubiquitous phenomenon, common in humans, model organisms and in the wild (Nussey et al. 2013; Belsky et al. 2015; Fontana and Partridge 2015). Aging is followed by death

and thus both processes (aging and death) are inevitably linked. However, aging is different from lifespan in that it explicitly refers to the decline in organismal functioning preceding death. Thus two individuals with the same lifespan can experience aging phases that differ in duration or intensity (Ricklefs 2010). Therefore lifespan and aging can be distinct phenomena (Williams 1999).

Because lifespan and aging are inherently linked, it is often assumed that both processes are consistently affected by the same factors (Williams 1999). For example, we may predict that longer-lived individuals may age later or at a slower pace. However, in humans, life expectancy has increased continuously since the 19th century, but it remains unclear to what extent this increase is accompanied by delays in aging (Christensen et al. 2009). Secondly, studies on model organisms in laboratory environments have shown that caloric and dietary restriction extend lifespan and can delay the onset of age-related pathologies such as type 2 diabetes, cancer and neurodegenerative diseases (Speakman and Mitchell 2011; Fontana and Partridge 2015). However, there are various examples in these same systems, showing that lifespan and aging can readily be uncoupled (Burger et al. 2007; Rueppell et al. 2007; Burger et al. 2010; Bansal et al. 2015). Thus, the assumption that aging and lifespan are in synchrony and affected by the same factors remains to be investigated (Williams 1999; Christensen et al. 2009; Kennedy et al. 2014; Bansal et al. 2015).

We therefore investigated the aging of individual zebra finches exposed the aforementioned brood size and foraging cost manipulations as part of section III of this thesis (**Chapters 7-11**). We then associated the above found experimental effects on lifespan with those on aging. Our starting hypothesis was the common assumption that the experimental group with the shortest lifespan aged fastest. The redness of the zebra finch bill **(Chapters 7 & 8)** is, in this context, a useful trait to study because it is a carotenoid-based sexual signal and therefore is expected to indicate individual 'quality' or 'physiological state' (Pérez-Rodríguez 2009; Simons et al. 2012). In brief, theory predicts that such costly signals can evolve when they are used as indicators of quality in mate choice (Zahavi 1975; Grafen 1990; Kotiaho 2001), as is the case for zebra finches (Simons and Verhulst 2011). In zebra finches it has, however, been suggested that bill color might be a poor indicator of quality in females (Price and Burley 1994). We thus first investigated to what extent the redness of the bill is an indicator of quality in male and female zebra finches, and found that males and females with redder bills live longer and reproduce more (**Chapter 7)**. These results thus show that for the zebra finch bill, redder is 'better'.

This cross-sectional association between bill redness and survival can arise because of two mutually non-exclusive explanations. On the one hand, it is possible that individuals with redder bills have an advantage over others at young age, creating a *between individual* change in population composition with age, i.e. selective disappearance. On the other hand, the cross-sectional association with survival might be due to aging, because bill color deteriorates with age *within individuals*. To separate between the contributions of these two processes, we collected longitudinal data of bill coloration. Between individuals we found that intermediate bill color lead to the longest lifespan, i.e. stabilizing survival selection. Within individuals, we found that bill color is maintained throughout life until a terminal decline in the last year before death (Fig. 5). Thus bill color showed aging. More generally, these studies illustrate the importance of using longitudinal over cross-sectional data when studying trait aging and their association with lifespan. That is because in the cross-sectional data (**Chapter 7**), we had found that individuals with the reddest bills live longest. However longitudinal data (**Chapter 8**) correctly show that this conclusion is confounded by within individual change and that individuals with intermediate redness lived longest. Thus, studying the individual aging and predicting lifespan is best done with longitudinal data, which is the approach used here below (Fig. 5).

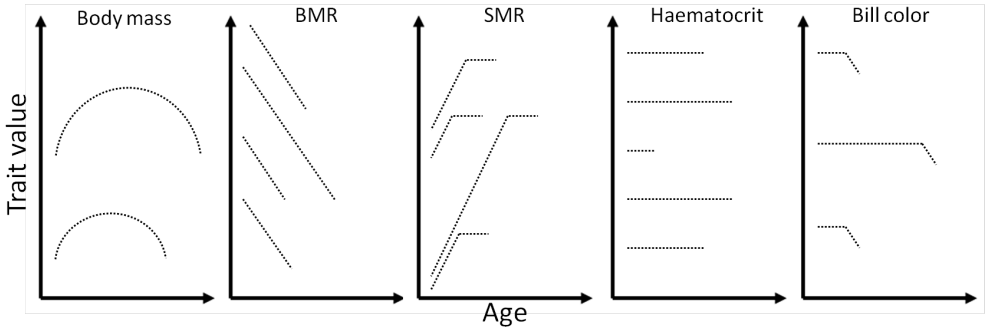

Fig. 5 Mosaic aging in zebra finches. Shown here is a schematic representation of the age trajectories for five traits longitudinally quantified in this study. Individuals with high body mass lived longer than those with low body mass, but within individuals most birds showed a quadratic association with age. For BMR and SMR there is no selective disappearance. Within individuals, BMR linearly declined with age, while SMR increased until final year. For hematocrit, we did not find any evidence for age associated changes or selective disappearance. Bill color shows stabilizing survival selection before the terminal decline in the final year. Analyses are based on more than 20.000 measurements on 597 individuals monitored for up to eight years.

Evolutionary theory predicts that traits within one organism should age in synchrony (Williams 1957; Maynard-Smith 1962). The rationale behind this is that any trait that causes early death should be selected against, while there might be little benefit in investing in perfect trait functioning until 'after death'. Unfortunately, this rationale

may well be overly simplified. Intuitively at least, it may seem likely that there are trait specific associations between age and survival and/or reproduction. This may arise for instance when an individual might benefit from altering its behaviour, physiology or life history decisions during late adulthood (McNamara et al. 2009). Various studies have indeed shown that an organism experiences heterogeneous declines in functioning with age between traits, tissues and cells, a phenomenon coined *´mosaic aging´* (Herndon et al. 2002; Cevenini et al. 2008; Walker and Herndon 2010; Baris et al. 2015; Hayward et al. 2015). For example, in *Drosophila*, muscular functioning shows profound declines in functioning with age, whilst the functioning of nervous system appears age-independent (Herndon et al. 2002). Thus, within one organism, traits differ in how they change with age and the origins of this mosaic remains poorly understood.

Furthermore, traits can age following various shapes, with declines being gradual, accelerating or terminal, i.e. be triggered by time before death rather than age per se. We further call the shape of how a trait changes with age during adulthood the '*age trajectory*'. Note that age trajectories can be distinct from aging because traits can improve with age especially during early adulthood, for which there are many examples (e.g. Rebke et al. 2010; Robinson et al. 2012). For example, in wild mammals a variety of age trajectories have been described for mass: quadratic associations with a maximum in bighorn sheep *Ovis Canadensis* (Nussey et al. 2011), accelerating declines in Roe deer *Capreolus capreolus* (Nussey et al. 2011), terminal declines in Soay sheep *Ovis aries* (Hayward et al. 2015), accelerating and terminal declines in European badgers *Meles meles* (Beirne et al. 2015) and in male Alpine marmots *Marmota marmota* (Tafani et al. 2013). The origins of the between-species variation in these age trajectories remain unknown. Actually, even less is known about the level of biological organization at which variation in age trajectories should be described. Is a trait's age trajectory fixed for a certain species or is it amenable to environment variation?

We thus investigated the age trajectories of several traits in zebra finches. Because the foraging cost manipulation changes an individual's energy balance, we chose to quantify a series of traits that are known to be affected by energy intake or energy turn-over. We started with mass and found that mass showed a quadratic age trajectory in males that is independent of our environmental manipulations. Quadratic age trajectories for mass have been described previously in humans (reviewed in Kuk et al. 2009) and in laboratory rodents (Yu et al. 1985; Murtagh-Mark et al. 1995; Turturro et al. 1999; Miller et al. 2002). Laboratory rats however also show terminal declines a few weeks before death (McDonald et al. 1996; Black et al. 2003). In females, we found a quadratic age trajectory for mass in the benign foraging environment, but a linear mass age trajectory

in the harsh foraging environment, the slope of which depended upon the developmental conditions. For females from benign developmental conditions, mass increased linearly with age, while females from harsh developmental conditions showed the opposite pattern. These results thus show that the age trajectory of mass is not fixed, but subject to environmental variation, extending back as far as during development.

We then investigated energetic expenditure. Basal metabolic rate (BMR) is the minimum energy expenditure of a post-absorptive adult animal measured during the rest phase at thermoneutral temperatures (IUPS Thermal Commission 2001). Standard metabolic rate (SMR) is the same as BMR, except that the animal is at a temperature below the thermoneutral zone, and hence SMR includes energy for thermoregulation. At first it may seem redundant to measure energy consumption at two different ambient temperatures, because these two measures will likely be correlated. In **chapter 10** we investigated this correlation. To do this we first needed to know to what extent these traits characterize an individual. This is done by quantifying the repeatability, i.e. proportion of total phenotypic variance that is caused by between individual variance (Falconer and Mackay 1996). Both BMR and SMR were repeatable over a period of years ($r \sim 0.3$), showing that individuals can be characterized based on these traits. However, the correlation between the traits was poor ($0.14 < r < 0.22$)[5], and thus BMR and SMR characterize different traits within an individual.

Once we knew that BMR and SMR are different traits, we could study aging of various components of the organism. In **chapter 11**, we quantified the age trajectories of BMR and SMR. We found that BMR declined with age (Fig. 5), and this is consistent with what was found other studies in birds and mammals (Elliott et al. 2015). In contrast to BMR, SMR increased with age until the terminal year (Fig. 5). This is new, and to our best knowledge, the first description of an SMR age trajectory. Thus BMR and SMR, two metabolic traits that quantify energy consumption and differ solely in the ambient temperature at which energy is consumed, age independently and in opposite directions. These results indicate that the aging of basal energy production is distinct from that of insulation and/or thermoregulation. For hematocrit, blood oxygen stores which can be important for metabolic activity (Petit and Vézina 2014), we found no evidence of any change with age, despite a high lifetime repeatability ($r \sim 0.6$). Together, the above results show that different components the zebra finch organism age at different rates and follow a variety of age trajectories. Thus zebra finches show mosaic aging.

[5] This refers to the phenotypic correlation. We note that here this weak phenotypic correlation was not due to the repeatability of ~0.3: correlation between SMRs at various ambient temperatures can be as high as 0.9 (see **chapter 10** for further explanation).

In our study, aging responses to our environmental manipulations were trait specific. Therefore, environmental factors affecting lifespan should be considered distinct from those that affect aging, which is similar to what has been suggested for genetic factors (Burger and Promislow 2006). Predicting for which traits an environmental variable that alters lifespan will also affect aging is currently difficult. One determinant factor is the shape of the age trajectory. For traits showing terminal declines, environmental factors that shorten lifespan will likely accelerate aging, as we found for SMR and for bill coloration. However, this assumes that the environment does not alter a trait's age trajectory, which was the case here for most traits except for mass. Therefore, the association between lifespan and aging is trait specific and depends on a trait's age trajectory, the environment and their interaction. I therefore believe that studying the age trajectories of a variety of traits is a fruitful approach to understanding the dynamics of aging, the factors affecting aging and the association between aging and lifespan.

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Chapter 2

What can long-lived mutants tell us about mechanisms causing aging and lifespan variation in natural environments?

Michael Briga & Simon Verhulst

Experimental Gerontology 71, 21-26

Abstract

Long-lived mutants of model organisms have brought remarkable progress in our understanding of aging mechanisms. However, long-lived mutants are usually maintained in optimal standardized laboratory environments (SLEs), and it is not obvious to what extent insights from long-lived mutants in SLEs can be generalized to more natural environments. To address this question, we reviewed experiments that compared the fitness and lifespan advantage of long-lived mutants relative over wild type controls in SLEs and more challenging environments in various model organisms such as yeast *S. cerevisiae,* the nematode worm *C. elegans*, the fruitfly *D. melanogaster* and the mouse *Mus musculus*. In competition experiments over multiple generations, the long-lived mutants had a lower fitness relative to wild type controls, and this disadvantage was clearest when the environment included natural challenges such as limited food (N=6 studies). It is well known that most long-lived mutants have impaired reproduction, which provides one reason for the fitness disadvantage. However, based on 12 experiments, we found that the lifespan advantage of long-lived mutants is diminished in more challenging environments, often to the extent that the wild type controls outlive the long-lived mutants. Thus, it appears that information on aging mechanisms obtained from long-lived mutants in SLEs may be specific to such environments, because those same mechanisms do not extend lifespan in more natural environments. This suggests that different mechanisms cause variation in aging and lifespan in SLEs compared to natural populations.

Introduction

Aging is the decline in physiological function with age, associated with decreasing survival probability and reproduction. Remarkable progress in our understanding of aging mechanisms has been achieved through the study of model organisms such as yeast *Saccharomyces cerevisiae*, the nematode worm *Caenorhabditis elegans*, the fruitfly *Drosophila melanogaster* and the mouse *Mus musculus* (e.g. Sprott and Austad 1996). An important tool in the study of aging mechanisms is the use of genetic mutants with an extended lifespan (Kenyon 2005, 2010; Partridge 2010; Gems and Partridge 2013). The effect of these genetic mutations can be enormous, with for example some mutants living up to 10 times longer than their wild type controls (Ayyadevara et al. 2009). Aging pathways identified in this way include those involved in stress responses and nutrient sensing such as the 'insulin/insulin-like growth factor 1 signaling' (IIS) pathway and the 'target of rapamycin' (TOR) pathway (Kenyon 2005, 2010; Fontana et al. 2010; Gems and Partridge 2013). The study of long-lived mutants has thus provided insight into key mechanisms that affect aging and lifespan.

Long-lived mutants are usually studied in standardized laboratory environments (SLEs), characterized by a constant climate, minimal exposure to pathogens, no opportunity to reproduce (depending on the species) and *ad libitum* food that can be obtained with little or no physical effort. Standardizing the environment has the advantage that it may reduce environmentally caused variation in aging and lifespan. More importantly, when the SLE provides an optimal environment the animals may achieve a lifespan that is close to their maximum, determined only by intrinsic causes. On the other hand, an intrinsic aging phenotype can only be defined against the background of the environment, because intrinsic aging factors interact with the environment to determine intrinsic aging rate (Stearns 1992; Flatt et al. 2013). Thus the lifespan achieved by long-lived mutants in SLEs is only one of the many phenotypes that characterize the specific long-lived mutant genotype, and mechanisms causing an extended lifespan in SLEs may not have a similar effect in more natural environments.

How the aging phenotype of a long-lived mutant varies between environments will depend on the physiological mechanism through which the extended lifespan is achieved. Given that SLEs lack most challenges faced by organisms in natural environments, the optimality theory of aging (Partridge and Barton 1993), an umbrella covering the antagonistic pleiotropy (Williams 1957) and disposable soma (Kirkwood 1977) hypotheses, suggests that the extended lifespan of long-lived mutants may at least in part be due to a reallocation of resources saved on mechanisms that enhance fitness

in natural environments (e.g. immune function, foraging, reproduction) to increased maintenance and repair (Fig. 1). If extended lifespans are achieved by saving resources that animals could not afford to save under more natural conditions, it is not clear how knowledge of the mechanisms giving these mutants an extended lifespan in SLEs will help understand variation in lifespan or the causes of aging in natural populations (including humans) where there would be strong natural selection against such savings. We thus question whether the mechanisms modulating lifespan in SLEs would be the same as those that explain variation in lifespan in the wild.

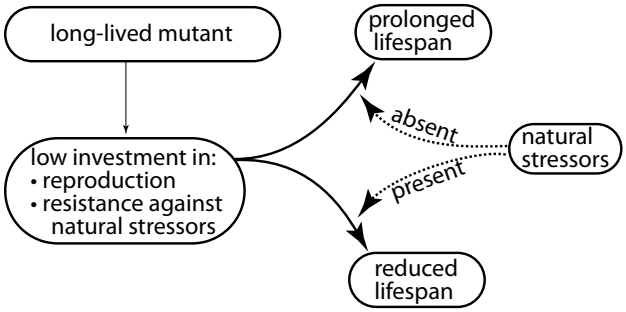

Fig. 1 Hypothesis, based on the optimality theory of aging (Partridge and Barton 1993) stating that the lifespan advantage of long-lived mutants is diminished in the presence of natural stressors that are as a rule absent from standard laboratory environments.

Given that much of our understanding of the mechanisms of aging comes from studies of long-lived mutants in SLEs, and that the environment can have profound effects on lifespan, we here ask to what extent insights from long-lived mutants in SLEs can be generalized to more natural environments. Is it possible that the longer lifespans of long-lived mutants are achieved at the expense of defenses against natural environmental challenges? And if so, what are the consequences for mechanisms involved in lifespan determination and variation in the wild? These questions are of importance when the aim is to apply insights from long-lived mutants in SLEs to other organisms such as humans, which are invariably exposed to a variety of environmental challenges. To address these questions we reviewed two kinds of studies. Firstly, we reviewed experiments that quantified the performance of long-lived mutants and their wild type controls on evolutionary timescales by measuring the fitness of both genotypes in either SLEs or more challenging environments. These studies carried out competition experiments, which consist of mixing two genotypes (the long-lived mutant and the wild type control) in a common environment (SLE or challenging) usually for several generations, after which the relative frequency of each genotype was quantified.

However, fitness (dis)advantages in competition experiments may have arisen through differences in lifespan, in reproduction or a combination of the two, and while competition experiments quantified fitness, they rarely quantified lifespan per se. In the second part, we therefore reviewed studies that quantified the lifespan advantage of long-lived mutants over the wild type controls in SLEs and environments containing more natural challenges. These experiments often lasted only one generation and excluded competition, i.e. long-lived mutant and wild type populations are not mixed. When the life-extending effect of mutations is largely independent of the environment, this indicates that the underlying mechanisms may be of general importance in causing variation in lifespan. Conversely, a strong dependence of the life extending effect on environmental conditions would give reason to question the generality of the mechanism causing the life extending effect in SLEs.

Material and Methods

To find papers that reported competition experiments including long-lived mutants, we searched the Web of Science database using the keywords *'long-lived mutant'* and *'evolution'* (last search on May 31st 2015). This search resulted in 42 articles, of which we selected all articles that had long-lived mutants compete with their wild type counterparts (Jenkins et al. 2004; Delaney et al. 2011; Savory et al. 2014). We then cross-searched all the references and citations of these articles.

For the lifespan studies, articles were only selected if the following criteria were met (i) a long-lived mutant had an extended lifespan in a SLE, (ii) an experimental manipulation of the environment affected the lifespan of either the long-lived mutant or the wild type control and (iii) an estimation of lifespan of the long-lived mutant and the wild type control in both environments. We searched the literature using (i) the above search and (ii) the Web of Science database using the keywords *'long-lived mutant'* and *'environment'* or *'long-lived mutant'* and *'natural'* (last search on May 31st 2015). In addition, we used influential reviews and perspective papers on long-lived mutants and genotype x environment interactions (Gems et al. 2002; Van Voorhies et al. 2006; Partridge and Gems 2007; Tatar 2007; Flatt et al. 2013; Tatar et al. 2014). For each of the three searches we searched all the references and citations of these articles before May 31st 2015 in the Web of Science database.

We define a stressor as a factor that shortens the lifespan of wild type controls and/or long-lived mutants relative to the lifespan in a SLE. When examining effects of stressors

on lifespan we distinguished between the application of short-term acute stressors (heat stress, UV-radiation, toxic chemicals) that cause more or less immediate death of part of the population (e.g. Barsyte et al. 2001; Clancy et al. 2001), and more moderate long-term stressors that were applied permanently. Long-lived mutants appear more resistant to short-term acute stressors than their wild type controls (see e.g. Zhou et al. 2011 for a review). Hence, when an environment is made more challenging by applying short-term acute stressors the lifespan advantage of the long-lived mutants may increase (Zhou et al. 2011). However, we considered such acute stressors to be generally outside of the range that animals under more natural conditions would encounter. Thus, we reviewed only studies that permanently applied more natural and/or moderate stressors, such as a more natural medium, food competition or exposure to pathogens. Note that in dietary restriction experiments, lifespan differences between long-lived mutants and wild type controls can also be environment dependent (Clancy et al. 2002; Gems et al. 2002; Tatar et al. 2014). Yet we did not consider dietary restriction to be a stressor or a natural challenge because it extends the lifespan of wild type controls. However dietary restriction experiments that used variety of diet concentrations can fulfill the challenging criteria if food dilution is applied to the extent that it shortens lifespan of the wild type controls (e.g. Broughton et al. 2010; Clancy et al. 2002; Tatar et al. 2014).

Several studies applied combinations of stressors, for example a variety of pathogens (Garsin et al. 2003), or different degrees of a stressor. To avoid pseudo-replication due to repeated testing, we restricted our analysis to those environmental manipulations that had the strongest effect on the lifespan of wild type controls, because these manipulations best represent a challenging environment.

Unfortunately, most studies did not statistically test genotype x environment interactions (Table S2), prohibiting a formal meta-analysis. However, given the results (e.g. Fig. 4), we see no reason to expect that a formal meta-analysis would change our findings.

Results

Competition performance of long-lived mutants

Very few competition experiments have been conducted in SLEs (n=3) and all have used *C. elegans* (Table S1). In two experiments, the relative fitness between the long-lived mutant and the wild type control did not differ and in one experiment the long-lived mutant went extinct while the wild type control persisted (Fig. 2). While the sample size is low, there is no evidence that long-lived mutants have a consistent competitive advantage or disadvantage over the wild type controls in SLEs.

We found five competition experiments carried out in more challenging environments, covering most model species (Table S1). In addition, we also found one study that carried out 49 competition experiments with long-lived yeast mutants *S. cerevisiae* (Delaney et al. 2011), which we discuss separately below. In all experiments, the challenge consisted of competition for food. The outcome of these experiments was consistent (Fig. 2): the frequency of the long-lived mutant decreased (Giorgio et al. 2012; Wit et al. 2013; Savory et al. 2014), and even went extinct in two out of five experiments (Jenkins et al. 2004; Walker et al. 2000). This outcome stands in contrast with what we found in SLEs, especially given that three out of these five experiments came from the same study as those from SLEs (Table S1). Thus, in competition experiments long-lived mutants have lower fitness relative to their wild type controls and this seems most pronounced in challenging environments.

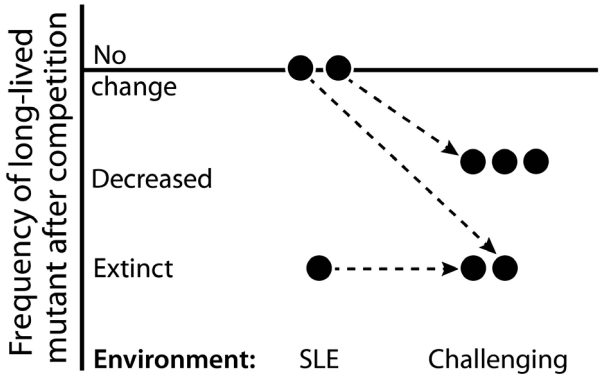

Fig. 2 Outcome of competition experiments between long-lived mutants and their wild type controls. The outcome is from the perspective of the long-lived mutant. Arrows connect experiments that were done in the same study. One additional study in yeast is discussed separately in the main text because it consisted of 49 experiments (Delaney et al. 2011). Studies are summarized in table S1. SLE: standardized laboratory environments.

In addition to the competition experiments discussed above, there is one study that comprised 49 experiments with 49 different long-lived yeast mutants (Delaney et al. 2011). In this study, 84% (41/49) of the long-lived mutants decreased in relative frequency (statistically significant for 32 mutants). In contrast, 16% (8/49) of the mutants increased in relative frequency (statistically significant for two mutants). Thus, the mutants were clearly outcompeted by the wild type yeast strain. In this study, the mutants differed strongly in the extent to which their lifespan was increased relative to wild type controls in the SLE (range 13-55% without competition). This allowed us to investigate whether the mutants with the largest lifespan advantage in a non-competitive environment also have the lowest fitness in a competitive environment. If

lifespan extension generally is achieved at the expense of competitive performance, we expect a negative correlation between the two variables. Indeed, yeast mutants with the largest lifespan advantage were, in evolutionary terms, least fit relative to the wild type controls in the competitive environment (Fig. 3). This finding confirms that extended lifespan is achieved at the expense of fitness in competitive environments. In conclusion, the competition experiments indicate that when having to reproduce and compete with wild type controls in the face of natural challenges such as food limitation, long-lived mutants have decreased fitness relative to wild type controls.

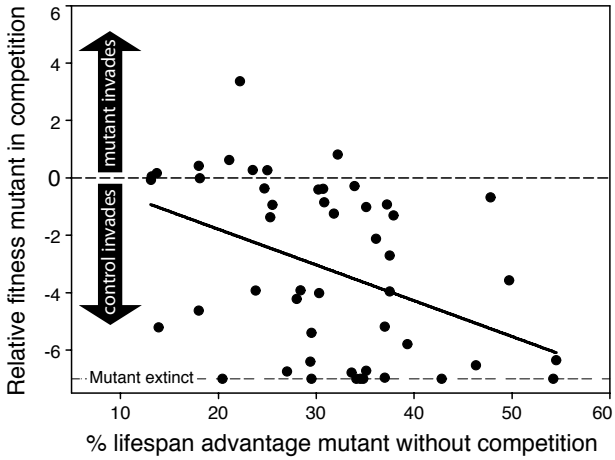

Fig. 3 Association between the lifespan advantage of 49 long-lived yeast mutants over controls in SLEs (standardized laboratory environments) and their fitness (dis)advantage in competition experiments. Relative fitness (RF) is defined as log base 2 ratio of mutant to wild type relative to the initial ratio, such that RF = 0 indicates no change in the ratio of mutant to wild type, an RF = 1 corresponds to twice as many mutant cells as wild type cells relative to the initial ratio, while an RF = -1 corresponds to twice as many wild type cells as mutant cells. A RF of -7 refers to extinction of the long- lived mutant. Competition experiments were carried out for all 49 mutants separately. Data from Delaney et al. (2011). Best fit: R^2=0.16, t=-3.13, p=0.003.

Lifespan of long-lived mutants in environments other than SLEs

The competitive disadvantage of long-lived mutants relative to their wild type controls can arise via diminished survival and/or diminished fecundity. It is a general finding, reviewed elsewhere, that long-lived mutants have diminished fecundity relative to their wild type controls (Flatt 2011; Kenyon 2005; Leroi et al. 2005; Partridge et al. 2005; Tatar 2010) although there are exceptions where the fecundity of both genotypes is similar (Rogina et al. 2003; Hwangbo et al. 2004). It is likely therefore that the reduced competitive ability of long-lived mutants is at least in part due to lower fecundity. However, lifespan was not monitored in the competition experiments, and the possibility

remains that a shortened lifespan of the long-lived mutants also contributed to the low competition success in more natural environments. To address this question we reviewed the studies that compared the lifespan advantage of long-lived mutants over their wild type controls in SLEs and in more challenging environments.

We found a total of 19 experiments in 10 studies where the lifespan of long-lived mutants relative to wild type controls was compared between SLEs and challenging environments, in three different species: *C. elegans*, *D. melanogaster* and *M. musculus*. Several studies exposed different populations to different stressors or different levels of a stressor. Following the pseudo-replication standards as explained in the *'Material and Methods'* section, we used 12 experiments in three species (Table S2). In 5 out of 12 experiments, the long-lived mutants lived significantly *shorter* than the wild type controls in the challenging environment (e.g. Mockett and Sohal 2006; Van Voorhies et al. 2005; Fig. 4). In another six experiments, the lifespan advantage of the long-lived mutants decreased, but long-lived mutants still lived as long as or longer than the wild type controls (e.g. Baldal et al. 2006; Broughton et al. 2010; Toivonen et al. 2007; Fig. 4). In only one case, the lifespan advantage of long-lived mutants over the wild type controls was larger in the challenging environment than in the SLE (Merino et al. 2015). Thus overall, the lifespan advantage of 'long-lived mutants decreased in the challenging environment in 92% (11/12) percent of studies and a two-tailed sign-test shows this deviation from 50:50 to be larger than expected by chance ($p=0.006$). Furthermore, we note that in studies with multiple levels of a stressor, the intensity of the stressor correlated negatively with the lifespan advantage of the long-lived mutants over the wild type controls. In other words, in response to high intensity stressors, the advantage of long-lived mutants over wild type controls was smaller than in response to low intensity stressors (e.g. Clancy et al. 2002). We anticipate therefore that in the studies where the long-lived mutants retained a lifespan advantage over the wild type controls in the challenging environment, long-lived mutants would end up living shorter than the wild type controls if the intensity of the challenge had been further increased. Thus, there is strong evidence that long-lived mutants cope less well with environmental challenges than the wild type controls.

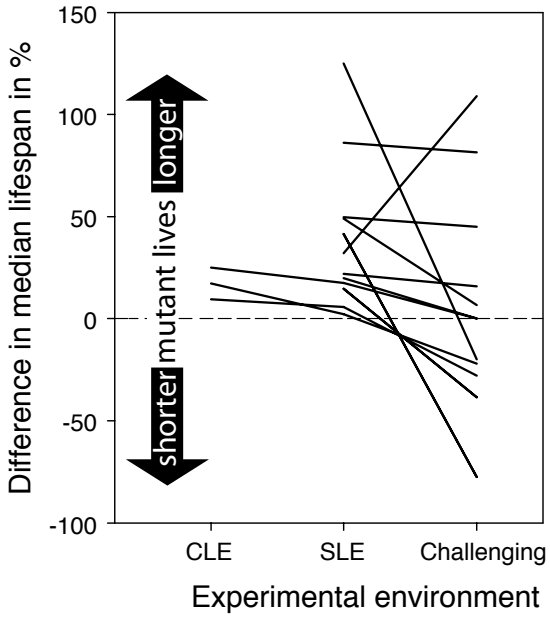

Fig. 4 Lifespan advantage of long-lived mutants over the controls is environment dependent. Lines connect environmental manipulations carried out within one study. CLE: Cafetaria style laboratory environment, SLE: Standardized laboratory environment, Challenging: environment was made more challenging in various ways as evidenced by a reduced lifespan of the control lines (see main text for details). Studies are summarized in table S2.

Of the studies listed above only two were on vertebrates (mice). One study was on Snell dwarf mice. This strain originated as a spontaneous mutation and animals homozygous for this mutation grow to approximately one third of the mass of their wild type siblings (Snell 1929). The impaired growth is due to defects in production of growth hormone, insulin-like growth factor-1 (IGF-1), thyroid hormones, and prolactin (reviewed e.g. in Bartke 2006). Snell dwarf mice were initially found to be a short-lived mutant due to increased susceptibility to infectious disease (Fabris et al. 1972). However, other laboratories later found that Snell dwarf mice had lifespans up to 40% longer than standard laboratory mice (Silderberg 1972; Shire 1973; Schneider 1976; Flurkey et al. 2001) when housing conditions were made more hygienic (Bartke 2006) and mutants were provided a companion mouse to keep them warm. This suggests that the increased lifespan of Snell dwarf mice might trade-off against the immune response and/or body temperature homeostasis. To our best knowledge, this dependence of the lifespan of Snell's dwarf mice on environmental conditions was not explicitly tested, but the contrasts are clear enough in our view to include this strain in Table S2. The second long-lived vertebrate mutant that was studied in a challenging environment was the *p66*^{Shc} knockout mouse. *P66*^{Shc} is a vertebrate protein that is involved in metabolism

and intracellular redox balance and its knockout results in mice that are leaner, more resistant to obesity and diabetes, with reduced oxidative stress and a 30% increased lifespan in SLEs (Migliaccio et al. 1999; Menini et al. 2006; Berniakovich et al. 2008; Fadini et al. 2010; Ranieri et al. 2010). However, in an outdoor enclosure where mice were exposed to natural variation in temperature, food competition and exposure to predators their survival advantage was overturned: after 8 months, 18% of controls were alive while only 5% of $p66^{Shc}$ knock outs were alive (Giorgio et al. 2012). Thus, the limited information available for rodents confirms the finding in invertebrates that the lifespan advantage of long-lived mutants is restricted to specific laboratory environments.

Lifespan in cafeteria environments

In the studies discussed above, the environment was made more challenging in different ways, for example by increasing the effort required to obtain a unit of food relative to SLEs. In contrast, a few studies decreased the effort required to obtain a unit of food, i.e. animals were offered a so-called 'cafeteria-style' laboratory environment (CLE). Such manipulations decrease lifespan (Ozanne and Hales 2004) and show strong similarities to the sedentary lifestyles that decrease lifespan in humans (Flegal et al. 2013). In *Drosophila,* CLEs induced an increase in calorie intake of up to 1.5 times that in SLEs and reduced the lifespan of controls and long-lived *Indy*, *chico* and *IPC KO* (insulin-producing cells knock out) mutants (Clancy et al. 2002; Wang et al. 2009; Broughton et al. 2010). In CLEs long-lived *Indy* mutants increased their lifespan advantage over that of controls (Wang et al. 2009). For *chico* and *IPC KO* mutants there was also an increase in lifespan advantage in CLEs relative to SLEs, but that increase was small, i.e. between 3 and 7% (Clancy et al. 2002; Broughton et al. 2010). CLEs consist of manipulations that make SLEs even more 'sedentary' (and thus are in the opposite direction to the experiments in which SLEs were made more challenging, Fig. 4). Thus, the few studies available suggest that long-lived mutants appear to increase their lifespan advantage relative to wild type controls (Fig. 4). This is consistent with our conclusion that the lifespan advantage of long-lived mutants over the wild type controls is most pronounced in environments with few environmental challenges.

Discussion

We investigated to what extent the performance of long-lived mutants depends on the environment in which they were studied, because this sheds light on the question whether mechanisms causing the extended lifespan may have similar effects in more natural environments. In competition experiments, the long-lived mutants almost always had

lower fitness relative to the wild type controls, especially in challenging environments (Fig. 2). It is well known that the fecundity of long-lived mutants is generally reduced (Flatt 2011; Kenyon 2005; Leroi et al. 2005; Partridge et al. 2005; Tatar 2010), but we find that the lifespan advantage of long-lived mutants is also diminished in more challenging environments (Fig. 4). This effect was such that the lifespan difference was reversed in 5/12 studies and we speculate that this proportion would increase further when environments are made more challenging, as graded dietary restriction studies in *Drosophila* suggest (Clancy et al. 2002; Tatar et al. 2014).

The observation that long-lived mutants are more susceptible to environmental challenges than the wild type controls suggests that they lack the required mechanisms to cope with such challenges. Indeed, in agreement with the optimality theory of aging (Partridge and Barton 1993), the extended lifespan of long-lived mutants may be due to a reallocation of resources saved on coping mechanisms (e.g. immune function) to increased maintenance and repair (Fig. 1). Unraveling the mechanisms that extend the lifespan of long-lived mutants is very interesting in itself. Yet the extended lifespans of long-lived mutants in SLEs are at least partially achieved by saving resources that animals could not afford to save under more natural conditions. Thus, in natural environments there would be strong natural selection against such savings and we therefore believe that variation in lifespan in natural populations (including humans) is unlikely to have the same mechanistic basis as that indicated by work on long-lived mutants in SLEs. The artificial conditions and selection pressures imposed by SLEs can do much to skew the physiological traits among model organisms that are relevant to the aging process in SLEs but not under natural conditions (Harshman and Hoffmann 2000; Sgrò and Partridge 2000; Linnen et al. 2001; Sgrò et al. 2013). This argument also applies when the underlying mechanism is not related to re-allocation of resources, because it is the finding that mechanisms can have the opposite effect on lifespan in more challenging environments that gives reason to question the relevance of these mechanisms in natural populations. Instead, with respect to aging mechanisms in natural environments, we believe there is a need for ecologically relevant manipulations that modulate lifespan and aging in a way that invokes mechanisms that have evolved naturally. Manipulation of reproductive effort or developmental conditions, which can both affect lifespan and aging (Lee et al. 2013, 2016; Boonekamp et al. 2014) come to mind as promising avenues to explore.

Our findings hold in all taxonomic groups where they were studied, including the nematode *C. elegans*, the fly *Drosophila*, and the mouse *Mus musculus*. Our review includes a variety of environmental challenges including exposure to pathogens, cold exposure

and competition for food or starvation (Table S2). Our review also included a variety of long-lived mutations involving multiple pathways. Several of these mutations (*Indy*, *chico*, *IPC KO* and $p66^{Shc}$) are one way or another involved in metabolism and energy balance. When these long-lived mutants are faced with food related challenges, genotype x environment interactions can be expected, but this does not make them less relevant given that food related challenges are common in nature. Further research is required to address whether metabolism-related mechanisms pathways extend lifespan in the wild.

More generally, we need to understand better which life-extending pathways are susceptible to which environmental challenges. This is important because insights gained from studying long-lived mutants in SLEs can provide an important source of inspiration for the development of interventions that postpone or slow down aging (Longo et al. 2015). Yet the trade-offs involved in extending the lifespan of long-lived mutants, and the environment dependent outcome of mutations that affect aging and lifespan, need to be taken into account for interventions to be effective (see also Kuningas et al. 2008; Vijg and Campisi 2008). We believe that ecologically relevant manipulations such as those mentioned above can uncover mechanisms and trade-offs involved in aging and lifespan variation and may provide essential insights for possible 'anti-aging' interventions.

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Supplementary information to:

What can long-lived mutants tell us about mechanisms causing aging and lifespan variation in natural environments?

Table S1 Overview of competition experiments with long-lived mutants carried out in various environments. The outcome of the competition experiment is from the perspective of the long-lived mutant. Abbreviations: NA: not applicable, NS: not significant.

Species	Mutant	Function	Outcome in SLE	Challenge	Outcome in challenging environment	Reference (Location)
S. cerevisae	49 genotypes	Various	NA	Cyclic starvation	84% decrease or extinct (65% siginificant); 16% increase (4% significant), no invading genotypes	Delaney et al. 2011 (Table 1)
C. elegans	*daf-2*	Insulin signaling	Extinct	Cyclic starvation	Extinct	Jenkins et al. 2004 (Fig. 1)
C. elegans	*age-1*	Insulin signaling	NS	Cyclic starvation	Extinct	Walker et al. 2000 (Fig. 1)
C. elegans	*age-1*	Insulin signaling	NS	Limited food	Decrease	Savory et al. 2014 (Fig. 1)
D. melanogaster	3 longevity lines	Unclear	NA	Field release with food searching	Decreased recapture probability	Wit et al. 2013 (Fig. 2, Table 5)
M. musculus	*p66Shc*	Various	NA	Outdoor enclosure with food competition	Decrease significantly within 1 or few generations. Wild type invaded to 75%	Giorgio et al. 2012 (Fig. 1)

Table S2 Overview of experiments in which the lifespan of long-lived mutants was compared with that of controls in SLEs and more challenging environments. Data was split in two types of environmental manipulations natural like challenges (top) and cafeteria style laboratory environments (CLE, bottom). To avoid pseudo replication of studies, per study we included only the experimental challenge that had the strongest negative effect on the lifespan of controls. Abbreviations: manip: manipulated, neg: negative, pos: positive, NST: not statistically tested, NS: not significant. For NST cases, where possible we derived statistical significance ourselves from the SE or SD given in manuscript.

					Lifespan [Days]					
					SLE		Challenging		Statistics G x E Interaction	
Study organism	Mutation	Function	Description environmental challenge	Trait	Controls	Mutants	Controls	Mutants		Reference
Environmental manipulation: natural challenge										
C. elegans	daf-2	Insulin signaling	Heat treated soil	Median	12	27	1	0.8	Yes	Van Voorhies et al. 2005 (Fig.2)
C. elegans	daf-2 (mean)	Insulin signaling	Pathogen	Median	13.1	24.4	2	3.63	NST	Garsin et al. 2003 (Table S1)
C. elegans	age-1	Insulin signaling	Pathogen	Median	13.1	19.6	2	2.9	NST	Garsin et al. 2003 (Table S1)
D. melanogaster	mth (heterozygote)	Stress response	Reproduction	Mean	26	31	23	23	Yes	Baldal et al. 2006 (Fig.3)
D. melanogaster	mth (heterozygote)	Stress response	Constant moderate heat stress	Mean	41	50	25	29	NST: Yes	Baldal et al. 2006 (Fig.3)
D. melanogaster	chico (homozygote)	Insulin signaling	Starvation	Mean	52	55	43	31	NST: Yes	Clancy et al. 2002 (Fig.1)
D. melanogaster	IPC KO (dilp2)	Insulin signaling	Starvation	Median	66	78	22	22	NST: Yes	Broughton et al., 2010 (Table 1)
D. melanogaster	Azot (homozygote)	Elimination of malfunctioning cells	Constant moderate heat stress	Median	25.9	34.2	7.8	16.3	NST	Merino et al. 2015 (Fig.7N vs. Fig.6Y)
D. melanogaster	indy206 (heterozygote)	Krebs cycle	Eliminating Wolbachia infection	Median	45	67	45	48	Yes	Toivonen et al. 2007 (Fig.5B)
D. melanogaster	mth (homozygote)	Stress response	Cold stress	Mean	138	141	5	3.9	NST: Yes	Mockett and Sohal 2006 (Table 1)
M. musculus	Snell dwarf mice	Insulin-like growth factor-1	Pathogen? Body Temperature homeostasis?	Mean	831	1178	600	135	NST: Yes	Bartke 2006 (p. 404); Fabris et al. 1972 (Fig.1); Flurkey et al. 2001 (Fig.1)
M. musculus	$p66^{Shc}$ (heterozygote)	Metabolism	Outdoors with food competition and predators	15% Survival	820	940	390	240	NST: Yes	Giorgio et al. 2012 (p. 163 paragraph 2); Migliaccio et al. 1999 (Fig.6)

Study organism	Mutation	Function	Description environmental challenge	Trait	Lifespan [Days] SLE Controls	SLE Mutants	Challenging Controls	Challenging Mutants	Statistics G x E Interaction	Reference
Environmental manipulation: CLE										
D. melanogaster	indy (homozygote)	Krebs cycle	CLE	Median	43	44	35	41	Yes	Wang et al. 2009 (Fig.1A; Table S1)
D. melanogaster	chico	Insulin signaling	CLE	Mean	52	55	42	46	NST: No	Clancy et al. 2002 (Fig.1)
D. melanogaster	IPC KO (dilp2)	Insulin signaling	CLE	Median	66	78	60	75	NST	Broughton et al. 2010 (Table 1)

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Part II

Population

Box A

Growing up in large broods impairs development in zebra finches

Michael Briga, Egbert Koetsier & Simon Verhulst

Study1: Growing up in large broods increases the effort made per item food reward
Experimental manipulation of developmental conditions are commonly done through changes in food abundance or brood size (Griffith and Buchanan 2010). In our study, we manipulated the brood size and here investigate the consequences on chick behaviour and growth. We paid particular attention to begging behaviour, because various studies have indicated that begging incurs costs, in terms of energy (Bachman and Chappell 1996; McCarty 1996; Moreno-Rueda 2007) or physiology. For example, in various bird species, experimental increases of begging behaviour were found to impair growth (Kilner 2001; Rodríguez-Gironés et al. 2001; Moreno-Rueda and Redondo 2011; Moreno-Rueda et al. 2012), immunocompetence (Moreno-Rueda 2010; Moreno-Rueda and Redondo 2011; Moreno-Rueda et al. 2012; Redondo et al. in press) and to increase oxidative stress (Moreno-Rueda et al. 2012). To investigate whether the brood size manipulation affected begging behaviour we recorded 7 small and 8 large brood nests. Recordings were done at two growth points, halfway through the chick stage and just before fledging, i.e. at the age of 7 and 15 days. We recorded on average 1.5 hour (95% CI 1-2 hours) per hour per age class, giving a total of 50 hours of recording. For each nest we quantified the time budget of two chicks. We found that chicks in large broods begged more (Fig. 1; $X^2=7.56$; $p=0.006$) and received less regurgitations per hour compared to chicks reared in small broods (Fig. 1; $F=8.14$; $p=0.01$). These results are consistent with other studies showing increased begging in chicks from large broods (Leonard et al. 2000; Neuenschwander et al. 2003; Kim et al. 2011). Thus, growing up in large broods increased the effort made per item food reward.

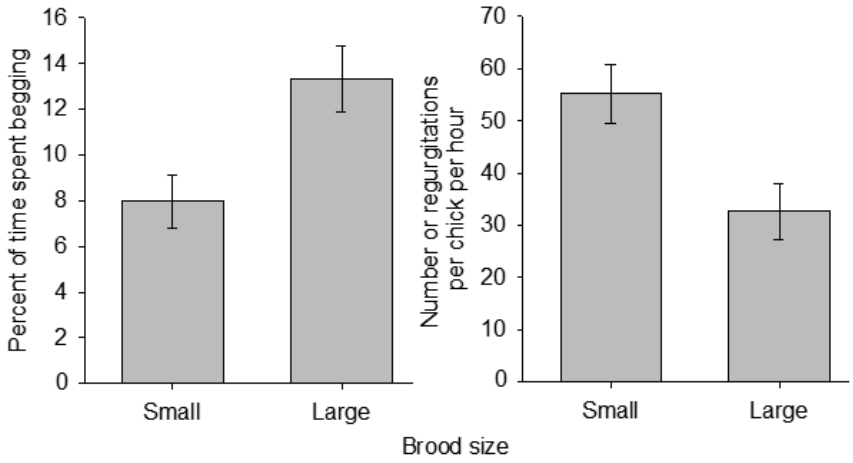

Fig. 1 Chicks in large broods spent more time begging (left), but nevertheless received fewer regurgitations from the parents (right). Shown are means per chick ± SE. Results are based on 50 hours of recording in 7 small and 8 large brood nests.

Study 2: Growing up in large broods impairs growth

Given that large broods increased the effort made per item food reward, we expected impaired growth in chicks from large broods. We quantified the growth curve by measuring chicks at three age stages: just before fledging (15 days) and at the age of 35 and 100 days, when birds are approximately fully grown. Data included here are 3263 measurements on 295 individuals from three breeding rounds in 2006, 2007 and 2008 and all these were allocated to the foraging cost treatment (Chapters 3-11). At each age, we measured weight and the length of tarsus, headbill and wing. We also devised a more general of structural body size, using the average of the tarsus and the headbill after transforming both to a standard normal distribution. As a control we weighed chicks before manipulation (day 5) and there was no difference in mass between chicks going to large broods or small broods (Fig. 2; $F=0.40$; $p=0.52$). All analyzes were performed in SAS JMP 7 using general linear models including as fixed effects brood size and age and as random effects individual, genetic father and genetic mother. Residuals of all models had a normal distribution and without outliers. To allow comparison of the effect of the brood size manipulation across ages and traits, we report the effect size as Cohen's d (Cohen 1988), which in brief, is the ratio of the difference between two groups over their standard deviation (Fig. 2). Confidence intervals were estimated following equations 15 and 16 in Nakagawa and Cuthill (2007). As a simple rule of thumb, an effect size between 0.1 and 0.5 is usually considered moderate (Cohen 1988), with 0.5 being the average effect size of published results in the fields of ecology and evolution (Moller and Jennions 2002). Note however that many studies with smaller effects do not make it till publication, i.e. there is a publication bias of positive, significant or 'stronger' results (Rosenthal 1979; Csada et al. 1996; Cassey et al. 2004; Fanelli 2010).

At the age of 5 days, i.e. before the brood size manipulation, there was no difference in mass between chicks that went to small or large broods ($F_{272}=0.04$; $p=0.92$; Fig. 2). The brood size manipulation had a major effect on mass at the age of 15 days: birds from large broods were 1.2 g lighter (11% at 10.0 g) than those from small broods ($F_{220.6}=51.2$; $p=0.0002$; Fig. 2). This effect decreased with age ($F_{780}=18.8$; $p<0.0001$; Fig. 2) and at the age of 100 days they were still 0.65 g (4% at 14.2 g) lighter which remained significant ($F_{246.5}=14.5$; $p=0.0002$; Fig. 2). Thus growing up in large broods impaired growth, which effect was followed by a partial compensation response. Note that the effect of the brood size manipulation on mass remained throughout adulthood at 0.56 g (Chapter 11).

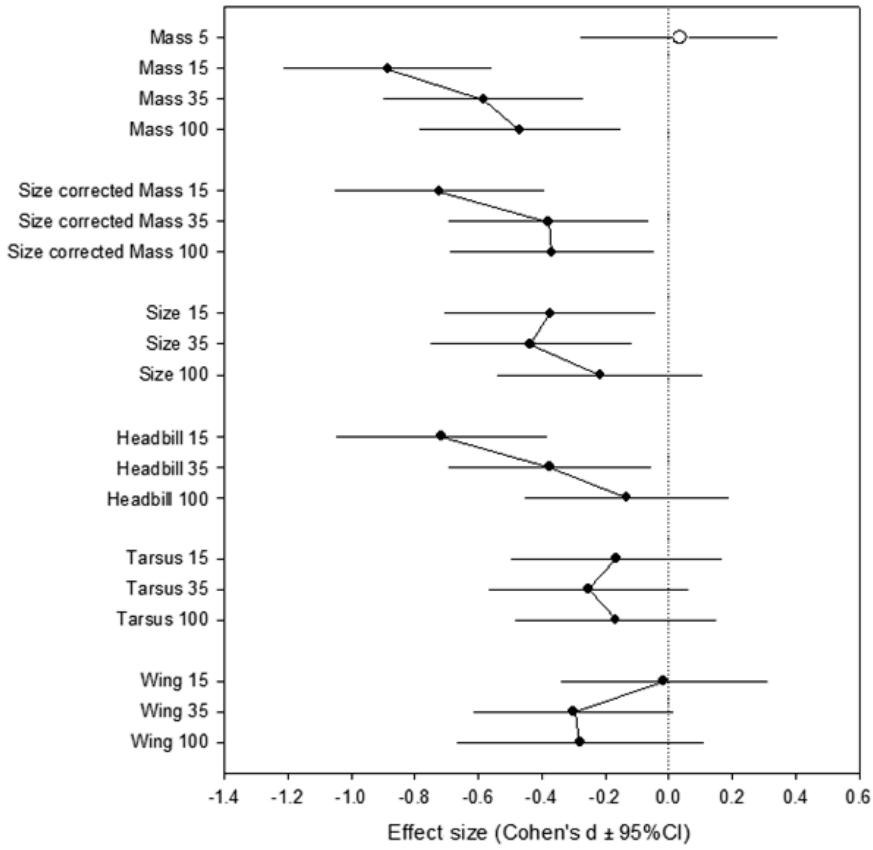

Fig. 2 Birds from large broods have impaired growth which effect decreases with age. Shown are effect sizes ± 95%CI, i.e. a standardized difference quantified as Cohen's d: $(m_{large}-m_{small})/SD$, with m_{large} and m_{small} being the mean value for birds from large and small broods respectively. Vertical dotted line shows d=0 no difference between birds from large and small broods and left of the vertical line shows better growth in small broods. Open dot shows an effect size on mass before manipulation, i.e. where there should be no brood size effect. Size refers to structural size, a standard normally distributed pooled measure of tarsus and headbill (see above).

The effects on mass can partially be mediated by size. Indeed, birds from large broods tended to have smaller tarsi ($F_{250}=2.83$; $p=0.09$) than birds from small broods and this was independent of age ($F_{502}=0.01$; $p=0.99$; Fig. 2). They also had smaller headbills, which effect was most pronounced at young age ($F_{248}=6.88$; $p=0.0092$) and decreased with age ($F_{466}=8.31$; $p=0.0003$; Fig. 2) until the age of 100 days at which headbills were slightly but not significantly smaller ($F_{248}=1.67$; $p=0.19$; Fig. 2). Overall young from large broods were smaller in size ($F_{250}=9.82$; $p=0.0019$), which effect weakly decreased with age ($F_{495}=2.12$; $p=0.12$; Fig. 2). Birds from large broods also had smaller wings ($F_{264}=4.58$; $p=0.03$), which effect did not change with age ($F_{471}=0.01$; $p=0.98$; Fig.

2). Taking the effects of size into account, the effect of the brood size manipulation on size corrected mass followed a very similar growth trajectory as that of mass, with the strongest effect at early growth (F_{225}=56.6; p<0.0001), followed by a partial compensation response (F_{492}=4.8; p=0.0088; Fig. 2). Note that the brood size effect on size corrected mass also remained throughout adulthood (Chapter 11). Thus, birds from large broods were lighter and smaller in size than birds from small broods and partially compensated in both mass and size.

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Chapter 3

Food availability affects adult survival trajectories depending on early developmental conditions

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Abstract

Food availability modulates survival in interaction with e.g. competition, disease and predators, but to what extent food availability in natural populations affects survival independent of these factors is not well known. We tested the effect of food availability on lifespan and actuarial senescence in a large population of captive zebra-finches by increasing the effort required to obtain food, reflecting natural contrasts in food availability. Food availability may not affect all individuals equally and we therefore created heterogeneity in phenotypic quality by raising birds with different numbers of siblings. Low food availability had no effect on lifespan for individuals from benign developmental conditions (raised in small broods), but shortened lifespan for individuals from harsh developmental conditions. The lifespan difference arose through higher baseline mortality rate of individuals from harsh developmental conditions, and despite a decrease in the rate of actuarial senescence. We found no evidence for sex specific environmental sensitivity, but females lived shorter than males due to increased actuarial senescence. Thus low food availability by itself shortens lifespan, but only in individuals from harsh developmental conditions. Our food availability manipulation resembles dietary restriction as applied to invertebrates, where it extends lifespan in model organisms and we discuss possible reasons for the contrasting results.

Introduction

In natural populations, food availability is a key factor in population dynamics and life history evolution, because survival and fecundity are thought to increase when food becomes more abundant (Martin 1987; Boutin 1990). However, the exact mechanisms through which food availability affect survival and reproduction remain unclear, because food abundance interacts with other ecological factors (McNamara and Houston 1987; Krebs et al. 1995; Prevedello et al. 2013). For example, an increase in food availability is likely to reduce starvation risk but also affects exposure to predators because animals are often more vulnerable when foraging, and high food abundance allows for a reduction in the time spent foraging. Thus an increase in food availability could affect survival primarily through an effect on predation rate, with a negligible contribution of altered starvation rate (McNamara and Houston 1987). Alternatively, increased food availability may increase the local density of conspecifics, which can result in an increase in the number of predators to the extent that per capita predation rate is increased (Gilroy and Sutherland 2007). That the relation between food availability and survival is complex is further illustrated by the finding that dietary restriction in laboratory animals generally *increases* survival and lifespan (Nakagawa et al. 2012). Perhaps due to a combination of these processes, a recent meta-analysis concluded that food supplementation in natural populations had no demonstrable effect on survival (Prevedello et al. 2013). Thus the extent to which food abundance *in isolation* affects survival in natural populations is an open question, at least in the food availability range where animals occur naturally. This is unfortunate, because insight in the mechanisms mediating demographic effects of food availability may be essential to predict such effects in our ever-changing world. Experiments are required to resolve this issue, and we here present the results of a large scale and long-term experiment in which we test for an effect of food availability on lifespan and ageing in zebra finches *Taeniopygia guttata*. An essential aspect of our approach is that we manipulated food availability in a way that mimics natural variation in food availability, i.e. by manipulating foraging costs (Koetsier and Verhulst 2011), here defined as the effort required to obtain a unit of food. Thus animals could respond to lower food availability by increasing their foraging effort, as they can in natural conditions. At the same time, in the foraging cost manipulation, density was controlled and there were no predators, and thus food availability effects on survival can be attributed to food availability per se.

Variation among individuals (individual heterogeneity) with respect to susceptibility to environmental factors such as food abundance can have interesting demographic consequences (Caswell 2001; Kendall et al. 2011; Plard et al. 2015). Environmental factors

contribute to the development of individual heterogeneity at all ages, but environmental conditions during development are thought to be of particular importance in determining individual heterogeneity in lifespan and, more generally, adult health (Lindström 1999; Hales and Barker 2001; Metcalfe and Monaghan 2001; Lummaa and Clutton-Brock 2002; Hanson and Gluckman 2014). The link between developmental conditions and lifespan can be complex when the effects depend on the environmental conditions in adulthood (Gilbert 2001; Monaghan and Haussmann 2015). For example, benign developmental conditions may yield high quality phenotypes that cope better with harsh conditions in adulthood relative to phenotypes from harsh developmental conditions (the 'silver spoon hypothesis'; Grafen 1988). In contrast, the match-mismatch hypothesis states that environmental challenges faced during development may prepare individuals to cope with similar environmental challenges during adulthood, while a mismatch my cause health problems (Bateson et al. 2004; Gluckman and Hanson 2004; Monaghan 2008; Hanson and Gluckman 2014). Lastly, specific stressors experienced during development may prime the development of resistance against such stressors in adulthood (known as a hormesis effect; e.g. Costantini et al. 2010). Experimental studies of developmental effects on lifespan have generally considered only high quality environmental conditions during adulthood (e.g. standard laboratory conditions), or considered animals in a (uniform) environment, and thus cannot distinguish silver spoon from match-mismatch scenarios. Such a test requires the independent manipulation of the environment during development and in adulthood in a crossover design, which needs a level of control that usually requires a laboratory setting. There are few such experiments and these have generally failed to find such interaction effects (Taborsky 2006; Barrett et al. 2009; Zajitschek et al. 2009; Auer 2010; Dmitriew and Rowe 2011; but see Saastamoinen et al. 2010). However, these studies all used species with indeterminate growth and/or developmental phases of flexible duration. Such developmental patterns increase the opportunity to mitigate effects of harsh developmental conditions in ways that are not open to species with determinate growth such as birds and humans. Thus to what extent lifespan is subject to match-mismatch effects versus silver spoon effects is unknown for species with determinate growth.

We here report the results of an experiment aimed to tease apart effects of foraging costs during development and in adulthood on lifespan and senescence. The zebra finch is a suitable species because it has determinate growth, and developmental conditions have previously been shown to affect the phenotype in ways that are important for adult health and lifespan (review: Griffith and Buchanan 2010). The experiment had a 2x2 design, independently manipulating foraging costs during development and adulthood, so that we could test for interaction effects between food availability at different life

stages. We manipulated the foraging costs during development by cross fostering chicks to either small or large broods (as in de Kogel 1997), which in a sense increases 'foraging costs' because chicks have to beg more per item food reward (Kilner 2001; Box A). In adulthood, we experimentally increased the flight costs per food reward (as in Koetsier and Verhulst 2011), and individuals were maintained in these conditions till natural death.

When age at death follows a Gompertz distribution, variation in lifespan can arise via two distinct but not mutually exclusive ways: a change in the (age independent) baseline mortality rate (vulnerability to the aging process) and/or a change in the age dependent mortality rate (actuarial senescence or 'aging rate'; Pletcher et al. 2000; Kowald 2002; Mair et al. 2003; Simons et al. 2013; Boonekamp et al. 2014). Identifying which parameter changed when there is a change in lifespan is informative because these effects are likely to be caused by different biological processes (Partridge et al. 2005; Boonekamp et al. 2015). We therefore analyzed our data in two steps. First, we identified treatment effects on lifespan using Cox proportional hazard (CPH) analyses (Cox 1972; Therneau and Grambsch 2000). Next, we evaluated the contribution of differences in age independent and age dependent mortality to the observed lifespan differences by fitting the Gompertz mortality function (Pletcher et al. 2000; Kowald 2002). In this way we tested effects of foraging costs during development and in adulthood on lifespan, and on the parameters that describe the mortality trajectory.

Material and Methods

Development

Birds for the experiment were reared by randomly mated pairs housed indoors on a 14:10 (hr) light-dark schedule at around 25°C and 60% humidity in a cage (LxHxD 80x40x40cm) with a nest box and nesting material (hay). Drinking water, sepia and a commercial tropical seed mixture were available at libitum. A teaspoon of fortified canary food (''eggfood'', by Bogena, Hedel, the Netherlands) was given three times a week till hatching of the first chick (no "eggfood" was given during the nestling phase to avoid possible diet variation between birds growing up in large and small broods). Nest boxes were checked daily. When the oldest chick of a brood was 4-5 days old, all chicks of that nest were cross-fostered randomly to small and large broods. We created experimentally small broods (89% with 2 chicks and 11% with 3 chicks) and large broods (80% with 6 chicks, 7% with 5, 9% with 7, and 4% with 8 chicks). These brood sizes are within the range observed in wild (Zann 1996). Behavioral observations (Box A) showed that birds

in large broods had to beg more per feeding from the parents, confirming that the effort required per unit of food was higher in large broods when compared to small broods. At the age of 15 days, birds were ringed and from the age of 35 days till approximately 120 days young were housed in larger indoor cages with up to 40 other young of the same sex and two male and two female adults for sexual imprinting.

At the age of 15 days, i.e. just before fledging, growing up in a large brood resulted in 1.4 g (12%) lower mass without altering the variance (SD: 1.4 in both groups; N=478; Fig. S1; see supp. information 1) in agreement with earlier reports (de Kogel 1997; Holveck and Riebel 2010). Selective disappearance of low quality individuals, reducing heterogeneity, can bias estimates of the long-term effects of developmental conditions, but survival between cross-fostering and age 120 days was high and independent of the number of siblings after cross-fostering (see supp. information 1 for details).

Adulthood

From an age of 120 days onwards, birds were housed in eight single sex outdoor aviaries (LxHxW: 310x210x150 cm) located in Groningen, the Netherlands (53° 13' 0" N / 6° 33' 0" E). Four aviaries had low foraging costs and four had high foraging costs, equally divided between sexes, and with a balanced spatial distribution. Thus all foraging costs / sex combinations were replicated. We manipulated foraging costs as in Koetsier and Verhulst (2011). In brief, in each aviary a food container (LxWxH: 120x10x60 cm) with 10 holes in the sides was suspended from the ceiling. In the low foraging cost (benign) treatment food containers have perches below the holes, while in the high foraging cost (harsh) treatment these perches are absent. Therefore, birds in the harsh foraging treatment need to fly from a distant perch to the food container, hover to get the seed(s) and fly back to the perch to consume it.

The adult phase of the experiment started on December 9th 2007 and we used data collected till January 1st 2015 in this paper. In each aviary we entered an approximately equal number of birds reared in small or large broods. Mass at ages 15 and 120 days did not differ between birds entered in the hard or easy foraging treatment (Table 1). As birds died, new birds, reared in small or large broods as described above, were added periodically to keep densities within aviaries within a limited range, which has the added advantage that this allows the separation of temporal effects from age effects in the statistical analyses. The starting population (2007) contained 235 birds, and the following numbers were added in subsequent years: 2008: 45, 2009: 41, 2011: 95, 2012: 62 and 2014: 27 (i.e. 270 birds added in total, bringing total birds in experiment to 505). Birds were entered in the aviaries when 3-4 months old, except for the first batch which

was 3-24 months old when the experiment started. The age at the start of the foraging cost experiment did not differ significantly among groups (Table 1), and we took age at start of the experimental treatment into account in the analysis. The first batch was housed in the same aviaries prior to the start of the experiment in 2007, but with food supplied in bowls on the floor. Before the start of the foraging cost experiment, all birds were trained on the harsh foraging treatment to ensure that birds in the benign and harsh adult foraging treatment were strictly comparable in case not all birds managed to cope with the harsh foraging treatment. Of the 562 birds trained, twelve died during training, equally divided among birds reared in small and large broods.

Table 1 Descriptive statistics [mean(SD)] of the experimental groups.

Developmental conditions:	**Benign**		**Harsh**	
Adult conditions:	**Benign**	**Harsh**	**Benign**	**Harsh**
N birds entered into adult experimental conditions	129	136	133	152
Proportion of males	0.50	0.50	0.51	0.53
Age (years) when entering adult experimental conditions	0.95 (0.55)	0.93 (0.54)	0.99 (0.60)	1.01 (0.60)
N Deaths on Jan 1st 2015	90	83	92	112
N Censored*	39	53	41	40
Mass (g) at age 15 days; during growth	11.4 (1.4)	11.2 (1.4)	10.2 (1.6)	9.7 (1.5)
Mass (g) at age 3 months; early adulthood	15.0 (1.5)	15.0 (1.6)	14.4 (1.6)	14.3 (1.6)

* Of the censored animals, 97.5 % outlived the experiment, the remaining 2.5 % died in accidents of various kinds.

Statistical analyses

Mortality may have been affected by fixed effects besides our manipulations (e.g. sex and starting age of the adult treatment). Furthermore, there are several random effects that may have affected mortality (birth nest, genetic mother, genetic father, rear nest, rear mother, rear father, birth batch and aviary). To identify whether these were of interest to take into account when testing for manipulation effects we used Cox Proportional hazard (CPH) analyses and model selection (Burnham and Anderson 2002; Burnham et al. 2011) based on the Akaike Information Criterion (AICc), to identify the model best supported by the data (see supp. information S2), for which as a rule of thumb, a change in AICc of -2 is considered significant (Burnham and Anderson 2002; Burnham et al. 2011). These analyses revealed that higher 'starting age' increased mortality, but there was no support for the inclusion of interactions between starting age and experimental manipulation (Table S2). We thus fitted parametric mortality models (see below) correcting for starting age (exponentially transformed and mean centered). CPH analyses also showed evidence for female biased mortality but not for sex-specific

experimental effects (Table S3). We thus fitted parametric mortality models excluding sex-specific experimental effects. Of all the random effects tested (see above), including or excluding them never altered any of the conclusions of the CPH analyses. Aviary was the best supported but still explained little variance ($p>0.10$) and we here report all CPH results including aviary as a random effect.

We employed parametric mortality models to quantify experimental effects on age dependent and age independent mortality components. Our sample sizes more than fulfill the minimum requirements for fitting mortality models (Promislow et al. 1999). Of the various mortality models fitted to our data, the Gompertz model fitted best (Table S4). We here further discuss two mortality models. In the exponential model the force of mortality at time t (M_t) is a constant k ($M_t=k$) and thus there is no senescence. In the Gompertz function ($M_t=Ae^{Bt}$ or, in the notation we use, $log(M_t)=log(A)+Bt$), the force of mortality at time t (M_t) is a function of baseline parameter A and increases exponentially with age according to the parameter B, which quantifies actuarial senescence. We used these Gompertz parameters to derive population characteristics such as (i) the mortality rate doubling time (MRDT), another measure of the rate of senescence, given by MRDT=0.693/B (Finch 1990, pp. 22-24), (ii) the life expectancy at start of the treatment and (iii) the standard deviation in ages at death(s) as a measure of lifespan inequality. Standard deviation of ages at death estimated based on simulations of populations of 10.000 individuals given the Gompertz parameter estimates (Table 2).

Table 2 Lifespan characterizations of the experimental groups. Time was taken to be time elapsed since birds entered the adult treatment.

Developmental conditions:	**Benign**		**Harsh**	
Adult conditions:	**Benign**	**Harsh**	**Benign**	**Harsh**
ln(Gompertz A), age independent mortality (±SE)	-2.21 (0.21)	-2.22 (0.20)	-1.91 (0.19)	-1.41 (0.16)
Gompertz B, age dependent mortality (±SE)	0.37 (0.07)	0.41 (0.06)	0.28 (0.06)	0.18 (0.06)
Mortality Rate Doubling Time [years]	1.78	1.69	2.48	3.85
life expectancy at start treatment [years]	3.28	3.21	3.20	2.66
standard deviation in age at death [years]*	1.67	1.60	1.83	1.79

* Simulation based using the Gompertz estimates in this table.

We fitted these models using the function 'basta' in the R package "Bayesian Survival Trajectory Analysis" (BaSTA; Colchero et al. 2012) which optimizes parametric survival functions using Markov Chain Monte Carlo (MCMC) procedures. Starting age was included as a covariate. We fitted the parametric mortality models using 4 parallel MCMC runs with 500.000 iterations, 100.000 burn in period and a thinning of 1000. Potential scale reduction factors (all less than the maximum of 1.1) and trace plots

(Fig. S2) indicated appropriate model convergences, and the levels of autocorrelation were low (<0.04). Parameter comparison between groups was done using the Kullback-Leibler discrepancy (KLD; Kullback and Leibler 1951; McCulloch 1989). KLD describes to what extent the posterior distributions of parameters between groups are similar and ranges from 0.5 (identical distribution) till 1 (no overlap).

Results

For brevity, we refer to the four experimental groups with BB, BH, HB, HH, where the first letter stands for benign (B) or harsh (H) developmental conditions (i.e. small or large brood size), and the second letter stands for benign (B) or harsh (H) foraging conditions in adulthood.

Lifespan

The foraging cost manipulation had little effect on the life expectancy of birds reared in benign developmental conditions (Fig. 1A; Table 2; BB vs. BH: ΔAICc>+0.67, Table S1A). In contrast, the foraging cost manipulation had a strong effect on the life expectancy of birds reared in harsh developmental conditions, with HH birds living six months (17%) shorter than HB birds (Fig. 1B; Table 2; HB vs. HH: ΔAICc=-3.61, Table S1B). The interaction between developmental and adult conditions was included in the best fitting model (ΔAICc=-1.22; Table S1E). Thus high foraging costs shortened lifespan, but only for birds that had experienced harsh developmental conditions.

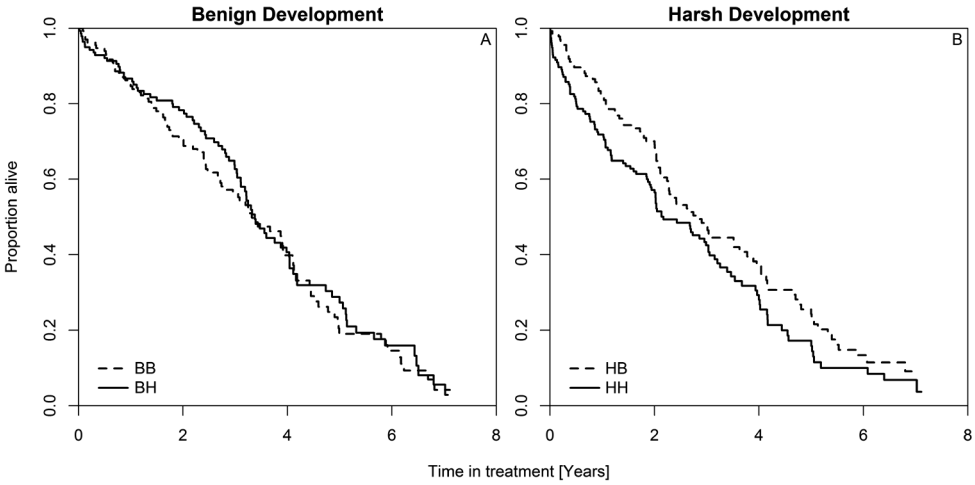

Fig. 1 High foraging costs shorten survival for birds from harsh (B) but not from benign (A) developmental conditions. Survival curves were corrected for age at start adult treatment (Table 1). Graphs for the four groups together are shown in Fig. S3A.

Senescence

To test for actuarial senescence, an increase in mortality rate with age, we compared the fit of the exponential model (that assumes a constant mortality rate with age) with that of the Gompertz model (that assumes an exponential change in mortality rate with age). The Gompertz model fitted the data better than the exponential model (ΔDIC=-241; Table S4) and showed that the risk of dying increased over time. Thus our population of zebra finches experienced significant actuarial senescence.

Having established that there was actuarial senescence we investigated whether changes in Gompertz baseline mortality rate and/or actuarial senescence could explain the experimental effects on lifespan. Baseline mortality rate (or 'vulnerability', the age independent '*A*' in the Gompertz equation) varied twofold between groups (Table 2). For birds from benign developmental conditions (BB vs. BH) foraging costs had little effect on Gompertz A (Fig. 2A) and posterior parameter distributions overlapped moderately (KLD=0.84; Fig. 2C). In contrast, for birds from harsh developmental conditions (HB vs. HH), high foraging costs increased Gompertz A (Fig. 2B) and there was no overlap in posterior parameter distributions (KLD=1.00; Fig. 2C). Thus, high foraging costs increased baseline mortality for birds that grew up in harsh relative to those from benign developmental conditions.

Age independent and age dependent mortality rate often correlate negatively (see discussion). Consistent with this general finding, the age dependent mortality rate (actuarial senescence or 'aging rate', '*B*' in the Gompertz equation) was higher for the experimental groups with low Gompertz A. For birds from benign developmental conditions (BB vs. BH) foraging costs had little effect on Gompertz B (Fig. 2A) and posterior parameter distributions overlapped moderately (KLD=0.82; Fig. 2C). In contrast, for birds from harsh developmental conditions (HB vs. HH), high foraging costs decreased Gompertz B (Fig. 2B) and there was virtually no overlap in posterior parameter distributions (KLD=0.99; Fig. 2C). In agreement with these findings, the mortality rate doubling time was longer for birds reared in harsh relative to those from benign developmental conditions, in particular when they lived in a harsh adult environment (HH; Table 2). Thus high foraging costs decreased age dependent mortality rate, but only for birds from harsh developmental conditions.

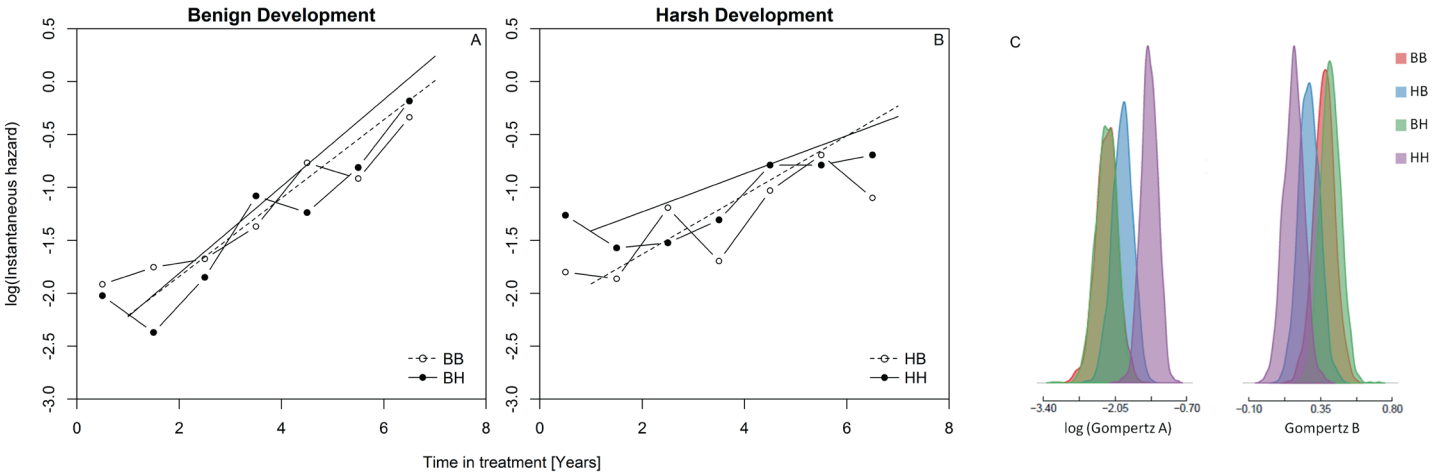

Fig. 2 High foraging costs had little effect on mortality rates for birds from benign (A) developmental conditions. In contrast, for birds from harsh (B), high foraging costs increased baseline mortality rate (Gompertz A) and decreased actuarial senescence (Gompertz B). Dots show mortality per half year. Lines show Gompertz fits. Graphs for the four groups together are shown in Fig. S3B. (C) Posterior distributions of Gompertz parameters overlap for birds from benign developmental conditions (BB and BH) but little for birds from harsh developmental conditions (HB and HH). Treatment abbreviations: B: benign conditions and H: harsh conditions, in chronological order, such that e.g. the BH group indicate benign developmental followed by harsh adult conditions.

A common measure of variation in lifespan is the standard deviation in age at death (SD), and other measures of lifespan inequality correlate well with the SD (van Raalte and Caswell 2013). We found that the SD of age at death was higher for birds from harsh developmental conditions, independent of foraging costs in adulthood (Table 2). Thus, while benign foraging costs mitigated the negative effects of harsh developmental conditions on mean lifespan, this was not the case for variation in lifespan.

Sex differences

Males lived on average one month longer than females (Fig. S4A; ΔAICc=-3.1; Table S3), but there was no evidence for sex-specific experimental effects (+1.0<ΔAICc<+6.5; Table S3). To understand whether the differences in lifespan between the sexes arose due to differences age independent and/or age dependent mortality rate, we fitted the Gompertz model per sex (Fig. S4B). The sexes did not differ in age independent mortality rate (Gompertz A; KLD=0.50; Fig. S4C) but females showed accelerated age dependent mortality rate relative to males (Gompertz B; KLD=0.93; Fig. S4C). Thus sexes differed in lifespan because females aged faster than males.

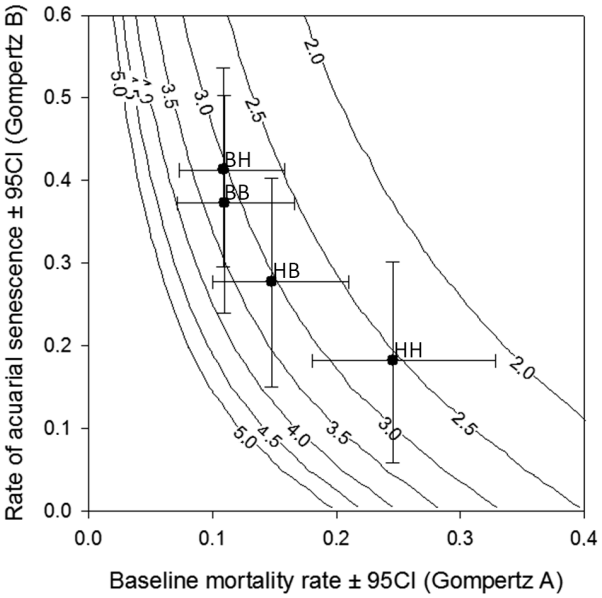

Fig. 3 Gompertz parameter estimates (±95CI) for the different treatment combinations. Iso-life expectancy lines show life expectancy (years) at start foraging treatment for different combinations of the two parameters.

Discussion

Whether food availability affects survival in natural populations has remained elusive due to interactions between food availability and factors such as predation and competition (Krebs et al. 1995; Prevedello et al. 2013). We therefore studied the effect of manipulated food availability on survival and lifespan in a setting where confounding effects of predation and competition were excluded. A unique aspect of our experiment is that we manipulated food availability in a vertebrate by increasing foraging costs, mimicking how animals experience natural variation in food availability. To our surprise, an increase in foraging costs that resulted in doubling of the time spent foraging (Koetsier and Verhulst 2011) had no effect on survival of birds that were reared in benign conditions (i.e. in small broods), despite the fact that the birds were housed in outdoor aviaries and were thus exposed to large fluctuations in ambient temperatures and hence energy needs (Briga and Verhulst 2015). In contrast, birds reared in harsh conditions (large broods) were susceptible to the foraging cost manipulation, attaining a shorter lifespan when facing increased foraging costs. Apparently, birds reared in benign conditions could compensate behaviorally or physiologically for the increase in foraging costs in a way that did not compromise their survival or lifespan, while birds reared in harsh conditions did not have this opportunity. Thus we conclude that the effect of food availability on survival and lifespan depends on the developmental history of the individual, with individuals in poorer conditions developing to be more vulnerable to an increase in foraging costs. To the best of our knowledge, this is the first experimental demonstration that foraging costs affect survival and lifespan of a vertebrate in a setting that fully excludes confounding effects of predation and competition.

The effect of food availability on lifespan has been well studied in laboratory animals (Nakagawa et al. 2012), but our study differs critically from this large body of work. We manipulated foraging costs in a way similar to dietary restriction (DR) as applied to invertebrates, where food is diluted in medium resulting in higher foraging costs per food reward. This approach contrasts with food availability experiments in vertebrates where food intake is restricted by providing less food (caloric restriction, CR), while food is unrestricted in DR experiments. CR and DR have very different effects on size and allocation of the energy budget (Carvalho et al. 2005; Wiersma et al. 2005), and to our best knowledge our experiment is the first study to investigate the effect of DR on ageing and lifespan in a vertebrate. DR as applied in our experiment did not extend lifespan, but it should be noted that we applied only two foraging cost levels, and therefore cannot rule out that other levels of foraging costs will have a different effect as previously found in invertebrates (Clancy et al. 2002).

Different predictions have been made with respect to the dependence of long-term fitness effects of developmental conditions on the environment experienced as adult. In a silver spoon scenario, individuals from good developmental conditions perform better than those from harsh developmental conditions (Grafen 1988) while according to the match-mismatch scenario individuals from harsh developmental conditions are better prepared to cope with similar challenges during adulthood (Bateson et al. 2004; Gluckman and Hanson 2004; Monaghan 2008; Hanson and Gluckman 2014). There is empirical support for both scenarios, and to some extent they are not mutually exclusive. Moreover, in practice one can only observe the net outcome of the different processes combined. Our results clearly point to the silver spoon hypothesis being the most important in our experiment, because individuals reared in small broods were less susceptible to an increase in foraging costs than those reared in large broods. This may at least in part be due to the fact that zebra finches, like other bird species, have determinate growth, which reduces the opportunity to adjust development to environmental conditions. The match-mismatch hypothesis was also tested using data of humans suffering famines, and also there the 'silver spoon' effect appeared to dominate the finding (Hayward et al. 2013). This lack of support for the match-mismatch hypothesis also fits the results of a taxonomically broad meta-analysis of experiments with similar designs to ours that measured a variety of traits (Uller et al. 2013) and with the results of (non-experimental) cohort studies in wild vertebrates (Douhard et al. 2014).

Different combinations of baseline mortality ('A') and actuarial senescence ('B') can result in the same lifespan (Fig. 3) and hence variation in lifespan can arise in different ways. How lifespan variation arises is of interest because it affects population demography, including for example, the proportion of old individuals in a population, and thereby the evolution of senescence and traits that are expressed late in life (Chantepie et al. 2015) and extinction risk (Robert et al. 2015). There is usually a negative association between the Gompertz parameters A and B among different populations of a species, known as the compensation law of mortality or Strehler-Mildvan correlation (Strehler and Mildvan 1960; Gavrilov and Gavrilova 2001; Simons et al. 2013), and we find a similar relation among the four groups in our experiment (Fig. 3). Figure 3 further shows that birds subjected to the HH treatment had shorter lifespan than birds subjected to the BH treatment due to a higher age-independent mortality rate, and despite a decrease in actuarial senescence. A difference in the same direction, but of considerably smaller magnitude, was found between birds in the HB and BB treatment groups. The negative effect of developmental conditions on lifespan was mediated via an immediate (vulnerability), rather than latent (actuarial senescence) mortality cost. This is interesting because the importance of developmental conditions for mortality

patterns in adulthood has not been fully resolved, even in humans (Kannisto et al. 1997; Doblhammer 2003; Finch and Crimmins 2004). Mortality rates of birds with different developmental backgrounds (B vs. H) converged to similar levels at high ages (Fig. 2), which is reminiscent of similar patterns in humans when comparing age dependent mortality rates between birth cohorts within a country or between developing and industrialized countries (Strehler and Mildvan 1960; Gavrilov and Gavrilova 1991; Edwards and Tuljapurkar 2005; Zheng et al. 2011; Beltrán-Sánchez et al. 2012; but see Yashin et al. 2002). Such a response can arise because of heterogeneity in population composition: in groups with high baseline mortality rate the more vulnerable individuals disappear at younger ages, leaving only less vulnerable individuals at old ages (Vaupel and Yashin 1985). It is noteworthy that while the developmental effects on life expectancy were reduced by benign conditions in adulthood, the effect on the standard deviation of age at death, a measure of lifespan inequality and population health (Edwards and Tuljapurkar 2005; van Raalte and Caswell 2013), was not (Table 2). Hence benign conditions in adulthood only partly mitigate effects of harsh developmental conditions.

Acknowledgements

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Supplementary information to:

Food availability affects adult survival trajectories depending on early developmental conditions

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Supplementary information S1: Effect of developmental conditions (brood size) on chick development and survival prior to the foraging cost manipulation

We used general linear models to analyze the effect of the brood size manipulation on chick mass during growth. Birth nest and rear nest were included as random effects. All analyses were done in R, v. 2.15.2 or later (R Core Team 2015) using the function 'lmer' in the package 'lme4' (version 1.1-7, Bates et al. 2015). Residuals were checked for normality and homogeneity of variance. Growing up in a large brood resulted in 1.4g (12%) lower mass at age 15 days, i.e. just before fledging (Fig. S1, N=477 X^2=71.4, p<0.0001) in agreement with earlier reports (de Kogel 1997; Holveck and Riebel 2010). Growing up in large broods shifted the whole distribution of chick weights downwards (Fig. S1) as shown by the similar standard deviations of 1.4 g for both groups. The difference in weight was due to differential growth, because at the time of the brood size manipulation there was no discernible difference in mass (N=523, X^2=1.00, p=0.27). At age 120 days (early adulthood), shortly before birds were housed in the experimental aviaries, individuals reared in large broods were 0.6g (4%) lighter than individuals reared in small broods (Fig. S1, N=508, X^2=15.1, p=0.0001). Standard deviations were similar for both groups (1.51 vs. 1.58 for small and large broods respectively). Thus growing up in poor developmental conditions impaired growth and this effect persisted into adulthood. Small and large broods thus reflect benign and harsh developmental conditions respectively.

We tested if manipulated brood size affected chick survival up to adulthood (3 months), including all manipulated chicks in the breeding batches from which birds were allocated to the foraging cost manipulation (n= 877 chicks in 293 nests). Of the 422 young reared in small broods, 21 (5.0 %) died before the age of 3 months. Of the 455 young reared in large broods, 41 (9.0%) died before the age of 3 months. Although there was a mortality difference in the expected direction, it is statistically far from being statistically significant (logistic regression: z=0.31, p=0.76). More importantly, the absolute difference is small, and we therefore consider it safe to assume that there was no bias from selective disappearance of individuals from large broods before the start of the foraging cost experiment during adulthood. Furthermore, the direction of the mortality difference is such that this will have decreased the difference in phenotypic quality between birds reared in small and large broods, making our statistical tests more conservative.

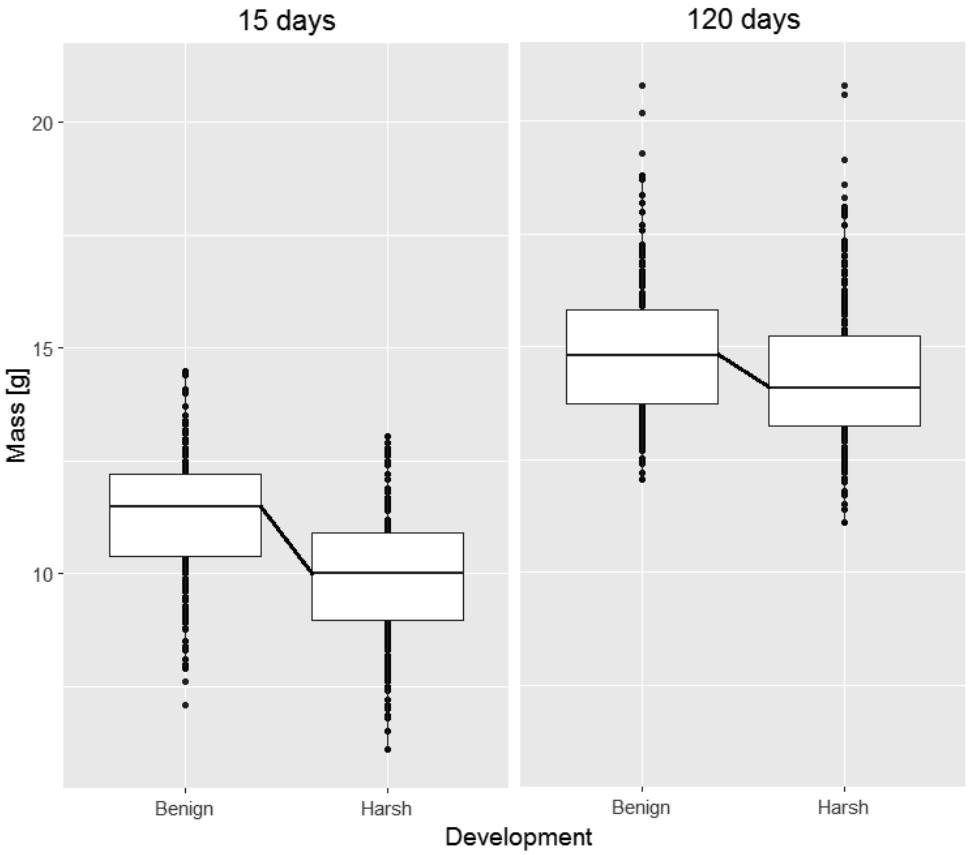

Fig. S1 Birds reared in large broods attained a lower mass as chicks (age 15 days, just before fledging,) and as young adult (age 120 days, i.e. just before the start of the manipulation of adult conditions, i.e. the foraging treatment). Boxplots show median, first and third quartiles and whiskers show 95% confidence interval.

Supplementary information S2: Cox proportional hazard analyses

To identify which covariates and/or random effects affected mortality in addition to the experimental treatments we performed survival analyses using the counting process formulation of the Cox proportional hazard (CPH) model (Cox 1972; Andersen et al. 1993; Therneau and Grambsch 2000). The counting process formulation allows the coefficient to be estimated at each time point and thus time-dependent covariates, such as age, can be included. Age was partitioned into 'starting age' and 'time in treatment', with day 1 for all birds being the day they started the foraging cost experiment, as advocated for randomized experiments (Fieberg and DelGiudice 2009). Survival was checked daily and as time base we therefore used daily intervals. Deaths that occurred due to accidents (N=7) and birds still alive were right-censored.

Analyses were done in R, v. 3.2.1 (R Core Team 2015) using the function 'coxme' in the package coxme (version 2.2-4; Therneau 2015). To find the model best supported by the data, we used the function 'dredge' of the package 'MuMIn' (Barton 2015). In brief, this is a hypothesis-based approach that generates, given a global model, subset models that best fit the data. This makes it possible to assess model support for each hypothesis. Model support is shown here by ranking all subset models within six AICc of the best model fit. CPH assumptions were checked for the best fitting models using scaled deviance and martingale residual plots (Cox 1972; Therneau and Grambsch 2000).

There was potential non-independence at several levels in our data set (shared birth nest, genetic mother, genetic father, rear nest, rear mother, rear father, birth batch and aviary), which we checked for by entering these factors as random effect. Note however that the experiment was balanced with respect to all these effects, except aviary, because adult treatment was varied at the aviary level. We therefore performed all analyses with aviary as random effect, and subsequently tested effects of all other potential random effects by adding these one at the time to the final model. Adding other random effects to the final model in no case improved model fit or otherwise altered the conclusions.

Table S1 Cox proportional hazard analyses of manipulations effects on lifespan. For birds from benign developmental conditions, there was little evidence that adult environment affected lifespan (Table S1A: ΔAICc=+0.7). In contrast, birds from harsh developmental conditions lived shorter in harsh than in benign adult environments (Table S1B: ΔAICc=-3.6). In the benign adult environment, the best fitting model did not include an effect of developmental conditions (Table S1C: ΔAICc≥+1.8). In the harsh adult environment, birds from benign developmental conditions lived longer than birds from harsh developmental conditions (Table S1D: ΔAICc=-10.9). The interaction between the developmental conditions and adult environment obtained moderate support (Table S1E: ΔAICc≥1.2). For table 1E, only models within 6 AICc of the best fitting model are shown. Values indicate model coefficients and are missing when the term was excluded from the model.

Note that these are Cox proportional hazards models and model coefficients are therefore hazard ratios relative to a baseline hazard, which always is a benign group. A hazard ratio of one implies no effect and for example a hazard ratio of 1.37 for *Devel.* (manipulation during development) means that the hazard rate increases with 37% between benign and harsh developmental conditions. Note that there is no main effect *Age* since it is included in the baseline mortality curve. All models included aviary as random effect. Results indicating how best to include *AgeStart* can be found in Table S2. Abbreviations: Devel.: Developmental conditions (i.e brood size manipulation); Adult: adult conditions (i.e. foraging cost manipulation); AgeStart: age at start of the foraging treatment. Interaction terms are indicated by *.

Table S1A Benign Devel. Model	Experimental manipulations			Age associated covariates [Year]				df	AICc	ΔAICc	weight
	Adult [Harsh]			AgeStart	AgeStart * Age		Adult *Age				
1				1.54	1.02			3	1824.1	0.00	0.49
2	0.92			1.55	1.02			4	1824.7	0.67	0.35
3	0.81			1.53	1.02		1.05	5	1826.4	2.34	0.15
Table S1B Harsh Devel. Model	**Experimental manipulations**			**Age associated covariates [Year]**				**df**	**AICc**	**ΔAICc**	**weight**
	Adult [Harsh]			**AgeStart**	**AgeStart * Age**		**Adult *Age**				
1	1.35			1.03	1.13			4	2212.5	0.00	0.62
2	1.51			1.03	1.12		0.95	5	2214.1	1.62	0.28
3				1.03	1.13			3	2216.1	3.61	0.10
Table S1C Benign Adult Model	**Experimental manipulations**			**Age associated covariates [Year]**				**df**	**AICc**	**ΔAICc**	**weight**
	Devel. [Harsh]			**AgeStart**	**AgeStart *Age**	**Devel. *Age**					
1				1.39	1.00			3	1940.1	0.00	0.56
2	1.37			1.36	1.01	0.88		5	1941.9	1.80	0.23
3	0.99			1.39	1.00			4	1942.1	1.99	0.21
Table S1D Harsh Adult Model	**Experimental manipulations**			**Age associated covariates [Year]**				**df**	**AICc**	**ΔAICc**	**weight**
	Devel. [Harsh]			**AgeStart**	**AgeStart *Age**	**Devel. *Age**					
1	2.53			0.97	1.23	0.79		5	2085.5	0.00	0.94
2	1.48			1.00	1.20			4	2091.2	5.68	0.06
3				1.03	1.21			3	2096.4	10.87	0.00
Table S1E All data Model	**Experimental manipulations**			**Age associated covariates [Year]**				**df**	**AICc**	**ΔAICc**	**weight**
	Devel. [Harsh]	**Adult [Harsh]**	**Devel. *Adult**	**AgeStart**	**AgeStart *Age**	**Devel. *Age**	**Adult *Age**				
1	1.56	0.91	1.48	1.17	1.09	0.83		9	4551.4	0.00	0.39
2	1.95			1.18	1.09	0.82		8	4552.6	1.22	0.21
3	1.95	1.13		1.17	1.09	0.82		8	4553.0	1.63	0.17
4	1.56	0.92	1.48	1.17	1.09	0.83	1.00	10	4553.4	1.99	0.14
5	1.95	1.18		1.18	1.08	0.82	0.98	9	4554.9	3.50	0.07

Table S2 Cox proportional hazard analyses to determine how to account for age at start of the experiment. The model best fitting the data did not include experiment-specific *AgeStart* effects. In contrast, model support for including *AgeStart* was strong, since excluding *AgeStart* gave the worst possible model (ΔAICc =14.73; model 11). *Age* and *AgeStart* variables are per year. Further table specifications as in Table S1.

Model	AgeStart effects [Year]					Experimental manipulations				df	AICc	ΔAICc	weight
	AgeStart	AgeStart *Devel.	AgeStart *Adult	AgeStart *Devel.*Adult *Treat	AgeStart *Age	Devel. [Harsh]	Adult [Harsh]	Devel. *Adult	Devel. *Age				
1	1.17				1.09	1.56	0.91	1.48	0.83	9	4551.4	0.00	0.27
2	1.42					1.53	0.90	1.49	0.84	8	4552.3	0.87	0.18
3	1.28	0.88			1.08	1.79	0.92	1.48	0.83	10	4552.8	1.42	0.13
4	1.12		1.08		1.09	1.57	0.84	1.47	0.83	10	4553.2	1.82	0.11
5	1.58	0.84				1.86	0.90	1.48	0.84	9	4553.3	1.84	0.11
6	1.38		1.06			1.53	0.85	1.48	0.84	9	4554.2	2.78	0.07
7	1.23	0.87	1.09		1.08	1.81	0.84	1.46	0.83	11	4554.6	3.19	0.06
8	1.53	0.84	1.07			1.87	0.84	1.47	0.84	10	4555.1	3.69	0.04
9	1.18	0.94	1.18	0.87	1.08	1.68	0.77	1.71	0.83	12	4556.5	5.08	0.02
10	1.46	0.90	1.17	0.86		1.73	0.76	1.73	0.84	11	4557	5.55	0.02
11						1.55	0.87	1.54	0.85	6	4566.1	14.73	0.00

Table S3 Cox proportional hazards analyses to show that sex dependent survival is independent of experimental manipulations. There was considerable model support for female biased mortality in the six best fitting models and model fit deteriorated when sex was not included (ΔAICc =3.1; model 7). Yet, the female biased mortality seems most pronounced at older ages since the 3 best fitting models also include a sex*age interaction (ΔAICc ≥1.8; model 4). In contrast, the model support sex-specific manipulation effects was weak: models 1 (best fitting) and 4 do not include interactions between sex and experimental manipulations. All '*Age*' and '*AgeStart*' terms are per year. Further table specifications as in Table S1.

Model	Sex specific effects [Male]					Experimental manipulations				AgeStart effects		df	AICc	ΔAICc	weight
	Sex [Male]	Sex *Devel.	Sex *Adult	Sex *Devel.*Adult	Sex *Age	Devel. [Harsh]	Adult [Harsh]	Devel. *Adult	Devel. *Age	AgeStart	AgeStart *Age				
1	1.04				0.89	1.51	0.88	1.51	0.85	1.15	1.09	9	4548.3	0.00	0.27
2	1.19	0.81			0.88	1.68	0.89	1.50	0.85	1.14	1.10	10	4549.3	1.03	0.16
3	1.02		1.03		0.89	1.51	0.87	1.51	0.85	1.15	1.09	10	4549.7	1.42	0.14
4	0.78					1.53	0.90	1.50	0.84	1.17	1.08	8	4550.1	1.83	0.11
5	1.16	0.81	1.04		0.88	1.68	0.88	1.50	0.85	1.14	1.10	11	4550.7	2.40	0.08
6	0.76		1.05			1.53	0.88	1.50	0.84	1.17	1.08	9	4551.4	3.12	0.06
7						1.56	0.91	1.48	0.83	1.17	1.09	9	4551.4	3.13	0.06
8	0.84	0.88				1.62	0.91	1.49	0.84	1.17	1.08	9	4551.7	3.42	0.05
9	1.10	0.92	1.19	0.79	0.88	1.58	0.82	1.68	0.85	1.14	1.10	12	4552.4	4.09	0.04
10	0.82	0.87	1.06			1.62	0.88	1.49	0.84	1.17	1.08	10	4552.9	4.67	0.03
11	0.77	0.97	1.18	0.81		1.55	0.83	1.65	0.84	1.17	1.08	11	4554.7	6.45	0.01

Supplementary information S3: Comparison of parametric mortality model fits

Table S4 Model selection results for parametric model fits using maximum likelihood approach of the R package fitdistrplus (Delignette-Muller and Dutang 2015). Shown numbers are AIC values (Akaike's 'An Information Criterion' (Burnham and Anderson 2002)). Results in bold are best fits. Multiple 'best fits' indicate that these models fit approximately equally well (ΔAIC<2; Burnham and Anderson 2002). Consistent with Bayesian methods, the Gompertz function fitted the data better than the exponential function.

Fitted model	**Hazard trend**	**Experimental Group**			
		BB	**HB**	**BH**	**HH**
Exponential	constant hazard	322.3	320.5	295.1	**338.2**
Weibull	monotonic slope	311.7	**317.7**	294.5	340.1
Gompertz	exponential slope	**305.1**	**315.9**	**285.8**	**337.7**
Gompertz-Makeham	exponential slope with 'extrinsic' term	**304.5**	318.0	**286.2**	**337.4**

Supplementary information S4: Gompertz fits with R package BaSTA

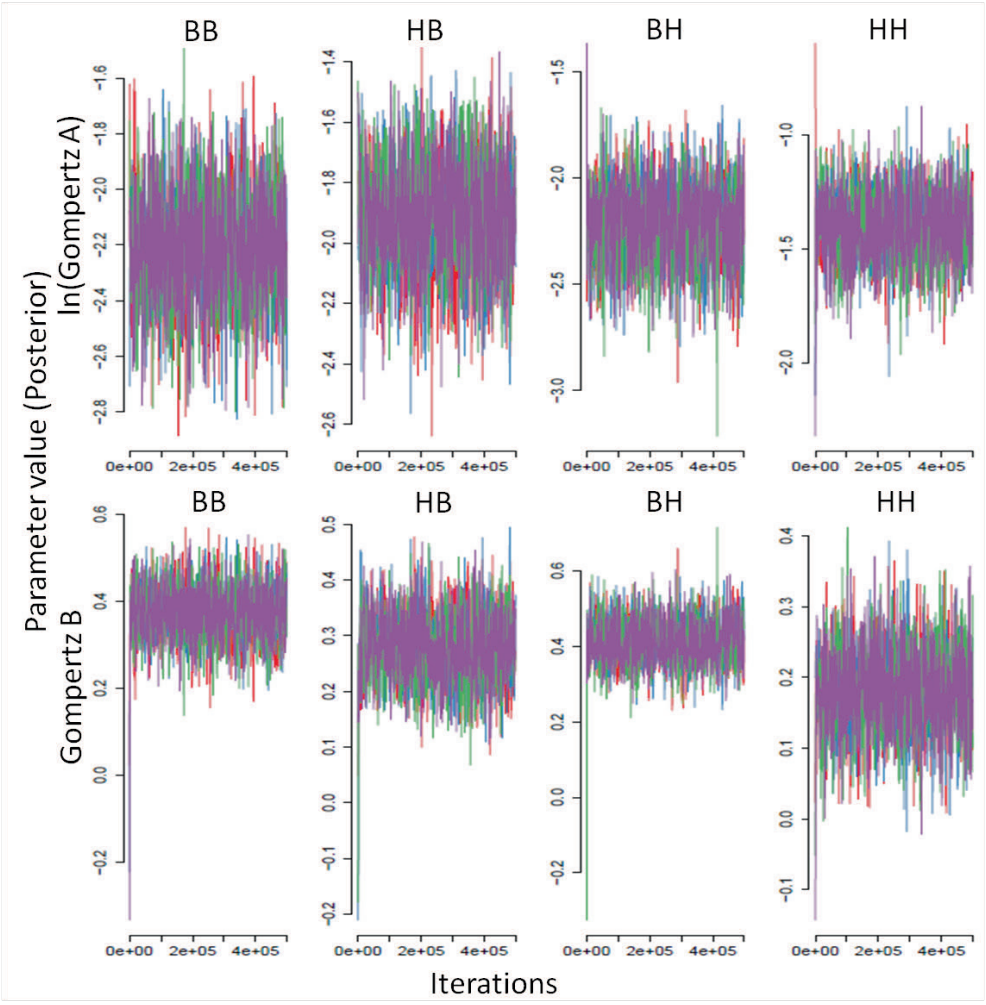

Fig. S2 Parameter trace plot of the MCMC optimization for Gompertz fits with BaSTA as in Fig 2. Settings were 4 parallel runs with 500.000 iterations, 100.000 burn in period and a thinning of 1000. Abbreviations: B: Benign and H: Harsh, in chronological order such that e.g. the HB group indicates harsh developmental followed by benign adult conditions. Note variation in Y-axes between panels.

Supplementary information S5: Comparison of survival and mortality of the four experimental groups

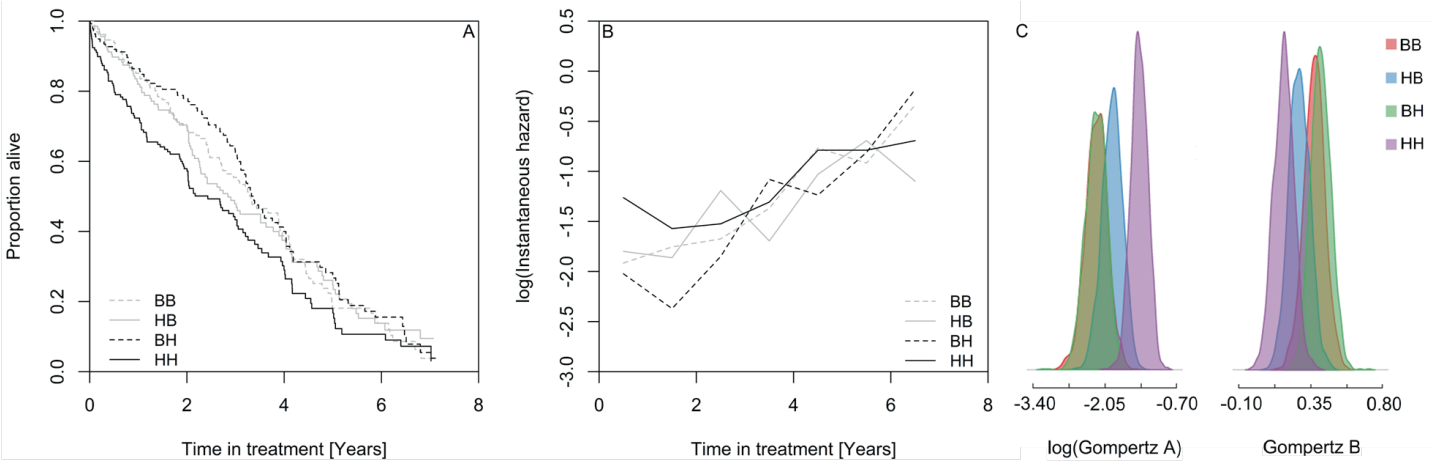

Fig. S3 Survival (A) and mortality (B) trajectories of the 4 experimental groups show that the HH group differs most from all other groups. Grey lines represent the benign adult environment, black lines the harsh adult environment, full and dotted lines show the benign and harsh developmental conditions respectively. Group abbreviations: B: benign conditions and H: harsh conditions, in chronological order, such that e.g. the BH group indicate benign developmental followed by harsh adult conditions.

Supplementary information S6: Sex-specific mortality trajectories

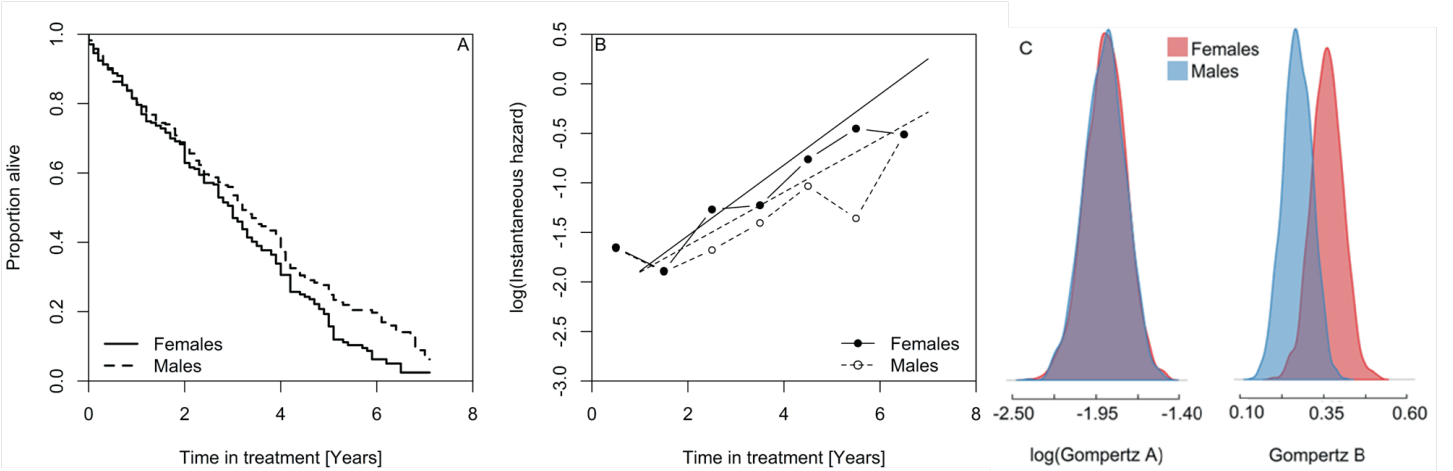

Fig. S4 Survival curve and instantaneous mortality rate in relation to sex. (A) Proportion of birds surviving since entering the adult treatment. (B) Instantaneous mortality rate as a function of time in treatment. In panel (B), dots represent mortality data and lines show Gompertz fits. (C) Posterior distributions of Gompertz parameters, showing that the sexes have very similar age independent mortality rate (Gompertz A; KLD=0.50) but that the rate of actuarial senescence is higher in females (Gompertz B; KLD=0.92).

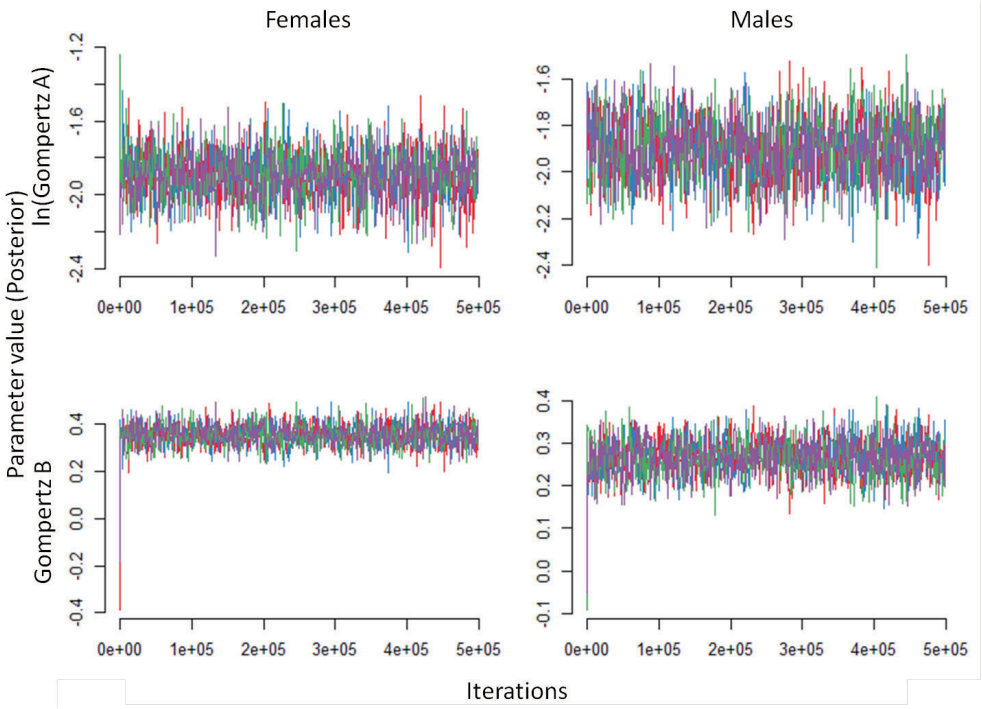

Fig. S5 Parameter trace plot of the MCMC optimization for Gompertz fits with BaSTA as reported in Fig. S4. Settings were 4 parallel runs with 500.000 iterations, 100.000 burn in period and a thinning of 1000.

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Chapter 4

Increased foraging costs impair reproduction and offspring development

Michael Briga & Simon Verhulst

Abstract

Foraging costs can be a major determinant of reproduction and offspring development. A previous study showed that zebra finches subject to experimentally increased foraging costs did not reproduce in winter. Here we investigate whether the birds facing this 'hard treatment' manage to reproduce in spring and how this treatment affects offspring development. We find that birds in the hard treatment manage to reproduce, but that they tend to start later, have smaller clutches and smaller broods relative to control birds in the 'easy treatment' that have cost free food. Increased foraging costs extended the duration of parental care at least twofold, but decreased offspring post-fledging development and survival. Thus high foraging costs decrease reproduction, induce seasonality in reproductive behavior and extend parental care. We discuss the relevance of foraging costs in the context of optimal parental care strategies and developmental plasticity.

Introduction

Environmental quality is crucial for population conservation. A major determinant of environmental quality is food abundance, which can have major effects on reproduction (Martin 1987; Boutin 1990; Prevedello et al. 2013; Ruffino et al. 2014) and on offspring development (e.g. Lindström 1999; Van de Pol et al. 2006; Griffith and Buchanan 2010). In captivity, such manipulations are often performed by decreasing food intake or food quality. In free living animals however, decreased food abundance often implies that an animal has to perform more effort per item food reward, i.e. food abundance affects foraging costs. The effects of increased foraging costs can be different from the effects of decreased food intake or food quality per se. For example, high foraging costs can decrease, but also increase an individual's energy consumption (reviewed in: Wiersma and Verhulst 2005) and induces physiological changes that are distinct from those of decreased food intake (Schubert et al. 2008). Thus, experimental manipulations of food abundance in captivity will benefit from manipulating foraging costs rather than food intake per se.

A few studies have investigated the effects of increased foraging costs on reproduction. In zebra finches, a mild manipulation of foraging costs by increasing chaff seed ratio revealed only a negative effect on egg laying interval (Wiersma and Verhulst 2005), but a previous study found also impaired offspring production (Lemon and Barth 1992). In mice, increased running wheel activity negatively affected litter mass (Schubert et al. 2009). We thus expect that increased foraging costs will negatively affect reproduction and perhaps offspring development.

For the foraging cost manipulation as described in Koetsier and Verhulst (2011), the effects on reproduction remain unknown. Briefly, this foraging cost manipulation is characterized by birds having to hover for food. Such a manipulation is different from earlier manipulations in zebra finches. For example, it considerably decreases body mass (Chapter 11), which was not the case for chaff/seed ratio manipulations (Lemon and Barth 1992; Wiersma and Verhulst 2005). Our previous study showed that zebra finches hovering for food skip reproduction in winter (Simons et al. 2014). This result suggests that birds facing high foraging costs may not be able to reproduce or, alternatively, that they constrain their reproduction to more favorable seasons. Here we investigate the effects of increased foraging costs on reproduction and offspring development by exposing birds to high foraging costs as in (Simons et al. 2014) in spring and quantifying the effects on latency of egg laying, clutch size, number of hatchlings, chick development and chick survival till early adulthood.

Material and Methods

Animals for this experiment were all coming from the experiment in (Simons et al. 2014). Briefly, all birds still alive after the experiment (N= 51 males and 53 females) were reintroduced in the aviary and foraging treatment they had been trained in before. Detailed information about the housing conditions and about foraging manipulation technique can be found in Simons et al. (2014) and in Koetsier and Verhulst (2011) respectively. Briefly, 60 males and 60 females (5 months<age<19 months) were randomly selected and housed in 4 outdoor aviaries (LxHxW, 310x210x150cm) in which tropical seed mix, cuttlebone, water, sand and grit were provided *ad libitum*. In each aviary, a food box was hanging from the ceiling. In the low cost treatment, the foodbox had perches. In the hard foraging treatment those perches were absent and birds had to fly from a distant perch, to the food box, hover to get the seed and fly back to distant perch to eat it. Spilled seeds were collected by a reception device birds could not access. Each aviary contained nest boxes (15 per aviary). Birds in both treatments build nests with *ad libitum* provided hay.

For unknown reasons, birds that were reintroduced in the hard treatment experienced mortality (N= 4 males and 9 females). Such a phenomenon may affect results for two reasons. The first reason is that the densities in hard treatment aviaries become lower than in the easy treatment. We solved this by randomly moving birds from the easy treatment aviaries to the hard treatment aviaries such that there were equal numbers of birds in each treatment. A second reason is that such mortality can create a bias in quality for the hard treatment, because the weakest birds died leaving only higher quality individuals. This scenario is not a problem for the interpretation of the results since we expect birds from the hard treatment to perform worse than birds from the easy treatment. Therefore, if selective disappearance is an issue, the actual treatment effect would only be larger those observed here. Alternatively, and less likely is that the birds that performed the best breeding in the experiment of Simons et al. (2014) died. This is not the case: clutch size of birds that died showed no difference with that of birds that remained alive (mean ± SD dead birds=1.17 ±1.28 eggs; live birds=1.43 ± 1.28 eggs; $F=0.43$, $p=0.51$). Therefore, we can conclude that the mortality of birds in the hard treatment did not create a bias in the reproductive traits between treatments.

Birds were allowed to breed from March 11th 2013 until May 27th 2013. Nests were checked twice a week. Chicks were ringed and first weighed before fledging, approximately at the age of 15 days, followed by regular weighing till moving at the mean age of 61 days (SD: 16 days) to juvenile aviaries, where food was cost free and in which birds that originated

from easy and hard treatments were mixed. Young moves were always paired for both treatments.

All analyses were done in R 3.0.2 (R Core Team 2013). Latency till egg laying and hatching are 'time to event data'. We therefore analyzed them with Cox proportional hazards (CPH) using the function 'coxme' in the package coxme (Therneau 2012) and the function 'coxph' in the package survival (Therneau 2013). Aviary was included as random effect, but this model fitted the data as well as a model without random effect ($0<\Delta AIC\leq+2$) and did not alter the conclusions regarding the fixed effects. Proportionality assumption was checked with the function cox.zph and using scaled deviance and martingale residual plots, but there were no violations. For comparing means of clutch size and number of hatchlings between groups we used non-parametric tests with the function 'Wilcox.test'.

Analyses of chick survival are also time to event data, and thus were done with CPH. CPH models assume that manipulation effects are proportional over time. This was not the case for survival data (Fig. 3). This can be solved by using the counting process formulation, which allows to include time-dependent covariates (Andersen et al. 1993; Therneau and Grambsch 2000). Several random effects can be included, namely aviary, birth nest and nest location (both nested in the aviary). We tested all these possibilities and the best fitting model is one with only birth nest as random effect ($\Delta AIC<-25$) and we thus report results of this model. In all analyses birds still alive on December 31st 2013 and one accidental death were censored.

Body mass analyses were done with linear mixed effect models using the function 'lmer' in the package lme4 (Bates et al. 2015). For this analysis, three random effects may be of importance: individual (individuals were weighted multiple times), birth nest (individuals can be siblings) and aviary (each aviary contains multiple nests). Some of these random effects can be nested within each other, e.g. individuals within nest and nest within aviary. We tested all these combinations of random effects and compared their model fit based on AIC. The best fitting model was one with two random effects, individual and birth nest, that were not nested ($\Delta AIC<-6.9$). Model residuals were checked with the function 'resid' and were normally distributed without any evidence for outliers.

Results

We here follow the reproduction of birds in both treatment groups. Birds in both treatments readily started laying. The high foraging cost group delayed the onset of laying with on average 9 days (Fig. 1A), but this difference was not significant (CPH $X^2(1)=2.84$; $p=0.09$). Laying was successful with 35 nests having a total of 142 eggs. There were more nests with eggs in the easy than in the hard treatment (26 vs. 24 respectively) and their clutches were significantly larger (median: 4 vs. 5 respectively; Fig. 2; Wilcoxon signed rank test $W=570$; $p=0.036$). Thus, high foraging costs had a minor negative effect on the onset of laying, but significantly decreased clutch size with 1 egg (20%) on average.

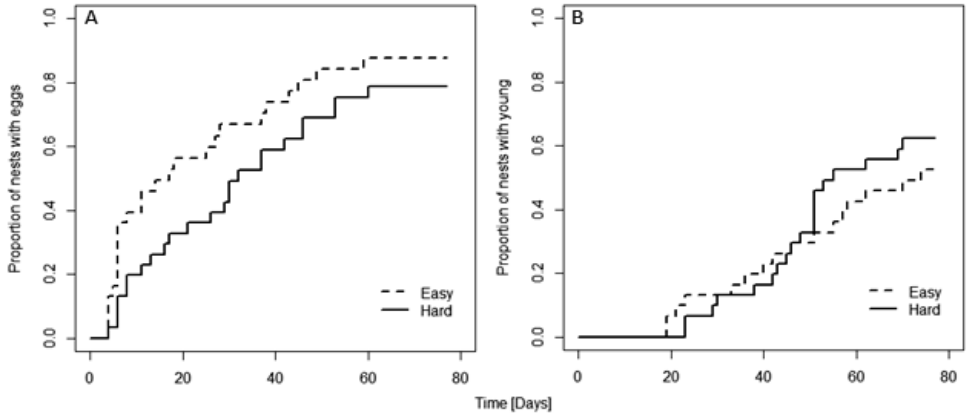

Fig. 1 Birds in the easy treatment start more nests and sooner than birds in the hard treatment (A). In contrast, both treatments had young at the same time (B).

There was virtually no difference in time till hatching between both treatments (mean 44 days for both groups; Fig. 1; CPH $X^2(1)=0.51$; $p=0.47$). Contrary to the expectation, we found more nests in the hard than in the easy treatment (19 vs. 16 respectively; Fig. 1B), but their brood size was reduced with one chick with a median of three and four young respectively (Fig. 2; Wilcoxon signed rank test $W=207.5$; $p=0.025$). Thus, high foraging costs had no effect on time till hatching but decreased the brood size of breeding parents with one chick (25%) on average.

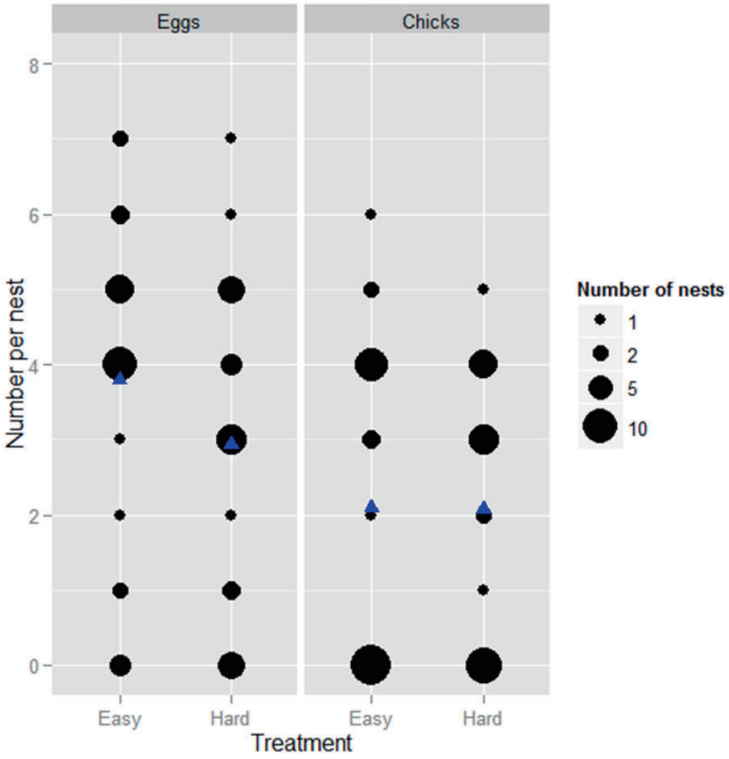

Fig. 2 Number of eggs (left) and young (right) per nest in easy and hard foraging treatments. Bubble area indicates the number of nests per dot. Blue triangles represent means per experimental group.

High foraging costs may negatively affect chick growth. When chicks were 15 days old, we found no difference in mass between chicks from the easy vs. harsh treatment (Fig. 3; N=105; F=0.035; p=0.85). At 35 days, young from the hard treatment were 0.73g (6.8%) lighter than young from the easy treatment, but this difference was not significant (Fig. 3; N= 83; F=3.43; p=0.19). At the age of 50 days, young from the hard treatment were on average 1.6g (11%) lighter than young from the easy treatment and this difference was significant (Fig. 3; N= 50; F=5.43; p=0.029). The interaction between age and treatment is significant (F=4.49; p=0.013). Thus, high foraging costs negatively affected young development during later growth.

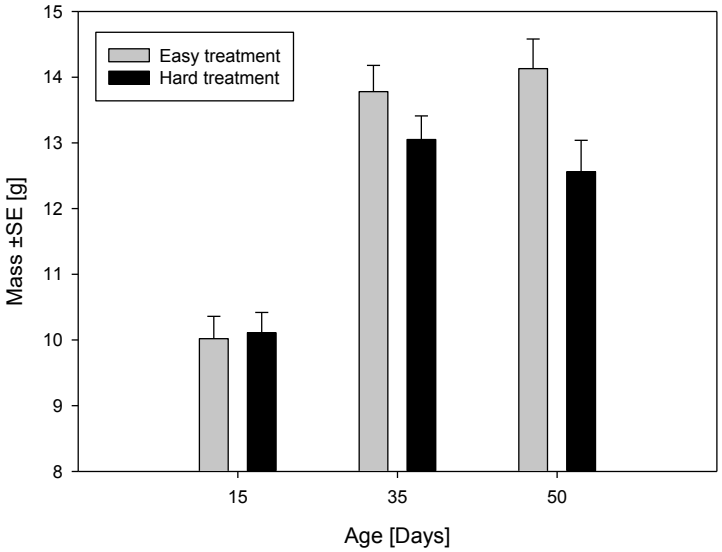

Fig. 3 High foraging costs impair post-fledging chick growth. The age of 15 days is just before fledging. At 35 days young feed independently under standard breeding procedures. The age of 50 days is the last age at which young were observed to be fed by parents in this experiment.

Overall, chick survival was overall low: 60% of the young reached the age of 3 months. For comparison, indoor breeding survival rates at the same age are as high as 91% (Chapter 3). This low survival may be particularly pronounced in the hard treatment. Indeed, young in the hard treatment experienced lower survival than young in the easy treatment (Fig. 4A; Table 1; $z=3.84$; $p=1.2E-04$) and this difference increased with age (Fig. 4A; Table 1; $z=3.68$; $p=2.3E-04$). In contrast, after moving to the cost free juvenile cages survival patterns were reversed (Fig. 4B; Table 1; $z=-4.11$; $p=3.9E-05$) and young from the hard treatment survived better relative to young from the easy treatment (post move data only: $z=-2.05$; $p=0.04$). Overall though, young from the easy treatment survived better than those of the hard treatment. Thus, young that grew up under high foraging costs experienced higher mortality, but this effect decreased when young moved to a low cost foraging environment.

To better understand the association between foraging costs and chick survival, we observed the foraging behaviour of young. Eighteen young were observed between the ages of 31 days till 57 days. We therefore observed 11 young in the hard treatment for a total of six hours. We observed a total of 62 feeding bouts. Of those, young fed independently only 3 times (5%), while adults were observed feeding these young 59 times (95%). In contrast, during more than three hours of observation of 7 young in

the easy treatment, there were 34 feeding observations, of which young fed 22 times independently (65%) while adults fed them on only 12 occasions (35%). Thus, increased foraging costs prolonged the dependency of young on parental feeding.

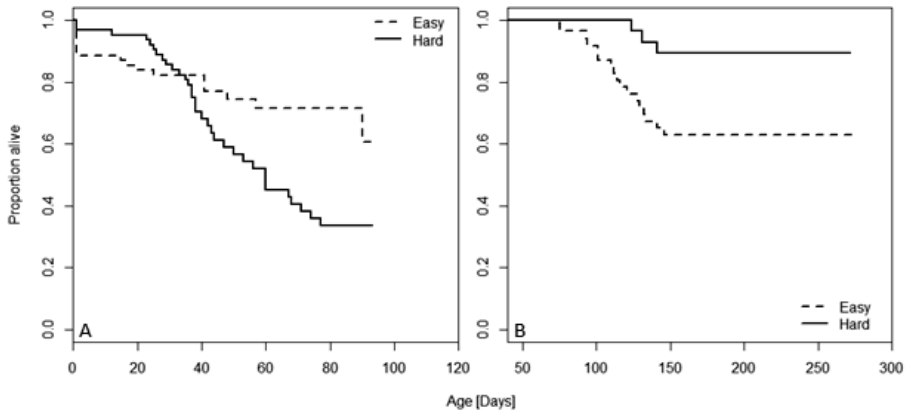

Fig. 4 High foraging costs decreased young survival (A) until the move to the cost-free-food aviaries (B), where the opposite pattern was observed.

Table 1 High foraging costs decrease young survival, an effect which increases with age. Note that is is a CPH analysis and coefficients are thus hazard ratios. Age is in days. Note that there is no main effect age since it is included in the baseline mortality curve. For. treat : foraging treatment. Move juv.: Move to juvenile aviaries.

	coef (SE)	exp(coef)	z	p
Foraging treatment [Hard]	5.00 (1.30)	148	3.84	1.20E-04
Move juv. [After]	-0.82 (0.95)	0.44	-0.87	0.39
For. treat * Age	0.054 (0.01)	1.06	3.68	2.30E-04
For. treat * Move juv.	-6.56 (1.60)	0.0014	-4.11	3.90E-05
Rejected terms				
For. treat * Age2	-0.00029	1.00	-1.21	0.22
Age * Move juv.	0.010	1.01	0.23	0.82
Random effects: σ^2_{nest} =2.52				

Discussion

In this study, we found that high foraging costs impair reproduction and offspring development. These results expand previous results of Simons et al. (2014), where birds facing high (but not low) foraging costs skipped egg laying. A likely explanation for this difference is that present study was conducted in spring while the study of Simons et al. (2014) was conducted in winter. Taken together, these results suggest that increased foraging costs induce seasonality in reproduction.

In our experiment, high foraging costs delayed the independence of young and prolonged the duration parental care. The duration of the prolongation is uncommon for zebra finches: previous manipulations expanded the parental care window with up to seven days (Rehling et al. 2012), while the adjustment shown here lasted at least 30 days, which is more than twice the natural duration of parental care in zebra finches in captivity (Rehling et al. 2012). Large prolongations of parental feeding have been described before, also in free living animals, as a strategy to cope with food shortage or to learn the required foraging skills (e.g. Burger 1980; Hunt et al. 2012). From a parent offspring-conflict theory perspective (Trivers 1974) alternative responses, such as brood desertion (e.g. Tveraa et al. 1997), are possible. Zebra finches in our experiment choose to prolong parental care. However, results on young growth and survival (Figs. 3 and 4) indicate that parents do not fully compensate for offspring needs. Thus the foraging cost manipulation applied here can be a useful tool to investigate predictions of theory on parental investment (Webb et al. 2002), including conflicts between parents (Houston et al. 2005) and parent-offspring conflict (Trivers 1974).

High foraging cost decrease offspring survival (Fig. 4A). However, this effect is reversed once birds move to a foraging cost free environment (Fig. 4B). This suggests that when going the juvenile aviaries young from the hard treatment are on average from better quality. This phenomenon can arise because the higher mortality in the hard treatment before moving wipes out the weakest individuals leaving on average individuals of better quality. This phenomenon can cause a compensation response in mortality (Vaupel 1979; Vaupel and Yashin 1985; Aalen 1988; Gavrilov and Gavrilova 2001; Zens and Peart 2003) which we also have found for the adults in our foraging cost experiment (Chapter 3). It is worth noting in this context that before fledging, young of both treatments have similar mass and survival (Figs. 3 and 4), indicating that on average young from both treatment groups may start off being of similar 'quality'. Thus, at later ages birds of the hard treatment can be on average of better quality than birds from the easy treatment due to selective disappearance of the weakest individuals.

The foraging cost manipulation can provide a useful method to investigate the consequences of poor environmental conditions during development. It impairs growth, in particular the later growth phase, and thus may constrain catch-up growth, which can have long lasting negative effects during adulthood (Metcalfe and Monaghan 2001; Criscuolo et al. 2008; Lee et al. 2013; Lee et al. 2015). A downside of this manipulation is the higher mortality of young in the high foraging cost treatment. This issue must be avoided when studying the effects of poor developmental conditions on adult physiology and survival.

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Chapter 5

The heuristic value of redundancy models of aging

Jelle J. Boonekamp, Michael Briga & Simon Verhulst

Experimental Gerontology 71, 95-102

Abstract

Molecular studies of aging aim to unravel the cause(s) of aging bottom-up, but linking these mechanisms to organismal level processes remains a challenge. We propose that complementary top-down data-directed modelling of organismal level empirical findings may contribute to developing these links. To this end, we explore the heuristic value of redundancy models of aging to develop a deeper insight into the mechanisms causing variation in senescence and lifespan. We start by showing (i) how different redundancy model parameters affect projected aging and mortality, and (ii) how variation in redundancy model parameters relates to variation in parameters of the Gompertz equation. Lifestyle changes or medical interventions during life can modify mortality rate, and we investigate (iii) how interventions that change specific redundancy parameters within the model affect subsequent mortality and actuarial senescence. Lastly, as an example of data-directed modelling and the insights that can be gained from this, (iv) we fit a redundancy model to mortality patterns observed by Mair et al. (2003; Science 301: 1731-1733) in *Drosophila* that were subjected to dietary restriction and temperature manipulations. Mair et al. found that dietary restriction instantaneously reduced mortality rate without affecting aging, while temperature manipulations had more transient effects on mortality rate and did affect aging. We show that after adjusting model parameters the redundancy model describes both effects well, and a comparison of the parameter values yields a deeper insight in the mechanisms causing these contrasting effects. We see replacement of the redundancy model parameters by more detailed sub-models of these parameters as a next step in linking demographic patterns to underlying molecular mechanisms.

1. Introduction

Unravelling the cause(s) of aging is often approached bottom-up, through the study of molecular mechanisms linked to lifespan. However, a full understanding of the aging process requires that these molecular mechanisms be linked up to the organismal level, which is challenging due to the involvement of multiple complex interacting processes (Chauhan et al. 2015; Kirkwood 2011; Kriete et al. 2010; Mc Auley et al. 2015). We propose that complementary top-down theoretical work in the form of data-directed modelling may contribute to this daunting task, and in this paper we discuss the potential of a specific form of reliability theory, redundancy models, to achieve this goal.

Many species across the tree of life, including humans, show an initial exponential increase of mortality rate with age and this feature of aging is well described by the Gompertz equation (Gompertz 1825). As a result, mortality is often modelled using the Gompertz equation, partitioning mortality into an age-independent (baseline) and age-dependent (aging rate) component. We acknowledge the value of this approach, and have applied it ourselves, because it allows more detailed conclusions regarding the effects of, for example, specific experimental interventions that modulate lifespan by investigating which Gompertz parameter is affected by the intervention (e.g. Boonekamp et al. 2014b; Gems et al. 2002; Pietrzak et al. 2015; Pletcher et al. 2000; Simons et al. 2013). On the other hand, the Gompertz equation also has limitations. Firstly, the exponential increase in mortality rate with age is not universal (Abrams and Ludwig, 1995; Jones et al., 2014). For example, in several species, including humans, the increase of mortality rate with age decelerates at older ages, leading eventually to late-life mortality plateaus (Carey et al. 1992; Gavrilov and Gavrilova 1991) that cannot be described with the Gompertz equation. Secondly, and perhaps more importantly in the present context, for a top-down theoretical investigation of aging mechanisms it is a necessity that mortality patterns emerge from the model, as opposed to being specified by the model. Thus the top-down approach to unravel aging mechanisms requires a mechanistic model.

Building on the work of Gavrilov and Gavrilova (1991, 2001), we explored the heuristic value of redundancy models in the investigation of aging mechanisms. Previous studies have used redundancy models primarily as a means to describe mortality patterns, and hence as an alternative to the Gompertz equation (Gavrilov and Gavrilova 2001; 1991; Milne 2008; Vural et al. 2014). We employ the same model structure in a different way, namely as a simple mechanistic model.

It has previously been argued that aging models should fulfill a number of criteria (Strehler and Mildvan 1960) to clarify the mechanisms which determine the lifespan of organisms (Gavrilov and Gavrilova 1991) and these criteria have been discussed in detail with respect to the redundancy model (Gavrilov and Gavrilova 1991). We will not re-iterate this discussion here, except to note that the redundancy model is flexible, and hence can be made to fit very different mortality patterns with age, including late-life mortality plateaus. Although, redundancy models have been qualitatively investigated (Gavrilov and Gavrilova 2001; 1991; Li and Anderson 2009; Milne 2008; Vural et al. 2014), they have rarely been fitted to empirical data (Vural et al. 2014) and, to our knowledge, redundancy model performance has not been formally compared to other models. Here, we quantitatively explore the explanatory power of redundancy models of aging. We show that redundancy models fit the data well and argue that this is a strength of redundancy models over non-mechanistic models because (i) when contrasting aging patterns can be understood within the framework of a single mechanistic model this indicates that the model may capture the essence of the aging process, and (ii) redundancy parameter inference may teach us something about the underlying mechanisms and can as such be used to develop new hypotheses.

2. The redundancy model

Redundancy models are based on reliability theory (Barlow et al. 1965) and assume that organisms consist of one or more blocks, that are each composed of one or more (redundancy) elements. Elements do not age themselves but fail over time with a constant rate due to damage. Blocks keep on functioning until the last remaining element fails and Gavrilov and Gavrilova (2001) showed that aging (i.e. an increase of mortality rate with age) emerges from the model due to redundancy exhaustion. Thus, redundancy in the present context indicates extra capacity to absorb damage, which can be seen as a form of organismal resilience. The organism dies when the *first* block fails, i.e when the last remaining element within *any* block fails. Blocks and redundancy elements are abstract concepts, but one can think of blocks as sets of tissues or cells, or possibly an organ, that is vital for survival. Redundancy elements can be thought of as the cells within an organ, or critical functions of cells, where damage to cells do not lead to the organ's failure until a certain threshold is reached (in the context of the model, the moment the last redundancy element fails). Element failure rate is usually assumed to be constant over time and equal across blocks, and we adopt this assumption. It would be a logical extension to let element failure rate depend on other variables such as for example the number of remaining redundancy elements, but such extensions of the redundancy model fall outside the scope of the present paper.

Under these assumptions and following eq. 9 in the paper by Gavrilov and Gavrilova (2001) the mortality rate of the organism can be specified by:

$$\mu(x) = mknqce^{-nq}e^{-kx}\sum_{i=1}^{n}\frac{nq^{i-1}(1-e^{-kx})^{i-1}}{(i-1)!\,(1-(1-e^{-kx})^{i})} \qquad (1)$$

where (m) is the number of blocks containing (n) redundancy elements that become damaged at rate (k) (i.e. in each time step a proportion (k) of the remaining redundancy elements is damaged), and c is a normalizing factor determined by (n) and (q) (see SI–1 for details). Additionally, it is assumed that not all redundancy elements are intact at maturation, i.e. all elements have a probability (q) to be damaged from the time point that mortality is studied. The initial number of redundancy elements within blocks is given by a Poisson distribution $\lambda = nq$. Gavrilov and Gavrilova (2001) gave as biological interpretation of (q) the accumulation of somatic damage before completion of the developmental period. However, the main effect of (q) in the model is to generate variation between individuals in the number of redundancy elements at the start of life (Fig. S1), and biologically that variation can be due to any factor that causes variation in development. For example, manipulated growth trajectories affect lifespan in sticklebacks (Lee et al. 2013), and growing up in an enlarged brood accelerates telomere shortening, which reduces survival in jackdaws (Boonekamp et al. 2014a). Both of these experimental effects can be thought of as reflecting an effect of developmental conditions on the number of redundancy elements an individual has at maturation.

Because it is an important feature of the redundancy model that it can describe mortality plateaus, we illustrate it in figure 1 by fitting a simple redundancy model to a particularly well known example of late-life mortality plateaus observed in a group of 1.2 million medflies (Carey et al. 1992). It can be seen that the redundancy model describes the observed mortality pattern well, and certainly better than the Gompertz model that we fitted for comparison (see figure 1 legend for details). Note, that simpler forms of redundancy models have also been developed (we fitted the two-parameter model (Gavrilov and Gavrilova 2001) for comparison to the data of Carey et al. (1992); see Fig. 1). The late-life mortality plateau is a shared feature of reliability-based models (Gavrilov and Gavrilova 2001; 1991; Li and Anderson 2009; Milne 2008; Vural et al. 2014). Nevertheless, that the redundancy model fits the medfly data well does not naturally follow from the fact that it can in principle describe such plateaus, because of constraints in the pliability of the model. We therefore consider the good fit an encouraging result.

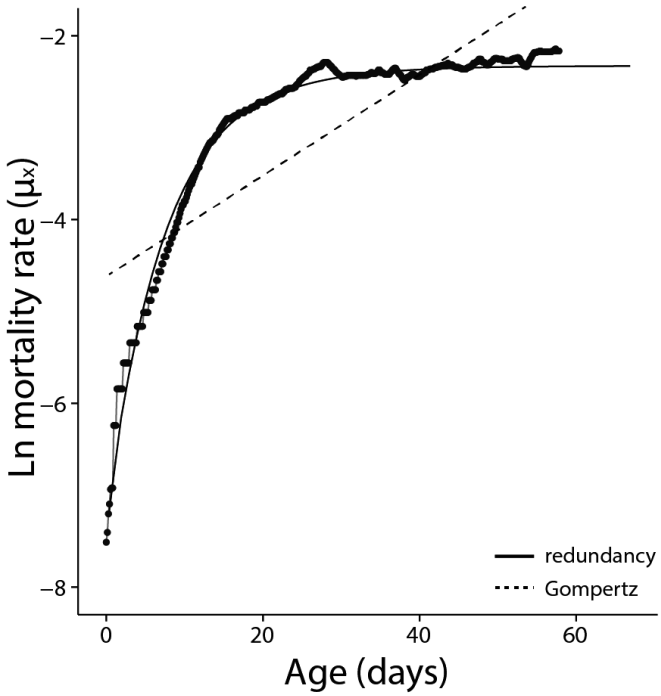


Fig. 1 Gompertz (dashed) and redundancy (solid) models fitted to the actuarial senescence pattern (shown in dots) of 1.2 million medflies (data extracted from Fig. 1C in Carey et al. 1992, using GraphClick). The Gompertz equation ($u(x)=Re^{ax}$) lacks the mortality deceleration property resulting in a low fit, while in comparison the redundancy model (using eq. 1) fits the data well. Fitted Gompertz parameters: $R= 0.01$, $a = 0.058$, fit: $R^2=0.61$; fitted redundancy parameters: $k=0.1077$, $m=1$, $n=71$, $q=0.1$, fit: $R^2=0.99$. For comparison, we also fitted the simplest 2-parameter redundancy model. This model assumes that organisms consist of 1 block containing (n) redundancy elements that fail at rate (k). The 2-parameter model also outperformed the Gompertz function ($n=3$, $k=0.084$, fit: $R^2=0.79$). Models were fitted using the nonlinear least squares function in R and R^2 values were calculated 'manually' using the standard formula: R^2 = 1 – residual sum of squares / total sum of squares.

In figure 2 we illustrate how each of the redundancy model parameters affects mortality rate and its dependence on age. We consider the effect of each parameter on the intercept, slope and plateau of the relation between age and mortality rate. The initial mortality rate (intercept) largely depends on the number of initial intact elements (i.e. qn) and the number of blocks, being lower with an increasing number of elements (n) and a lower number of blocks (m) (Fig. 2). The rate of increase of mortality with age (actuarial senescence) depends mainly on the element failure rate (k) (Fig. 2). The level of the mortality rate plateau at late ages is equal to the product of failure rate (k) times the number of blocks (m), and is equal to (k) when there is only one block ($m=1$). This is so because blocks have only one redundancy element left near the end of life, and the probability of death is then given by (k). Mortality rate increases with the number

of blocks because the probability of redundancy exhaustion within *any* block increases with the number of blocks. As a result, increasing the number of blocks decreases the proportion of the cohort that reaches the age range of the mortality plateau due to the higher overall mortality. The initial number of redundancy elements (n) has no effect on the mortality plateau. Insight into the effects of the different parameters is best gained by experimenting with them, and to this end we have included an R script in the supporting information (supp. information 6 "an R script for redundancy demography").

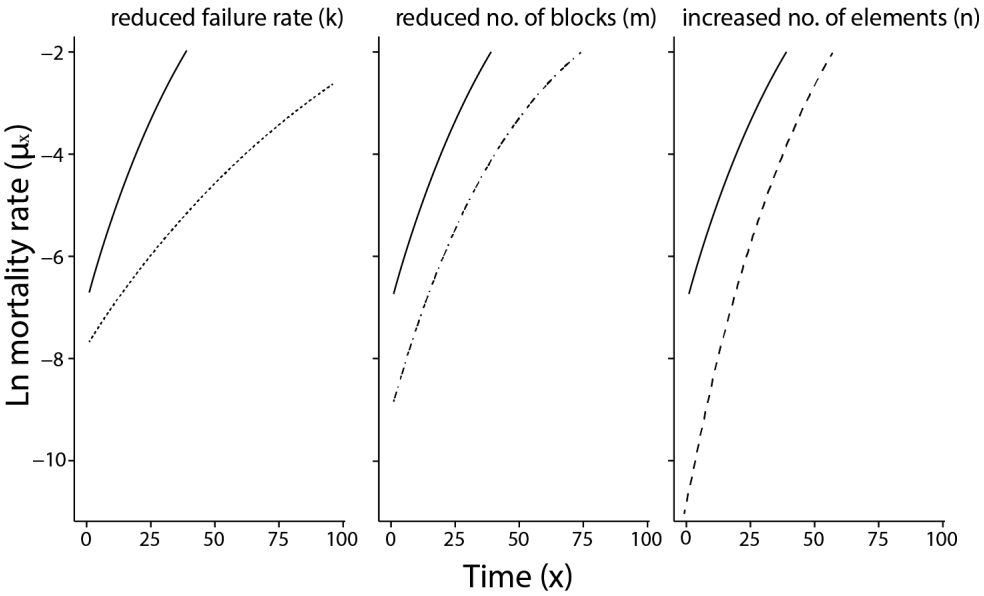


Fig. 2 The effect of the redundancy model parameters (using eq. 1) on age-dependent mortality rate: element failure rate (k), number of blocks (m) and elements (n). The high mortality rate lines (solid) are identical in the three panels (k=0.0169, m=500, n=115). The low mortality rate lines (dashed) were obtained by changing one parameter at a time (to k=0.0073; m=60; n=156 respectively). Initial damage was invariant at 90% (q=0.1) and age is in arbitrary units. These parameter combinations approximately describe mortality trajectories of fruit flies (see section 4). Note that there is no clear mortality plateau in these graphs as in Fig.1, because with the chosen parameters it is not realistic to extend the age-axis sufficiently to reach that stage.

The Gompertz equation is the most widely used distribution for fitting mortality data. We therefore explored how variation in parameters of the Gompertz equation is reflected in the parameters of the redundancy model with the aim to improve the interpretability of redundancy model parameters. The element failure rate parameter (k) best reflected the Gompertz slope and both the redundancy parameter (n) and the number of blocks parameter (m) reflected the Gompertz intercept (see supp. information 3 for details and supplementary figures and tables).

The redundancy model is phenotypic in nature rather than genetic. This does not imply that genetic variation cannot be accommodated; we see genetic variation as being expressed in different values of the model parameters. Investigating how genotypes associated with aging and/or lifespan affect these parameter values would gain further top-down insight in the mechanisms mediating such genetic effects.

Formal models differ in the level of detail, and model choice is, amongst other considerations, the outcome of the trade-off between generality, precision and realism (Levins 1966). We here choose to present only simple redundancy models, at the expense of realism, in that model components represent abstract entities rather than traits that can be measured directly. We see this as a stepping-stone towards more realistic models, in which redundancy model parameters are gradually replaced by realistic sub-models of these parameters.

A key difference between redundancy models and real biological systems is that the latter have repair, mitigating damage, while repair is so far absent from the redundancy model. The element failure rate in the models should therefore be thought of as net failure rate, i.e. the damage remaining after repair, rather than gross failure rate (Glaser 2009). This contrast may be of importance, at least in some cases, and it would be of interest therefore to model damage and repair explicitly and let element failure rate be the process emerging from this balance.

3. Interventions

In this section we utilize the redundancy model to make predictions regarding the effects of lifestyle changes and medical intervention on actuarial senescence and remaining lifespan. These predictions may help unravelling mechanisms underlying empirical results of aging interventions.

Instantaneous mortality rate can change during life due to changes in environmental circumstances, lifestyle, disease or medical interventions. Given the redundancy model framework, such interventions can affect mortality either through an effect on element failure rate (k), or an effect on somatic redundancy (n). Furthermore, the effect of an intervention may depend on the age at which it occurs. We investigated effects of interventions on age-dependent mortality rate and remaining lifespan with an individual based simulation (see supp. information 4 for details on simulation procedures). For simplicity, our approach is limited to the investigation of effects of changes in element

failure rate and the number of redundancy elements, but more complex changes can also be envisioned.

Reducing the element failure rate (by 50%, from 0.02 to 0.01) had two distinct effects: (i) a reduction in the age specific mortality rate immediately after the intervention and (ii) a reduction in the rate of actuarial senescence (mortality increase with age) from the moment of intervention onwards (Fig. 3). Identical interventions were modelled to onset at ages 20, 40 and 50 (early, mid and late life in our simulation) to investigate their consequence for remaining life expectancy. Not surprisingly, the effect on life expectancy of an intervention causing a reduction of element failure rate was larger on an absolute scale when started earlier in life (Table 1).

Restoring a single redundancy element also had two distinct effects: (i) a large instantaneous reduction in the mortality rate at the moment of the intervention, followed by (ii) an increase in the rate of actuarial senescence relative to the control group (shown in black) (Fig. 3). The mortality reduction is caused by the restoration of one damaged element, which instantly increases the system's capacity to absorb damage. However, this beneficial effect is compensated by a subsequent increase in actuarial senescence, a process known as mortality rate convergence. Mortality rate convergence arises because systems with more redundancy elements receive more damage per unit of time relative to systems with few elements, causing convergence of redundancy state and mortality trajectories over time (Boonekamp et al. 2013; Gavrilov and Gavrilova 2001). The effect on life expectancy of the restoration of one redundancy element was largest at an intermediate age (Table 1).

The implication of these findings is that different types of interventions have different effects on life expectancy, depending on the age at which the intervention took place. This may help to understand why some interventions appear to have larger effects early in life. For example, quitting smoking had larger beneficial effects when started earlier in life (Taylor et al. 2002), which qualitatively matches the effect of element failure rate on mortality. Restoring a redundancy element could reflect medical intervention, e.g. cardio surgery, which has relative large effects during middle or late life. Note that we cannot quantitatively compare the effects of interventions of element failure rate versus redundancy due to the lack of knowledge on the biology of these parameters, but the difference in dependency on age can be inferred from our simulations because the pattern differs qualitatively (Table 1).

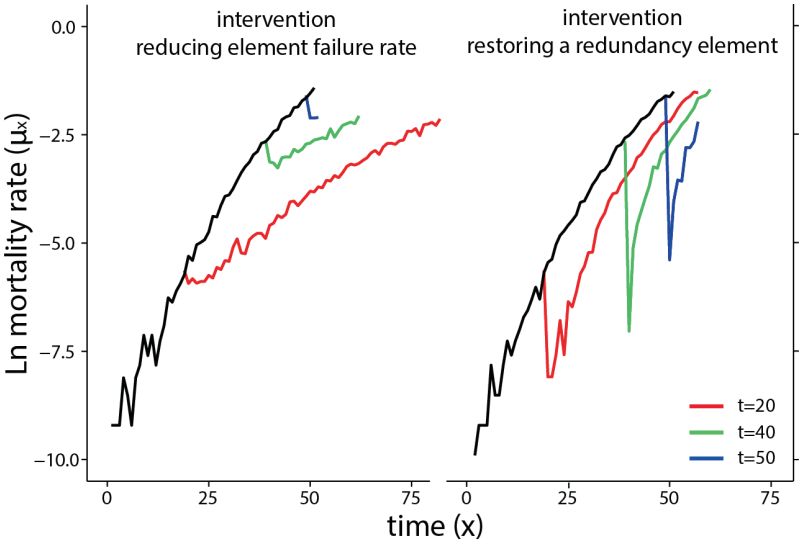


Fig. 3 The effect of an intervention that either reduces element failure rate (left panel) or restores redundancy (right panel) on age-dependent mortality. Data were obtained through individual based simulations (see SI–4 for details). Intervention reduced element failure rate from 0.02 to 0.01 (left panel) or restored 1 redundancy element per block (right panel) at ages 20 (red), 40 (green), or 50 (blue). The initial (control) mortality groups (black lines) were generated for 10.000 individuals with q=0.1, m=500, n=152 and k=0.02. Age at death was used for the calculation of life tables and life expectancy (Table 1).

Table 1 The effect of interventions that reduce element failure rate (k) or restore redundancy elements (n) on life expectancy at age x (e_x). Element failure rate (k) was reduced from 0.02 to 0.01 and redundancy was restored with a single element. The life expectancy difference denotes the difference between no intervention versus intervention. Life expectancy was calculated on the basis of life tables generated with the individual based simulations. See legend of Fig. 3 for parameter settings and other details.

intervention		life expectancy (e_x)		
type	*moment (x)*	*no intervention*	*with intervention*	*difference*
rate (k)	20	18.81	38.98	20.17
	40	4.48	10.74	6.26
	50	0	1.55	1.55
redundancy (n)	20	19.07	25.91	6.84
	40	4.53	13.01	8.48
	50	0	4.33	4.33

4. An example of fitting the redundancy model to real data

Dietary restriction (DR) and modulation of environmental temperature are classic tools to modulate lifespan. DR restriction extends lifespan in many species (Fontana et al. 2010; Nakagawa et al. 2012), and the same is true of low environmental temperature in ectotherms (Pearl 1928). Such effects can arise either through an effect on instantaneous mortality rate, an effect on ageing (actuarial senescence), or a combination of the two. Mair et al. (2003) carried out a clever experiment to distinguish between these hypotheses using *Drosophila*. They applied both treatments, and for each treatment switched flies between the low/high lifespan regimes at different ages. They concluded that DR affected instantaneous mortality rate without affecting aging, while the temperature manipulation affected only aging. However, the mechanism(s) that causes the effect of DR on mortality risk are not fully understood and Mair et al. (2003) did not discuss how these effects might arise. We here explore whether the observed effects can be described with the redundancy model, as a step towards a better understanding of the mechanisms causing the observed complex patterns. Given that the results of the two experimental interventions are very different it is a challenge to generate both effects with one model. Hence, confirmation of whether the redundancy model can explain both effects would support the hypothesis that redundancy models of aging capture the essence of the mechanisms causing the observed variation in actuarial senescence and lifespan.

Our approach was as follows. For each treatment (temperature / dietary restriction) we first fitted the redundancy model (eq. 1; see supp. information 5 for details on fitting procedure) to the continuous treatment groups (i.e. the flies that were not switched between treatments). Our aim here was to let the high and low lifespan models differ in only one biologically plausible parameter chosen a priori. A good fit however does not necessarily imply that the redundancy parameter difference between the control groups resembles the essence of the mechanisms causing these effects, because such differences can possibly also be achieved with other model adjustments. The data of Mair et al. (2003; from hereon we refer just to 'the experiment') included switches between the high and low mortality regimes. This provides us with the opportunity to investigate whether the parameter differences between treatments also explained the observed pattern following switches between treatments. To this end, we simulated the experimental switches between the high / low lifespan treatment parameters at the various time points as in the experiment, and compared the predictions derived in this way to the results observed in the experiment.

Manipulation ambient temperature

Because the redundancy element failure rate parameter (k) has the largest effect on the rate of actuarial senescence (Fig. 2) we hypothesised that this parameter was modified by ambient temperature in the experiment. We therefore constrained the model by allowing only the element failure rate parameter (k) to vary between the two continuous treatments (i.e. (q), (m), and (n) were optimised but kept identical) when fitting the redundancy model to the 27 C° and 18 C° temperature groups (data from Fig. 3A in Mair et al. 2003). The best fit combined over the two continuous treatments was $R^2 = 0.92$ and 0.89 to the 27 C° and 18 C° groups respectively. Results of individual based simulations illustrate the good fit of the model to the control group mortality patterns (Fig. 4a – black and red lines). Note that these fits were not optimal due to the restriction of keeping the parameters other than (k) equal, but our purpose was to test whether temperature increased mortality via an effect on (k), requiring that the other parameters are kept constant. Individual based simulations of the experimental switches between temperature regimes reproduced the data well (green, blue, and purple lines), regardless of the direction of the switch (Fig. 4a; $R^2 = 0.70$, pooled over the four switch experiments, two in each direction). It is worth emphasizing that the predicted effects of the switches were simulated using the parameters fitted only on the continuous treatments. That these predictions fit the data of the switches well suggests that the effect of ambient temperature on actuarial senescence in *Drosophila* can be well described by the redundancy model via changes in the element failure rate parameter (k).

Dietary restriction

The DR effect is complex and could affect mortality via several physiological pathways. We therefore devised multiple models of the DR effect with increasing complexity to compare which redundancy-based mechanism best fit the observed pattern. As with the temperature manipulations, for each of these models we first fit the redundancy model (eq. 1) to the fully fed and DR groups (Fig. 1A in Mair et al. 2003) and subsequently tested whether the required adjustment to accommodate the DR effect in the continuous treatment groups yielded predictions for the switching experiments that fitted the observed results.

A simple mechanism by which DR could reduce mortality risk is through a reduction in the net element failure rate (i.e. reducing parameter (k)). The logic behind this model adjustment is that DR causes reallocation of resources from reproduction towards somatic maintenance, which is in line with a reduced net element failure rate. However, as shown in the analysis of the temperature manipulations (Fig. 4a), element failure rate modulates the slope of actuarial senescence, which is not in qualitative agreement

with the data. The fit to the control groups (Fig. 4a – black and red lines) is nevertheless reasonably high (R^2 = 0.9), but breaks down when we simulate the switch experiments (R^2 = -0.43; Fig.4b – green, blue and purple lines). Thus the DR effect cannot be explained by a change in element failure rate alone.

It has been hypothesised that the life-extending effect of DR is due to animals re-allocating resources from reproduction towards somatic maintenance when resource intake is insufficient for reproduction (Partridge et al. 2005; Shanley and Kirkwood 2000). Fruit flies require yeast for reproduction and the absence of yeast during DR by itself causes females to remain reproductive-inactive (Carey et al. 1998; Good and Tatar 2001). It seems reasonable therefore to assume that DR reduced mortality in Mair's experiment at least in part through a diminishing effect of DR on reproduction (Carey et al. 1998; Good and Tatar 2001). We therefore looked at ways to accommodate this effect in the redundancy model.

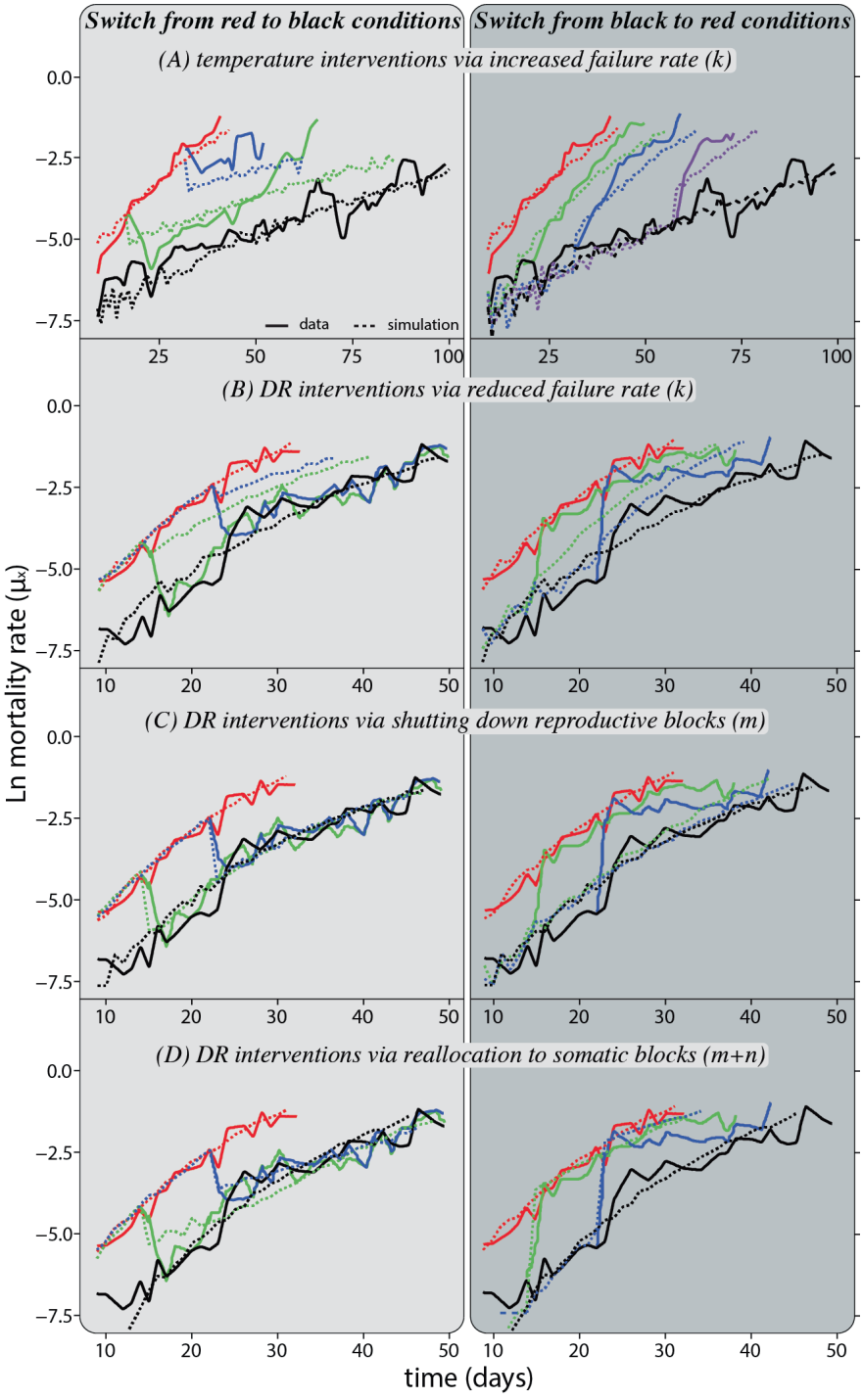
We hypothesized that organisms consist of two different types of blocks (reproductive and somatic blocks) and that DR reduces the element failure rate only in reproductive blocks. This adjustment is in line with the idea that the functions affected by reproduction will sustain little damage in DR conditions because there is little investment in reproduction. To investigate this hypothesis we first fitted the model to the fully fed group (R^2 = 0.97; Fig. 4c – red line; see SI–5 for details). Subsequently, we fitted the model to the DR group (Fig. 4c – black line), by optimizing the number of reproductive blocks in which the element failure rate was reduced (from k=0.0298 to almost zero: k=0.0001), while constraining the other parameters to the level fitted to the control group. The good fit (R^2 = 0.95) to the continuous DR group is encouraging but the individual based simulations of the treatment switches fitted well in only one of the two directions (Fig. 4c – green and blue lines). The simulation fitted the switch from fully fed to DR conditions very well but failed to reproduce the reversed switch resulting in a relatively poor fit over the four switch experiments combined (R^2 = 0.46; Fig. 4c). This is because when DR diminishes damage accumulation in reproductive blocks, subsequent damage accumulation in these blocks after switching does not increase mortality rate instantaneously as in the experiment due to the lag in damage accumulation relative to reproductive blocks in the fully fed group. We conclude therefore that, given the context of our model, the mechanism by which DR reduces mortality is more complex than the reduction of damage accumulation in reproductive blocks.

When implementing the re-allocation hypothesis we reduced element failure rate in reproductive blocks and as a next step, we simultaneously reallocated redundancy

elements from reproductive blocks towards somatic blocks (Fig. 4d). Redundancy is the resilience to withstand aging damage and therefore redundancy reallocation is another way (next to changes in element failure rate) to model reallocation from reproduction towards somatic maintenance. The model adjustments fitted the data of the continuous treatment groups well (Fig. 4d; red line – fully fed: R^2 = 0.97; black line – DR: R^2 = 0.95). More importantly, individual based simulations based on these adjustments fitted well to the switches in both directions (Fig. 4d – green and blue lines; R^2=0.81, pooled over 4 switches in two directions). We emphasize again that the model parameters were optimized to fit the data of the continuous treatment groups only, and we are fitting the data of the experimental switches to predictions based on the fit to the continuous treatment groups. Reallocation of redundancy elements when switching from DR to fully fed instantly increases the mortality rate due to the reduction of redundancy in the somatic blocks. We conclude that re-allocation of redundancy elements from reproductive to somatic blocks within the context of the redundancy model can explain the DR effect on the mortality pattern observed by Mair et al. (2003).

The redundancy model as we applied it is devoid of any mechanistic detail. It is encouraging therefore that these simple models were able to reproduce the contrasting effects of DR and environmental temperature on the pattern of actuarial senescence. This indicates that the interdependencies of blocks, elements, and damage may, in an abstract way, capture the essence of the aging process in *Drosophila*.

Fig. 4 The redundancy model fitted to aging experiments on fruit flies. Individual based simulation based on the redundancy model (dashed lines) optimized to reproduce the observed actuarial senescence patterns of *Drosophila* (solid lines) that were either subjected to ambient temperature manipulations (4A) or dietary restriction (DR; 4A–D) (data extracted from Figs. 1 and 3 in Mair et al. (2003)). Solid black and red lines represent the groups in which temperature (panel A; red = 27°C, black = 18°C) and diet conditions were kept constant (panels B to D; red = fully fed, black = DR). These red and black lines are termed the continuous treatments, and they are identical in the left and right figures within each panel. Solid green, blue, and purple lines reflect switches of experimental conditions at different time points from one continuous treatment group to the other: from red to black (left panel); from black to red (right panel). Dashed red and black lines reflect individual based simulations using redundancy parameter settings fit to the continuous treatment data. Dashed green, blue, and purple lines reflect switches in redundancy parameters from one continuous groups parameters to the others. Parameter values: panel A: m=500, n=110, q=0.1, k=0.0163 (27°C) or 0.0064 (18°C); panel B: m=500, n=140, q=0.0298, k=0.0298 (fully fed) or 0.0193 (DR); panels C–D: m=500, n=140, q=0.0298, k=0.0298 and where DR was modelled to reduce (k) to 0.0001 in 380 out of 500 blocks (panel C). In panel D, 2 redundancy elements were reallocated from reproductive to somatic blocks in addition to the parameter interventions in panel C. See SI–5 for details on the fitting procedures, and SI–4 for individual based simulation procedures. ➔


Switch from red to black conditions
Switch from black to red conditions
(A) temperature interventions via increased failure rate (k)
(B) DR interventions via reduced failure rate (k)
(C) DR interventions via shutting down reproductive blocks (m)
(D) DR interventions via reallocation to somatic blocks (m+n)
data
simulation
Ln mortality rate (μx)
time (days)
0.0
−2.5
−5.0
−7.5
10
20
30
40
50
25
50
75
100

5. Redundancy in the wild

The antagonistic pleiotropy theory of senescence proposes that senescence could evolve when alleles conferring a fitness benefit early in life also confer a detrimental effect on fecundity and/or survival late in life (Williams 1957). Such alleles may for example increase investment in reproduction early in life, at the expense of the reproductive output late in life (an effect known as the costs of reproduction). When investment in reproduction is made at the expense of the system's redundancy, e.g. through an increase in element failure rate, this is a mechanism by which a negative association could arise between early reproduction and late life survival. We view redundancy to be conceptually similar to the disposable soma in the disposable soma theory of aging (Kirkwood 1977) which is a special case of the antagonistic pleiotropy theory of aging (Kirkwood and Holliday 1979). Thus the redundancy model is compatible with one of the main evolutionary theories of aging.

Senescence patterns show large variation both between and within species (Bouwhuis et al. 2010; 2012; Nussey et al. 2013). Much of this variation has been attributed to ecological factors (e.g. extrinsic mortality rate) in combination with life-history trait optimization, but it is largely unclear how these factors mechanistically link up to the aging process. It would therefore be of interest to investigate to what extent variation in lifespan and mortality patterns between natural populations can be attributed to redundancy vs. element failure rate variation (or a combination of the two).

When applying the redundancy model to natural populations, it is important to realise that the model is only concerned with intrinsic sources of mortality, while in natural populations the mortality pattern is also shaped by interactions between internal state (redundancy) and the environment, and extrinsic mortality independent of state. For example, the ability to withstand environmental adversity may be higher when remaining redundancy is high, but presently such interactions between environmental conditions and internal state are not included in the model. So without further extension, the model is best suited to analyse mortality patterns of populations of humans and laboratory animals where extrinsic factors such as famine and predators are less important. The model can be fitted to aging in natural populations as it is, but the parameter estimates would be a mixture of external and intrinsic mortality effects. It would be more informative therefore to build a shell around the redundancy model that introduces extrinsic effects and perhaps also interactions between extrinsic factors and redundancy state or element failure rate. This may initially be as simple as the multiplication of age dependent mortality with an extrinsic (age independent) mortality factor.

Mortality rate does not always increase with age. In many species mortality rate declines with age early in life, before maturation, but in addition there are species where mortality rate continues to decline over a large part of their lives, for example because they become less vulnerable due to an increase in size. This phenomenon can be thought of as negative senescence (Vaupel et al. 2004). Within the context of the redundancy model this effect can be achieved through an increase of the number of redundancy elements with age (Milne 2008), which is conceivable in species showing continuous growth. It may also accommodate the typical u-shaped mortality that is observed in humans, among other species, where the initial decline in mortality rate is caused by growing redundancy due to development (Milne 2008).

To illustrate how the redundancy model might elucidate unexplained aging characteristics we here briefly discuss some examples, mainly from our own work, where other concepts known to us did not yield a satisfactory explanation.

The association between telomere length and mortality diminishes with age in humans, which finding does not fit the concept that telomere length is a measure of biological age in its most simple form (Boonekamp et al. 2013). Instead, we showed that telomere length can be interpreted as measure of somatic redundancy: the diminishing association between telomere length and mortality with age fitted the redundancy model, because variation in redundancy between individuals diminishes with age, causing the remaining redundancy to be a poor predictor of mortality (Boonekamp et al. 2013). Furthermore, we could make the prediction that the association between telomere shortening rate and mortality should increase with age (i.e. because when telomere shortening rate reflects element failure rate it strongly determines the late-life mortality plateau), and evidence confirming this prediction is emerging (Epel et al. 2009), but more studies are needed to verify this point.

In wild jackdaws (a small corvid), we found that effects of reproductive effort on survival became apparent only after birds had been subjected to multiple years of manipulation (Boonekamp et al. 2014b); a single year of reproductive effort manipulation did not noticeably affect mortality. The latter finding may be the general pattern (with exceptions) because a recent meta-analysis showed that on average there is no discernible survival cost of increased reproductive effort when birds are manipulated in a single year (Santos and Nakagawa 2012). Thus, it appears that birds have a buffer to resist high effort for one season. We think of the buffer in this explanation as redundancy, and an increase of reproductive effort as in increase in element failure rate, where an increase in element failure rate for a single season does not yet exhaust redundancy to the extent that it

yields a significant effect on survival. A prediction based on this interpretation is that experiments that manipulate reproductive effort for a single season without a discernible effect on survival until the next year (Santos and Nakagawa 2012) may find an effect at the end of life of life, i.e. on lifespan. This could arise when the effort manipulation did induce a difference between experimental categories in remaining redundancy level, but this difference is only translated in a survival effect at the end of life, when redundancy levels are low. To our best knowledge this hypothesis has not yet been tested.

Lastly, it was recently shown that malaria infection had a delayed effect on lifespan in great reed warblers, but no discernible immediate effect on mortality (Asghar et al. 2015). As in the Jackdaw example discussed above, a redundancy buffer to withstand such a physiological challenge might explain this pattern. This interpretation is supported by the finding that malaria accelerated telomere shortening (Asghar et al. 2015), because we previously showed that human telomere length variation can be interpreted as a measure of somatic redundancy (Boonekamp et al. 2013). These three examples suggest an explanatory power provided by the redundancy model not (yet) offered by other models.

6. Concluding remarks

Our main aim with this paper is to advocate more interaction between theoretical and empirical work in aging research through data-directed modelling. In this context we see modelling as complementary method for hypothesis testing regarding mechanisms causing the observed patterns (Servedio et al. 2014), and as a way to integrate theoretical and empirical perspectives. In this integration process there is a part to play for models that differ strongly in level and realism, ranging from top-down models as presented in this paper, to very detailed biochemical models (e.g. Mc Auley et al. 2015). When it comes to the top-down approach, we like to emphasize that we have at present little reason to advocate the redundancy model over other models (e.g. Li and Anderson 2009; Milne 2008; Pletcher and Neuhauser 2000; Vural et al. 2014), if only because a formal comparison with the performance of other models is lacking. Nevertheless, there are factors speaking in favour of the redundancy model. Firstly, there is the practical point that at least so far it seems to do the job we adopted it for. Another important criterion the redundancy model fulfils is that, at least to us, the redundancy concept is intuitively plausible, in that the model components can reasonably be mapped onto real biology. Lastly, with three parameters (we keep initial damage mostly constant), the model is tractable to an extent that is difficult to attain with more complicated models.

We see three main routes to take this work forward. Firstly, it would be of interest to fit the redundancy model to other data sets. This may yield more insights in those data sets and the experiments that generated them, and may be of interest for comparative analyses, for example to explain the difference in fitness costs of senescence between birds and mammals (Bouwhuis et al. 2012), and to explain the large range of mortality patterns seen more generally in nature (Nussey et al. 2013). Fitting the model to existing and new data sets may also help to identify the model's limitations. This in turn would be useful input to develop better fitting models; finding out what changes are necessary to yield a better fit is in itself of interest. Moreover, this exercise would teach us something about how many models we would need to describe mortality patterns in a larger number of species – that one size fits all is unlikely. Secondly, the redundancy model is only one of an infinite number of possible mechanistic models, and a competition between such models is clearly desirable to see which model serves our purpose best, and model performance should be formally compared. Lastly, a logical next step would be to replace parameters in the model with sub-models of those parameters. For example, coming back to the data of Mair et al. (2003) discussed in section 4, redundancy element failure rate could be made a function of physiological variables or molecular processes that change in response to ambient temperature.

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Supplementary information to:

The heuristic value of redundancy models of aging

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Supplementary information S1: Normalizing factor *c*

Following Gavrilov and Gavrilova (2001) the normalizing factor in eq.1 of our main paper is given by:

$$c = \frac{1}{1 - e^{-qn} - e^{-qn} \sum_{i=n+1}^{\infty} {(nq)^i}/{i!}}$$

Supplementary information S2: Figure S1

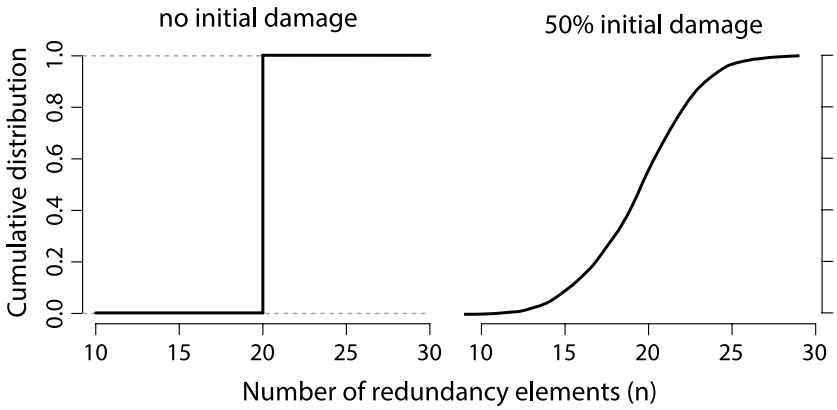

Fig. S1 The effect of the initial damage parameter q on the (cumulative) distribution of the initial number of redundancy elements. Shown are two examples: no damage in the left panel (q=1), and 50% damage in the right panel (q=0.5). Note that the average number of redundancy elements does not differ between these examples because the number of pre-initial damage elements n was doubled in the right panel. Thus initial damage primarily affects the variation in number of initial redundancy elements in the population and only affects the mean when n is not adjusted accordingly.

Supplementary information S3: Relationships between Gompertz and redundancy model parameters

Because the Gompertz distribution is much used, we here we explore how variation in parameters of the Gompertz distribution (Gompertz 1825) is related to the parameters of the redundancy model. To this end, we fitted the redundancy model to individual based data simulated with Gompertz equations with different parameter values, and evaluated how parameters in the fitted redundancy model changed to accommodate the change in the Gompertz parameters (Table S1).

Individual lifespans (n = 20,000 subjects) were generated using the two-parameter Gompertz equation for mortality $\mu(x) = Re^{ax}$. Three cohorts were simulated based on different values of *R* and *a* (shown in red, blue, and black). The different Gompertz parameters that we used are arbitrary due to that time is in arbitrary units (i.e. days, weeks, etc.). Lifespan data were generated up to the age where 90% of the cohort had died to maintain accuracy. Instantaneous mortality rate in the simulated lifespan data was calculated using the epiR package in R.

The Gompertz function decomposes actuarial senescence into two parameters where parameter (*R*) reflects the intercept (also known as 'initial mortality rate', and 'vulnerability') and parameter (*a*) the slope of actuarial senescence (known as aging rate). When fitting the redundancy model to data simulated using the Gompertz equation, the first thing to note is that there is room for parameters (*q*) and (*n*) to compensate each other. This is so because the number of initial elements in each block is given by *q***n*. Thus when fitted to data simulated with our reference Gompertz parameters (line 1 in Table S1), a combination (*q*, *n*) of (0.1, 115) fits the simulated data about equally well as the combination (0.8, 15), with little difference in *k* between these models (Table S1). It is for this reason that we have held (*q*) invariant (at 0.1) in most models here and in the main paper.

When changing the intercept (*R*) in the Gompertz equation (Fig. S2), and fit the redundancy model to these data, we find that generally speaking it is the combined number of initial redundancy elements that is adjusted to achieve a high fit. However, because the initial number of redundancy elements is jointly determined by *q, m* and *n,* this adjustment can be achieved in different ways (Table S1). When we constrained 3 out of 4 redundancy parameters to the optimal value when fitted to the reference Gompertz cohort, optimizing the 4th parameter (either *n* or *m*) to fit the cohort with the different intercept, this yielded a fit of $R^2 > 0.93$ (Table S1 – lines B,C).

Table S1 Redundancy model fitted to data generated by the Gompertz equation with low and high intercepts and slopes. Letters in the last column denote models where only one parameter was allowed to vary (A-failure rate; B-blocks; C-redundancy) while the others were constrained at the level in the first line of the table. The purpose of this exercise was to investigate to what extent variation in the one parameter would be sufficient to adjust the fit of the redundancy model to the different Gompertz slopes and intercepts. Note that q was set at either 0.1 or 0.8 and not optimized separately due to the fact that q and n compensate for each other.

Gompertz		redundancy				goodness of fit	
R (intercept)	*a (slope)*	*q (init. dam.)*	*m (blocks)*	*n (elements)*	*k (failure rate)*	R^2	
high (0.0015)	steep (0.120)	0.1	500	115	0.0169	0.99	
		0.8	500	15	0.0174	0.99	
high (0.0015)	shallow (0.038)	0.1	500	115	0.0073	0.88	A
		0.1	500	102	0.0058	0.99	
		0.8	500	13	0.0057	0.99	
low (0.00015)	steep (0.120)	0.1	60	115	0.0169	0.93	B
		0.1	500	156	0.0169	0.96	C
		0.1	500	147	0.0154	0.99	
		0.8	500	19	0.0158	0.97	

When changing the aging parameter (a) in the Gompertz equation, the main adjustment required in the redundancy model is to the failure rate (k) (Table S1). This adjustment was however not sufficient to obtain maximum fit (R^2=0.88 versus 0.99, Table S1), because failure rate also has a small effect on initial mortality rate (the intercept). Thus when Gompertz parameter (a) becomes more shallow, and (k) decreases to accommodate the lowered rate of actuarial senescence, the initial number of redundancy elements needs to be decreased to maintain the mortality rate intercept at the same level and thus yield a good fit to the data (R^2=0.99, Table S1, Fig. S2).

It is worth noting that the redundancy variation inducing parameter (q) (Fig. S1) is crucial to yield a good fit to data that fit the Gompertz equation. This is so because (q) causes there to be individuals with low initial redundancy in the population. These individuals are necessary to increase the initial mortality rate, which makes the mortality pattern nearly linear on a log scale as in the Gompertz equation (Gavrilov & Gavrilova 1991).

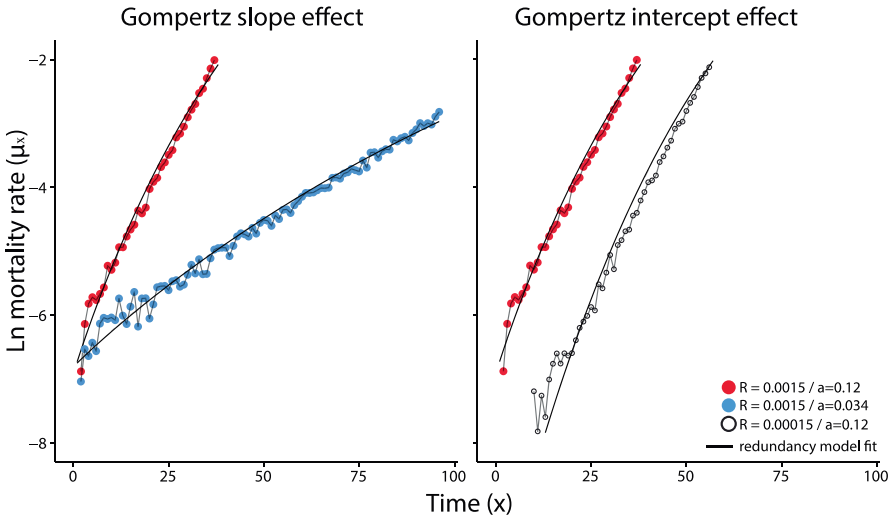

Fig. S2 The redundancy model optimized to fit Gompertz generated data. Gompertz parameters are shown in the figure legend. Redundancy parameters were: fit to red (k=0.0169, m=500, n=115, q=0.1); fit to blue (k=0.0058, m=500, n=102, q=0.1); fit to black (k=0.0154, m=500, n=147, q=0.1). The redundancy parameter (q) was invariant at 0.1 (reflecting high initial heterogeneity in the number of elements) and time is in arbitrary units.

Supplementary information S4: Individual based simulation procedures

Here we describe the general procedures for individual based simulations that we used to produce figures 3 and 4 (based on eq. 1) in the main paper.

In the redundancy model it is assumed that individuals die when their last remaining redundancy element (in any given block) is damaged. This is also how we generated mortality in our individual based simulations.

First, a matrix was generated defining the redundancy architecture for each individual in the simulation, i.e. for each individual, m number of blocks containing n number of elements were defined. Second, a distribution of initial damage was generated at $t = -1$ to induce the necessary heterogeneity in the number of intact elements at $t = 0$. Initial damage to elements was defined to occur with probability P(1-q) and we used random numbers drawn from U(0-1) to determine whether an element was damaged or not (i.e. when $q > U$(0-1)). Likewise, it was determined for every time step from $t > 0$ onwards whether an element would become damaged using failure probability k (i.e. when $k < U$(0-1)). Individual lifespans were recorded when the last remaining element in any block would fail, by definition causing death.

Simulations were done in R with groups of 10,000 individuals to achieve reliable early- and late-life mortality data. Instantaneous mortality rates were extracted from the simulated lifespan data using the epiR package in R.

Supplementary information S5: Redundancy model fitting procedures

Here we describe how the redundancy parameters q, n, m, and k (equation 1 in the main paper) were optimized to fit the data of Mair et al. (2003). Fitting was done by minimizing the sum of squared differences between the fitted equation and the individual data points using the standard non-linear least squares function – i.e. nls() – in R. Note that the procedure is general and can be applied to any dataset. Although we did not do so, instead of fitting to the raw data it would also be possible to fit the redundancy model to a moderately smoothed data set, using e.g. the running mean, to reduce sensitivity of the fitting procedure to stochastic variation in the data.

The four redundancy parameters have partly overlapping effects on mortality, in particular q, m and n that together largely determine the intercept of the relation between age and mortality (see section 2 in main paper). It can therefore be difficult to optimize all four parameters simultaneously due to convergence problems. Parameters were therefore optimized by fitting the failure rate parameter k for an array of values of m, n and q (m and n are discreet values). This procedure results in an array of goodness of fit measures from which we selected the parameter combination with the lowest least squares value, indicating the best fit. We explored the complete parameter space for q and k, i.e. between 0-1, but note that the value of q had no effect on the goodness of fit as long as $q < 1$ (see supp. information 2 for a discussion on the function of parameter q). Therefore, we chose to optimize redundancy parameters using a fixed value of q (we used q=0.1). For both n and m we limited the range from 1 to 500, because extending the range further yielded ever-smaller increases in model performance (now increases of $R^2 < 0.001$ per unit of parameter space).

Calculation of R^2

We computed R^2 values based on the standard formula R^2=1 – residual sum of squares / total sum of squares. This implies that identical mortality trajectories between the data and the model fit or simulated data result in $R^2 = 1$. Poor fits between simulated and observed data can result in values below zero because the total variance in the observed data can be lower than the sum of squared differences between the simulated and the observed data.

Supplementary information S6: An R script for redundancy demography

The following R script can be used to explore effects of the parameters in the redundancy model on mortality rate and how it changes with age. The output consists of a plot with two panels. In the top panel you will see Ln mortality rate as a function of time for two different sets of redundancy parameters (to be modified as you see fit). In the bottom panel you will see the hazard ratio as a function of time of one of the mortality functions relative to the other mortality function. Please ensure that the required packages (ggplot2, gtable) are installed. When changing parameter settings please take note of the constraints on the parameter ranges.

```
#install.packages('ggplot2')

#install.packages('gtable')



library(ggplot2)

library(gtable)



rm(list=ls())

rm(list = ls(all = TRUE))



#Redundancy parameter settings for the red curve

n1<-50  #number of elements in any block (min=1)

m1<-100  #number of blocks (min=1)

k1<-0.01 #failure rate of elements (min=0, max=1)

q1<-0.1  #proportion of initial intact elements (min>0 , max<1)
```

```
#Redundancy parameter settings for the black curve

n2<-100  #number of elements in any block (min=1)

m2<-100  #number of blocks (min=1)

k2<-0.01 #failure rate of elements (min=0, max=1)

q2<-0.1  #proportion of initial intact elements (min>0 , max<1)


#NOTE THAT PARAMETER q SHOULD NEVER BE SET TO 1, BECAUSE THE
EQUATION REQUIRES VALUES <1.

#THE FUNCTION WILL WORK WITH q=1, BUT THE OUTPUT WILL BE WRONG.


#functions used for fitting.

f<-function(x)

 {

  j=0

  jj=n1

  cj=0

  aj=0

  while(j<n1)

  {j=j+1

   aj<-aj+(((n1*q1) ^ (j-1))*((1-exp(-k1*x)) ^ (j-1)))/(factorial(j-1)*(1-(1-exp(-
k1*x)) ^ j))
```

```
 }
 while(jj<100)
 {jj=jj+1
  cj<-cj+(((n1*q1) ^ jj)/factorial(jj))
 }
 C<-1/(1-exp(-(n1*q1))-exp(-(n1*q1))*cj)
 R<-m1*C*k1*(n1*q1)*exp(-(n1*q1))*exp(-(k1*x))
 log(R*aj)
}


f2<-function(x)
{
 j=0
 jj=n2
 cj=0
 aj=0
 while(j<n2)
 { j=j+1
  aj<-aj+(((n2*q2) ^ (j-1))*((1-exp(-k2*x)) ^ (j-1)))/(factorial(j-1)*(1-(1-exp(-
k2*x)) ^ j))
```

```
}
while(jj<100)
{jj=jj+1
 cj<-cj+(((n2*q2)^jj)/factorial(jj))
}
C<-1/(1-exp(-(n2*q2))-exp(-(n2*q2))*cj)
R<-m2*C*k2*(n2*q2)*exp(-(n2*q2))*exp(-(k2*x))
log(R*aj)
}

x <- seq(1,100)
y <- f(seq(1:100))
set1<-cbind(x,y,1,1)
y <- f2(seq(1:100))
set2<-cbind(x,y,2,1)
y<-f(seq(1:100))/f2(seq(1:100))
set3<-cbind(x,y,3,2)
data<-as.data.frame(rbind(set1,set2,set3))
```

```
theme<- theme_update(
 panel.grid.major=element_line(colour="grey"),
 panel.grid.minor=element_line(colour=NA),
 #panel.background = element_rect(colour = NA,fill=NA,size=1),
 axis.line=element_line(colour="black",size=1),
 axis.title.x=element_text(size=20,face="bold",hjust=0.5,vjust=0.5,angle=0),
 axis.title.y=element_text(size=20,face="bold",hjust=0.5,vjust=0.5,angle=90),
 axis.text.x=element_text(colour="black",angle=0,size=15),
 axis.text.y=element_text(colour="black",angle=0,size=15),
 axis.ticks=element_line(colour="black",size=1),
 axis.ticks.margin=unit(0.1,"cm"))

#--Define axis labels:
xlabel <- "Time"
ylabel <- "Ln mortality rate (ux)"

#facet labels
my_labeller <- function(var, value){
 value <- as.character(value)
```

```
 if (var==”V4”) {
  value[value==”1”] <- “actuarial senescence”
  value[value==”2”]  <- “hazard ratio”
 }
 return(value)
}

p<-ggplot(data, aes(x, y)) +
 geom_line(data=subset(data, data[,3]==1),color=”red”, lwd=2) +
 geom_line(data=subset(data, data[,3]==2),color=”black”, lwd=2)+
 geom_line(data=subset(data, data[,3]==3),color=”black”, lwd=2)+
 facet_grid(V4~.,scales=c(“free”),labeller=my_labeller) +
 theme(strip.text.y = element_text(size=16, angle=270),
    strip.background = element_rect(colour=NA, fill=NA))+
 xlab(xlabel)+
 ylab(ylabel)
p+ggtitle(“redundancy demographics”) +
 theme(plot.title = element_text(face=”bold”,size=20))
```

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Chapter 6

Large diurnal temperature range increases bird sensitivity to climate change

Michael Briga & Simon Verhulst

Scientific Reports 5, 16600

Abstract

Climate variability is changing on multiple temporal scales, and little is known of the consequences of increases in short-term variability, particularly in endotherms. Using mortality data with high temporal resolution of zebra finches living in large outdoor aviaries (5 years, 359.220 bird-days), we show that mortality rate increases almost two-fold per 1°C increase in diurnal temperature range (DTR). Interestingly, the DTR effect differed between two groups with low versus high experimentally manipulated foraging costs, reflecting a typical laboratory 'easy' foraging environment and a 'hard' semi-natural environment respectively. DTR increased mortality on days with *low* minimum temperature in the easy foraging environment, but on days with *high* minimum temperature in the semi-natural environment. Thus, in a natural environment DTR effects will become increasingly important in a warming world, something not detectable in an 'easy' laboratory environment. These effects were particularly apparent at young ages. Critical time window analyses showed that the effect of DTR on mortality is delayed up to three months, while effects of minimum temperature occurred within a week. These results show that daily temperature variability can substantially impact the population viability of endothermic species.

Introduction

Climate change affects the abundance and distribution of populations through changes in both mean and variability of climatic variables (Coulson et al. 2001; Boyce et al. 2006; Parmesan 2006; Jenouvrier et al. 2008; van de Pol et al. 2010; García-Carreras and Reuman 2013; Thompson et al. 2013). When investigating climate variability, usually time scales of months or years are considered (Easterling et al. 2000; Schar et al. 2004; Rahmstorf and Coumou 2012; O'Gorman 2014), but climatic variability over much shorter timescales, typically days, has also changed in recent decades, at least on a regional scale (Vose et al. 2005; Solomon et al. 2007; Stocker et al. 2013; Wang and Dillon 2014). For example, average diurnal temperature range (DTR), the difference between maximum and minimum temperature within one calendar day, has increased more than 2°C since approximately the 1960's in Mexico, Bolivia, Patagonia, Madagascar, Indonesia, central Russia and the Western Himalaya, while other areas have experienced up to equally large decreases, for example in north-eastern Canada, north and central Africa and the Eastern Himalaya (Yadav et al. 2004; Englehart and Douglas 2005; Vose et al. 2005; Jhajharia and Singh 2011; Wang and Dillon 2014). Climate change is thus also associated with changes in temperature variability on short time scales.

DTR responses independent of mean temperature can occur following Jensen's inequality (Jensen 1906; Ruel and Ayres 1999): when there are nonlinear associations between a system and its environment, mean system state will change in response to increased environmental variation even when the environment mean remains constant (Fig. 1). Increasing DTR has been shown to reduce population viability of ectotherms (Raffel et al. 2012; Paaijmans et al. 2013; Clavijo-Baquet et al. 2014; Zeh et al. 2014; Levy et al. 2015), although the strength and direction of the effect can depend on the (mean) temperature (Bozinovic et al. 2011; Vasseur et al. 2014). Endotherms might also be susceptible to DTR but knowledge of DTR effects in endotherms is restricted to humans, where the elderly experience up to 3% increase in hospital admissions and 1% increase in mortality per 1°C increase in DTR (Kan et al. 2007; Song et al. 2008; Cao et al. 2009; Liang et al. 2009; Tam et al. 2009; Lim et al. 2012). However, elderly humans behave very differently from endotherms in natural environments that are permanently exposed to natural variation in temperature. Thus the demographic and ecological consequences of changes in DTR in endotherms in natural environments remain unknown.

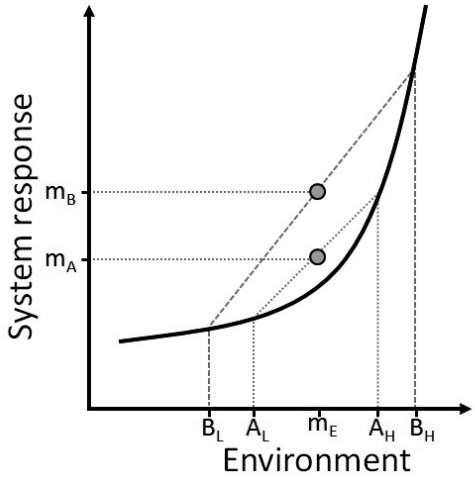

Fig. 1 Illustration of Jensen's inequality in a convex environment-system response scenario. Two environments (A and B) with the same mean state m_E can both be in one of two states (A_L & A_H; B_L & B_H) with equal frequency but with different ranges ($range_A < range_B$). It can be seen that because of the convex pattern the mean system response differs between environments A and B ($m_A < m_B$) despite A and B having the same average.

We used high-resolution (daily) mortality data to investigate the association between DTR and the survival of zebra finches (n=476) housed in outdoor aviaries, and hence exposed to natural variation in temperature (Fig. 2 A & B). Our population has resided in captivity for generations, but the species is originally widely distributed in Australia. The natural variation in DTR in our aviaries is entirely within the natural range (Plummer et al. 1995). The minimum temperature (MinT) can be lower in the Netherlands than in Australia (Zann 1996), but in various areas of Australia zebra finches regularly experience MinT below zero (Immelman 1965; Kikkawa 1980; Zann 1996) and our results were still supported when conditions outside the zebra finches natural range were excluded.

The timeframe over which one investigates effects of climatic variables on mortality (or any other trait) can be chosen in different ways. For example, one can arbitrarily choose to average climatic variables over one or two weeks before each day. However, such arbitrary choices may not reflect the timeframe over which the biological effects occur, and this approach implicitly makes the unlikely assumption that all days within the selected time interval have equal effects on the phenomenon that is studied. To resolve this issue we calculated the (weighted) time window over which climatic variables best correlated with mortality using a technique recently introduced by van de Pol and Cockburn (2011).

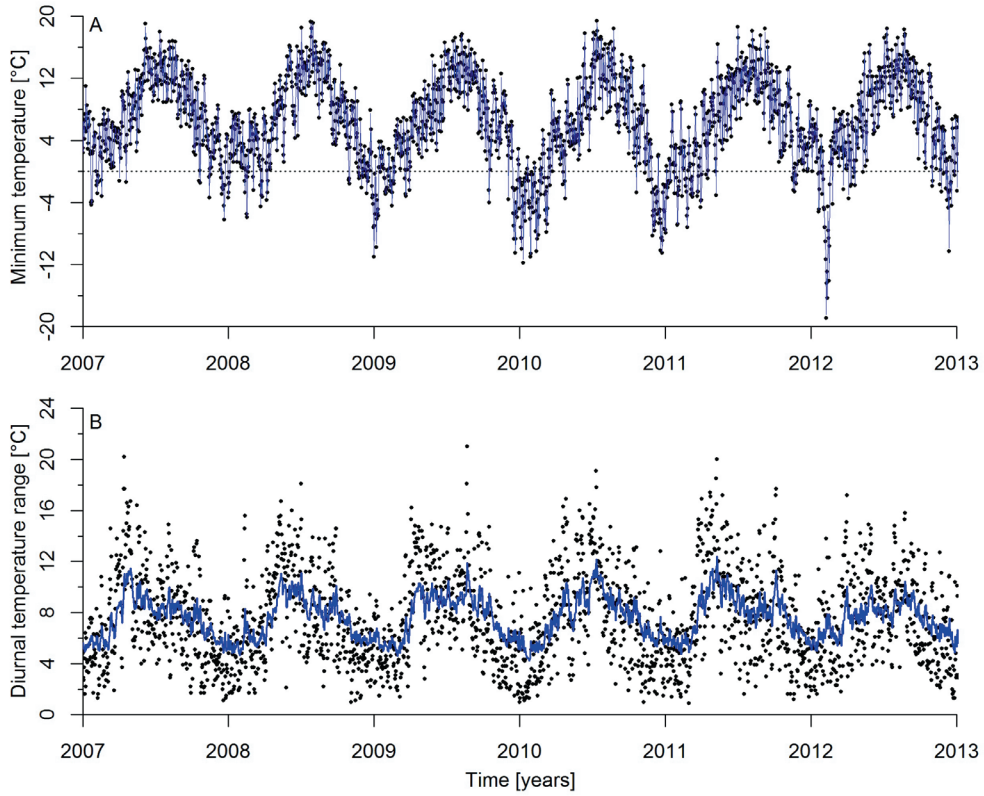

Fig. 2 Minimum temperature (A) and diurnal temperature range data (B) during the study period. Black dots represent the actual data, while blue lines depict the weighted data that are used in the analyses (table 1) based on the weight functions in Fig. 3. Dotted horizontal lines are references lines at 0°C for MinT and 8°C for DTR.

Data were collected over 5 years in the context of an experiment in which we manipulated environmental conditions during development (brood size) and in adulthood (foraging costs) in a 2x2 design with the primary aim to study effects on ageing and lifespan. Based on published results in ectotherms and humans (Kan et al. 2007; Song et al. 2008; Cao et al. 2009; Liang et al. 2009; Tam et al. 2009; Lim et al. 2012; Raffel et al. 2012; Paaijmans et al. 2013; Clavijo-Baquet et al. 2014; Zeh et al. 2014), we hypothesized, before analyzing the data, that large DTR could increase mortality. We further hypothesised that the strength of a DTR effect may depend on current or past environmental quality. Large broods are a poor developmental environment that causes pervasive negative effects during adulthood in many species (de Kogel 1997; Lindström 1999; Metcalfe and Monaghan 2001; Griffith and Buchanan 2010; Boonekamp et al. 2014), and hence this manipulation allows us to investigate whether effects of DTR depend on phenotypic quality. In laboratory environments no effort has to be made

to obtain food. In contrast, in more natural environments animals experience foraging costs. The foraging cost experiment thus allows us to compare DTR effects on mortality between a typical 'easy' laboratory foraging environment, with low foraging costs, and a 'hard' semi-natural foraging environment, with high foraging costs. Our expectation is that, since DTR represents a challenge, DTR will have more pronounced effects on mortality in animals that experience(d) poor quality environments.

Material and Methods

Birds and housing

All birds used in this study were reared and housed at the University of Groningen, the Netherlands (53° 13' 0" N / 6° 33' 0" E). Birds were bred indoors in single housed pairs housed in 80 x 40 x 40 cm (l x h x d) cages with two perches, a wooden nestbox and abundant nesting material (hay). Food (tropical seed mixture), water, grit and cuttlebone were provided ad libitum. In addition, the birds received one teaspoon of fortified canary food ("eggfood", by Bogena, Hedel, the Netherlands) 3 times a week, until hatching of the first chick. Birds were cross-fostered when the oldest chick in a brood was 5 days old to broods that were either small (2 young, sometimes 3) or large broods (6 young, sometimes 5 or 7). Birds reared in a large brood attained lower body mass during growth and this effect persisted into adulthood (Briga et al. submitted). Young were removed from the parental cage when 35 days old and housed in indoor aviaries until they were entered in the experiment at 3-4 months of age.

Adults were housed in eight large outdoor aviaries (LxHxW 310x210x150 cm) and subject to a foraging cost manipulation as described previously (Koetsier and Verhulst 2011). Briefly, in each aviary a food box was attached to the ceiling, with holes in the sides from which food (tropical seed mixture) could be obtained. In the easy foraging environment (4 aviaries) the food box has perches beneath the holes, while in the hard foraging environment these were removed (also 4 aviaries), forcing birds to fly and hover for seeds. Water (for drinking and bathing), grit and cuttlebone were provided ad libitum. In addition the birds received 1.25 g of fortified canary food (''eggfood'', by Bogena, Hedel, the Netherlands) per individual per week in three portions given on different days.

Each aviary contained 15-30 birds of one sex (4 aviaries of each sex). To maintain numbers within a limited range, new birds were periodically added to replace dead birds. The first batch was 3-24 months old when the experiment started and variation

in age when entering the experiment ('start age') was therefore included as variable in all analyses. The first batch was kept in similar housing as in the experiment until the experiment started.

The foraging experiment was conducted from Dec 9th 2007 till Jan 1st 2013. During this period, 478 birds were entered in the experiment of which 285 died a natural death and 7 died an accidental death. In all analyses, accidental deaths and birds still alive were censored, but treating accidental deaths as natural deaths did not change the conclusions (results not shown).

All methods and experimental protocols were carried out under the approval of the Animal Experimentation Ethical Committee of the University of Groningen, license 5150A. All methods were carried out in accordance with these approved guidelines.

Temperature data

Temperature data (Fig. 1) were collected at the weather station of Eelde, approximately 7 km from the aviaries (http://www.knmi.nl/klimatologie/), where temperature was recorded 1.5m above ground, every hour with accuracy of 0.1°C. DTR is the difference between maximum (MaxT) and minimum (MinT) temperature within one day. Both MinT and DTR measured at the weather station correlate well with the measurements at the aviaries (N=1196, r=0.96 and 0.83 for MinT and DTR respectively; Supplementary Fig. S2).

Apparent effects of DTR on mortality could instead be caused by minimum or maximum temperature, because DTR will be higher when either minimum or maximum temperature has an extreme value. To resolve this issue, we included MinT as a covariate in addition to DTR in all analyses and also tested the interaction between MinT and DTR. Alternatively, we could have included MaxT instead of MinT. However, DTR = MaxT – MinT, and thus when MinT and DTR are given the corresponding MaxT is known. Hence having DTR in the model with either MinT or MaxT is mathematically equivalent. To confirm this point we reran the best fitting model in Table 1 with MaxT instead of MinT which as expected confirmed the importance of DTR on mortality.

Statistical analyses

Survival was analysed using the counting process formulation of the Cox proportional hazard (CPH) model (Cox 1972; Andersen et al. 1993; Therneau and Grambsch 2000) in R (R Core Team 2014), version 3.0.1 with the function 'coxph' of package survival (Therneau 2013), version 2.37-4. The counting process formulation allows the

coefficient to be estimated at each time point and thus time-dependent covariates, such as minimum temperature, DTR and age can be included. Time was portioned into daily intervals for all analyses.

When analysing effects of climatic variables on system responses, the time window over which the climate variable affect system response needs to be identified. However, this time is usually not known. Should the temperature be quantified as a (weighted) mean over the preceding day, week or month? To resolve this, we identified the time window over which each climate variable affected survival using a flexible time window approach (van de Pol and Cockburn 2011). In brief, this method uses a maximum-likelihood optimization procedure to estimate a weight function over a time window that creates weighted temperature variables that best describe the variation in mortality data. As weight function we used a three-parameter Weibull function. Weight functions may differ between treatments climate variables, and we thus estimated weighing functions for each climate variable separately. To estimate the strength of the difference in time windows between climatic variables, we used the weight function of one climatic variable to construct the other weighted climatic variable. We then compared the fit of this model relative to the fit of the model with the best fitting weight function. Model fits were compared using Akaike Information Criterion (AICc). Weight functions of the climate variables did not differ between treatments ($0.1<\Delta AICc<0.5$) and hence all analyses were carried out using the weight functions as in Fig. 3 in both treatments.

Except for the first batch to enter the adult phase of the experiment, other batches were housed indoors prior to being entered into the experiment. These birds were thus not exposed to the outdoor climatic variables before starting the foraging cost experiment and their mortality cannot be included in the survival analyses for the length of the period that the weighted climatic variable was calculated. Given the results of the time window analysis (Fig. 3), we excluded the first month of survival data after birds were entered in the foraging cost experiment. As a control we also ran the final model with (i) all data included and (ii) three months of data excluded, and both gave results that were consistent with those reported here (results not shown).

We used a model selection approach to find the model best supported by the data. To this end we followed Burnham and Anderson model selection approach (Burnham and Anderson 2002; Burnham et al. 2011), based on Akaike Information Criterion (AICc) with the function 'dredge' of the package 'MuMIn' (Barton 2013). In brief, this is a hypothesis-based approach that generates, given a global model, subset models that best fit the data. This makes it possible to assess model support for each hypothesis

tested. Model support is shown here by ranking all subset models within 4 AICc of the best model fit. Weighted DTR and MinT were mean centered in all analyses.

The counting process formulation of the CPH model allows for non-proportionality by including the interaction between the main effect and time or age. Other assumptions of the CPH models were fulfilled as indicated by scaled deviance and martingale residual plots. Age was square-root transformed to fit the assumption of a linear age effect on mortality. Because, we found virtually no support for sex-specific mortality or for sex-specific DTR effects (see section 3 of the supplementary material), sexes were pooled in all analyses. Many random effects can potentially be included in these analyses: birth nest, genetic mother, genetic father, rear nest, rear mother, rear father, (birth) batch and aviary. We ran all models with aviary as random effect. We previously verified that including other random effects in CPH models did not improve the models (results not shown).

Results

The climatic data are shown in Fig. 2. Estimating the time frame over which the climatic variables best explained mortality showed that the effects were most pronounced the day preceding the event (i.e. the survival or death of an individual), accounting for 77% and 15% of the weight for MinT and DTR respectively (Fig. 3). The time window over which MinT affected mortality was much shorter than DTR ($8.9<\Delta AICc<12.5$, see methods for details on test). For MinT, (almost) 80% of the effect was captured the day before the event, while reaching 80% for DTR required 3 months (Fig. 3). Thus MinT had an immediate effect on bird mortality in comparison with DTR for which the effect was delayed.

DTR and MinT both affected mortality but in an interaction, which received strong support: in both foraging environments all models within 4 AICc of the best fitting model contained the interaction between DTR and MinT (Table 1). However, the sign of the interaction term depended on foraging environment (Supplementary Table S3), which we discuss in more detail below. This three-way interaction (Treat*DTR*MinT) is well supported since it was included in all 14 best fitting models (Supplementary Table S3). In the case of the best fitting model this interaction had a $X^2=10.41$ 1 , $p=0.0013$, and removing it from the best fitting model decreased model fit by 9.2 AICc. Furthermore, all selected models contained an interaction between age and DTR, indicating a changing DTR effect with increasing age (Table 1; Supplementary Table S2).

Because in natural populations most birds are young, we here focus the presentation on young birds (but note that models in Table 1 are based on the complete data set). Details of age-specific changes are discussed in section 4 of the supplementary material.

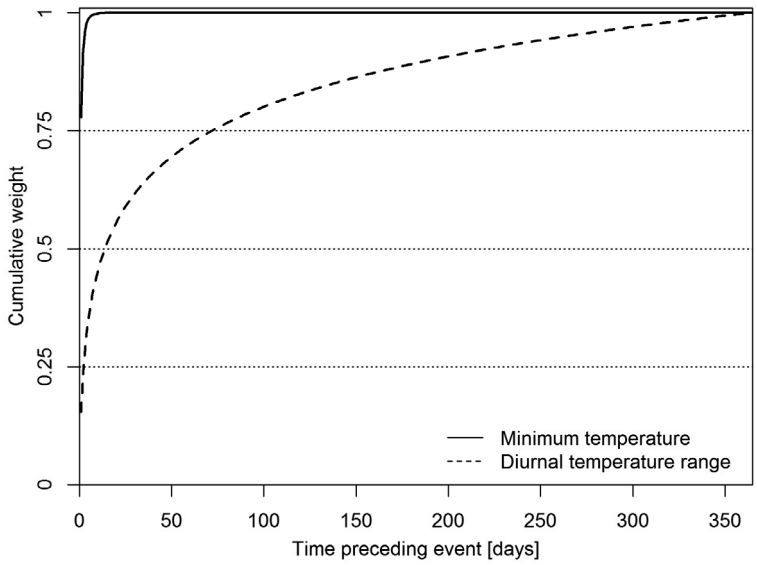

Fig. 3 Cumulative weight functions (Weibull fits) that were best supported by the data. X-axis refers to time before the event (i.e. either survival or death of an individual) in days. Y-axis shows the cumulative weight or "influence" of the climatic variable on the event. For example for MinT, weights add up to 100% within 5 days, which means that all the minimum temperature of the 5 days prior to the event determine event outcome. For DTR the effect is delayed: 100% of the weight needs more than 3 months to accumulate. Horizontal dotted lines are reference lines at given weights.

In the easy foraging environment, the effect of DTR was most pronounced on cold days (Fig. 4A, Supplementary Fig. S4A, Table 1): birds experienced an up to ten-fold increase in mortality over the DTR range in our dataset. Evidence for this is robust since all selected models (Table 1) included DTR and the interaction between DTR and MinT. Thus, in the easy foraging environment, we found that large DTR increased mortality on cold days, but not on warm days.

In the semi-natural foraging environment the evidence for an interaction between DTR and MinT was also robust: excluding the interaction decreased model fit with at least 3.3 AICc and models without the interaction all had weights ≤0.04 (Table 1). Note however that coefficients for the DTR*MinT interaction were in the opposite direction compared to the easy environment (Table 1). Indeed large DTR increased mortality on warm days, but not on cold days (Fig. 4B, Supplementary Fig. S4B). Note that for DTR

the coefficient in the best fitting model is larger in the semi-natural than in the easy environment on days with MinT of 6°C, which is the mean MinT at our study location (2.04 vs. 1.36 respectively in the best fitting model). This implies an increase of 1°C DTR has a stronger effect in the semi-natural than in the easy foraging environment (increases in mortality rate per °C DTR of 104% vs. 36% respectively). Thus, in the semi-natural foraging environment large DTR increased mortality on warm days but not on cold days, opposite to the pattern in the easy foraging environment.

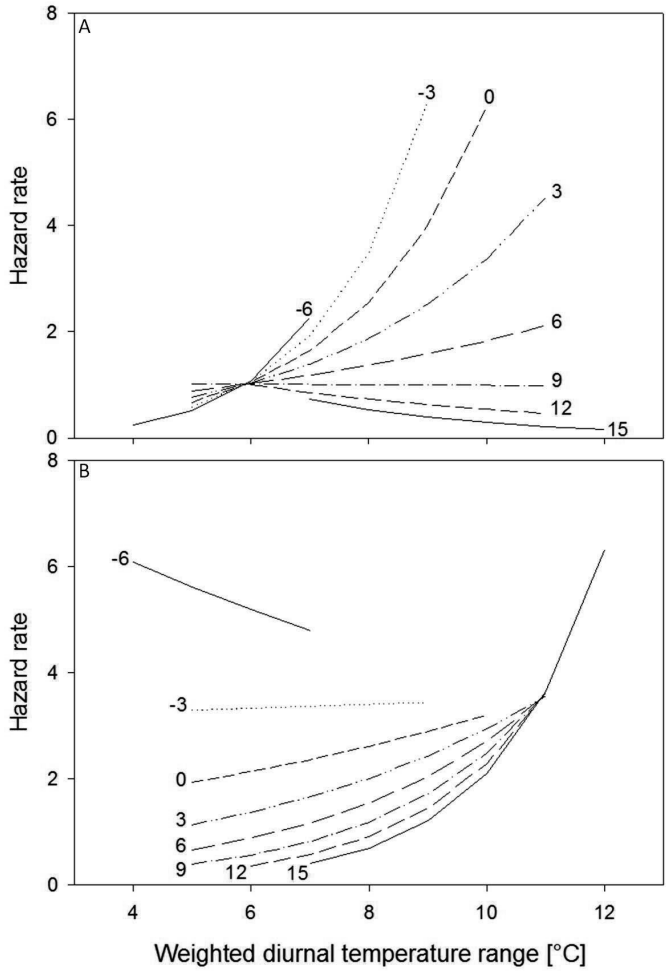

Fig. 4 Hazard rate in relation to minimum temperature and diurnal temperature range (DTR) in easy (A) and semi natural (B) foraging environment for the best fitting model of Table 1. Fitted lines represent hazard rates for different weighted minimum temperatures (temperatures plotted at line ends) calculated for individuals with a study start age at start of 1 year old (population mean) and 0.36 years in study (at which 90% of the population is alive). Lines cover 95% of the data range. See Supplementary Fig. S4 for a contour plot with data distribution and hazard rates.

Table 1 Cox proportional hazard models in relation to climate, age and experimental treatments, with the focal variables indicated by grey shading. Data shown are coefficients (or NA for variables not in the model, and + for included random terms). All models within 4 AICc of the best model are shown, ordered by AICc. Model weights are relative to all fitted models. Note that model coefficients are hazard ratios: a hazard of 1 implies no effect and a hazard ratio of 1.25 for 'AgeStart' means that the hazard rate increases 25% per year. There is no main effect 'age' because it is included in the baseline mortality. AgeStart: Age at start of the foraging experiment in years.

Easy foraging	Temperature variables [°C]			Age related correction variables [Years]				Developmental variables			Random term	Model Fit			
environment Model	DTR	MinT	DTR*MinT	AgeStart	AgeStart*Age	DTR*Age	MinT*Age	Brood Size	Brood Size *DTR	Brood Size *Age	Aviary	df	AICc	ΔAICc	weight
1	1.36	0.89	0.953	1.25	NA	0.74	1.06	NA	NA	NA	+	10	1269	0.00	0.09
2	1.22	0.95	0.951	1.24	NA	0.83	NA	NA	NA	NA	+	9	1269	0.03	0.09
3	0.98	0.95	0.951	1.22	NA	NA	NA	NA	NA	NA	+	8	1270	0.65	0.07
4	1.40	0.88	0.952	1.69	0.81	0.73	1.07	NA	NA	NA	+	11	1270	1.35	0.05
5	1.24	0.95	0.950	1.56	0.85	0.83	NA	NA	NA	NA	+	10	1271	1.62	0.04
6	1.36	0.89	0.953	1.26	NA	0.74	1.06	0.99	NA	NA	+	11	1271	1.86	0.04
7	1.22	0.95	0.951	1.24	NA	0.83	NA	0.99	NA	NA	+	10	1271	1.89	0.04
8	1.38	0.89	0.953	1.27	NA	0.74	1.06	1.13	NA	0.91	+	12	1271	2.08	0.03
9	0.97	0.91	0.952	1.23	NA	NA	1.03	NA	NA	NA	+	9	1271	2.08	0.03
10	1.23	0.95	0.951	1.25	NA	0.83	NA	1.13	NA	0.91	+	11	1271	2.08	0.03
11	1.20	0.95	0.951	1.25	NA	0.83	NA	0.98	0.97	NA	+	11	1271	2.31	0.03
12	0.98	0.95	0.951	1.47	0.88	NA	NA	NA	NA	NA	+	9	1271	2.39	0.03
13	1.24	0.95	0.951	1.26	NA	0.82	NA	1.12	0.97	0.90	+	12	1272	2.59	0.03
14	0.98	0.95	0.951	1.22	NA	NA	NA	0.99	NA	NA	+	9	1272	2.59	0.03
15	1.35	0.89	0.953	1.26	NA	0.74	1.06	0.98	0.97	NA	+	12	1272	2.66	0.02
16	1.39	0.89	0.953	1.27	NA	0.73	1.06	1.12	0.97	0.91	+	13	1272	2.81	0.02
17	0.98	0.95	0.951	1.23	NA	NA	NA	1.13	NA	0.91	+	10	1272	3.12	0.02
18	1.40	0.88	0.952	1.70	0.81	0.73	1.07	0.99	NA	NA	+	12	1272	3.22	0.02
19	1.24	0.95	0.950	1.56	0.85	0.83	NA	0.99	NA	NA	+	11	1272	3.49	0.02
20	0.97	0.95	0.951	1.22	NA	NA	NA	0.98	0.97	NA	+	10	1272	3.53	0.02
21	1.41	0.88	0.952	1.63	0.84	0.73	1.06	1.12	NA	0.91	+	13	1273	3.66	0.01
22	0.97	0.91	0.951	1.52	0.86	NA	1.04	NA	NA	NA	+	10	1273	3.75	0.01
23	1.25	0.95	0.950	1.50	0.88	0.82	NA	1.12	NA	0.91	+	12	1273	3.85	0.01
24	1.22	0.95	0.951	1.57	0.85	0.83	NA	0.98	0.97	NA	+	12	1273	3.94	0.01
Semi-natural environment Model	**DTR**	**MinT**	**DTR*MinT**	**AgeStart**	**AgeStart*Age**	**DTR*Age**	**MinT*Age**	**Brood Size**	**Brood Size *DTR**	**Brood Size *Age**	**Aviary**	**df**	**AICc**	**ΔAICc**	**weight**
1	2.04	0.88	1.03	1.34	NA	0.50	1.05	1.15	NA	NA	+	11	1247	0.00	0.19
2	1.82	0.93	1.04	1.33	NA	0.56	NA	1.31	NA	0.91	+	11	1247	0.13	0.18
3	2.06	0.88	1.03	1.35	NA	0.49	1.05	1.31	NA	0.91	+	12	1247	0.21	0.17
4	1.76	0.93	1.04	1.04	1.22	0.58	NA	1.32	NA	0.90	+	12	1248	1.33	0.10
5	1.99	0.88	1.03	1.16	1.12	0.50	1.05	1.15	NA	NA	+	12	1249	1.70	0.08
6	1.99	0.88	1.04	1.09	1.18	0.51	1.05	1.32	NA	0.90	+	13	1249	1.70	0.08
7	1.79	0.93	NA	1.34	NA	0.55	NA	1.15	1.00	NA	+	10	1250	3.32	0.04
8	1.82	0.92	NA	1.35	NA	0.55	NA	1.30	0.99	0.91	+	11	1251	3.57	0.03

While there is strong evidence that growing up in large broods negatively affects lifespan in the semi-natural environment (Table 1), there is little support for the hypothesis that brood size manipulation affects vulnerability to DTR in either environment (Table 1).

DTR varied seasonally (Fig. 2), and the association between DTR and mortality can therefore be confounded with other climatic variables with similar seasonal variation as DTR. We captured the seasonal variation of climatic variables by adding photoperiod as a covariate to the best models of Table 1 and Supplementary tables S2, S3 and S4. Photoperiod was in no case significant ($0.23<X^2<2.29$, $0.13<p<0.63$), never improved the model fit ($0.3<\Delta AICc<6$), and the effect on the model coefficients of DTR, Min T or their interaction was negligible. We therefore conclude that DTR contributes to mortality independently of the seasonal variation of other (climatic) variables.

Discussion

A large DTR substantially increased mortality rate and this effect was modulated by minimum temperature, age and environment, but not by developmental conditions. That DTR affects mortality is relevant because climate change is associated with changes in temperature variability on short time scales, i.e. days (Vose et al. 2005; Solomon et al. 2007; Stocker et al. 2013). Yet, to our knowledge this is the first study on the mortality consequences of changes in DTR in a non-human endotherm. Our study shows that changes in DTR can potentially pose a threat to the population viability of endotherms and that this threat is most apparent in semi-natural environments. Note however that our study exploited natural variation in DTR and hence we cannot exclude the possibility that other climatic variables contributed to the observed patterns.

Our results indicate that responses to changes in climate variability differ considerably between laboratory and semi-natural environments, in that foraging costs determined the temperature range at which birds are most susceptible to large DTR. DTR increased mortality more on days with *low* minimum temperature in an easy foraging environment, but more on days with *high* minimum temperature in a semi-natural foraging environment. A possible reason for such environment dependent effects is heat stress, which in this experiment can arise in the semi-natural foraging environment because of the combination of high temperatures with unavoidable heat production through increased foraging effort, which can have major effects on bird behaviour and physiology (du Plessis et al. 2012). Muscular exercise decreases heat tolerance because it generates heat which needs to be dissipated to avoid for example mitochondrial and immune

dysfunction, DNA damage, organ failure and even death (Walsh and Whitham 2006; Yan et al. 2006; Jimenez et al. 2008; Paul et al. 2009; Speakman and Król 2010; Sawka et al. 2011; Gamo et al. 2013). Such interaction effects are important when estimating the biological consequences of climate change (Parmesan et al. 2013). Our results indicate that climate change experiments in laboratory conditions may not simply underestimate impacts of climate change, but may provide completely contradictory results to natural conditions. Since climate change is associated with increases in minimum temperatures (Solomon et al. 2007; Stocker et al. 2013), our result also suggests that the DTR effect in natural populations may become more important in a warming world.

Associations between DTR and survival changed with age (Fig. S3). The dependence of the DTR effect on age may be due to individual heterogeneity in combination with selective disappearance: individuals that are sensitive to large DTR die and thus only birds that are relatively DTR insensitive remain at old age. It is worth noting that in natural populations the majority of individuals are young (Jones et al. 2014) and hence natural populations are likely to be more susceptible to DTR effects than our relatively protected study population.

We estimated the time window over which climate variables affected mortality, and found this to differ considerably between climatic variables, with MinT having a more immediate effect than DTR. The contrast between these time windows indicates that these climatic variables affect mortality through mechanisms that operate on different time scales. That MinT affected mortality on a short time scale is likely to reflect limits on the instantenous capacity to generate heat. We are less certain regarding the mechanism through which DTR affects mortality. However, birds adjust physiologically their energy allocation to ambient temperatures within days (Swanson and Olmstead 1999; Swanson 2001; Vézina et al. 2006; Bouwhuis et al. 2011) and short term temperature variation increases daily energy expenditure (Pendlebury 2004). The delayed DTR effect may thus reflect increased vulnerability due to the cumulative physiological acclimatization costs when DTR is high for a prolonged period.

In conclusion, our results show that DTR strongly affects avian mortality. DTR effects on mortality have previously been demonstrated in one other endotherm, humans, but our finding of an almost two-fold increase in mortality per °C DTR substantially exceeds the 1-3% increase in hospital admissions and 1% increase in mortality found in humans (Kan et al. 2007; Song et al. 2008; Cao et al. 2009; Liang et al. 2009; Tam et al. 2009; Lim et al. 2012). We note however that time windows over which DTR affects human mortality have to our knowledge not been quantified, and by definition such an analysis

would yield stronger DTR effects than hitherto reported. In humans, large DTR is associated with cardiovascular and respiratory dysfunctions, causing increased hospital admissions and mortality (Song et al. 2008; Liang et al. 2009; Tam et al. 2009; Lim et al. 2012) but whether the same mechanisms causes the DTR effects in birds remains to be established. Understanding the physiological mechanisms involved in the DTR effect is of interest in its own right, and may help predict which and when populations are most at risk. However, regardless of the underlying physiological mechanisms, our results, together with those found in humans, show that DTR effects are important for survival and hence for understanding and predicting population responses to climate change (Jenouvrier 2013).

Acknowledgements

Egbert Koetsier, funded by an NWO Vici-grant to S.V., set up the experiment, and over the years numerous students contributed to running the experiment. We thank the anonymous reviewers for their useful comments and suggestions.

Supplementary information to:

Large diurnal temperature range increases bird sensitivity to climate change

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Supplementary information S1: Information on temperature variables

Table S1 Summary statistics of weather variables used in this analysis.

	MinT		DTR	
Year	Mean	SD	Mean	SD
2007	7.3	5.0	7.4	3.6
2008	6.8	5.6	7.5	3.6
2009	6.3	5.8	7.7	3.7
2010	4.6	7.0	7.7	3.8
2011	6.5	5.6	7.9	4.0
2012	6.1	6.3	7.4	3.3

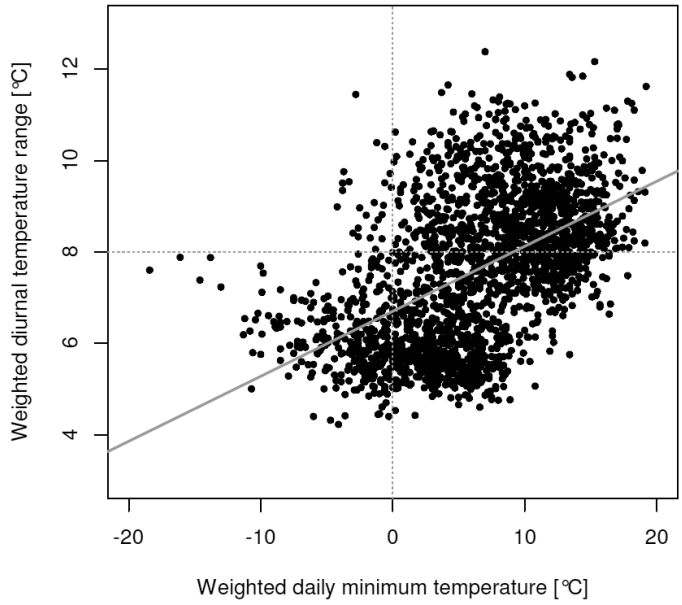

Fig. S1 Diurnal temperature range data plotted against minimum temperature. Grey line shows correlation (r=0.51).

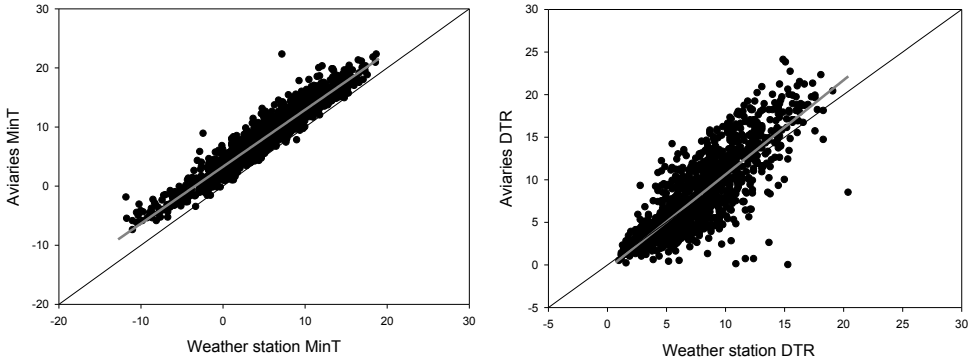

Fig. S2 Consistency between temperatures measured at the nearby weather station at Eelde and at the aviaries. Correlations are strong with r=0.96 and 0.83 for MinT and DTR respectively. Diagonal line shows x=y, grey line shows the fitted regression line. Note that we used weather station data over aviary data because of missing climate data for measurements at the aviaries.

Supplementary information S2: Model support for foraging environment specific DTR effects.

This analysis consists of 3 steps:

(i) Model support for age specific associations between DTR and bird mortality (table S2). We thus analysed these patterns for young (<median age) and old birds (>median age) separately.

(ii) In young birds there is strong support for treatment specific effects of DTR (table S3),

(iii) Old birds show treatment specific associations between DTR and bird mortality while DTR overall has a positive effect on survival (table S4).

Table S2 Associations between DTR and bird mortality weaken with age (as indicated by the grey column). Table shows model support based on AICc criteria, including all model fits on data within 4AICc of the best model. Table shows model support based on AICc criteria, including all model fits on data within 4AICc of the best model. The best model fit for each treatment is given in the top row. For fitting variables coefficients are given. Non fitting variables have coefficient NA. Grey columns emphasize the variables to use for the take home message. Note that these are a Cox proportional hazards models and model coefficients are thus hazard ratios. A hazard ratio of one implies no effect and for example a hazard ratio of 1.35 for 'AgeStart' means that the hazard rate increases 35% per year. Note that there is no main effect age since it is included in the baseline mortality curve. AgeStart: Age at start of the foraging experiment in years.

All data Ages	Temperature variables [°C]			Foraging treatment variables [Hard]				Age related correction variables [Years]					Random term	Model Fit			
Model	DTR	MinT	DTR *MinT	Treat	Treat *DTR	Treat *MinT	Treat*DTR *MinT	AgeStart	AgeStart *Age	DTR *Age	MinT *Age	Treat *Age	Aviary	df	AICc	ΔAICc	weight
1	1.81	0.88	NA	0.96	0.78	NA	NA	1.35	NA	0.58	1.07	NA	+	7	2716	0.00	0.12
2	1.77	0.88	0.99	0.73	0.85	0.99	1.04	1.34	NA	0.59	1.06	NA	+	10	2717	0.31	0.11
3	1.80	0.88	NA	0.92	0.81	0.98	NA	1.35	NA	0.58	1.06	NA	+	8	2718	1.49	0.06
4	1.80	0.88	1.00	0.96	0.78	NA	NA	1.35	NA	0.58	1.06	NA	+	8	2718	1.85	0.05
5	1.82	0.88	NA	1.07	0.78	NA	NA	1.35	NA	0.58	1.07	0.91	+	8	2718	1.97	0.05
6	1.83	0.87	NA	0.95	0.78	NA	NA	1.43	0.96	0.57	1.07	NA	+	8	2718	1.98	0.05
7	1.78	0.88	0.99	0.83	0.85	0.99	1.04	1.34	NA	0.58	1.06	0.90	+	11	2719	2.26	0.04
8	1.79	0.88	0.99	0.73	0.85	0.99	1.04	1.42	0.96	0.58	1.06	NA	+	11	2719	2.29	0.04
9	1.55	0.94	0.99	0.73	0.86	0.99	1.04	1.32	NA	0.67	NA	NA	+	9	2720	3.15	0.03
10	1.57	0.94	NA	0.96	0.78	NA	NA	1.33	NA	0.67	NA	NA	+	6	2720	3.16	0.03
11	1.77	0.88	NA	0.93	NA	0.96	NA	1.35	NA	0.59	1.06	NA	+	7	2720	3.25	0.02
12	1.78	0.88	NA	NA	NA	NA	NA	1.35	NA	0.58	1.06	NA	+	5	2720	3.30	0.02
13	1.79	0.88	0.99	0.92	0.81	0.98	NA	1.35	NA	0.58	1.06	NA	+	9	2720	3.32	0.02
14	1.81	0.88	NA	1.03	0.81	0.98	NA	1.35	NA	0.58	1.06	0.92	+	9	2720	3.47	0.02
15	1.82	0.88	NA	0.92	0.81	0.98	NA	1.43	0.96	0.58	1.07	NA	+	9	2720	3.48	0.02
16	1.81	0.88	1.00	1.07	0.78	NA	NA	1.35	NA	0.58	1.06	0.91	+	9	2720	3.82	0.02
17	1.82	0.87	1.00	0.95	0.78	NA	NA	1.44	0.95	0.57	1.07	NA	+	9	2720	3.83	0.02
18	1.83	0.88	NA	1.07	0.78	NA	NA	1.43	0.96	0.57	1.07	0.91	+	9	2720	3.95	0.02

Table S3 Young birds show treatment specific associations between DTR and bird mortality (as indicated by the grey column).

Data Young	Temperature variables [°C]			Foraging treatment variables [Hard]				Age related correction variables [Years]					Random term	Model Fit			
Model	DTR	MinT	DTR *MinT	Treat	Treat *DTR	Treat *MinT	Treat*DTR *MinT	AgeStart	AgeStart *Age	DTR *Age	MinT *Age	Treat *Age	Aviary	df	AICc	ΔAICc	weight
1	1.22	0.91	1.00	0.68	1.02	1.01	1.11	1.22	NA	NA	NA	NA	+	8	1191	0.00	0.08
2	1.20	0.91	1.00	0.67	1.02	1.01	1.11	0.74	1.97	NA	NA	NA	+	9	1192	0.65	0.06
3	1.20	0.86	1.00	0.68	1.01	1.01	1.11	1.22	NA	NA	1.08	NA	+	9	1192	0.80	0.06
4	1.21	0.91	1.00	1.25	1.05	1.01	1.12	1.22	NA	NA	NA	0.41	+	9	1192	1.06	0.05
5	1.18	0.86	1.01	0.68	1.01	1.01	1.11	0.69	2.12	NA	1.09	NA	+	10	1192	1.17	0.05
6	1.53	0.84	1.01	0.68	1.00	1.01	1.11	1.23	NA	0.70	1.13	NA	+	10	1192	1.22	0.05
7	1.35	0.91	1.00	0.67	1.02	1.01	1.11	1.23	NA	0.86	NA	NA	+	9	1193	1.62	0.04
8	1.20	0.91	1.00	1.27	1.04	1.01	1.12	0.73	2.01	NA	NA	0.40	+	10	1193	1.63	0.04
9	1.19	0.87	1.00	1.22	1.04	1.01	1.12	1.22	NA	NA	1.08	0.44	+	10	1193	1.98	0.03
10	1.18	0.86	1.00	1.24	1.03	1.02	1.12	0.69	2.16	NA	1.09	0.43	+	11	1193	2.29	0.03
11	1.54	0.84	1.01	1.23	1.01	1.02	1.12	1.23	NA	0.69	1.13	0.43	+	11	1193	2.35	0.03
12	1.41	0.85	1.01	0.68	1.00	1.02	1.11	0.82	1.71	0.78	1.13	NA	+	11	1194	2.55	0.02
13	1.23	0.91	1.00	0.67	1.01	1.01	1.11	0.75	1.91	0.97	NA	NA	+	10	1194	2.64	0.02
14	1.36	0.91	1.00	1.26	1.04	1.01	1.12	1.23	NA	0.85	NA	0.41	+	10	1194	2.64	0.02
15	1.24	0.91	NA	NA	NA	NA	NA	1.25	NA	NA	NA	NA	+	3	1194	2.89	0.02
16	1.22	0.86	NA	NA	NA	NA	NA	1.25	NA	NA	1.09	NA	+	4	1194	3.42	0.02
17	1.22	0.91	NA	NA	NA	NA	NA	0.76	1.98	NA	NA	NA	+	4	1194	3.50	0.01
18	1.23	0.91	1.00	1.27	1.04	1.01	1.12	0.75	1.93	0.96	NA	0.40	+	11	1195	3.61	0.01
19	1.41	0.85	1.01	1.25	1.01	1.02	1.12	0.81	1.74	0.77	1.12	0.42	+	12	1195	3.65	0.01
20	1.20	0.85	NA	NA	NA	NA	NA	0.70	2.16	NA	1.10	NA	+	5	1195	3.68	0.01
21	1.55	0.84	NA	NA	NA	NA	NA	1.27	NA	0.70	1.14	NA	+	5	1195	3.88	0.01

Table S4 Old birds do not show treatment specific associations between DTR and bird mortality (as indicated by the grey column Treat*DTR*MinT). DTR overall has a positive effect on survival (grey column DTR).

Data Old	Temperature variables [°C]			Foraging treatment variables [Hard]				Age related correction variables [Years]					Random term	Model Fit			
Model	DTR	MinT	DTR *MinT	Treat	Treat *DTR	Treat *MinT	Treat*DTR *MinT	AgeStart	AgeStart *Age	DTR *Age	MinT *Age	Treat *Age	Aviary	df	AICc	ΔAICc	weight
1	0.74	1.38	NA	0.82	NA	0.92	NA	1.40	NA	NA	0.82	NA	+	6	1534	0.00	0.07
2	0.73	1.35	NA	0.82	NA	0.92	NA	3.73	0.57	NA	0.83	NA	+	7	1534	0.37	0.06
3	0.74	1.36	NA	0.34	NA	0.93	NA	1.40	NA	NA	0.83	1.68	+	7	1535	1.51	0.03
4	0.74	1.38	NA	0.80	0.91	0.93	NA	1.40	NA	NA	0.82	NA	+	7	1535	1.67	0.03
5	0.74	1.39	0.99	0.82	NA	0.92	NA	1.40	NA	NA	0.82	NA	+	7	1535	1.74	0.03
6	0.55	1.45	NA	0.82	NA	0.92	NA	1.41	NA	1.19	0.80	NA	+	7	1536	1.84	0.03
7	0.73	1.34	NA	0.36	NA	0.93	NA	3.68	0.57	NA	0.84	1.64	+	8	1536	1.94	0.03
8	0.73	1.35	NA	0.79	0.91	0.93	NA	3.73	0.57	NA	0.83	NA	+	8	1536	2.03	0.03
9	0.73	1.37	0.99	0.81	NA	0.92	NA	3.79	0.56	NA	0.83	NA	+	8	1536	2.04	0.03
10	0.48	1.46	NA	0.82	NA	0.92	NA	4.00	0.55	1.28	0.80	NA	+	8	1536	2.06	0.03
11	0.74	1.37	NA	0.80	0.76	NA	NA	1.39	NA	NA	0.83	NA	+	6	1537	2.89	0.02
12	0.74	1.36	NA	0.32	0.91	0.94	NA	1.40	NA	NA	0.83	1.73	+	8	1537	3.13	0.02
13	0.73	1.34	NA	0.80	0.76	NA	NA	3.72	0.57	NA	0.84	NA	+	7	1537	3.23	0.01
14	0.74	1.38	0.99	0.34	NA	0.92	NA	1.40	NA	NA	0.82	1.67	+	8	1537	3.26	0.01
15	0.55	1.44	NA	0.34	NA	0.93	NA	1.41	NA	1.20	0.80	1.70	+	8	1537	3.34	0.01
16	0.74	1.39	0.99	0.79	0.92	0.93	NA	1.40	NA	NA	0.82	NA	+	8	1537	3.44	0.01
17	0.55	1.45	NA	0.80	0.91	0.93	NA	1.41	NA	1.20	0.80	NA	+	8	1537	3.50	0.01
18	0.73	1.34	NA	0.33	0.91	0.94	NA	3.68	0.57	NA	0.84	1.68	+	9	1537	3.56	0.01
19	0.56	1.46	0.99	0.82	NA	0.92	NA	1.41	NA	1.18	0.79	NA	+	8	1537	3.60	0.01
20	0.74	1.36	NA	0.20	0.77	NA	NA	1.39	NA	NA	0.83	2.27	+	7	1537	3.61	0.01
21	0.48	1.45	NA	0.35	NA	0.93	NA	3.96	0.55	1.29	0.80	1.65	+	9	1537	3.62	0.01
22	0.73	1.35	0.99	0.36	NA	0.92	NA	3.75	0.57	NA	0.83	1.63	+	9	1537	3.63	0.01
23	0.72	NA	NA	0.80	0.76	NA	NA	5.33	0.46	NA	NA	NA	+	5	1537	3.68	0.01
24	0.48	1.46	NA	0.79	0.91	0.93	NA	4.01	0.55	1.29	0.80	NA	+	9	1537	3.71	0.01
25	0.73	1.36	0.99	0.79	0.92	0.93	NA	3.80	0.56	NA	0.83	NA	+	9	1537	3.73	0.01
26	0.49	1.47	0.99	0.81	NA	0.92	NA	4.06	0.54	1.27	0.79	NA	+	9	1537	3.76	0.01
27	1.41	NA	NA	0.80	0.76	NA	NA	4.08	0.53	0.66	NA	NA	+	6	1537	3.80	0.01
28	0.75	0.98	NA	0.82	NA	0.92	NA	5.28	0.47	NA	NA	NA	+	6	1538	3.95	0.01

Supplementary information S3: Model support for the absence of sex specific effects.

Table S5 Weak support for sex-specific associations between DTR and bird mortality (as indicated by the grey column) in the easy foraging environment.

Easy treatment	Temperature variables [°C]			Sex specific variables [Male]			Age related correction variables [Years]				Random term	Model Fit			
Model	DTR	MinT	DTR*MinT	Sex	DTR*Sex	Sex*Age	AgeStart	AgeStart*Age	DTR*Age	MinT*Age	Aviary	df	AICc	ΔAICc	weight
1	1.36	0.89	0.95	NA	NA	NA	1.25	NA	0.74	1.06	+	10	1269	0.00	0.06
2	1.22	0.95	0.95	NA	NA	NA	1.24	NA	0.83	NA	+	9	1269	0.03	0.06
3	0.98	0.95	0.95	1.63	NA	0.59	1.22	NA	NA	NA	+	10	1269	0.42	0.05
4	0.98	0.95	0.95	NA	NA	NA	1.22	NA	NA	NA	+	8	1270	0.65	0.05
5	1.22	0.95	0.95	1.63	NA	0.59	1.23	NA	0.83	NA	+	11	1270	0.68	0.05
6	1.36	0.89	0.95	1.63	NA	0.59	1.25	NA	0.75	1.06	+	12	1270	0.88	0.04
7	0.98	0.95	0.95	0.78	NA	NA	1.22	NA	NA	NA	+	9	1270	1.31	0.03
8	1.40	0.88	0.95	NA	NA	NA	1.69	0.81	0.73	1.07	+	11	1270	1.35	0.03
9	1.22	0.95	0.95	0.79	NA	NA	1.24	NA	0.83	NA	+	10	1270	1.52	0.03
10	1.24	0.95	0.95	NA	NA	NA	1.56	0.85	0.83	NA	+	10	1271	1.62	0.03
11	0.98	0.95	0.95	1.70	1.11	0.58	1.21	NA	NA	NA	+	11	1271	1.67	0.03
12	0.97	0.91	0.95	1.63	NA	0.59	1.22	NA	NA	1.03	+	11	1271	1.79	0.03
13	1.20	0.95	0.95	1.67	1.10	0.59	1.23	NA	0.84	NA	+	12	1271	1.90	0.02
14	1.35	0.89	0.95	1.67	1.10	0.59	1.25	NA	0.75	1.06	+	13	1271	2.03	0.02
15	0.97	0.91	0.95	NA	NA	NA	1.23	NA	NA	1.03	+	9	1271	2.08	0.02
16	0.98	0.95	0.95	1.63	NA	0.59	1.49	0.87	NA	NA	+	11	1271	2.12	0.02
17	1.36	0.89	0.95	0.78	NA	NA	1.25	NA	0.74	1.06	+	11	1271	2.20	0.02
18	1.40	0.88	0.95	1.63	NA	0.59	1.70	0.80	0.73	1.07	+	13	1271	2.24	0.02
19	1.24	0.95	0.95	1.63	NA	0.59	1.57	0.85	0.83	NA	+	12	1271	2.26	0.02
20	0.98	0.95	0.95	NA	NA	NA	1.47	0.88	NA	NA	+	9	1271	2.39	0.02
21	1.22	0.95	0.95	0.81	1.10	NA	1.24	NA	0.83	NA	+	11	1272	2.67	0.02
22	0.98	0.95	0.95	0.80	1.10	NA	1.22	NA	NA	NA	+	10	1272	2.74	0.02
23	0.97	0.91	0.95	0.78	NA	NA	1.23	NA	NA	1.03	+	10	1272	2.75	0.02
24	1.37	0.89	0.95	0.81	1.10	NA	1.26	NA	0.74	1.06	+	12	1272	2.78	0.02
25	0.97	0.91	0.95	1.71	1.11	0.58	1.22	NA	NA	1.03	+	12	1272	2.97	0.01
26	0.98	0.95	0.95	0.78	NA	NA	1.50	0.87	NA	NA	+	10	1272	3.01	0.01
27	1.24	0.95	0.95	0.78	NA	NA	1.57	0.85	0.83	NA	+	11	1272	3.14	0.01
28	0.98	0.95	0.95	1.70	1.11	0.58	1.50	0.86	NA	NA	+	12	1272	3.35	0.01
29	1.39	0.88	0.95	1.67	1.10	0.59	1.71	0.80	0.74	1.07	+	14	1272	3.40	0.01
30	0.97	0.91	0.95	1.63	NA	0.59	1.54	0.85	NA	1.04	+	12	1272	3.42	0.01
31	1.22	0.95	0.95	1.67	1.10	0.59	1.57	0.84	0.83	NA	+	13	1272	3.51	0.01
32	1.41	0.88	0.95	0.78	NA	NA	1.70	0.81	0.73	1.07	+	12	1273	3.59	0.01
33	NA	0.95	NA	1.61	NA	0.59	1.21	NA	NA	NA	+	8	1273	3.71	0.01
34	0.97	0.91	0.95	NA	NA	NA	1.52	0.86	NA	1.04	+	10	1273	3.75	0.01

Table S6 No sex-specific effects in the association between DTR and bird mortality (as indicated by the grey column) in the hard foraging environment.

Hard treatment	Temperature variables [°C]			Development	Sex specific variables [Male]			Age related correction variables [Years]				Random term	Model Fit			
Model	DTR	MinT	DTR *MinT	Brood size	Sex	DTR *Sex	Sex *Age	AgeStart	AgeStart *Age	DTR *Age	MinT *Age	Aviary	df	AICc	ΔAICc	weight
1	2.04	0.88	1.03	1.15	NA	NA	NA	1.34	NA	0.50	1.05	+	11	1247	0.00	0.50
2	1.99	0.88	1.03	1.15	NA	NA	NA	1.16	1.12	0.50	1.05	+	12	1249	1.70	0.21
3	1.82	0.93	1.04	1.16	NA	NA	NA	1.30	NA	0.56	NA	+	10	1252	5.31	0.04

Supplementary information S4: Age specific DTR effects

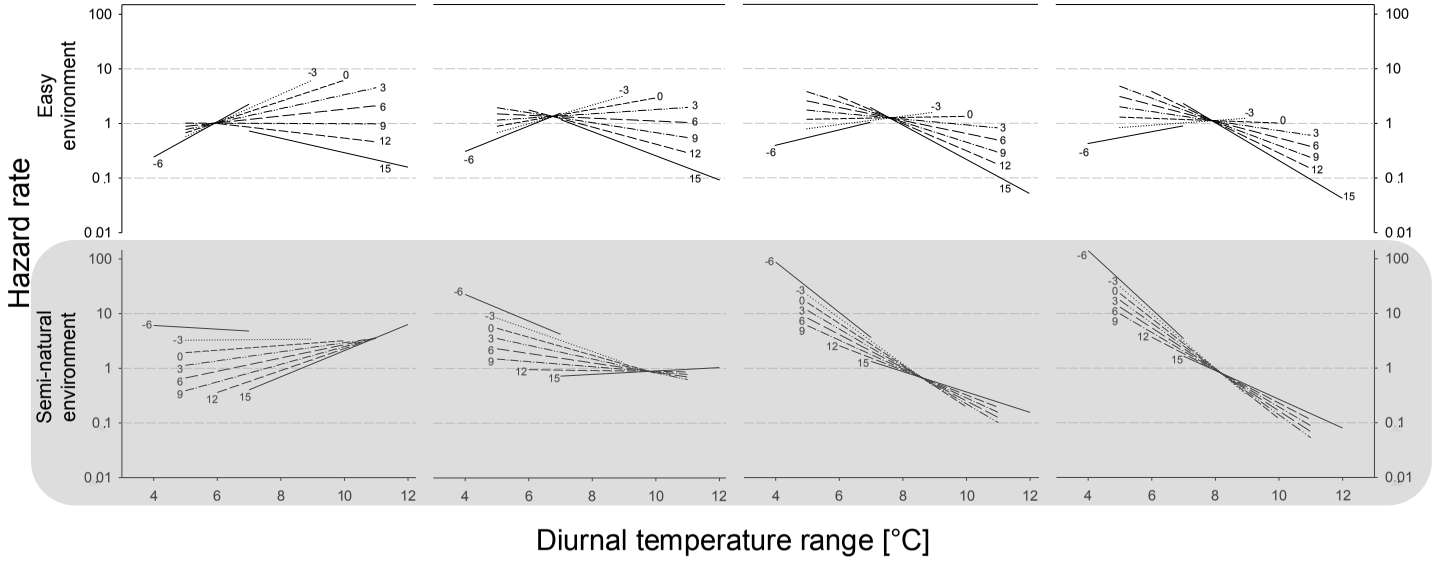

Fig. S3 Associations between DTR and hazard rate change with age for the easy foraging environment (top graphs in white) and semi-natural foraging environment (lower graphs in grey). From left to right: fitted lines for the ages of 0.36, 1.65, 4.00 and 5.07 years (at which 90%, 70%, 30% and 10% of the population was alive respectively). Lines within each graph show days with different minimum temperatures, indicated by numbers next to each line. Grey horizontal lines are reference lines at hazard rates of 0.1, 1 and 10. Graphs can be interpreted as follows: (i) In the easy foraging environment, young birds suffer high mortality when cold days get warmer (large DTR). In contrast, old birds remain unaffected by DTR on cold days, but do best when warm days get warmer. (ii) In the semi-natural foraging environment, high mortality occurs for young birds when warm days get warm (large DTR), but for old birds days that do not get warm are most lethal (small DTR). This suggests that DTR affects mortality via different ways in young versus old birds. For example, old birds in the semi-natural foraging environment, the DTR effect is likely due to cold exposure.

Supplementary information S5: Isolines representation of association between DTR and mortality

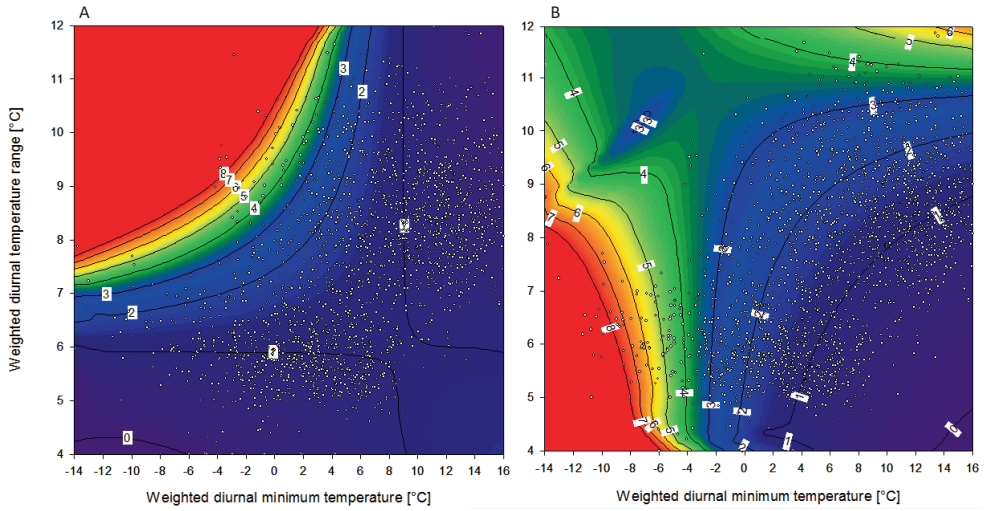

Fig. S4 Effects of natural variation in minimum temperature and diurnal temperature range on the hazard rate of zebra finches in easy foraging treatment (A) and semi natural environment (B). Isolines connect data with the same relative hazard rate and are the result of the model (Table 1, calculated for the age of 0.36 years as in Fig. 4) which is based on the daily observation of survival of 229 (A) and 246 (B) individuals from December 9th 2007 till January 1st 2013. Grey dots represent weighted weather data.

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Part III

Individual

Chapter 7

Bill redness is positively associated with reproduction and survival in male and female zebra finches

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Abstract

Sexual traits can serve as honest indicators of phenotypic quality when they are costly. Brightly colored yellow to red traits, which are pigmented by carotenoids, are relatively common in birds, and feature in sexual selection. Carotenoids have been linked to immune and antioxidant function, and the trade-off between ornamentation and these physiological functions provides a potential mechanism rendering carotenoid based signals costly. Mutual ornamentation is also common in birds and can be maintained by mutual mate choice for this ornament or by a correlated response in one sex to selection on the other sex. When selection pressures differ between the sexes this can cause intralocus sexual conflict. Sexually antagonistic selection pressures have been demonstrated for few sexual traits, and for carotenoid-dependent traits there is a single example: bill redness was found to be positively associated with survival and reproductive output in male zebra finches, but negatively so in females. We retested these associations in our captive zebra finch population without two possible limitations of this earlier study. Contrary to the earlier findings, we found no evidence for sexually antagonistic selection. In both sexes, individuals with redder bills showed higher survival. This association disappeared among the females with the reddest bills. Furthermore, females with redder bills achieved higher reproductive output. We conclude that bill redness of male and female zebra finches honestly signals phenotypic quality, and discuss the possible causes of the differences between our results and earlier findings.

Introduction

Sexual traits can serve as indicators of quality and require costs to facilitate honest signaling (Grafen 1990; Zahavi 1975). Red and yellow secondary sexual traits are found throughout vertebrates and are relatively common, especially in birds (McGraw 2006*a*). These traits have in some species been shown to feature in sexual selection (e.g. in birds: Jawor et al. 2003; Simons and Verhulst 2011; Sundberg 1995*a*; Toomey and McGraw 2012) and are of specific interest because in most birds they are pigmented by carotenoids (Olson and Owens 2005). In search of the costs maintaining honest advertisement of quality via yellow and red traits, carotenoids have been linked to antioxidant and immune status signaling (Lozano 1994; Pérez-Rodríguez 2009; von Schantz et al. 1999). Carotenoid-dependent traits may therefore signal phenotypic quality by advertising the ability to allocate carotenoids away from physiological functions towards sexual coloration.

Ornamentation of both sexes is also relatively common in birds. Mutual mate choice can maintain the ornamentation of both sexes (Amundsen 2000). Or it can be maintained via a correlated response to selection on the other sex (Amundsen 2000), which can cause intralocus sexual conflict (Bonduriansky and Chenoweth 2009; Chenoweth and McGuigan 2010; van Doorn 2009). Most genes are carried across generations in both males and females, but the selection pressures acting on these genes can differ in strength and even in sign between the sexes, i.e. sexually antagonistic selection. For sexual traits there are few examples of sexually antagonistic selection (Björklund and Senar 2001; Price and Burley 1994; Robinson et al. 2006) and to our best knowledge there is only one example for carotenoid dependent ornaments: bill redness of the zebra finch (*Taeniopygia guttata*) was positively related to survival and reproductive success in males, but negatively so in females (Price and Burley 1994 note that the survival relationship was non-significant in males). Given that the genetic correlation of bill redness is high ($r = 0.93$; Schielzeth et al. 2012), intralocus sexual conflict is plausible.

Zebra finch bills derive their red color from carotenoids and males have redder bills than females (McGraw, 2004; McGraw et al. 2003). Within males, bill redness reflects recent environmental (Eraud et al. 2007) and immunological challenges (Alonso-Álvarez et al. 2004*a*; Cote et al. 2010*a*; Gautier et al. 2008), and correlates positively with immune functioning (Birkhead et al. 1998; 2006). These signaling attributes of bill redness may be why there is female preference for this trait (Simons and Verhulst 2011). In contrast, male mate choice in relation to female bill coloration has been little studied (Simons and Verhulst 2011) and relatively little is known about the possible signaling value of

female bill coloration. Two studies reported females with redder bills to deposit more carotenoids in their eggs (Bolund et al. 2009; McGraw et al. 2005*a*), which is associated with increased hatching success (McGraw et al. 2005*a*). This suggests that also in females redder bills may be associated with higher phenotypic quality.

We tested the associations of female and male bill coloration with reproduction and survival, as did Price and Burley 1994, but our study differs from theirs in two main aspects. Firstly, in the study of Price and Burley the birds were reproducing, which may have confounded the estimated association of bill color with survival when bill color affects reproduction and reproduction in turn affects survival. We therefore examined the association between bill color and survival in single sex aviaries, in which birds could not reproduce. In mixed sex aviaries we examined the relationship between bill color and reproductive success. Secondly, Price and Burley selected birds with extreme bill colors for their study, which can lead to erroneous conclusions when the associations of bill color with survival and reproduction are not linear. We therefore did not select particular phenotypes for our study, and thus also included the intermediate phenotypes. Contrary to the results of Price and Burley we found no evidence for sexually antagonistic selection: individuals with redder bills of both sexes showed higher survival, and females with redder bills achieved higher fledgling production. Our findings thus substantiate signaling of physiological state by male zebra finch bill coloration and we show that it does so similarly in females.

Material and Methods

Bill color measurement

Bill color measurements were performed using digital photography (Sony DSC-F707). Pictures were taken of the top of the bill in controlled light conditions, on a Kaiser photography table equipped with four Philips Photocrescenta 150 watt light bulbs, with manually fixed camera settings. Digital cameras often do not respond linearly to the amount and spectral properties of light (Pike 2011; Stevens et al. 2007). We corrected for this using a calibration set of color patches (Munsell glossy finish collection, with published spectra from the Joensuu Spectral Database, http://cs.joensuu.fi/~spectral/databases/. Accessed 2012 June 17) to obtain a simulated reflectance spectrum from the digital images using Wiener estimation (Stigell et al. 2007). This methodology uses *a priori* information on the spectral reflectance of training objects (e.g. Munsell patches) captured by the digital camera RGB response (i.e. the sensors in the digital camera with spectral sensitivity to "red", "green" and "blue") to create an estimation

matrix using Wiener estimation (Stigell et al. 2007), via cross-correlation between the obtained RGB values of each patch and the known corresponding spectral reflectance of the training objects. By using not only the single RGB values, but also their polynomials an improved fit to the original spectra can be obtained (Stigell et al. 2007). We used 3rd-order polynomials of the obtained RGB values as input. The estimation matrix can then be used when capturing other objects than the training set to obtain simulated spectra. We did this per pixel of the bill and averaged these simulated spectra to obtain the simulated spectrum across the bill. These spectra are thus corrected for non-linearity in the response of the digital camera to light given that the estimation matrix is derived from known spectra of training objects.

The spectra we obtained showed a characteristic profile for red traits: little reflection from blue toward green, increasing reflection and leveling off in the red part of this spectrum (i.e. a sigmoid shape). From this spectrum we calculated the inflection point, as a measure of hue, using non-linear fitting of a 4-parameter sigmoid curve. Chroma of the bill was calculated as the summed reflectance between 600-700 nm divided by the summed reflectance of 380-700 nm. The bill was selected automatically from each picture using cluster analysis, which was manually checked and corrected for any inaccurate selections (which occurred in < 1% of the pictures). All these procedures were implemented in Matlab software (code available upon request).

Both chroma and hue measures were highly repeatable as estimated in a separate set of male and female birds of which we took two pictures a minute apart (hue: $r = 0.997$; chroma: $r = 0.990$; n = 30). Additionally we validated our method in this set of birds from which we obtained simulated reflectance spectra from photographs and reflectance spectra assessed with a spectrophotometer (BLK-C-100 spectrophotometer, SL4-DT (Deuterium/Tungsten) light source, R600-8-UV-VIS reflectance probe, StellarNet, FL). Estimates of both hue and chroma correlated strongly between both methods (hue: $r = 0.92$, n = 31; chroma: $r = 0.77$, n = 31). Chroma and hue covaried strongly in both directly measured ($r = 0.88$, n = 31) and simulated spectra ($r = 0.96$, n = 31). In the following we will present the results based on the measure of hue only. Analyses with chroma as dependent gave qualitatively the same results. Moreover, the majority of previous studies on zebra finch bill coloration used a Munsell color chip system which is primarily based on hue (Birkhead et al. 1998; Burley and Coopersmith 1987). As a control for ambient and technical conditions in which the photographs were taken we included the yellow patch of a Kodak color chart in each picture and extracted hue from this patch in the same way as for the bills. When light conditions or camera sensitivity would change, due to a factor we could not control, this will affect both the color of the

bill and the patch in the same picture. In none of the analyses was the hue measured from the Kodak chart correlated with the hue of the bill in the same picture ($p > 0.36$).

Survival

Birds were housed in four outside aviaries (L * W * H: 320 * 150 * 225 cm), two with males (n = 72, 36 per aviary) and two with females (n = 68, 32 and 36 per aviary). Before the experiment started individuals were kept in unisexual groups of similar density as in the experimental setting and the birds had no breeding experience. Food (tropical seed mixture), water, grit and cuttlebone were provided *ad libitum*. In addition the birds received fortified canary food ("eggfood", by Bogena, Hedel, the Netherlands) in weighed portions (0.42 gram/bird, 3 times a week; control treatment as described in Koetsier and Verhulst, 2011). All bill coloration measures were taken in November 2008, after which survival was monitored till December 2011. During this period new birds were introduced into the aviaries replacing individuals that had died, to maintain a relatively constant density throughout the experiment. This experiment started in December 2007, but due to low mortality in the first year and addition of birds in 2008, our sample size to assess correlates with survival was largest in 2008. Mortality (82 cases) was recorded daily and was analyzed using proportional hazards models (using the Survival package in R, function "coxph", R Development Core Team, 2011). We tested for violations of the proportional hazards assumption using the function "cox.zph" and by scaled Schoenfeld residual plots. We detected no violations of this assumption. Deaths, which occurred within 48 hours after handling for experimentation (n = 9), or birds that were terminated for various welfare reasons (n = 6) and birds still alive were censored. Note, when both these categories of deaths were treated as natural deaths, this did not qualitatively change the results. Parameters included in the model were: aviary (as random term, using the function "frailty"), age at the time of bill measurements (mean age = 659 ± SD 329 days, range = 151-1028 days), sex, bill hue (mean centered per sex), bill hue squared and bill hue interactions with sex. In this study the rearing brood sizes of the birds were either standardized to 2 or 6 (de Coster et al. 2011). Although this did not affect either survival (when included as factor in the full model, $p = 0.55$) or bill color ($p = 0.97$), brood size was retained in the proportional hazards models as strata. Age at the time of bill measurement was also not related to bill coloration ($p = 0.66$). These birds are the control treatment of a larger experiment (de Coster et al. 2011), in which context the birds were blood sampled for 2-3 times per year and respirometry measurements were taken 1-2 times a year, but otherwise these birds were left undisturbed.

Reproduction

This experiment was initiated in April 2009, when a mixed-sex group of adult zebra finches, previously housed in unisexual groups of similar density as in the experiment and thus without previous breeding experience, was housed in two outdoor aviaries (dimensions as in the survival measurements). Reproduction was facilitated by providing a surplus of nest boxes (20) per aviary, and nest material (hay). Offspring were removed from the aviaries at around 35 days of age, when they are usually nutritionally independent. The food regime was essentially the same as in the survival measurements, except that here egg food was provided *ad libitum*. Also in this set the birds were blood sampled for 2-3 times per year and respirometry measurements were taken 1-2 times a year in the context of other experiments, but otherwise left undisturbed. We investigated the relationship between initial bill color and subsequent fledgling production. Bill coloration was measured of two batches of females (mean age = 415 ± SD 110 days, range = 182-737 days), before they were introduced to the aviaries in spring about one year apart (April 2009, n = 22; June 2010, n = 13). Follow up consisted of two subsequent summers for each batch (2009-2010 and 2010-2011) in which parentage was assessed by observations of chick feeding through one-way mirrors. Parentage of clutches that did not hatch was thus not assessed. Individual birds were identified by the use of color bands (colors used: black, cyan, green, white, yellow; band color was not associated with either reproduction ($X^2(4) < 8.25$, $p > 0.08$) or bill hue ($X^2(4) < 5.87$, $p > 0.21$) as tested within both sexes). Bill color did not differ between batches ($t = 0.47$, $df = 17$, $p = 0.68$), but total fledgling production, broods produced and fledging per brood within the two breeding seasons of follow up differed between batches (all were higher in batch 2, $p < 0.05$, Table 3.S1) and were left-skewed (but not Poisson distributed). Therefore we standardized these measures by dividing them by their median per batch. Mortality occurred and therefore longer-lived females had a wider window of opportunity to reproduce. To correct for this we divided fledgling production by the number of days available for breeding and further standardized this by dividing it by its median per batch. Days available for breeding was defined as the part of the year at which other birds had nestlings and when the focal bird was alive. These two relationships were assessed for significance using rank correlations. In a similar fashion we analyzed correlates of male (n = 25, mean age = 674 ± SD 334 days, range = 370-1384 days) bill color for which we only had measurements of the first batch. Pair formation was investigated in the first batch, because in this group information on bill coloration was available for both sexes. Only the first pair-bond that resulted in hatchling production in the first breeding season was considered, to avoid complication of re-pairing after deaths of partners and unknown bill coloration of males introduced in the second breeding season, and we examined whether bill coloration

influenced the likelihood of pair formation. During their rearing all the birds entered in the aviaries were allowed imprinting opportunity on adults of both sexes for at least 100 days after birth, which may be important in shaping to what extent zebra finches use bill coloration in mate choice (Simons and Verhulst 2011). Because we did not assess extra-pair paternity, which can be as high as 29% in this species in aviary contexts (Burley et al. 1996; Forstmeier et al. 2011), the results on male reproduction are considerably less reliable than those on females.

Ethics Statement

The research presented here has been approved by the animal welfare ethics committee of the University of Groningen (according to Dutch law), under license number 5150.

Results

Survival

Survival of individuals with redder bills was higher (Fig. 1, Table 1; negative estimates indicate lower risk of death), and equally so in both sexes as indicated by the non-significance of the interaction between sex and bill hue (Table 1). To investigate whether the observed relationship was linear we additionally tested for quadratic associations of bill hue with survival. The interaction of this quadratic term with sex was significant (Table 1). Within males only the linear term was significant (Table 2), whereas within females we detected a significant quadratic term (Table 3, Fig. S1). The optimum of this quadratic relationship is 0.74 nm above the female average (mean = 583.3 ± SD 4.8) of bill hue. To test for negative survival selection we split the dataset into bill hue below and above this estimated optimum. In females showing redder bills than the optimum we did not detect significant negative survival selection with respect to bill hue (Table 3). However in females with bill hue less red than the estimated optimum we found higher survival with increasing bill hue (Table 3). As expected, higher age at measurement was associated with increased risk of death (Tables 1-3).

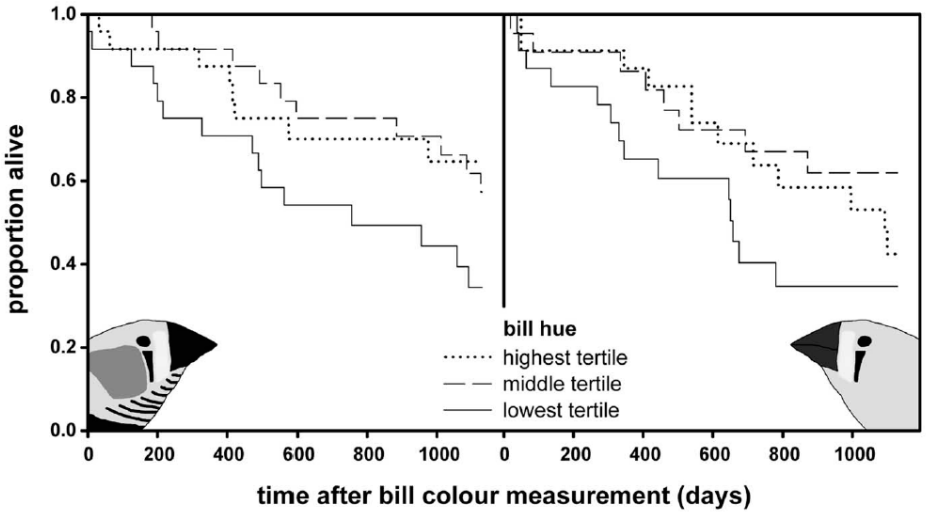

Fig. 1 Bill hue and survival. Survival of males (left panel) and females (right panel) in relation to bill hue categories (tertiles). Note that data are shown for bill hue tertiles but bill hue was entered as continuous variable in the analyses. In both sexes individuals with low redness survive worst. In females a quadratic relationship of survival with bill hue was detected (see main text).

Table 1 Proportional hazard models including both sexes.

model	parameter	estimate	s.e.	p value
without quadratic term	bill hue	-0.090	0.026	0.00029
	sex	-0.21	0.25	0.39
	age at measurement	0.00097	0.00041	0.015
	sex X bill hue (omitted)	-0.0059	0.051	0.91
with quadratic term	bill hue	-0.037	0.037	0.32
	bill hue^2	0.024	0.0066	0.00027
	sex	0.45	0.39	0.25
	age at measurement	0.0010	0.00042	0.013
	sex X bill hue	-0.10	0.071	0.14
	sex X bill hue^2	-0.031	0.010	0.0023

Table 2 Proportional hazard model within males.

parameter	estimate	s.e.	p value
bill hue	-0.1	0.034	0.0056
bill hue^2 (omitted)	-0.0067	0.0076	0.38
age at measurement	0.00066	0.00061	0.28

Table 3 Proportional hazard models within females.

model	parameter	estimate	s.e.	p value
all females	bill hue	-0.04	0.038	0.32
	bill hue^2	0.026	0.0076	0.00081
	age at measurement	0.0013	0.00058	0.028
females with hue < optimum	bill hue	-0.26	0.093	0.0051
	age at measurement	0.0028	0.001	0.0043
females with hue > optimum	bill hue	0.22	0.15	0.13
	age at measurement	0.00015	0.00079	0.85

Reproduction

Fledgling production increased with bill redness in females (Fig. 2 right panel, $r_s = 0.46$, $p = 0.005$). This effect was not solely due to a higher survival rate of redder females, because it remained significant when fledgling production was divided by the number of days available for breeding due to survival differences ($r_s = 0.33$, $p < 0.05$). The increase in fledgling production was equally due to a higher rate of brood production (i.e. broods produced which resulted in hatchlings) and a larger number of fledglings produced per brood because these components of fledgling production correlated equally with bill color (rate of brood production: $r_s = 0.295$, $p = 0.09$; fledglings per brood: $r_s = 0.290$, $p = 0.10$). Within males no significant relationships were detected between bill redness and the measures of reproductive success we tested above in females (Fig. 2 left panel; range $r_s = -0.25 \mid -0.14$, $p > 0.23$). The likelihood of ending up in a pair after introduction was higher for females that exhibited redder bills ($X^2(1) = 5.44$, $p = 0.02$, $n = 22$), but we did not detect such a relationship in males ($X^2(1) = 0.96$, $p = 0.33$, $n = 25$) and within pairs male and female bill hue did not correlate ($r = 0.08$, $n = 12$, $p = 0.81$).

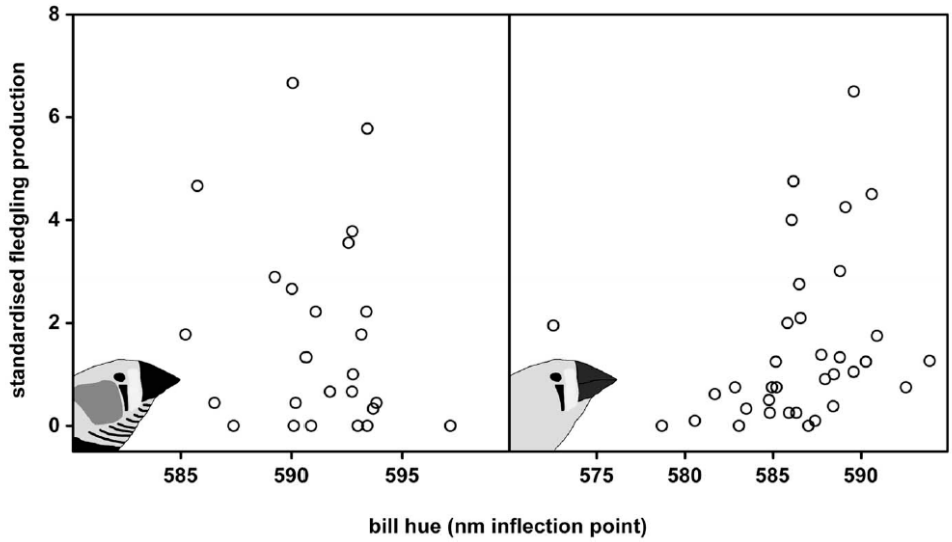

Fig. 2 Bill hue and fledgling production. Fledgling production (standardized by dividing it by the median fledgling production per batch, thus not corrected for differences in longevity; see main text) of males (left panel) and females (right panel) in relation to bill hue. Only in females was redder bill hue significantly associated with reproductive success (see main text).

Discussion

Male and female zebra finches with redder bills showed increased survival, in particular among birds with bills that were less red than average (Fig. 1). In other bird species male sexual ornaments have also been linked to survival, reviewed in (reviewed in Jennions et al. 2001). However, for carotenoid dependent traits there are relatively few examples (Figuerola and Senar 2007; Hill 1991; Hõrak et al. 2001; Nolan et al. 1998) and evidence is particularly sparse in females with only two published studies that we are aware of (Hõrak et al. 2001; Price and Burley 1994). Our findings thus substantiate signaling of phenotypic quality by zebra finch bill coloration. This contradicts an earlier report of females with less red bills showing the highest survival rates (Price and Burley 1994). In females, but not in males, we detected that the relation between bill color and survival leveled off at higher redness (Fig. S1), with no significant relation among females with the reddest bills. Although we do not detect significant negative selection against redder bills in females it may be suggestive of sexually antagonistic selection revealing itself among the reddest females. This would also fit with the observation of Burley and Coopersmith (1987), in which male zebra finches were shown to prefer females with intermediate bill hues.

Females with redder bills also produced more fledglings, contrary to earlier findings (Price and Burley 1994). This, together with increased survival of redder females suggests positive selection for bill redness in both males and females, instead of sexually antagonistic selection as reported by Price and Burley (1994). This discrepancy may be due to several reasons. The first reason may be a matter of sample size and follow up. In Price and Burley's study the sample size for survival was lower (n = 30 males and n = 30 females vs. n = 72 males and n = 68 females in our study) and follow up was shorter (1.7 years vs. 3.1 years). Second, for their experiment Price and Burley selected the least red and reddest individuals from a larger population. When relationships are non-linear, as we demonstrated for the association between female coloration and survival, the findings will be strongly influenced by the criteria used to select different subsets. Third, survival in Price and Burley's study was measured under *ad libitum* reproduction, which may affect the relationship of bill coloration with survival. We avoided this issue by studying survival in a setting without reproduction, but for comparability with the study of Price and Burley also tested the association between bill color and survival among the breeding birds. In the batch of females under *ad libitum* reproduction for which we had the longest follow up (n = 22, 15 deaths, survival follow up: 2.8 years) the associations were similar (linear term: -0.40 ± s.e. 0.15, p = 0.007; quadratic term: 0.05 ± s.e. 0.038, p = 0.17) to those we report for single-sex housed females in our survival study. Within males we did not detect significant associations of bill hue with survival (n = 25, 11 deaths, survival follow up: 2.8 years, linear term: 0.14 ± s.e. 0.13, p = 0.30). Interestingly Price and Burley also found no significant association of bill hue and survival within males contrary to females. This suggests that within males the association between bill hue and survival is lost under *ad libitum* reproduction. In continuing our *ad libitum* reproduction experiment we will increase our sample size to test this hypothesis. Fourth, we cannot exclude the possibility that there are population differences (caused by e.g. husbandry, origin of birds, environmental differences) in the relations we studied.

Given that we found no evidence for sexually antagonistic selection for bill coloration we expected assortative mating instead of possible disassortative mating. In accordance with this expectation we found that redder females were more likely to be engaged in pair formation, possibly mediated by male choice, but in our limited sample we do not find evidence for assortative mating. This may be attributed to assortative mating among extra-pair copulations, which we did not establish in this study. We conclude that bill coloration of male and female zebra finches signals phenotypic quality. This suggests that in both males and females the deposition of carotenoids into bill coloration ensures signal honesty.

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Supplementary information to:

Bill redness is positively associated with reproduction and survival in male and female zebra finches

Table S1 Presented are the medians per batch of the reproduction measures we analyzed, along with the non-parametric test for differences between batches.

variable	batch 1	batch 2	Wilcoxon test
fledgings produced in the two seasons of follow up	4	21	p = 0.0028
broods (which included hatchlings) produced in the two seasons of follow up	2	8	p = 0.011
fledglings per brood	1.5	3	p = 0.016

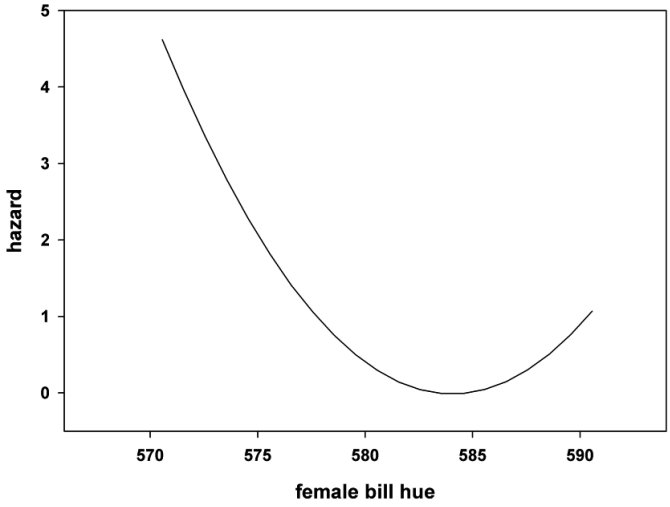

Fig. S1 Predicted hazard from the model including all females (Table 3.3). The predicted relationship is plotted for the range of bill hues observed within this specific set of females. Hazard rate sharply drops when bill hue increases, but levels off and tends to increase at the highest bill hues (see main text).

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Chapter 8

Stabilising survival selection on pre-senescent expression of a sexual ornament followed by a terminal decline

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Abstract

Senescence is a decrease in functional capacity, increasing mortality rate with age. Sexual signals indicate functional capacity, because costs of ornamentation ensure signal honesty, and are therefore expected to senesce, tracking physiological deterioration and mortality. For sexual traits, mixed associations with age and positive associations with life expectancy have been reported. However, whether these associations are caused by selective disappearance and/or within-individual senescence of sexual signals, respectively, is not known. We previously reported that zebra finches with redder bills had greater life expectancy, based on a single bill colour measurement per individual. We here extend this analysis using longitudinal data, and show that this finding is attributable to terminal declines in bill redness in the year before death, with no detectable change in pre-senescent redness. Additionally, there was a quadratic relationship between pre-senescent bill coloration and survival: individuals with intermediate bill redness have maximum survival prospects. This may reflect that redder individuals overinvest in coloration and/or associated physiological changes, while below average bill redness probably reflects poorer phenotypic quality. Together this pattern suggests that bill coloration is defended against physiological deterioration, because of mate attraction benefits, or that physiological deterioration is not a gradual process, but accelerates sharply prior to death. We discuss these possibilities in the context of the reliability theory of ageing and sexual selection.

Introduction

One of the most intriguing things about life is that it will inevitably end. Almost all organisms age and at first glance this is a paradox. Death by ageing reduces the opportunity to reproduce and thereby reduces Darwinian fitness (Williams 1957). The disposable soma theory (Kirkwood and Holliday 1979; Ricklefs 1998) explains how ageing can increase fitness, postulating that investments to increase reproduction are achieved at the expense of investment in somatic repair and maintenance. Physiological deterioration, not fully countered by somatic repair and maintenance, leads to a decline in functional capacity with age, i.e. senescence. On a demographic level this results in accelerating (intrinsic) mortality with age (Ricklefs 2010). Mortality risk is therefore predicted to be closely matched by deterioration of physiological parameters, i.e. "condition" (Ricklefs 2010). In other words, physiological parameters directly related to increased mortality risk are predicted to senesce in concordance with demographic increases in mortality rate.

The correlation between age-specific declines in reproductive performance – a measure of condition – and mortality rate varies widely between species, however, suggesting that physiological markers of performance need not always track mortality rate (Burger and Promislow 2006; Bouwhuis et al. 2012). Thus alternatively, individuals may maintain their physiological variables at a similar level until death, when intrinsic causes of death are of a catastrophic nature (Ricklefs 2010; Nussey et al. 2011). Prior to death this may result in rapid physiological declines – terminal declines – apparent in for example reproduction (Coulson and Fairweather 2001; Rattiste 2004). A different explanation of a lack of concordance between mortality senescence and the physiology measured is that the variable measured is not causally linked to mortality (Simons 2015), or that the physiological variable is defended against gradual senescence. The short-term reproductive benefit of investing in the maintenance of e.g. sexual attractiveness may offset the benefit of investing in other aspects of the soma, e.g. immune function, with longer-term reproductive benefits. The fitness return of investments with long-term benefits is reduced by the risk of extrinsic mortality (Kirkwood and Holliday 1979; Ricklefs 1998) and hence physiology associated with long-term benefits is predicted to senesce relatively sooner.

Sexual selection has resulted in exaggerated traits (Andersson and Iwasa 1996) that can serve as sexual signals (Kokko et al. 2006). The signalling value of a trait increases when cheating is effectively precluded and when it reveals information about aspects of physiology that underlie phenotypic quality (Hill 2011). We may therefore expect traits

that feature in mate-choice to closely follow demographic senescence, and hence be a biomarker of ageing. This expectation will however depend on the honesty of the sexual signal in question and may change if trade-offs maintaining signal honesty shift with age. Also if the benefits and/or costs of investing in sexual ornamentation change with age, or if an investment yields strong current reproductive benefits, sexual signals could show catastrophic rather than gradual senescence.

Associations with age have been reported for a diverse array of sexual traits. Cross-sectional studies have reported both increasing (Budden and Dickinson 2009; Laucht and Dale 2012) and declining (Garratt et al. 2011; Edler and Friedl 2012) signal expression with age. However, relationships with age estimated from cross-sectional analyses can be caused by selective disappearance from the population rather than reflect changes with age within individuals (van de Pol and Verhulst 2006; Kervinen et al. 2015). Statistically separating within- and between-individual variation is required to obtain unbiased estimates of changes with age within individuals, and the few studies of this kind mainly reported increased sexual signalling with age (Delhey and Kempenaers 2006; Nussey et al. 2009; Judge 2010; Val et al. 2010; Evans et al. 2011; Kervinen et al. 2015). It therefore seems that we still know little about the details of the expression of sexual signals in relation to ageing despite its relevance for life-history evolution and sexual selection. Interpreting analyses that do not separate within- and between-individual variation is complicated further because sexual trait expression is generally found to be positively associated with survival (meta-analysis in Jennions et al. 2001). On the population level a positive relationship between trait expression and survival can come about via terminal declines of sexual signals, variation between individuals in senescence or associations with the level of pre-senescent sexual signal expression (Reed et al. 2008).

Here, we dissect these intricate relationships between mortality and sexual signal senescence in zebra finches (*Taeniopygia guttata*) using longitudinal data, allowing us to separate between- and within-individual variation. Zebra finches form stable pair-bonds (Silcox and Evans 1982), but re-pair readily if a partner is lost. Extra-pair paternity in the wild is low (Birkhead et al. 1990) and reproductive success depends strongly on biparental care (Royle et al. 2006). Sexual selection for traits that honestly indicate quality, parental care and longevity could aid in the life determining choice of who to mate. Male and female zebra finches exhibit bills that are a colourful orange to deep red, pigmented by carotenoids (McGraw 2004), which have to be acquired exclusively from the diet and are associated with immunocompetence and oxidative stress state (Simons et al. 2012b). Male bill colour is subject to female choice, as we recently showed using

meta-analysis across ten separate studies (Simons and Verhulst 2011), and is positively associated with longevity (Simons et al. 2012a). Positive associations of bill redness of females with survival and fledging production suggest that male choice for redder females will also yield benefits (Simons and Verhulst 2011; Simons et al. 2012a). One could question whether in a captive situation where food is freely accessible carotenoids are limiting. However, birds increase in colouration when supplemented with carotenoids in captivity as well, and carotenoids and carotenoid-dependent signals are associated with physiological parameters (Simons et al. 2012b). Comparative evidence suggests that carotenoid acquisition can underlie honest sexual signalling (Simons et al. 2014a). Furthermore, carotenoid supplementation can affect later reproduction in the same captive environment we use in this study (Simons et al. 2014b). These considerations have led us to interpret bill colouration as an indicator of physiological state (Pérez-Rodríguez 2009), also in our captive environment. We therefore analysed patterns of ageing and investigated the contribution of terminal effects in bill redness and its association with mortality in both male and female zebra finches.

Material and Methods

Experimental setup

For six consecutive years (2007-2012) we took bill colour measurements (n = 1200) around mid-November each year of males (n = 224) and females (n = 220) from our population of zebra finches housed in eight unisex outdoor aviaries (L * W * H: 320 * 150 * 225 cm). Individual birds have been added multiple times to this experiment thereby replacing individuals that died (median longevity of a zebra finch in our population is ≈ 3.7 years). This maintained the total population of birds around 200 individuals. All birds were bred within our own facility and should be considered domesticated zebra finches (for more information see: Briga and Verhulst 2015). These birds are used in a long-term experiment investigating the relationships between survival, a foraging costs treatment (easy or hard foraging) (Koetsier and Verhulst 2011), and early rearing conditions (raised in small or large broods) (de Coster et al. 2011). In the hard foraging treatment, individual birds have to hover in front of a feeding hole to obtain seeds (tropical seed mixture, *ad libitum*), whereas in the easy condition there is a perch allowing effortless access to the seed. Small brood (2 chicks) and large broods (6 chicks) were created by cross-fostering broods at an age of 5 days under forced pairing in individual indoor breeding cages (L * W * H: 40 * 80 *40 cm). Cuttlebone, grit and water were provided *ad libitum* and the birds received fortified canary food ("eggfood", by Bogena, Hedel, the Netherlands) in weighed portions (Koetsier and Verhulst 2011). The birds

were left undisturbed until natural death, except for blood sampling and respirometry measurements several times a year in the context of other non-experimental studies (always in equal measure for all treatments and ages). For identification all birds were banded with a numbered aluminium ring. The aviaries were inspected daily and deaths recorded until the end of December 2014. In our previous study of the association of bill colour with survival we used only one bill colour measurement and restricted ourselves to the easy foraging condition of this experiment to avoid possible unknown confounding effects (Simons et al. 2012a). Here we tested the associations of the foraging treatment and early rearing conditions, and their interaction, with longitudinal bill colour measurements, as outlined below in the statistical analysis and results section. However we did not detect any associations with the two treatments and therefore present results across the whole population of the experiment.

Bill colour measurement

Measurements of bill colouration were taken as described previously (Simons et al. 2012a). In brief, bills were digitally photographed (camera: Sony DSC-F707) with fixed camera settings and in a controlled lighting environment. Birds were manually restrained on top of a foam mould and the top of the bill was photographed. Digital cameras can respond to light and light composition in a non-linear fashion (Stevens *et al.*, 2007). We corrected for this using a calibration set of colour patches (Munsell glossy finish collection) with known spectra obtained from the Joensuu Spectral Database (http://cs.joensuu.fi/~spectral/databases/) to generate simulated reflectance spectra from the digital images (Stigell et al. 2007). Bills were automatically selected from the pictures using thresholding and cluster analysis. All these selections were manually checked and corrected in the few instances when the automatic selection procedure failed. From these bills simulated spectra were obtained and we calculated the inflection point, which is a measure of hue, using non-linear fitting of a 4-parameter sigmoid curve. All the above procedures were programmed and run in Matlab software. We validated the above method with direct measurement of reflectance, using a spectrophotometer (BLK-C-100 spectrophotometer, SL4-DT (Deuterium/Tungsten) light source, R600-8-UV-VIS reflectance probe, StellarNet, FL), in a subset of 31 birds. Measures of hue obtained with this method and hue from the simulated spectra of digital pictures correlated strongly ($r = 0.96$). Repeatability of our method was high ($r = 0.997$), estimated by taking two pictures from the same individual in close succession (Simons *et al.*, 2012a).

Statistical analysis

We used mixed models implemented in R (R Development Core Team 2011) to analyse variation in bill colour. In our models we included average age across the measures of

an individual and the difference in age from this average age for each measurement (Δage), to separate within- and between-individual effects (van de Pol and Verhulst 2006). The effect of Δage (centered around the average age at measurement) provides an estimate of the within individual slope of age against bill colour independent of selective disappearance. The term average age tests the effect of age across individuals, and is thus dependent on effects of selective disappearance. In addition, we investigated terminal effects by fitting a binomial factor coding for whether an individual died a natural death in the subsequent year or not. All these models included a random effect at the intercept for each individual and a random effect of slope for Δage across individuals. Neglecting to include random-slopes in mixed models is likely to result in erroneous conclusions (Schielzeth and Forstmeier 2009). We included two additional random intercepts in the mixed models: the year in which measurements were taken and the birth nest (210 individual nests) of the individuals.

Within the analyses of bill hue senescence we tested for main effects of foraging treatment and rearing brood size (and their interaction) and for interactions with the independent variables included in these models of the foraging treatment and rearing brood size (and their interaction). We selected the best model among the models that contained our hypothesized variables of interests (see result section) using a best subsets approach, i.e. fitting all possible variables combinations, using the MuMIn package in R, based on BIC (Bayesian information criterion). In practice this resulted in the models that excluded terms (ΔBIC > 2.3) related to both the foraging treatment and rearing brood size.

To assess relationships of trait values with survival, we fitted right-censored cox proportional hazards (Survival package in R, "coxph"). Censored cases included birds that were still alive, died within 48 hours after handling for experimentation or by accident (n = 34), and birds that were terminated for various welfare considerations (n = 12). Violations of the proportional hazards assumption were tested using the "cox.zph" function and by plotting scaled Schoenfeld residual plots. No such violations were detected.

To contrast cross-sectional population level analyses with within-individual analyses, we also analysed survival on a yearly basis, by estimating the difference in bill hue between survivors and birds that died in the subsequent year. These estimates we summarized across years using a fixed-effects meta-analysis (Viechtbauer 2010), and corrected the associated confidence interval of the average effect for the dependence within the data due to multiple measures from the sample individual (Higgins et al. 2008). This entailed

inflating the associated standard error by multiplying it by the square root of the fraction of the dependent sample size (the number of measurements) over the independent sample size (the number of unique individuals). We investigated both male and female bill colouration and all models were tested separately for each sex.

Results

Mortality and bill colour on the population level

To contrast the results of a cross-sectional analysis with the within-individual analyses that follow, we first tested for the six separate years of our study whether the individuals that died in the subsequent year following our measurement had lower bill hues (Fig. 1). We find that for both males ($z = -2.40$, $p = 0.016$) and females ($z = -1.73$, $p = 0.08$) lower bill hues are associated with lower survival in the subsequent year (Fig. 1).

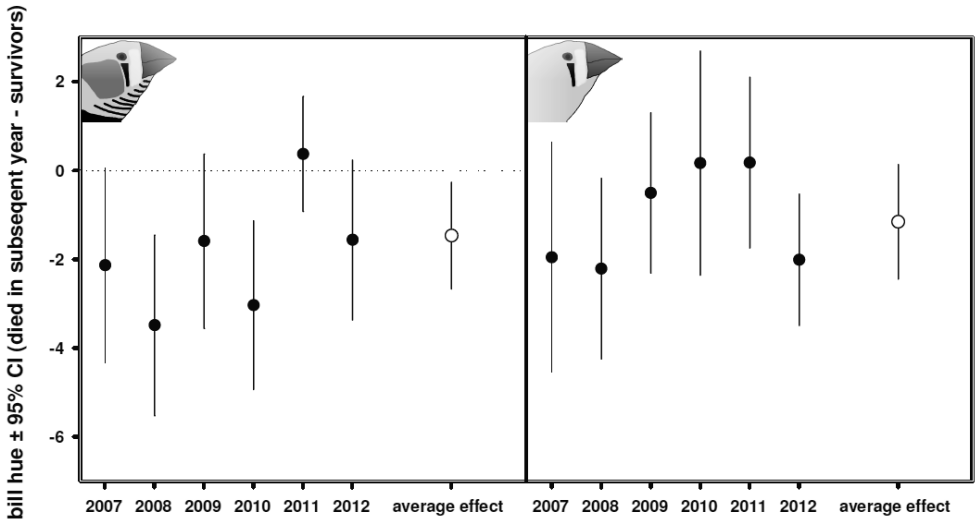


Fig. 1 Estimated bill hue difference between the individuals that died in the subsequent year and those that survived for each year of the study (filled circles), and the average effect across the years of measurements (open circles). In both males (left panel) and females (right panel) lower bill hue was associated with mortality in the subsequent year. The error bars indicate 95% confidence intervals and the dotted horizontal line at zero indicates no difference in bill hue between individuals that died in the subsequent year and the survivors.

Within- and between-individual associations with age

We first investigated the dependency of bill colour on age separating within- and between-individual effects (Table 1A), which included average age (between-individual

effect) and Δage (within-individual effect). The necessity to examine between and within-individual effects of age simultaneously was evident from the result; because for males we found a significant decrease in bill hue with age within-individuals, but a significant positive slope between-individuals. This indicates selective disappearance of individuals with low bill hues from the population, causing an increase of average bill hue with age. Within females the same pattern emerged, but is not significant, but note that the standard errors of the Δage and average age estimate do not overlap (Table 1A) which is indicative of significant selective disappearance in females as well (van de Pol and Verhulst 2006).

Terminal effects

Next we investigated terminal effects, by adding a factor indicating whether the bird died in the subsequent year following the bill colour measurement or not (Table 1B). We omitted the last bill colour measurements of birds that were censored (see methods) from this analysis, because we do not know whether these birds would have died a natural death in the year following the last measurement or not. In both sexes death was preceded by a drop in bill hue, although note that this effect was significant in males, but 45% smaller in females and statistically only a trend (Table 1B). Because in these models some individuals are only measured once or twice, this causes Δage and "died in subsequent year" to code for essentially the same change in these individuals, not allowing the model to separate the two. Moreover not all individuals in this set have died yet, also potentially biasing the results, because in these individuals the terminal effect cannot be estimated. Therefore we also tested the terminal effect in a truncated dataset, including only birds for which three or more measurements were available and that had died (Table 1C). Also in this set we find, although only for males, that imminent death is accompanied by a drop in bill hue (Fig. 2). In both sexes, the parameter estimate of Δage is reduced in magnitude and becomes non-significant when we include the terminal effect in the models, suggesting that bill hue does not change prior to the terminal decline that precedes death. This also suggests that there is no selective disappearance with respect to bill colouration other than through the decline in colouration associated with imminent death.

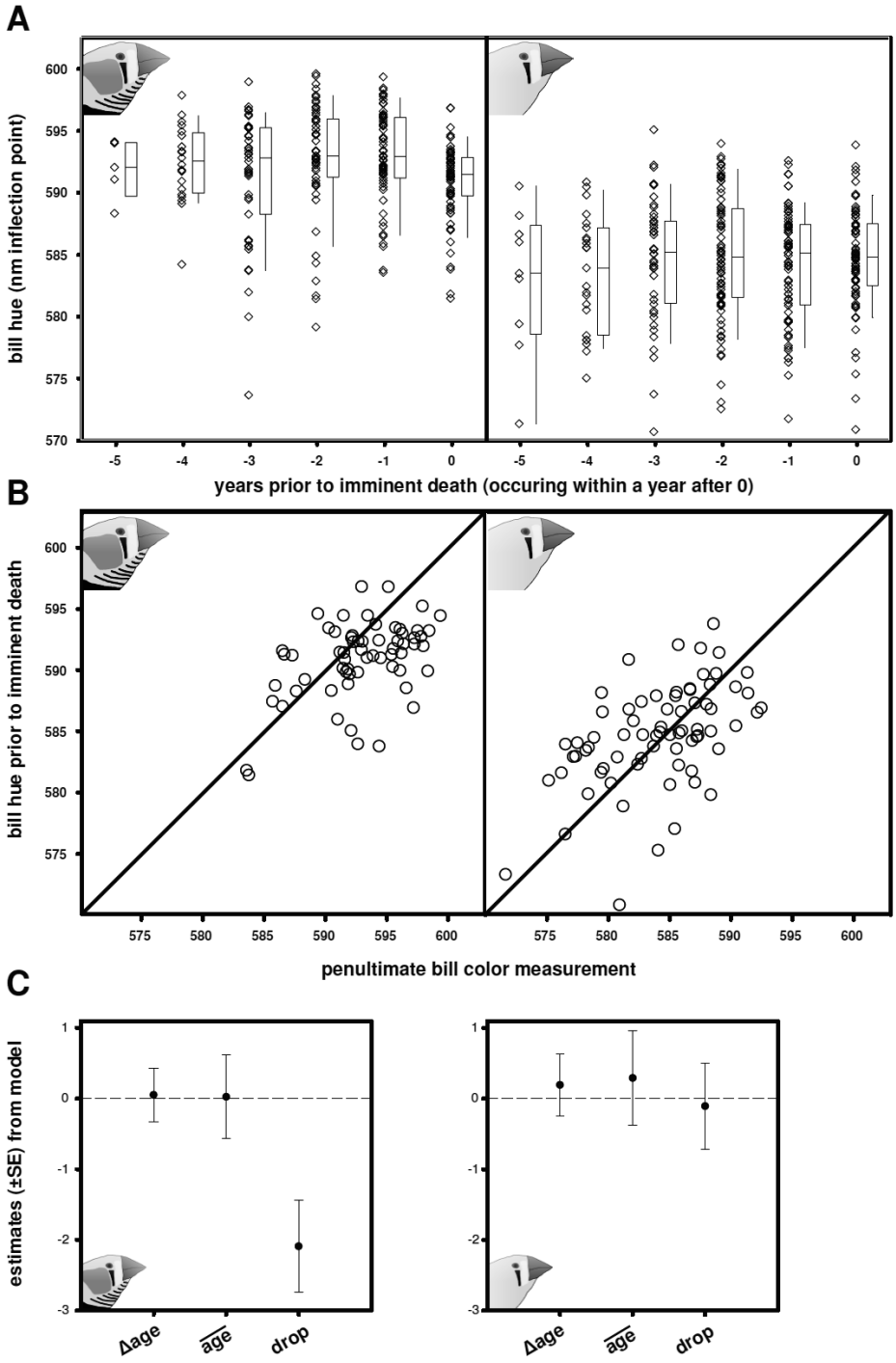
We refrained from including quadratic age terms, because in our dataset the number of individuals with three measurements or more is limited. Moreover the inclusion of a quadratic age term would complicate the independent estimation of a terminal effect and would require further restriction of the dataset (from data in Table 1C). However, it is not unusual for ornamentation to increase with age early in life, and to test for such an effect we investigated whether birds in their first year of life had lower bill hue. By

adding 'first year' as factor to the model presented in Table 1B, but found no evidence for such an effect in either females (estimate -0.14 ±0.52, p = 0.79) or males (estimate -0.15 ±0.48, p = 0.74). To scale the magnitude of the terminal decline in bill hue we calculated the repeatability of pre-senescent bill hue (males, 0.36 ±0.08, p < 0.0001; females, r = 0.50 ±0.07, p < 0.0001) and the standard deviation of the penultimate measurement prior to death (males, SD = 4.0; females, SD = 4.6). The terminal decline we detect in males thus reduced bill hue by 0.53 SD (Table 1C) and pre-senescent bill colouration was repeatable between years.

Table 1 A) Bill hue modelled as a function of within- (Δage) and between-individual (average age) effects of age. **B)** The model of bill hue presented in table 1A, but extended with a factor coding for the last measurement prior to natural death (= 1 when it died in the subsequent year, = 0 when it did not). Note that measurements in the year prior to censoring are excluded from this dataset. **C)** The model presented in table 1B with the selection from the dataset including only individuals that were measured at least three times and died a natural death.

A	**term**	**estimate (±s.e.)**	**p**
males (n = 224 birds, 616 measurements)	Δage	-0.59 (0.18)	0.0015
	average age	0.62 (0.24)	0.011
females (n = 220 birds, 584 measurements)	Δage	-0.41 (0.22)	0.06
	average age	0.26 (0.28)	0.36
B			
males (n = 217 birds, 591 measurements)	Δage	-0.24 (0.21)	0.26
	average age	0.43 (0.25)	0.086
	died in subsequent year	-1.44 (0.40)	0.0004
females (n = 213 birds, 561 measurements)	Δage	-0.09 (0.27)	0.74
	average age	0.17 (0.29)	0.56
	died in subsequent year	-0.79 (0.44)	0.074
C			
males (n = 63 birds, 257 measurements)	Δage	0.052 (0.38)	0.89
	average age	0.025 (0.59)	0.96
	died in subsequent year	-2.09 (0.65)	0.0015
females (n = 72 birds, 292 measurements)	Δage	0.19 (0.44)	0.67
	average age	0.29 (0.67)	0.66
	died in subsequent year	-0.11 (0.61)	0.86

Fig. 2 Longitudinal patterns in bill hue. **A)** Bill hue drops in the year prior to imminent death in males (left panel) with no evidence for senescence in both sexes prior to this point. Data are raw data and box plots from a subset of individuals that all died a natural death and were measured for three or more years (see Table 1C). **B)** Drops in bill hue visualized on the individual level. Pre-senescent bill hue, measured in the year penultimate to the year of death, is plotted against the last measurement prior to death. Outside this mixed model context (Table 1C) matched pairs t-tests resulted in the same conclusions (males: t_{62} = -4.26, p < 0.0001; females: t_{71} = 0.99, p = 0.32). **C)** Estimates of Δage, average age and terminal declines in the year prior to imminent death from the model presented in Table 1C. ➔


A
bill hue (nm inflection point)
600
595
590
585
580
575
570
-5
-4
-3
-2
-1
0
years prior to imminent death (occuring within a year after 0)
B
bill hue prior to imminent death
penultimate bill color measurement
C
estimates (±SE) from model
1
0
-1
-2
-3
Δage
age
drop

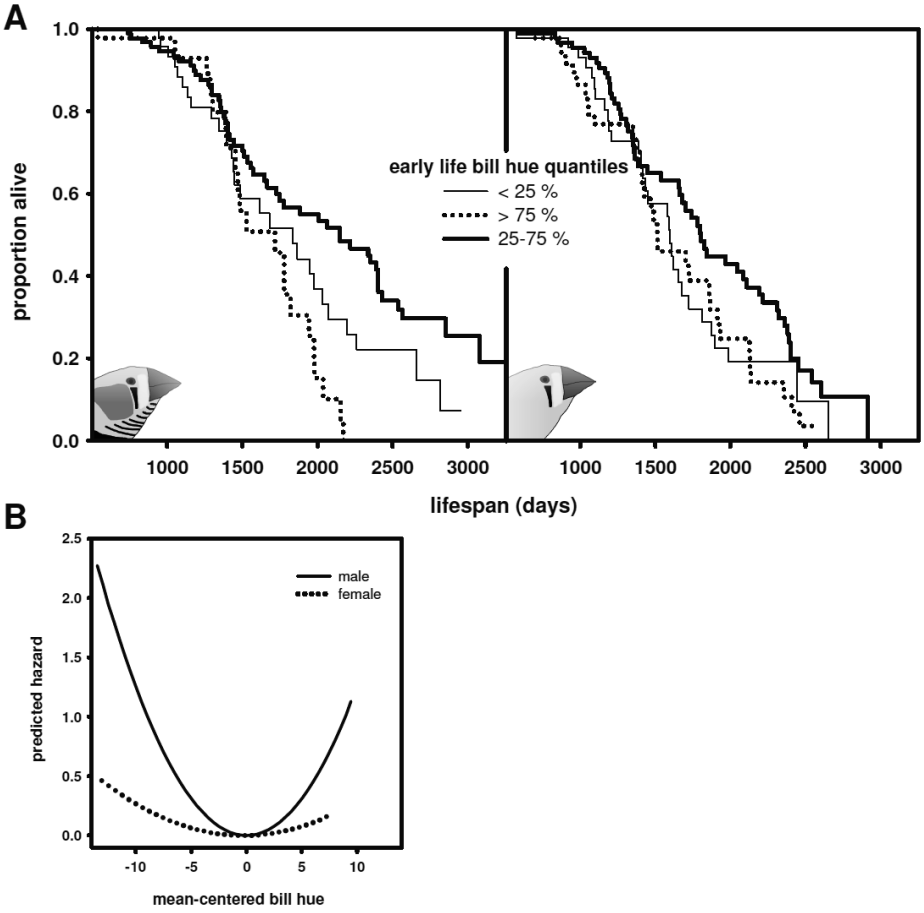
Association between pre-senescent bill hue and survival

Given that bill hue did not systematically change with age before a terminal decline preceding death, a distinction can be made between pre-senescent and senescent bill hue. To examine whether pre-senescent bill hue is associated with survival we used the last measurement prior to the year that was followed by death or censoring in the subsequent year, corrected for measurement year in a mixed model. We only included one data point per individual instead of an average, to avoid regression to the mean biasing our estimates (the longest living individuals would have more measurements, and hence through stochastic effects an average closer to the population mean), however associations with survival using an estimated average pre-senescent bill hue per individual were very similar (data not shown). We entered pre-senescent bill hue values (mean centered per sex) into a Cox proportional hazards survival analysis in which we tested both linear and quadratic effects. We found that the data were best described by the quadratic term of bill hue alone in males (Table 2), indicating better survival of individuals with a bill hue close to the average (Fig. 3). In females this pattern was similar in shape but smaller in magnitude and not statistically significant (Table 2, Fig. 3). The linear term of bill hue was small for both males (estimate: 0.06 ± 0.034, $p = 0.06$) and females (estimate: 0.023 ± 0.024, $p = 0.35$). Note that in the models that did include the linear term of bill hue the quadratic term of bill hue was also significant in males ($p = 0.0002$) and again not significant in females ($p = 0.22$). To test whether this pattern is driven by stronger directional selection at one or the other side of this optimum, analyses of the associations with survival in the least red and reddest half of the data were conducted. We detected significant negative survival selection at both ends of the intermediate bill hue in males (Table 2). These results indicate that mortality is lowest for individuals with pre-senescent bill hue close to the average (Fig. 3B) and increases when pre-senescent bill hue deviates more from the average in either direction (Table 2).

Fig. 3 Pre-senescent bill hue and survival. **A)** Survival patterns of sub-groups that differed in pre-senescent bill hue (the yearly bill hue measurement prior to the last measurement before natural death or censoring). Plotted are quantiles but note that the proportional hazard models (Table 2) were based on continuous data. Also note that the plot only contains individuals for which pre-senescent bill hue could be assessed and that bill colouration measurements started in adulthood (> 160 days), explaining the high survival at young ages in the plot. Therefore the x-axis of the plot was truncated to the shortest lifespan observed in the set for visualization purposes. **B)** Predicted quadratic relationships with bill hue and hazard of death from the proportional hazard models in which bill hue was entered as continuous variable (Table 2), plotted for the full range of the underlying data. Intermediate bill hues are associated with higher survival. ➔

Table 2 Proportional hazard models estimating the relationship between pre-senescent bill hue and survival prospects. The full sets contained 180 females (64 censored), 184 males (83 censored). Note that the significance of the quadratic effects reported here are not dependent on the exclusion of the linear term from the models (see text).

	term	estimate (±s.e.)	p
males	pre-senescent bill hue^2	0.0127 (0.004)	0.0018
females	pre-senescent bill hue^2	0.0028 (0.0031)	0.38
males (only least red half of data)	pre-senescent bill hue^2	0.011 (0.0048)	0.022
	pre-senescent bill hue	-0.121 (0.054)	0.026
males (only reddest half of data)	pre-senescent bill hue^2	0.030 (0.011)	0.002
	pre-senescent bill hue	0.238 (0.090)	0.008
females (only least red half of data)	pre-senescent bill hue^2	0.0003 (0.0038)	0.93.
	pre-senescent bill hue	-0.026 (0.046)	0.58
females (only reddest half of data)	pre-senescent bill hue^2	0.012 (0.0069)	0.086
	pre-senescent bill hue	0.118 (0.060)	0.048



Discussion

In summary, we find that bill hue drops sharply when death is imminent without prior signs of improvement or senescence, and that individuals with average pre-senescent bill hue have the best survival prospects (schematic overview in Fig. 4). Associations within females are in the same direction as in males, but weaker and hence not statistically significant in all analyses, despite a significant association between bill hue and survival also in females (Fig. 1; Simons et al. 2012*a*). We therefore tentatively conclude that qualitatively the same pattern holds in females as in males, but less strongly, and therefore more data are required to find statistically significant results. The positive associations of bill colour with survival we reported earlier (Simons *et al.*, 2012a) can thus be attributed to the combined effect of lowered survival of individuals that have low pre-senescent bill hue and the drop in bill hue associated with imminent death.

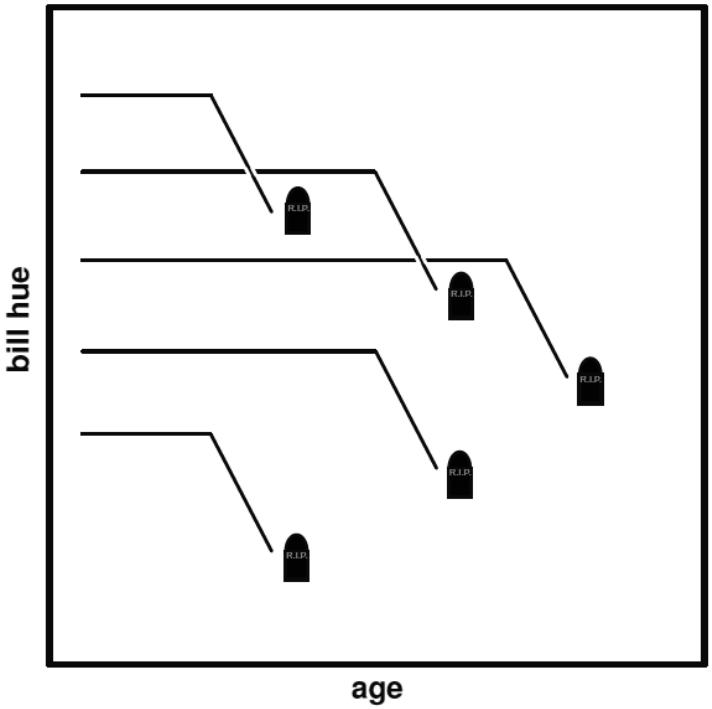


Fig. 4 Schematic representation of the main results. The separate lines depict hypothetical individuals with different bill hues and lifespans. Bill hue drops prior to imminent death (as indicated by the gravestones). There is no evidence of senescence before this drop. Individuals with intermediate bill hue in early life (before the drop in bill hue) survive longest. Note that these associations were stronger and statistically significant within males and weaker but similar in direction within females.

Positive associations between ornament expression and survival have often been reported (Jennions et al. 2001), but it remains to be investigated whether the underlying pattern of negligible senescence, a terminal decline and stabilising survival selection we find in our study is also general. We know of only one other report of a similar pattern: Common guillemots (*Uria aalge*) show declines in breeding success in the last years prior to death and pre-senescent breeding success shows a quadratic relationship with reproductive lifespan, with longest reproductive lifespans for the individuals with average early-life reproductive output (Reed et al. 2008). The multiple steps of analysis required to arrive at our and Reed et al.'s conclusions may be a reason why similar results have not been reported in other species. The generality of this pattern for fitness linked traits therefore warrants more study.

Our results have implications for mate choice, because they indicate that declines in bill hue signal imminent death and hence potential mates with low bill hue should be avoided. This strategy would yield benefits, because individuals with low bill hue have lowered short-term (Figs 1, 2) and long-term (Fig. 3) survival prospects, which put breeding attempts at risk because zebra finches depend strongly on biparental care (Royle et al. 2006) and re-mating can be costly (Ens et al. 1993; van de Pol et al. 2006). Yet, the reddest individuals also suffer from reduced survival probabilities (Fig. 3). This could indicate that these reddest individuals overinvest into their ornaments and associated physiology, reducing their survival, in line with the disposable soma theory (Kirkwood and Holliday 1979). Overinvestment into the ornament yields increased attractiveness (Simons and Verhulst 2011), possibly because it obscures the terminal decline in bill hue to potential mates (Fig. 4). The costs of losing a mate could be a functional reason to avoid the reddest males in mate choice. Non-directional preferences as previously shown for mate choice by zebra finches males (Burley and Coopersmith 1987), are a possible solution to avoid potential mates that overinvest in their ornamentation (Chenoweth et al. 2006). Note however that female zebra finches do prefer males artificially manipulated to display super red bills (beyond the natural range) (Burley and Coopersmith 1987). It is tempting to speculate that the possible differences between male and female choice evolved to match differential investment into reproduction in females and males (Chenoweth et al. 2006). Yet, in mate-choice in general, and also in the zebra finch (Simons and Verhulst 2011), the exact shape of preference functions are rarely tested, possibly because mate-choice experiments are hard to do (Bell et al. 2009). Note that reduced survival does not need to be directly related to overinvestment in the ornament. It could also be that these reddest individuals have larger reproductive capacities, and associated physiological adaptations, which may be only slightly offset by reduced survival. For instance, we have earlier reported higher fledgling production by

the redder females (Simons et al. 2012a). Reduced survival of the individuals exhibiting the reddest pre-senescent bills does therefore not necessarily point to cheating, but can also represent a different life-history strategy.

The zebra finch bill therefore provides different information at different life stages (Fig. 4). This nuance is likely not exclusive to the zebra finch bill but could be a general property of sexual signals (Candolin 1999). Intermediate pre-senescent bill hue is associated with highest survival, whereas in general the most "yellow" individuals survive worst because bill hue drops when death approaches. Phenotypic correlations (e.g. immunocompetence, condition, behaviour) with sexual traits (e.g. colouration) likely differ in strength and perhaps even sign between these life-history stages and this may explain why these associations are relatively weak (Nakagawa et al. 2007; Simons et al. 2012b). Hence we might rather expect mates to monitor bill coloration changes in their partner and use this information to decide on divorce or reproductive investment. Indeed experimentally reducing foot coloration after pair-bond formation of blue-footed booby males reduced female courtship behaviour and propensity to copulate (Torres and Velando 2003). Mate-choice for a first or novel social or sexual partner is likely based on avoidance of individuals with low bill hues, and on choice for redder bills, in all likelihood driven by the expected association with reproductive capacities or the benefit of producing more attractive offspring. In the captive single sex conditions in which the zebra finches in this study were kept these sexually selected benefits were not acting and we cannot exclude that the birds may have modulated sexual signalling accordingly. The birds could however not know that they would spent their lives without reproductive opportunities and this is probably also the reason they kept their signalling efforts up, or for reasons of intrasexual competition.

On the individual level mortality risk is effectively tracked by terminal declines in bill hue. Yet bill hue before the terminal decline does not senesce and individuals with intermediate pre-senescent bill hue survive best (Figs. 2, 3, 4). Prior to the terminal decline, bill hue does not signal physiological deterioration underlying mortality. This finding is also illustrated by the fact that we did not find effects of the foraging or the rearing brood size treatment on bill hue, even though these treatments do affect survival rates (Chapter 3). Although we tested for confounding effects of our foraging treatment and brood size manipulation within the current data set (see above), we cannot exclude that associations would be different under a harsher environmental manipulation or in the field. On a more positive note, compared to other work investigating relationships with sexual signalling in a control lab environment only, we can generalise our results further because they hold across our range of mild manipulations of environmental

quality in early and adult life. Potentially harsher and more immediate manipulations of physiological state than rearing brood size and foraging treatment, like an immune challenge (Alonso-Álvarez et al. 2004) and cold exposure (Eraud et al. 2007) have in contrast been shown to reduce zebra finch bill colouration. Bill colouration is thus likely defended against physiological deterioration, probably because of its attractiveness benefits, except when facing immediate severe physiological challenges.

Alternatively, it may be that physiological deterioration underlying senescence is not a gradual process but accelerates sharply prior to death. Indeed, fecundity in black-legged kittiwakes (*Rissa tridactyla*), common gulls (*Larus canus*), and common guillemots (*Uria aalge*) has been found to also drop prior to imminent death (Coulson and Fairweather 2001; Rattiste 2004), but also more complicated terminal effects, interacting with age, on reproduction have been reported (Torres et al. 2011; Hammers et al. 2012). Yet other studies do not find these effects in for example great tits (*Parus major*) (Bouwhuis et al. 2009) and mute swans (*Cygnus olor*) (McCleery et al. 2008), where reproductive senescence was found to be gradual. It would be illuminating to unravel to what extent these different senescence trajectories on the demographic level are paralleled by different physiological senescence trajectories, because both the absence and the presence of such parallels would provide information on the ageing process.

Physiological markers that are correlated to mortality risk, such as telomeres, can potentially be revealing in this respect (Boonekamp et al. 2013; Simons 2015). Interestingly, telomere shortening also accelerates sharply prior to imminent death in jackdaws (*Corvus monedula*) (Salomons et al. 2009). Telomeres are DNA/protein structures at the end of chromosomes, are sensitive to oxidative stress, decline in length with age (Riethman 2008), and in humans behave as a biomarker of somatic redundancy (Boonekamp et al. 2013). Reliability theory of ageing postulates that the soma is composed of redundant units, which fail at a certain rate, and when redundancy is depleted the organism dies (Gavrilov and Gavrilova 2001). Usually failure rate of redundancy units is assumed to be constant (Gavrilov and Gavrilova 2001; Boonekamp et al. 2013), yet this does not need to be the case (Simons et al. 2013). Terminal declines in physiological parameters like telomere length, reproduction and sexual signalling shortly before death may indicate that failure rate increases shortly before death, or represent a physiological collapse when redundancy is almost exhausted. This exemplifies that research on connections between changes with age in biomarkers (Boonekamp et al. 2013; Simons 2015) of physiological functioning and demographic patterns of deaths may prove highly fruitful in understanding the biology of ageing. Sexual ornaments may be excellent traits to study these connections, because of their intimate relationship with physiological state.

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Chapter 9

Baseline glucose in adulthood is higher in birds exposed to adverse developmental and adult environments and shortens lifespan

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Submitted

Abstract

Epidemiological research in humans highlights the association of high baseline glucose levels with numerous pathologies and lifespan. Causes and consequences of individual variation in baseline glucose level are therefore of interest as a factor linking environmental conditions to lifespan and health. We tested to what extent baseline blood glucose level is a repeatable trait in adult zebra finches, and whether glucose level was associated with age, manipulated environmental conditions during development (rearing brood size) and adulthood (foraging cost), and lifespan. We found that: (i) repeatability of baseline glucose level was 30%, both within and between years. (ii) Having been reared in a large brood and living with higher foraging costs as adult were both associated with higher baseline glucose. Baseline glucose was low when ambient temperature was high and foraging costs were low, indicating that baseline glucose is regulated at a lower level when energy turnover is low. (iii) Survival probability decreased with increasing baseline glucose. We conclude that baseline glucose level is an individual trait negatively associated with survival, which increases due to adverse environmental conditions during development (rearing brood size) and adulthood (foraging cost). Glucose may be therefore part of the physiological processes linking environmental conditions to lifespan.

Introduction

Glucose regulation is a key aspect of homeostasis maintenance and difficulties maintaining such balance associate with detrimental effects. For example, experimentally induced lower glucose caused death within 3 to 4 days in chickens (*Gallus gallus domesticus*) (Akiba et al. 2010) and high blood glucose is associated with lower survival in humans (see e.g. Barr et al. 2007; Rao et al. 2011). Blood glucose concentration is modulated depending on life-history stage and environmental factors in birds and mammals. Glucose levels are higher in birds during demanding stages such as early development, courtship and parental care (Brzęk et al. 2010; Gayathri et al. 2004; Lill et al. 2002) and at old age (Ferrer and Dobado-Berrios 1998; Prinzinger and Misovic 2010). Blood glucose is also affected by ambient temperature and day length, which can also be interpreted as responses to variation in energy turnover (Bairlein 1983; Remage-Healey and Romero 2000; Schradin et al. 2015). Thus blood glucose level is an important but complex trait, and in particular in birds little is known about causes and consequences of its individual variation.

Adverse developmental conditions have long-term effects on offspring fitness prospects in many species, including both birds (Gustafsson et al. 1995; de Kogel et al. 1997; Lindström 1999; van de Pol et al. 2006; Reid et al. 2010; Boonekamp et al. 2014), and mammals (Ozanne and Hales 2004; Kerr et al. 2007; Plard et al. 2015). The mechanism(s) mediating these effects have not yet been resolved, particularly in birds. However, it is evident that direct or indirect nutritional restriction (i.e. mediated through sibling competition) has long-term effects on fundamental physiological processes. For example, restrictive nutrient conditions during development induce higher glucose levels in adult mammals (Jackson 1990; Gluckman et al. 2005; Burns et al. 1997; Fernandez-Twin et al. 2005; Gardner et al. 2005; Fagundes et al. 2007), and associate with higher energy metabolism in adult birds (Verhulst et al. 2006; Criscuolo et al. 2008; Schmidt et al. 2012). However, whether adverse developmental conditions have long-term effects on glucose homeostasis in adult birds has to our best knowledge not been investigated.

Food availability is a key ecological variable that can have major consequences reproduction and survival (e.g. Schubert et al. 2009; Verhulst et al. 2004). Food availability also affects blood glucose levels. For example, partial food deprivation in captive birds initially resulted in lower glucose levels, while prolonged starvation and refeeding both resulted in higher glucose levels (Savory 1987; Lone and Akhtar 1988; Alonso-Alvarez and Ferrer 2001; Rodríguez et al. 2005; Khalilieh et al. 2012). However, in natural conditions, food availability variation usually takes the form of variation in

the effort required per item of food obtained (i.e., foraging cost). Such variation may have effects on physiology that are very different from the response to food-deprivation and thus little is known about the effect of naturalistic variation in food availability on glucose levels.

In this study, using adult zebra finches *Taenopygia guttata* living in outdoor aviaries, we investigated causes and consequences of individual variation in baseline blood glucose level (for brevity, from here on referred to as 'baseline glucose'). More specifically, we investigated whether baseline glucose is (i) repeatable within individuals over weeks and years, (ii) affected by variation physical ambient conditions (i.e. temperature, day length), (iii) related to mass and age, (iv) affected by the developmental environment (either large or small rearing brood size), foraging costs (either low or high foraging cost) and their interaction, and (v) associated with survival probability. Considering previous findings in other species, we predicted baseline glucose to be a repeatable trait, subject to physical environmental variables (e.g., temperature, day length) and positively associated with age and mass. To evaluate the effect of brood size and foraging costs on baseline glucose, we manipulated brood size shortly after hatching to experimentally produce small and large broods, and during adulthood we exposed these offspring to a lifelong condition of either low or high foraging cost (Koetsier and Verhulst 2011). We previously found that in particular individuals exposed to the combination of harsh developmental (large brood size) and adult (high foraging cost) environments have lower survival (Briga and Verhulst 2015). Based on this finding, and the literature on the link between glucose and survival (Barr et al. 2007; Rao et al. 2011), we predicted birds experiencing adverse environmental conditions, either during development or in adulthood, to have higher baseline glucose, and among them, those birds exposed to both harsh environments to have the highest levels.

Material and Methods

Birds and Housing

Birds were from the zebra finch breeding colony of the University of Groningen. Individuals were reared in 'breeding cages' of 80 x 40 x 40 cm with a nest-box and nesting material (hay). Each breeding cage contained a single reproductive pair which had unrestricted access to cuttlebone, water and sand. Breeding pairs were food supplement (egg food, Bogena, Hedel, the Netherlands) regularly until the chicks hatched. Nests were checked daily around the expected date of hatching.

During the study, adult birds were housed in eight single sex outdoor aviaries (L x H x W: 310 x 210 x 150 cm) located in Groningen, the Netherlands (53° 13' 0" N / 6° 33' 0" E). Each aviary contained 15 - 25 birds. At the time of glucose measurements subjects were 0.4 - 8.4 years old (mean ± s.e.m.: 3.3 ± 0.11 years). All birds were provided with a tropical seed mixture available *ad libitum* (but see below), unrestricted access to cuttlebone, water and sand, and were supplemented with 0.42 g of egg food per bird three times per week.

Experimental treatments

We manipulated developmental conditions by cross-fostering all chicks to create broods that were either small (2 chicks) or large (6 chicks). Cross-fostering took place when the oldest chick of a birth nest was 4 - 5 days old. Resulting brood sizes were within the range observed in wild zebra finches (Zann 1996). In this species, being raised in large broods has been shown to impair growth and result in a smaller size at adulthood and shorter survival (De Kogel 1997; Tschirren et al. 2009), also in our experimental birds (Briga and Verhulst 2015). Growing up in a large brood thus constitutes an adverse developmental environment. After nutritional independence, from the age of 35 days until approximately 120 days (when sexually mature), young were housed in larger (L x W x H: 153 x 76 x 110 cm) indoor cages with up to 40 other young of the same sex and two male and two female adults (tutors for sexual imprinting) until the start of the adult treatment.

During adulthood we manipulated aviaries to have either low or high foraging costs (4 aviaries each, 2 per sex, 8 aviaries in total) as described in (Koetsier and Verhulst 2011). Each aviary was equipped with a food container (L x W x H: 120 x 10 x 60 cm) with 10 holes in the sides to access food, which was suspended from the aviary ceiling. In the low foraging cost treatment food containers had perches beneath the holes, whereas in the high foraging cost treatment these perches were removed. Hence, when perches were absent, birds were forced to fly from a distant perch to the food container and back for each seed. Seeds spilt by birds while feeding were collected by a duct, and hence were not accessible for the birds. Birds facing high foraging costs have lower survival (Briga and Verhulst 2015), thus a high foraging cost condition constitutes an adverse environment.

Mass and Size

Body mass was measured monthly for all the birds and the measurement closest to the blood sampling session (< 15 days) was taken for statistical analyses (birds were not weighed at sampling to minimize potential effects of prolonged handling on baseline

glucose). As body mass increased through day, for statistical analysis we used the residuals of the linear regression of body mass on hour of measurement.

Structural size was measured when birds reached 100 days old and corresponds to a combined measurement of the tarsus and the head-bill length, both standard normally distributed using the equation: (standardized tarsus + standardized head-bill)/2. Residual body mass was calculated as the residuals of the linear regression of body mass on structural size.

Glucose

Blood sampling was carried out on the same colony of birds in two periods: (i) July 1st - August 9th, 2012, and (ii) August 23rd - October 2nd, 2014. Sample size was 171 birds (0.4 - 6.6 years old; mean ± s.e.m.: 3.1 ± 0.16 years) in the first sampling period (2012), and 135 birds (0.9 – 8.3 years old; mean ± s.e.m.: 3.4 ± 0.17 years) in the second one (2014). To check the repeatability of baseline glucose, 59 birds were sampled twice within the same year (31 in 2012 and 28 in 2014) and 78 from the 171 birds sampled in 2012 were re-sampled in 2014. The exact same sampling protocol was followed in both sampling periods (see below).

Before sampling, birds were taken out from their aviary and individually housed in a small (L x W x H: 40 x 40 x 15 cm) box without access to food or water. The box was placed in a dark room, at the same ambient temperature as the aviary, for 30 minutes together with two other boxes containing birds from the same aviary. The aim of this procedure was to yield baseline glucose values independent of recent food consumption. A pilot study, conducted using this method on a different sample of birds of the same colony, showed that intra-individual baseline glucose stay in a relative stable state between 30 and 60 minutes after the capture (B. Montoya et al. unpublished data). Consequently, to reduce stress associated to alimentary, social and motor restriction we used the minimum waiting time within this interval. After 30 minutes a 70 μL blood sample was taken from the brachial vein and collected in heparinized capillaries. Immediately after sampling, blood was diluted 30x in a heparin (500 IU/mL) - 0.01% EDTA solution and frozen until glucose measurement.

For the measurement of whole blood baseline glucose we performed the Hoffman's ferricyanide method using a Thechnicon autoanalyzer (Beckman Coulter LX20PRO). Blood samples taken in 2014 were analyzed in duplicate obtaining an ICC of 72.4% (n = 324 measurements, 95% C.I. 64.5, 79%). Statistical calculations were done on the average of the two duplicates.

Statistical analyses

All statistical analyses were performed using R version 3.1.2 (R core team 2014). Glucose values were ln transformed prior to analysis to obtain a normal distribution. Additionally, when year was not included in the model, glucose values were mean centered by year. Linear mixed models were fitted using the function lmer of the package lme4 (Bates et al. 2015). To control for stochastic variation associated to the specific sampling session, we included the factor "sampling *day"* as a random term in all models. Furthermore, to correct for daily climatic fluctuation associated with seasonal variation, we used the continuous variable called sampling *date* (day number in the year when the sample was taken, counting on from January 1st). In all fitted models, repeated measures per individual were accounted for by including individual identity as a random term. We verified that the data met the assumptions underlying the statistical tests.

Survival analysis was performed fitting a Cox proportional hazards (CPH) model (coxme package; Therneau 2012). Because age at sampling was not proportional, we stratified sampling age into two groups of equal range. All survival analyses were checked for the proportionality assumption, using Schoenfeld residuals and the *'cox.zph'* function. Linearity and influential data points were checked with Martingale and deviance residuals respectively.

Results

Repeatability

We calculated the repeatability of baseline glucose on two levels: within years and between years. Data for the repeatability estimates were collected in two sessions in each of the two years (i.e. four sessions in total). Because average baseline glucose differed between these four sessions ($P < 0.0001$), we used deviations from the session average for the repeatability calculations. Within-year repeatability (intra-class correlation coefficient) was 29.7% (Fig. 1a; n = 57 individuals, 95% C.I.: 2.7- 56.7%). Similarly, between-years repeatability was 27.4% (Fig. 1b; n = 81 individuals, 95% C.I.: 4.7- 50.1%). Thus baseline glucose is an individual characteristic of zebra finches, even over a period of years.

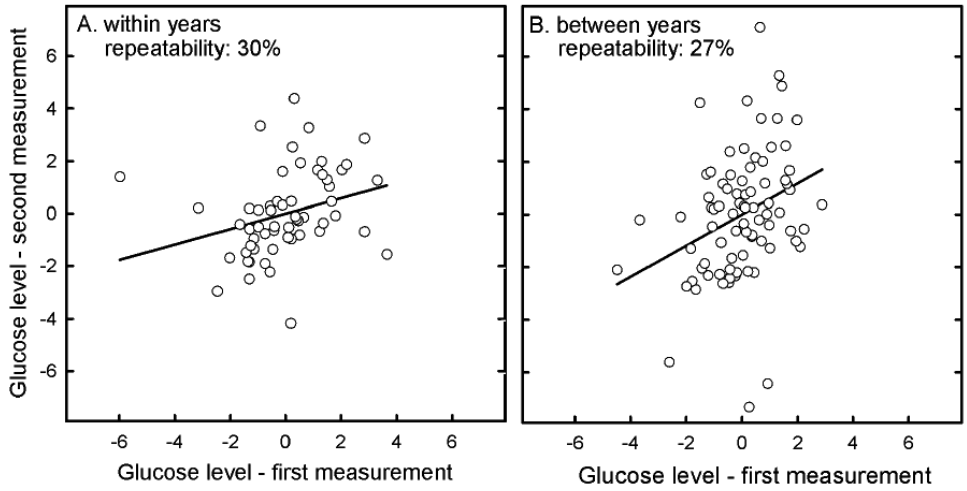

Fig. 1 Individual repeatability of baseline glucose levels (mM) within years (A) and between years (B). Plotted data show second measurement plotted against the first measurement in both cases. Data points are deviations from the mean glucose level in each of the measurement sessions to account for within and between year variations in average glucose level. Intra-class correlation coefficient, within years n = 57 individuals, 95% C.I.: 2.7- 56.7%, between years n = 81 individuals, 95% C.I.: 4.7- 50.1%.

Physical environment

Before examining the association between baseline glucose with experimental treatments, age and survival, we evaluated whether baseline glucose were related to year of sampling and ambient variables (time of the day, time of the day squared, day length and ambient temperature). Baseline glucose was almost 20% lower during the first of the two study years (Fig. 2a; $F_{1, 229.51} = 33.18$, $P < 0.001$; first year mean ± s.e.m.: 12.98 ± 0.10 mM; second year: 16.03 ± 0.18 mM). Furthermore, baseline glucose was lower at higher temperatures and longer days (Table 1; Fig. 2). Considering these results, and to increase statistical power, we included sampling day as a random factor in models of subsequent sections. This factor included environment fluctuation associated with temperature, day length and other potentially unidentified sources of variation among sampling days.

Sex, age, size and body mass

There was a non-significant tendency for males to have higher baseline glucose than females (effect of sex added to minimal model in Table 1; males had 1.50% higher baseline glucose than females; $F_{1, 217.18} = 3.23$, $P = 0.07$). However, sex did not explain a significant portion of the variation as a main factor or in interactions ($P > 0.10$) in the analyses reported below, and was therefore excluded from all models.

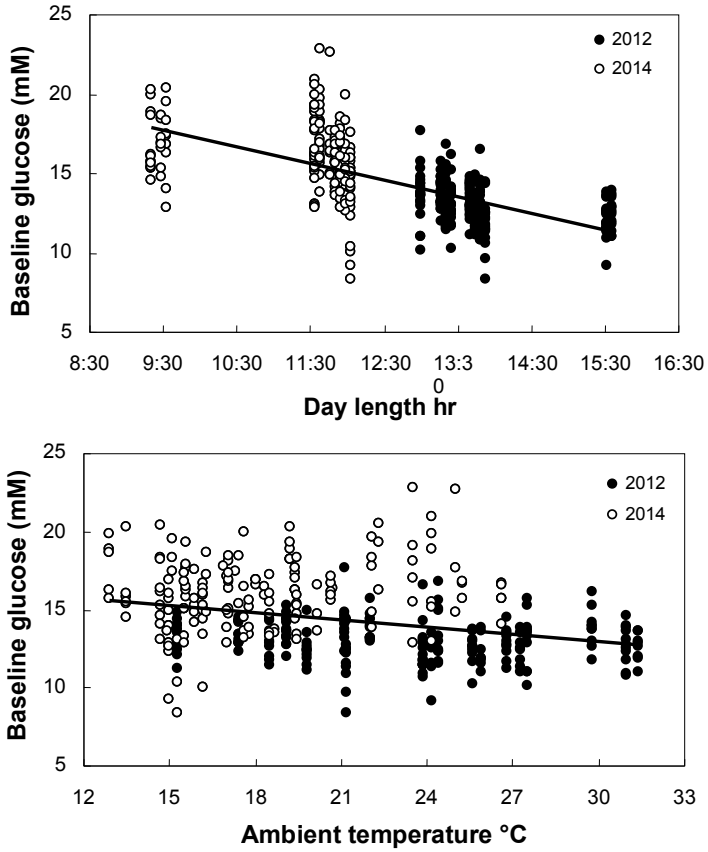

Fig. 2 Baseline glucose level (mM) in relation to day length (A) and ambient temperature (B). Closed circles correspond to birds sampled in 2012 and open circles to birds sampled in 2014. General linear mixed model, n = 366 samples collected on 227 individuals (Day length $F_{1,262.87}$ = 42.81, $P < 0.001$; ambient temperature $F_{1,329.77}$ = 6.71, $P = 0.01$).

Table 1 Plasma baseline glucose level (mM, ln transformed) and its association with physical environment factors.

Fixed effects	**Coefficient (± s.e.)**	**Den DF**	***F***	***P***
Temperature	0.004 ± 0.001	329.77	6.71	0.010
Day length	-1.15 ± 0.18	262.87	42.81	<0.001
Year (relative to 2012)	0.11 ± 0.02	229.51	33.18	<0.001
Rejected terms				
Time of the day	-0.05 ± 0.08	336.89	0.41	0.52
Time of the day^2	-0.13 ± 0.54	291.26	0.059	0.81
Random effects	**Variance**	**Standard deviation**		
Bird identity	0.29	0.54		

* n = 366 samples collected on 227 individuals.

Aging has been associated with a decline in baseline metabolic rate in our study species (e.g. Moe et al. 2009), also in our study population (Chapter 11), yet we found no association between age (or age squared) and baseline glucose (Table 2). Neither were there significant interactions between age and brood size or foraging conditions (Table 2) or with sex ($F_{1,310.86} = 0.26$, $P = 0.61$).

Table 2 Relationship between age and glucose level (mM, mean centered by year).

Variable (Fixed effect)	**Coefficient (± s.e.)**	**Den DF**	***F***	***P***
Foraging treatment (High cost)	0.24 ± 0.10	200.34	5.58	0.02
Rearing brood size (Large brood)	0.22 ± 0.11	206.88	4.50	0.04
Rejected terms				
Age	0.01 ± 0.02	267.90	0.22	0.64
Age^2	-0.004 ± 0.01	256.12	0.14	0.70
Foraging treatment (H) * Age	-0.06 ± 0.05	309.03	1.72	0.19
Rearing brood size (L) * Age	-0.02 ± 0.05	315.25	0.13	0.71
Random effects	**Variance**	**Standard deviation**		
Bird identity	0.27	0.52		
Sampling day	0.19	0.43		

*n = 366 samples collected on 227 individuals.

There is usually a strong positive association between energy turnover and body mass (e.g. Rønning et al. 2007), and we therefore evaluated the association between baseline glucose and body mass. Variation in body mass arises through variation in structural body size, and because individuals of a given structural body size can have different body composition e.g. muscle mass and energy reserves. We thus separated effects of size and body composition by splitting mass in two components: structural body size, and residuals from the regression of mass on body size. We explored for an association between baseline glucose and body mass or size, adding these variables one by one to a model that included only sampling day and bird identity (random effects). There was no association between baseline glucose and body mass ($F_{1,305.02} = 0.40$, $P = 0.52$), residual body mass ($F_{1,295.7} = 0.24$, $P = 0.62$) or structural size ($F_{1,197.85} = 0.23$, $P = 0.63$). Similarly, there was no significant interaction of sex with body mass ($F_{1,316.06} = 2.79$, $P = 0.10$), residual body mass ($F_{1,291.41} = 3.54$, $P = 0.06$) or structural size ($F_{1,199.08} = 0.11$, $P = 0.74$). We noted though that males with higher residual body mass tended to have higher baseline glucose ($\beta = 0.14 \pm 0.08$), whereas in females there was an opposite tendency.

Developmental and adult environments

Given that baseline glucose is an individual characteristic, because it was a repeatable trait, we tested whether it was affected by our permanent experimental manipulations i.e. rearing brood size and adult foraging effort. Because baseline glucose differed significantly between years, even after controlling for variation of the physical environment (i.e. temperature, day length; Table 1), we analyzed baseline glucose values mean centered by year (i.e. the mean value of the sampling year was subtracted from each observed glucose value). Additionally, we included sampling day as random term to control for within year variation in the physical environment.

Baseline glucose was higher in birds reared in large broods, and in birds exposed to high foraging costs (Table 2, Fig. 3) while there was no significant interaction between brood size and foraging cost ($F_{1,207.78} = 0.04$, $P = 0.84$). One could speculate that individual observations within aviaries are not statistically independent, but adding aviary identity as random effect to the model did neither explain significant variation (REML, $P = 0.22$), nor cause substantial changes in the model, indicating statistical independence of individual measurements within aviaries.

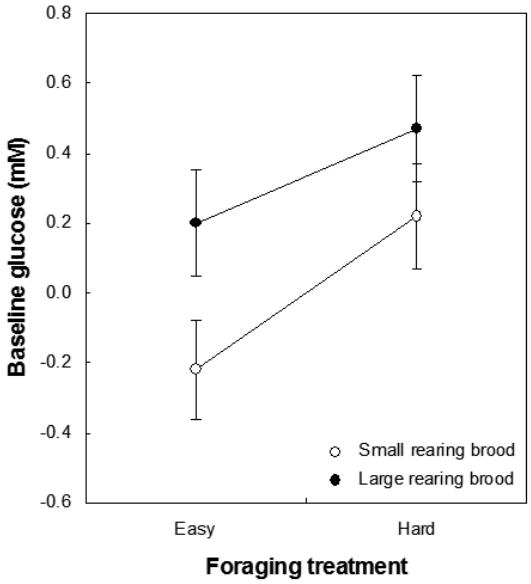

Fig. 3 Manipulated environmental conditions (rearing brood size and foraging treatment) and model estimates of baseline glucose (mM ± s.e.m.; data mean centered by year). Open circles correspond to birds reared in small broods (2 chicks), and closed circles correspond to birds reared in large broods (6 chicks). General linear mixed model, n = 366 samples collected on 227 individuals (Foraging treatment $F_{1,200.34} = 5.58$, $P = 0.02$; rearing brood size $F_{1,206.88} = 4.50$, $P = 0.04$).

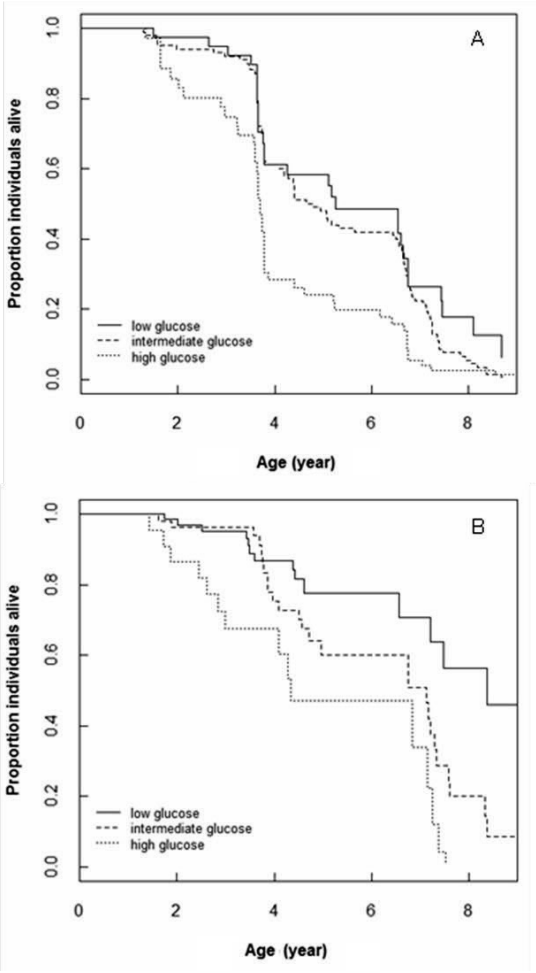

Fig. 4 Plasma baseline glucose level and survival. Panel A shows birds sampled in 2012 and panel B individuals sampled in 2014. In both panels, solid line corresponds to low glucose level (mM, lower quartile), dashed line to intermediate glucose (two middle quartiles pooled), and dotted line to high glucose (upper quartile). Note that this grouping is for illustrative purposes only; glucose level was treated as a continuous variable in the analysis and years were pooled. Cox proportional hazards model, n = 304 samples collected on 225 individuals of which 176 died (residual glucose $z = 2.32$, $P = 0.02$; see also Table 3).

Survival

By January 2016, 120 of the 170 birds sampled in 2012 and 56 of the 134 birds sampled in 2014 had died. Note that because some birds were sampled twice, of the 225 birds sampled in total, 176 had died. Only the first sample of each sampling year was included in the survival analysis for individuals that were resampled later in the same year. In the survival analyses, to correct glucose measurements for variation associated with

sampling session and seasonal fluctuation in ambient temperature, we used the residuals of the linear regression of sampling date and ambient temperature on baseline glucose. Birds with the highest baseline glucose had lower survival probability and this pattern was very similar in the two sampling years (Fig. 4). This result did not change when we controlled for the two experimental manipulations and their interaction (Table 3), or the manipulations without the interaction (data not shown). We also tested for a quadratic survival association with baseline glucose, but this did not improve the model fit (β = -0.002 ± 0.012, P = 0.88).

Table 3 Relation between residual glucose* (mM) and mortality probability, fitted with a Cox proportional hazards model.

Fixed effects	**Exp(coef) ± s.e.**	***z***	***P***
Residual glucose	1.10 ± 0.04	2.39	0.17
Rearing brood size (Large brood)	0.95 ± 0.05	-0.99	0.32
Foraging treatment (High cost)	1.15 ± 0.45	0.31	0.76
Rearing brood size * Foraging treatment	1.02 ± 0.08	0.25	0.80
Random effects	**Variance**	**Standard deviation**	
(Bird identity)Aviary	0.16	0.40	

* For the cox proportional hazards model residual glucose was calculated from the linear regression of sampling day and ambient temperature on baseline glucose. n = 304 samples collected on 225 individuals of which 176 died in the study period.

Discussion

Significant repeatability of traits implies consistent variation between individuals in the level at which the trait is regulated. Individual variation in baseline glucose was repeatable, and repeatability within and between years was almost indistinguishable, at 30 and 27% respectively. Estimates of baseline glucose repeatability in other species appear to be rare, but our estimates are close to the repeatability value of 32% found on average for physiological traits (Wolak et al. 2012). Furthermore, our estimate falls within the range reported for other physiological traits in zebra finches, 18-46% (Rønning et al. 2005; Careau et al. 2014). Thus individual zebra finches can be characterized by their baseline glucose.

Baseline glucose was regulated at different levels depending on multiple current and historical environmental conditions. With respect to current environmental conditions, baseline glucose was lower in the low foraging costs treatment, when ambient temperature was higher, and when day length was longer, as previously reported for various bird and mammal species (Bairlein 1983; Remage-Healey and Romero 2000;

Schradin et al. 2015). Higher ambient temperature and low foraging costs are likely to result in lower energy expenditure (Wiersma and Verhulst 2005; Koetsier and Verhulst 2011), and these findings therefore indicate that baseline glucose is regulated at a lower level when energy turnover is low. Long day length was also associated with low baseline glucose, suggesting that the rate of energy expenditure is lower on long days, possibly because the birds have more day light hours to fulfill their energy needs. Body mass and age did not explain variation in baseline glucose, while these factors are known to affect the rate of energy turnover in the nocturnal phase (e.g. Moe et al. 2009). Nonetheless, during the active phase animals have ample leeway for energetic compensation, e.g. by altering body temperature or activity pattern, and whether mass and age affect energy turnover during the active phase in our study species is not yet known.

In addition to effects of current environmental conditions, baseline glucose was also differentially regulated depending on historical environmental conditions, with birds reared in large broods having 11% higher baseline glucose than birds raised in small broods (Fig. 3). This finding is consistent with the proposed long-term effects of early-life nutritional restriction on glucose homeostasis in mammals (Desai et al. 1996; Gardner et al. 2005; Fagundes et al. 2007). The effect of being reared in a large brood was independent of the current foraging costs experienced, because early and adult life manipulations did not significantly interact to affect baseline glucose in adulthood (Table 2). The absence of this interaction contrasts with predictions following from the thrifty phenotype hypothesis, according to which one would expect the response to nutritional stress in adulthood to depend on the nutritional stress during development following a match-mismatch pattern (Hales and Baker 1992, 2001; Gluckman et al. 2005: Hanson and Gluckman 2014). However, our finding is in agreement with the lack of broad support for the match-mismatch hypothesis emerging from a recent meta-analysis, summarizing effects on a wide range of traits, including physiology, reproduction and survival, in animals and plants (Uller et al. 2013).

High baseline glucose was associated with higher mortality (Fig. 4). A positive association between baseline glucose and mortality is well known in humans (see for example Barr et al. 2007; Rao et al. 2011), but we are not aware of studies in other species testing for this relationship. In our study population, we previously found that birds reared in large broods and exposed to high foraging costs achieved a shorter lifespan than birds subjected to all other treatment combinations (Briga and Verhulst 2015). One of our aims in studying physiological variation in our study population was to identify physiological processes that vary in parallel with the experimental lifespan effects. However, the treatments and their interaction did not significantly affect survival in the

present data set (Table 3; removing baseline glucose from the model does not change this result), which contrasts with our findings in the complete data set (Briga and Verhulst 2015). This is not unexpected, because the experimental survival effects were most conspicuous in early adulthood, and birds in the present data set were generally older because the experiment had been running for four years when the data for the present paper were collected. Thus to what extent the experimental survival effects can be attributed to processes reflected in baseline glucose cannot be inferred from the present data set. Instead we can conclude that baseline glucose is associated with mortality over and above mortality effects of the environmental manipulations.

An increase in baseline glucose with age has previously been reported in other bird species (Ferrer and Dobado-Berrios 1998; Prinzinger and Misovic 2010), but despite a substantially larger sample size we found age to be unrelated to baseline glucose in our cross-sectional analysis. Paradoxically, this finding, in combination with the positive relationship between baseline glucose and mortality, points towards there being a longitudinal increase of baseline glucose within-individuals. Otherwise the selective disappearance of individuals with high baseline glucose individuals would have generated a negative cross-sectional association between age and baseline glucose. A longitudinal study is required to test this interpretation, which we unfortunately cannot yet do with our data because we have only two time points at which birds were sampled, and hence we cannot distinguish age from year effects.

Given that elevated baseline glucose is associated with increased mortality, our results raise the question as to why individuals regulate their baseline glucose at high levels when exposed to harsh environments. High baseline glucose was shown to enhance performance during physically and cognitively demanding activities in other species (Rodríguez et al. 2009; Gilsenan et al. 2009). Thereby short-term benefits of high baseline glucose may outweigh any long-term survival cost. In line with this interpretation, baseline glucose may more generally reflect individual position in the slow-fast life-history continuum. Studies on the association of baseline glucose with other life-history traits, in particular reproductive investment, are required to test this hypothesis.

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Chapter 10

Individual variation in metabolic reaction norms over ambient temperature causes low correlation between basal and standard metabolic rate

Michael Briga & Simon Verhulst

Abstract

Basal metabolic rate (BMR) quantifies an individual's minimal energy consumption at thermoneutral ambient temperatures (Ta). It is generally assumed that BMR is indicative of the minimum energy consumption at sub-thermoneutral Ta, but this assumption has remained untested. Using a new statistical approach to distinguish between-individual from within-individual correlations, we quantified the individual consistency in metabolic rate at thermoneutrality (BMR), below thermoneutrality (standard metabolic rate, SMR) and across Ta in zebra finches. Mass, BMR and SMR were repeatable over lifetime ($r \sim 0.30$) and thus individuals can be characterized by these traits. Correlations between BMR and SMR were always weak ($r < 0.37$), indicating that BMR and SMR are regulated independently between and within individuals. In contrast, the between-individual correlation of SMR at various sub-thermoneutral Ta was high ($r = 0.91$), showing that individuals can consistently be ranked according to their SMR. Thus individuals differ consistently in their metabolic reaction norms across Ta. We show that these differences can at least in part be explained by body temperature (Tb) regulation: when facing cold Ta's some individuals lower their Tb more relative to others that maintain a higher increase in metabolic rate. Harsh environments decreased the repeatabilities of BMR and SMR but not of mass as shown by an ecologically relevant foraging cost manipulation, but the above conclusions were environment independent. Thus, BMR is not indicative of SMR. Associations between minimal energy consumption and other life history traits are thus best studied at Ta's that are representative of the living environment.

Introduction

Energy is an essential resource for reproduction and survival. Therefore, metabolic rate, an individual's rate of energy turn over, can affect many life history traits. One component of energy turnover is basal metabolic rate (BMR), i.e. the minimum energy expenditure of a postabsorptive adult animal measured during the rest phase at thermoneutral temperatures (McNab 1997; IUPS Thermal Commission 2001). Thermoneutral temperatures are defined as the ambient temperatures (Ta) at which body temperature (Tb) regulation is achieved without regulatory changes in metabolic heat production or evaporative water loss (IUPS Thermal Commission 2001). BMR has been studied in association with many traits such as growth, reproduction, personality, oxidative stress, senescence and survival (Careau et al. 2008; Biro and Stamps 2010; Bouwhuis et al. 2011; Burton et al. 2011; Bouwhuis et al. 2014). It is often implicitly assumed that individual variation in BMR is representative of individual variation in daily energy expenditure (DEE), which includes all energy requiring processes such as self-maintenance, thermoregulation and behaviour. However, this correlation is generally weak in birds and mammals ($0<R^2< 0.23$; Meerlo et al. 1997; Fyhn et al. 2001; Speakman et al. 2003; Tinbergen and Wiersma 2003; Tieleman et al. 2008; Careau et al. 2012). Thus the assumption that individual variation in BMR reflects variation in DEE, and hence can be interpreted as index of total energy turnover, is not well supported.

Multiple hypotheses can be formulated to explain why DEE and BMR are only weakly correlated. The hypothesis we study here builds on the fact that BMR is measured at thermoneutrality, while DEE is measured at Ta as experienced in natural environments, which is often below thermoneutrality. Here, we investigated to what extent BMR is indicative of metabolic rate at Ta's below thermoneutrality. To this end, we repeatedly measured BMR and standard metabolic rate (SMR) in the same individuals and under the exact same conditions except that Ta was below the thermoneutral zone during SMR measurements. If individual differences in thermoregulatory response to a lower Ta are small, individual variation in BMR will be strongly correlated with SMR (Fig. 1A). In this case BMR and SMR can be considered two different expressions of the same trait. Alternatively, individuals may differ in their thermoregulatory response to the extent that the correlation between BMR and SMR is absent (Fig. 1B). In this case BMR and SMR can be considered different traits, and this would at least partly explain the low correlations observed between BMR and DEE.

The extent to which individual variation in BMR and SMR is repeatable will set an upper limit to the extent that BMR and SMR can be correlated. For example, in the extreme

case that the repeatability of BMR or SMR is zero, the correlation between BMR and SMR can only be zero. In this context, repeatability is the proportion of total phenotypic variance that is caused by between individual variance (Falconer and Mackay 1996). Thus traits can only be correlated when they are repeatable.

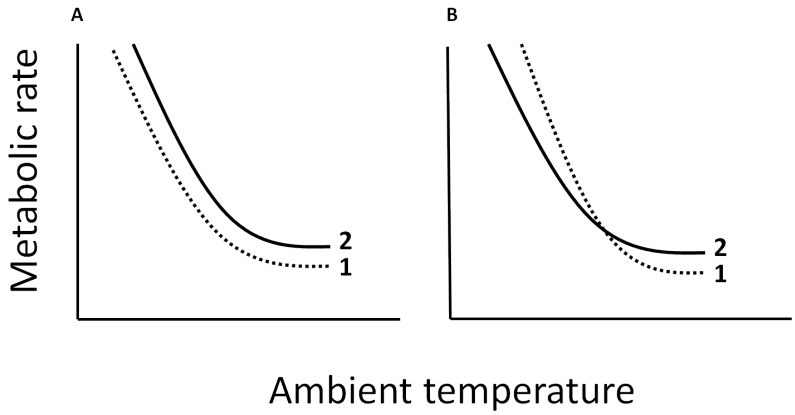

Fig. 1 Schematic representation of the metabolic rate of two individuals (1 and 2) in relation to ambient temperatures up to the upper critical temperature. Figure A represents the case where individuals differ little in their metabolic response to a decrease in ambient temperature, generating a high correlation between BMR and SMR. Figure B represents the alternative scenario in which there is variation between individuals in their metabolic response to a decrease in ambient temperature, generating a low correlation between BMR and SMR.

When multiple traits of an individual are each measured multiple times, phenotypic correlations between traits can arise via two mutually non-exclusive ways (Lynch and Walsh 1998; Dingemanse and Dochtermann 2013). The first possibility is that the individual mean values of one trait correlate with individual mean values of the other trait, i.e. between individual correlations. If between individual correlations are high, individual rankings according to either BMR or SMR will be similar (Fig. 1A), which is not the case when these correlations are low (Fig. 1B). The second possibility is that the change in value of one trait over time correlates with the change in value of the other trait, i.e. within individual correlations. For example, in our metabolic rate dataset, within individual correlations reflect changes in metabolic rate between seasons and between years. A high within individual correlation could imply that an individual regulates BMR and SMR as one system, while a weak correlation could indicate that BMR and SMR are regulated independently. Decomposing trait correlations into between vs. within-individual correlations is thus informative since both levels refer to biologically distinct processes.

Here we first quantified the repeatability of body mass, BMR and SMR of 361 zebra finches housed in outdoor aviaries measured more than 3000 measurements collected at ambient temperatures ranging from 5°C till 39°C (Fig. 2). Next, we investigated the within and between individual correlations between BMR and SMR. When exposed to a lower Ta, homoeothermic organisms balance three interrelated physiological components: metabolic rate, insulation and Tb (McNab 1980). For example large differences between Ta and Tb increase heat loss, and one way to minimize this loss is by down regulating its Tb (Körtner et al. 2000; Geiser 2004; Angilletta et al. 2010). Thus Tb adjustments are an important determinant of metabolic responses to lower Ta. To investigate the role of Tb responses in the between individual correlation between BMR and SMR we measured Tb of a subset of individuals at multiple Ta's. We then correlated within individual changes in MR with changes in Tb in response to colder Ta.

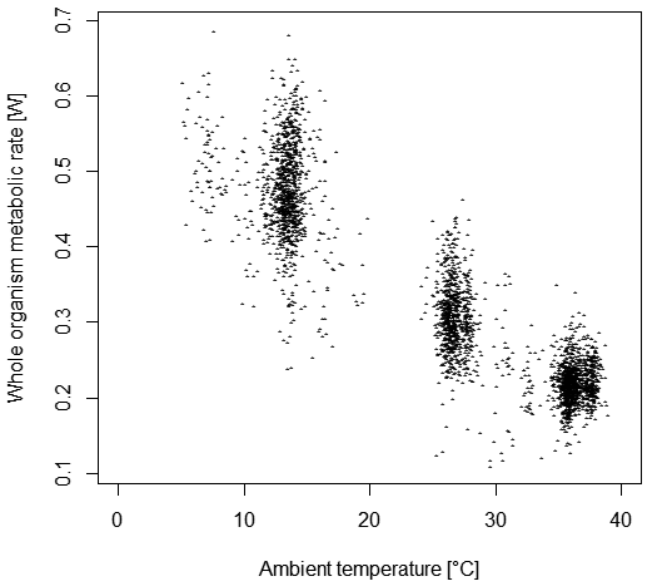

Fig. 2 Overview of the variation in whole organism metabolic rate as a function of ambient temperature (N=3059 measurements on 361 individuals).

Repeatabilities of and correlations between traits can be environment specific: between - and within individual variance depend among others on environmental characteristics, genetic composition and data collection methods (e.g. measurement error). Our results are thus inherently environment specific and confined to the specificity of a captive population. Free-living animals however are commonly exposed to foraging costs which can fundamentally alter animal physiology and life history traits (Schubert et al. 2008;

Schubert et al. 2009; Prevedello et al. 2013; Ruffino et al. 2014; Briga and Verhulst 2015a; Briga and Verhulst 2015b). To increase the resemblance of our population to that of a free-living population, we randomly exposed half of our population to a high foraging costs using a lifelong manipulation of flight costs per food reward (Koetsier and Verhulst 2011), which for example decreases survival (Chapter 3). How environmental quality affects trait repeatabilities and correlations is not well known. Reviews on the repeatability of metabolic rate have not included an environmental quality component (Nespolo and Franco 2007; Versteegh et al. 2008; White et al. 2013). One study has shown that metabolic rates are more repeatable in a laboratory animals than in their free-living conspecifics (Auer et al. 2016), but these environments differ in environmental quality as well as in variability and in population genetic composition. However, experimental studies on heritability, i.e. the additive genetic component of between individual variation, have shown that on average heritability increases with environmental quality (Charmantier and Garant 2005; Visscher et al. 2008). Even though the association between heritability and repeatability can be somewhat complex (Dohm 2002), we therefore expect a positive association between repeatability and environmental quality. Thus, how environmental quality affects the repeatability of and correlations between metabolic traits is not well known, but based on heritability studies we expect a positive association.

Material and Methods

Birds and housing

The birds measured here are part of a long-term experiment investigating the relationships between foraging costs and survival. Information about the foraging cost manipulation can be found in Koetsier and Verhulst (2011). Birds were housed in eight unisex outdoor aviaries (L * W * H: 320 * 150 * 225 cm) located in Groningen, the Netherlands (53° 13’ 0” N / 6° 33’ 0” E). The experiment started in December 2007 and birds entered the set-up when at least three months old and remained there till natural death. Each aviary contained an approximately equal number of birds (15-25) and to keep bird densities within a limited range, we regularly entered new birds to replace those that died. Food (tropical seed mixture), cuttlebone, grit and water were provided *ad libitum* and the birds received 0.42 g/bird of fortified canary food (“eggfood”, by Bogena, Hedel, the Netherlands) three times a week. During development these birds had been subjected to a brood size manipulation. The manipulated brood sizes were within the natural range (Zann 1996) and did not affect either BMR, SMR (Chapter 11). This manipulation will therefore not be considered here. The foraging cost manipulation

did affect repeatability estimates and in some cases the BMR-SMR correlations, and we therefore present results for both groups pooled and separately.

Body mass and body size

Between December 2007 and July 1st 2014, 395 birds were weighed a total of 11.106 times, i.e. up to 82 times per bird (Fig. S1A). Size measurements were done at the average age of 133 days (SD: 33 days). As a measure of structural body size we used the average of the tarsus and the headbill after transforming both to a standard normal distribution.

Metabolic rate

Between December 2007 and April 2013, 3059 metabolic rate measurements were taken of 361 birds. Birds were measured up to 25 times (Fig. S1B) between the ages of 0.4 and 7.2 years. Birds that were measured only once were not included in these analyses because their within individual variance cannot be estimated. Measurements were concentrated in spring and autumn. Measurements were done at ambient temperatures (Ta) ranging from 5°C till 39°C (Fig. 2), but most measurements were centered around three Ta's of 36°C for BMR (±3°C) and for SMR at 26°C (±3°C) and 12°C (±3°C), all of which were measured each season (Table 1). The MRs at these three Ta categories are further abbreviated as BMR, SMR26 and SMR12 respectively. 36°C is within the zebra finches' thermoneutral zone (Calder, 1964 and see below), the ambient temperatures at which Tb regulation is achieved without regulatory changes in metabolic heat production or evaporative water loss and we measure the basal metabolic rate (BMR). 26°C and 12°C are below the thermoneutral zone, at which birds increase metabolic rate for heat production in order to maintain their body temperature and we refer to this as standard metabolic rate (SMR). As expected (Scholander et al. 1950), MR increased with lower Ta (Fig. 2). Note that also the SD of MR increased with colder Ta (Table 1). This increase in SD was proportional to the increase in mean value since the coefficients of variation remained similar across all Ta (Table 1).

Overnight energy expenditure was measured using an open flow respirometer situated in a dark acclimatized room kept at the desired ambient temperature. Up to sixteen individuals per night were taken from the aviaries on average at 18:10 (SD=01:17), weighed (±0.1 g) and randomly transferred to one of sixteen 1.5 l metabolic chambers in a dark climate room. This room was kept and continuously monitored at the above-mentioned temperatures with multiple PT100 temperature sensors, one located in the room recording continuously and one in each metabolic chamber recording at each MR measurement. Technical specification of the equipment can be found in Bouwhuis et

al. (2011). In brief, the air-flow through the metabolic chambers was controlled at 25 l/h by mass-flow controllers (5850S; Brooks, Rijswijk, the Netherlands) calibrated with a bubble flow meter. Air was dried using a molecular sieve (3 Å; Merck, Darmstadt, Germany) and analyzed by a paramagnetic oxygen analyzer (Servomex Xentra 4100; Crowborough, UK). During measurements each metabolic chamber or reference outdoor air was sampled every 8 min for 60s to stabilize measurement levels. In each sampling, we measured O_2 and CO_2 concentrations. Oxygen consumption was calculated using Eq. (6) of Hill (1972). An energy equivalent of 19.7 kJ/l oxygen consumed was used to calculate energy expenditure in watt (W). Metabolic rate was taken to be the minimum value of a 30-min running average, which included 3–6 measurements per individual. The first measurement hour was excluded to minimize potential effects of handling stress and incomplete mixture of air in the metabolic chamber. Body mass for the metabolic rate measurements was calculated as the average of the before and after measurement values.

Table 1 Description of the metabolic rate dataset at the ambient temperature ranges for which most data were collected. Note that the complete dataset is larger (N=3059 measurements on 361 individuals) and includes measurements at other temperatures than the intervals considered here (Fig. 2).

Whole population	**SMR12**	**SMR26**	**BMR**
Temperature range [°C]	9 till 15	23 till 29	32.5 till 39
Date first measurement	16-apr-08	19-apr-08	16-dec-07
Date last measurement	12-apr-13	14-apr-13	15-apr-13
Number of birds	215	201	267
Number of measurements	868	693	1076
Mean metabolic rate [W]	0.48	0.31	0.22
SD metabolic rate [W]	0.067	0.048	0.030
CV	0.14	0.15	0.13
Benign environment			
Date first measurement	18-apr-2008	30-jun-2008	16-dec-07
Date last measurement	9-apr-2013	14-apr-2013	15-apr-13
Number of birds	112	103	129
Number of measurements	467	361	534
Harsh environment			
Date first measurement	24-apr-2008	19-apr-2008	18-dec-07
Date last measurement	12-apr-2013	13-apr-2013	6-apr-13
Number of birds	103	98	138
Number of measurements	401	332	542

The thermoneutral zone of the zebra finch was previously identified as ranging from 29.5°C till 40°C and the minimum oxygen consumption was measured at 34.9°C (Calder 1964). Yet, these estimates were based on 72 measurements and larger datasets may find a narrower thermoneutral zone. In our database, we found a quadratic association between Ta and MR within the range of 32.5°C and 39°C (Table 1), with the minimum MR being at 34.2°C. Within this Ta range the effect was minimal: the difference between maximum and minimum BMR was minor at 0.016W. We can therefore be confident that our metabolic rate measurements at 36°C were in the thermoneutral zone and can be considered BMR measurements.

Body temperature

During autumn-winter 2011 we collected body temperature (Tb) data at the end of 550 respirometry measurements (mean time 9:46 h; SD=0:38) of 189 individuals using a Omega® Thermocouple Thermometer Type T smoothened with Johnson & Johnson® lubrication gel. All measurements were done within 30 seconds of handling and the temperature reading was done within 5 seconds after entering the probe in the cloaca, at which time point Tb was stable.

Statistical analyses

The repeatability is the ratio of the between-individual variance over the total phenotypic variance. We used the linear mixed model approach (sensu Nakagawa and Schielzeth 2010): in a mixed model with body mass or metabolic rate as the dependent variable the between individual variance is estimated by including individual identity as a random effect, while the total phenotypic variance is the sum of the variance explained by individual identity and the residual variance. We did this using a Bayesian approach (Dingemanse and Dochtermann 2013) with the R package MCMCglmm (Hadfield 2010) in R v. 3.1.1 (R Core Team 2014) with flat improper priors with $1.5*10^5$ iterations, 10000 burn-in and a thinning interval of 100. This yielded Markov chain Monte Carlo (MCMC) sample sizes of at least 1000 with low levels of autocorrelation (mean R=-0.002 with all R<0.1). Bayesian results were consistent with those of the frequentist approach (with restricted maximum likelihood and maximum likelihood), using the functions (i) 'lmer' of the package 'lme4' (Bates et al. 2015), (ii) 'lme' of the package 'nlme' (Pinheiro et al. 2014) and (iii) 'rpt' of the package 'rptR' (Nakagawa and Schielzeth 2010). For Bayesian estimates we report 95% credible intervals (95CI) and for frequentist estimates 95% confidence intervals (95CI), which were estimated by non-parametric bootstrapping (Nakagawa and Schielzeth 2010). P-values were obtained using permutations tests and likelihood ratio tests (Nakagawa and Schielzeth 2010). To test for differences between repeatabilities we used t-tests with the number of individual identities as conservative

sample size. Covariation between traits were analyzed using a Bayesian approach (Dingemanse and Dochtermann 2013). We performed a trivariate analysis (SMR12, SMR26 & BMR) with the function 'mcmcglmm' (Hadfield 2010) using uninformative inverse Wishart priors. The effects of the season, foraging cost manipulation on trait values were analyzed using general linear mixed models, 'lmer' of the package 'lme4' (Bates et al. 2015) including individual as random effect. Residuals were checked with function 'resid' and all had a normal distribution without outliers.

Results

Body mass: repeatabilities

Repeatability of body mass was high at 0.69 (0.66<95CI<0.72; Fig. S1A; N=11.106 measurements on 395 individuals). A modest part of the between-individual variation was due to variation in body size: body mass repeatability conditional on size was 0.1 lower at 0.59 (0.56<95CI<0.63; Fig. 3B vs. C). Results for the metabolic rate subset, using only pre-measurement body mass and the whole dataset were similar (data not shown). All estimates differed from 0 (LR>2287, p<0.0001). Hence, the zebra finches in our population can be characterized by their size adjusted body mass.

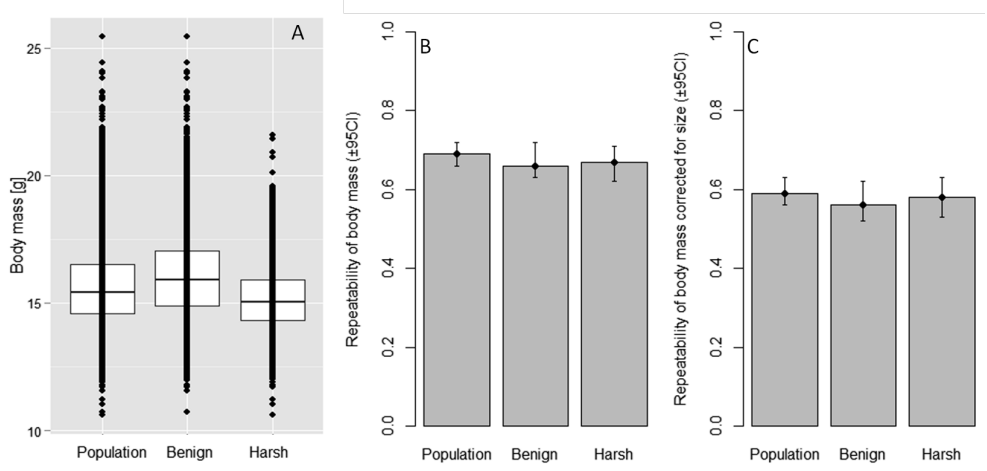

Fig. 3 Environmental quality affects body mass (A) but not its repeatability estimates (B & C). 'Population' refers to all the birds pooled, 'Benign' and 'Harsh' refer to the subset of the population that lived in benign and harsh environments respectively. Repeatability estimates are determined by between and within individual variances. These variance estimates can be found in table S1. Note in panel A the smaller data range of body mass in harsh environment. This is due to both smaller within and between individual variance (Table S1).

Birds exposed to high foraging costs birds were 5% lighter compared to birds in low foraging cost aviaries (Fig. 1A) and this difference was highly significant (F_{396}=42.4, p=2e^{-10}). Mass correlates well with size (r=0.56; Chapter 11) and when controlling for size the mass difference persisted (F_{393}=47.7, p=2e^{-11}). Thus high foraging costs negatively affected body mass.

Environmental conditions can affect between and within-individual variance, and thus repeatability is conditional on the environment. The harsh environment was characterized by smaller between and within-individual variance components than the benign environment (Table S1) and this difference was significant (paired t=4.94, p=0.016). However, repeatability estimates of body mass and size corrected body mass in both environments were similar (~0.60; Fig. 3B & C) because the between and the within-individual variance components changed to the same extent. Thus environmental quality did not affect the repeatability of body mass, but individuals in the harsh environment experienced smaller body mass variation between and within individuals.

Metabolic rate: repeatabilities

Whole organism BMR had a repeatability of 0.51 (0.45<95CI<0.57), in the range of previously published results (Nespolo and Franco 2007; Versteegh et al. 2008; White et al. 2013). Part of this repeatability will be due to the high repeatability of body mass, which correlates positively with metabolic rate (r=0.60). Indeed, when body mass was added to the statistical model, repeatability of BMR conditional on body mass was halved to 0.27 (Fig. 4C). This decrease was due to a decrease in between individual variance while the within individual variance remained little affected (Table S2). Both repeatability estimates differed significantly from 0 (LR>86, p<0.0001). Thus the zebra finches in our population can be characterized by their BMR.

Pooling all the subthermoneutral measurements (from 5°C till 32°C) and including Ta in the model, whole organism SMR had a repeatability of 0.41 (0.33<95CI<0.44). Adding body mass to the model decreased the repeatability of SMR with approximately 25% to 0.31 (Fig. 4C). The repeatabilities within the narrower Ta ranges of 26°C and 12°C, i.e. SMR26 and SMR12, were slightly higher than for SMR across the whole subthermoneutral range (Fig. 4B & C). All these estimates were significantly larger than 0 (LR>263, p<0.0001). The repeatability of whole organism SMR was slightly lower than that of whole organism BMR (Fig. 4B; t=2.33, p=0.02), while repeatabilities of mass corrected BMR and SMR were indistinguishable (Fig. 4C; t=0.75, p=0.45). Thus the zebra finches in our population can be characterized by their SMR as well as by their BMR.

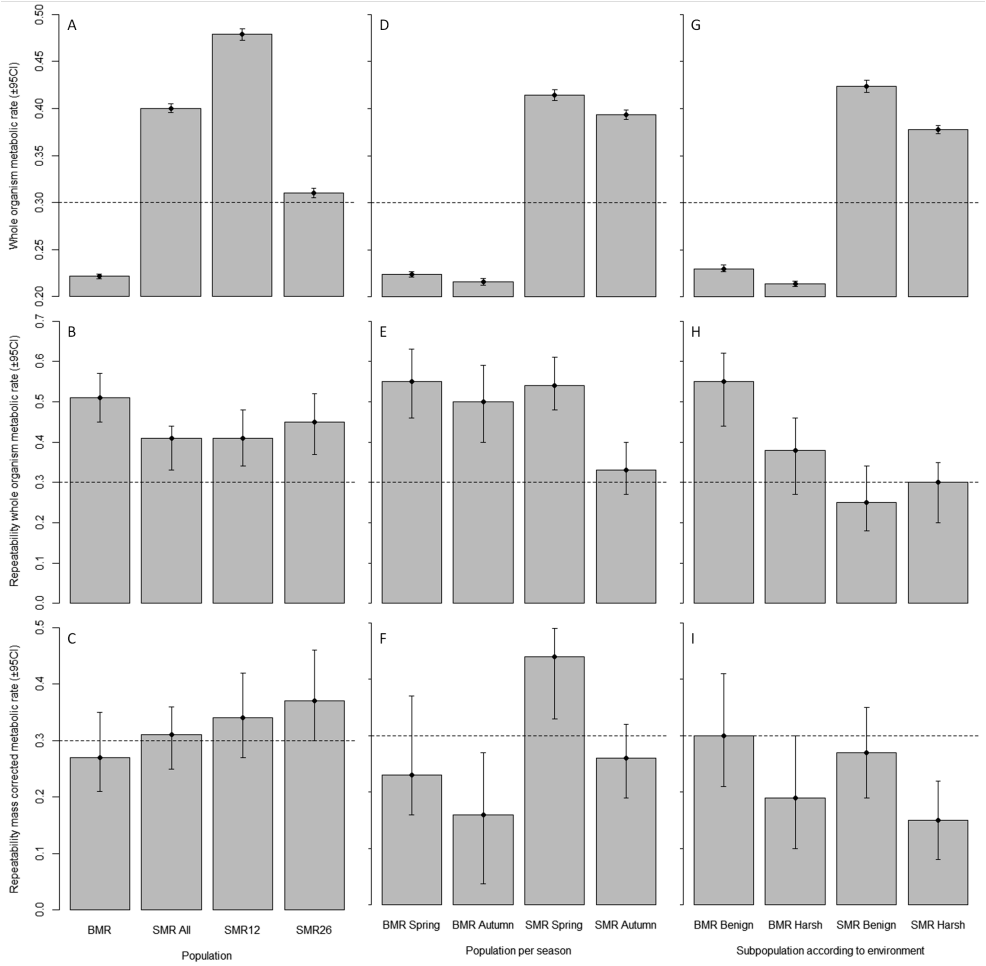

Fig. 4 Overview of basal metabolic rate (BMR, i.e. at thermoneutral temperatures) and standard metabolic rate (SMR, i.e. subthermoneutral) and their repeatability estimates (±95CI). Top graphs (A, D, G) show mean MR, middle graphs (B, E, H) repeatability of whole organism values, lower graphs (C, F, I) repeatability of mass corrected values. The horizontal dashed lines at 0.3 serve as a reference line. Differences in repeatability estimates between traits or datasets can arise due to changes in either between or within individual variance. Variance estimates for each of these estimates can be found in table S2.

The above repeatability estimates of SMR across the whole subthermoneutral range suggest that individuals can be characterized by their SMR reaction norms over Ta. If this is the case, then a random slope model will fit the data better than a random intercept model. In contrast, if individuals cannot be characterized by their SMR reaction norm then the random slope model will provide a worse fit over the random intercept model. We found that a random slope model fitted the data better than a random intercept model

for whole organism SMR (ΔAICc=-41) and for mass corrected SMR (ΔAICc=-15). Thus individuals can be characterized by their SMR reaction norm over Ta.

Data were collected in spring and autumn each year between 2008 and 2013. Metabolic rate can show seasonal flexibility, and this was also the case here: metabolic rate in spring was higher than in autumn and this was valid for both BMR and SMR (Fig. 4D; BMR: $F_{874}=82.8$; $p<2.2e^{-16}$; SMR: $F_{1763}=144$; $p<2.2e^{-16}$). Year round repeatability estimates are a mixture of between and within seasonal repeatability. Therefore metabolic rate may reflect different traits between seasons, as was the case in great tits (Bouwhuis et al. 2011). To investigate this, we quantified the repeatability of spring and autumn metabolic rate separately. If repeatabilities of season specific metabolic rates are higher than those of year round data, then metabolic rates between seasons reflect different traits. In contrast, if repeatabilities are within the same range, then metabolic rates can be considered as one trait all year round. Mass-corrected BMR per season tended to have lower repeatability than whole year BMR at ~0.20 (Fig. 4C vs. 4F) but the difference was not significant ($t<1.70$, $p>0.09$). In contrast, repeatability of mass-corrected SMR in spring was approximately 25% higher relative to whole year data at 0.42 (Fig. 4C vs.4 F; $t>2.8$, $p<0.005$) due to lower within individual variance (Table S2). Repeatability of mass-corrected SMR in autumn was the lowest at 0.25 (Fig. 4E & F; $t>3.92$, $p<0.0001$) due to lower between individual variance than spring SMR or year round data (Table S2). These results thus show that despite seasonal changes in metabolic rate and its repeatability, seasonal and year round mass-corrected BMR and SMR have similar repeatabilities ($0.25< r <0.42$). In our dataset BMR and SMR can thus be considered as a trait all year long despite seasonal phenotypic flexibility.

Individuals facing high foraging costs reduced their whole organism BMR and SMR by 8% and by 11% respectively (Fig. 4G; BMR: $F_{265}=42$, $p=4e^{-10}$; SMR: $F_{268}=136$, $p<2.2e^{-16}$), in line with findings in the literature (Wiersma and Verhulst 2005; Schubert et al. 2009). Some of that effect may be explained by a lower body mass in the harsh environment (Fig. 3A), but the effect of environment remained 5% and 8% for mass-corrected BMR and SMR respectively (BMR: $F_{248}=20.9$, $p=0.77e^{-6}$; SMR: $F_{245}=84.8$, $p<2.2e^{-16}$). The effect of foraging costs on metabolic rate was more pronounced on mass-corrected SMR than on BMR ($F_{2838}=115.1$, $p<2.2e^{-16}$). In contrast, the foraging cost effect was similar for mass-corrected SMR12 and SMR26 ($F_{1409}=1.40$, $p<0.24$). Thus, birds from harsh environments lowered their minimal energy expenditure, and this was most pronounced at temperatures below thermoneutrality.

The repeatabilities of mass corrected BMR and SMR in the harsh environment were lower than in the benign environment (Fig. 4H & I, $t>26$, $p<0.0001$). This effect arose because between individual variance was decreased in the harsh environment, while the within individual variance remained independent of environmental quality (Table S2). Harsh environments also decreased the individual characterization of SMR reaction norms over Ta: the model fit improvement of a random slope model over a random intercept model was better in the benign than in the harsh environment whole organism SMR: $\Delta AICc=-6.8$; mass corrected SMR: $\Delta AICc=-3.3$). Thus a harsh environment decreased the extent to which individuals can be characterized by their metabolic rate and by their metabolic reaction norms over Ta.

Metabolic rate: correlations at multiple ambient temperatures

We started by investigating correlations at the phenotypic level. Phenotypic correlations between SMR12 and SMR26 were moderate (0.52 and 0.43 for whole organism and mass-corrected values respectively, Table 2, Fig. S2) and differed significantly from 0 ($t>11.45$, $p<2.2e^{-16}$). In contrast, the phenotypic correlations between BMR and any of SMRs were weaker and to a considerable extent generated by variation in body mass (Table 2, Fig. S2). However, mass corrected correlations were still significantly positive ($t>3.45$, $p<0.0006$). These patterns were true for the benign and harsh foraging environment alike (Table 2). The phenotypic correlations appeared somewhat stronger in the benign than in the harsh foraging environment, but the difference was not significant (paired $t=0.90$, $p=0.41$). Thus, at the phenotypic level BMR and SMR correlate, but the SMRs at multiple Ta correlate better with each other than with BMR.

The phenotypic correlations above can be the result of between and/or within individual correlations and we here distinguished the contributions from both levels. Correlations at the within individual level were always weak and often not distinguishable from 0, irrespective of environmental quality (Table 2). This shows that in our zebra finches an individual changes its BMR and its SMRs between measurement sessions (i.e. seasons and years) independently from one another.

The between individual correlations between SMR12 and SMR26 were higher than the phenotypic correlations and remained close to one even when correcting for body mass (Table 2). In contrast, the between individual correlations between mass-corrected BMR and any of the mass-corrected SMRs were weaker at approximately 0.30 (Fig. 5, Table 2), but remained significantly positive ($t>4.18$, $p<0.005$). This shows that correlations between SMRs at the between individual level are strong, but the correlations between BMR and SMR are weak. Therefore the ranking of individuals according to their BMR

differs from the ranking according to their SMR (Fig. 5) and our results best fit the scenario in Fig. 1B over that in Fig. 1A.

Between individual correlations might be exacerbated due to persistent variation in environmental quality. However, the high correlations between mass corrected SMR12-26 held in both foraging cost groups (Table 2). Similarly, the correlations between mass corrected BMR and any of SMRs were weak in both foraging cost groups. Thus our results were independent on the manipulations of environmental quality.

Table 2 Correlations (± 95CI) between metabolic rate measurements at multiple levels. Overall phenotypic correlations are a combination of both between and within individual correlations. Correlations are estimated using Bayesian methods. See Fig. S3 for scatterplots.

		Correlation level	
Whole population	**Overall phenotypic**	**Between individual**	**Within individual**
Whole organism			
SMR12-SMR26	0.56 (0.50-0.61)	0.95 (0.88-0.99)	0.25 (0.14-0.33)
BMR-SMR12	0.41 (0.34-0.47)	0.71 (0.61-0.83)	0.09 (-0.02-0.19)
BMR-SMR26	0.45 (0.38-0.51)	0.69 (0.54-0.79)	0.22 (0.15-0.35)
Mass corrected			
SMR12-SMR26	0.43 (0.36-0.49)	0.91 (0.79-0.98)	0.12 (0.02-0.22)
BMR-SMR12	0.14 (0.06-0.22)	0.37 (0.11-0.57)	0.04 (-0.06-0.15)
BMR-SMR26	0.22 (0.14-0.29)	0.34 (0.02-0.51)	0.20 (0.10-0.31)
Benign environment			
Whole organism			
SMR12-SMR26	0.46 (0.37-0.55)	0.93 (0.63-0.99)	0.33 (0.23-0.46)
BMR-SMR12	0.38 (0.28-0.47)	0.70 (0.48-0.88)	0.19 (-0.02-0.31)
BMR-SMR26	0.46 (0.26-0.46)	0.64 (0.37-0.91)	0.30 (0.14-0.41)
Mass corrected			
SMR12-SMR26	0.36 (0.26-0.46)	0.89 (0.48-0.98)	0.25 (0.11-0.37)
BMR-SMR12	0.14 (0.03-0.25)	0.18 (-0.21-0.54)	0.11 (-0.03-0.27)
BMR-SMR26	0.12 (0.001-0.23)	-0.06 (-0.46-0.39)	0.16 (0.04-0.31)
Harsh environment			
Whole organism			
SMR12-SMR26	0.30 (0.19-0.40)	0.84 (0.64-0.99)	0.04 (-0.11-0.17)
BMR-SMR12	0.24 (0.13-0.35)	0.79 (0.53-0.97)	-0.01 (-0.19-0.11)
BMR-SMR26	0.40 (0.30-0.49)	0.76 (0.53-0.97)	0.21 (0.08-0.34)
Mass corrected			
SMR12-SMR26	0.19 (0.07-0.30)	0.80 (0.43-0.98)	0.04 (-0.13-0.14)
BMR-SMR12	0.05 (-0.06-0.17)	0.50 (-0.19-0.98)	-0.02 (-0.17-0.11)
BMR-SMR26	0.28 (0.17-0.38)	0.43 (-0.22-0.96)	0.27 (0.14-0.41)

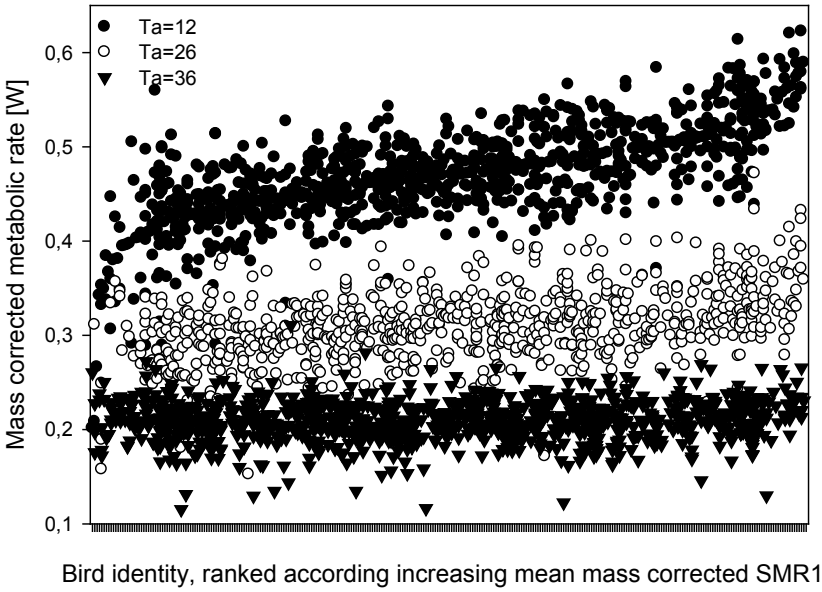

Fig. 5 Repeated measures of mass corrected metabolic rate within and across ambient temperatures. Birds were measured at three ambient temperatures (Ta) of 12°C, 26°C and 36°C and are ordered along the X-axis according to increasing mass corrected metabolic rate at 12°C. To avoid overlap between measurements of different ambient temperature categories, mean metabolic rate per ambient temperature group was added to all residuals. While metabolic rate increases to the right of X-axis for SMR12 and SMR26, this is not the case for BMR. This illustrates that SMR 12 and SMR 26 are repeatable, but that an individual's mean SMR correlates weakly with its mean BMR.

Body temperature

Birds can decrease their Tb in response to lower Ta and this was also the case in our zebra finches (Fig. 6A; $F_{329}=632$, $p<0.0001$; N=493 measurements on 177 individuals). Given that individuals living in harsh environmental conditions can be expected to minimize energy consumption, we expected more hypothermia in harsh relative to a benign environment. This was indeed the case at Ta of 12°C (Fig. 6B, $F_{136}=9.30$, $p=0.0027$) and at thermoneutral Ta (Fig. 6B, $F_{146}=9.23$, $p=0.0028$), but not at Ta of 26°C (Fig. 6B, $F_{111}=0.60$, $p=0.44$). The interaction between environment and Ta was significant ($F_{351}=3.30$, $p=0.038$). Thus, individuals decrease their Tb in response to lower Ta's and this is more pronounced in harsh than in benign environments.

Given that when facing decreasing Ta, an individual balances metabolic rate, insulation and Tb (McNab 1980), we investigated whether the weak between individual correlation between BMR and SMR may be associated with individual differences in Tb. We did this at both the between individual level and the within individual level. At the between individual level, we correlated MR with Tb. Thermal physics predicts that, everything

else remaining equal, individuals with low SMR also have a lower Tb. At Ta of 12°C zebra finches with lower Tb also had lower SMR (Fig. S3, N=191, r=0.23, p=0.0006). At Ta of 26°C this association was less strong (Fig. S3, N=127, r=0.13, p=0.07), while there was no association between Tb and BMR (Fig. S3, N=175, r=0.052, p=0.47). The differences in the Tb-MR associations between Ta categories were significant (F_{438}=3.55, p=0.03). Thus, SMR was associated with Tb but BMR was not.

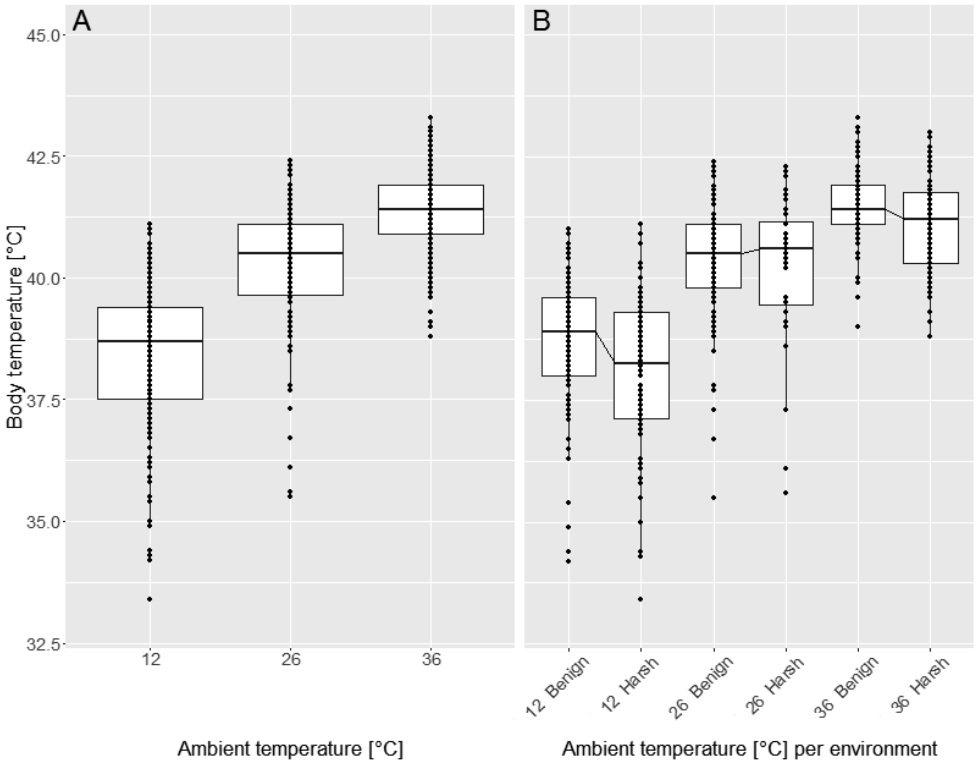

Fig. 6 Body temperature declines in response to colder ambient temperature (A) and this is more pronounced for birds in harsh foraging environments (B). Boxplots show median and 25th and 75th percentiles.

At the within individual level, we explored whether there was a negative correlation between change in MR and change in Tb when the same individual was measured at multiple Ta. We found such a negative correlation when Ta declined from the 36°C to 12°C (Fig. 7, N=137, 0.0001<p<0.04). However, there was no such correlation when Ta declined from 36°C to 26°C (Fig. S4A, N=99, 0.15<p<0.93), nor when Ta declined from 26°C to 12°C (Fig. S4B, N=105, 0.11<p<0.29), although the differences in these associations were not significant (F=2.18, p=0.11). These results indicate that individuals differ in their response to cold Ta: some individuals increase their metabolic

rate more than others and thereby keep more constant body temperatures, while others become more hypothermic. The weak between individual correlation between BMR and SMR (Table 2) can thus in part be explained by individual differences in Tb regulation.

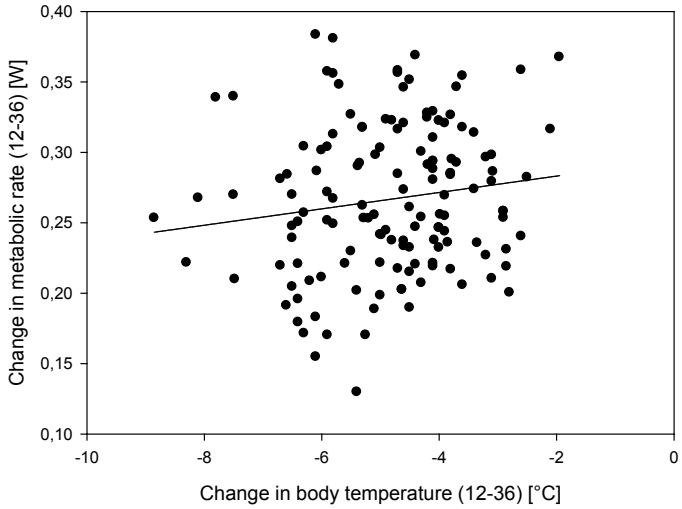

Fig. 7 Individuals trade-off change in metabolic rate for change in body temperature when facing the ambient temperature declines below themroneutrality.

Discussion

SMRs at multiple ambient temperatures correlated are almost perfectly between individuals. In contrast, correlations between BMR and SMR were weak and thus individuals with high BMR do not necessarily have high SMR (scenario Fig. 1B). This weaker correlation can at least partially be explained by individual variation in metabolic reaction norms: when facing cold ambient temperatures some individuals lower their body temperature more relative to others that maintain a higher increase in metabolic rate.

Repeatabilities

The repeatabilities of whole-organism MR of 0.4 till 0.5 found here are consistent with those in the literature, with most repeatability values for whole-animal BMR ranging between 0.30 and 0.80 (Nespolo and Franco 2007; Versteegh et al. 2008; White et al. 2013; Auer et al. 2016). Our repeatabilities of mass corrected MR, ranging between 0.3 and 0.4 are also consistent with earlier studies in birds, although perhaps on the lower

range relative to that found in earlier zebra finch studies which were between 0.3 and 0.6 (Rønning et al. 2005; Vézina and Williams 2005; Careau et al. 2014). Our dataset covers a larger time range (up to 5.5 years) than previous studies, which deflates trait repeatability (White et al. 2013; Auer et al. 2016). Furthermore, our birds are housed outdoors and thus exposed to a wider range of environmental variation than birds housed indoors, which may further decrease repeatabilities relative to indoor housed subjects exposed to less environmental variability (Auer et al. 2016). Thus, the repeatabilities of our MR measurements are within the range one would expect based on earlier studies.

How environmental quality may affect the repeatability of metabolic rate is not well known. For heritability however there are various predictions (Hoffmann and Merilä 1999), although on average there is a positive association between environmental quality and heritability (Charmantier and Garant 2005; Visscher et al. 2008). The results of our experimental manipulation of foraging costs are consistent with these earlier findings: high foraging costs decreased the repeatability of BMR and SMR, but not of body mass. In all cases did the harsh environment decrease the between-individual variance in traits, but for body mass also the within individual variance was decreased. A harsher environment thus decrease the differences in trait values between individuals, and for mass also the phenotypic flexibility within individuals (sensu Piersma and Drent 2003). These results are different from those in which zebra finches were exposed to a harsh environment during development, via dietary restriction of the parents, which appeared to increase between individual variation in a range of traits, including BMR (Careau et al. 2014). The effect of harsh environments during development on between and within individual trait variance may be distinct from those imposed during adulthood. Our results can be considered consistent with the result of a recent meta-analysis showing that metabolic rates are more repeatable in a laboratory animals than in their free-living conspecifics (Auer et al. 2016). In this study however, environments differed in environmental quality, environmental variability, population genetic composition and lacked experimental manipulations. Our experiment thus indicates that at least part of the higher repeatability in laboratory environments (relative to the wild) can be explained by improvements in environmental quality.

BMR and SMR as independent traits

To our best knowledge our study is the first to quantify the correlation between BMR and SMR. At the between-individual level, BMR and SMR correlated poorly (Table 2), indicating that BMR and SMR are essentially different traits. This raises the question whether one should characterize an individual's energy consumption or minimum cost of self-maintenance as BMR or SMR and in our view this may depend on the purpose of

the study. For example, imagine that one finds an effect of environmental quality on an individual's SMR. One would want to know whether the environmental manipulation affects minimum energy consumption or conductance. This requires measuring both BMR and SMR. However, imagine a study in which the one wants to associate an individual's minimum energy expenditure with other life history traits. Conductance is an inevitable determinant of an individual's minimum levels of energy expenditure and such studies would benefit from quantifying SMR rather than BMR only. Because SMRs at all sub-thermoneutral Ta correlate almost perfectly with each other (Table 2), (intraspecific) individual differences in SMR are equally well characterized at all Ta and it does not matter at which Ta SMR is measured. To quantify interspecific differences in SMR however the problem might be more intricate because species live at different mean Ta and thus interspecific variation in SMR might be caused by Ta solely. A possible option might be to quantify a standard level of conductance for all species, for example by always quantifying SMR a number of degrees below the thermoneutral zone. Thus, we believe insight can be gained on the role of energy expenditure in, for example, life history by quantifying SMR instead of BMR.

The within individual correlations are considerably lower than the between individual correlations and in many cases indistinguishable from zero (Table 2). This means that an individual changes its BMR and SMR (i.e. conductance) independently across sampling sessions, i.e. across seasons and years. Given that Tb is an important factor determining conductance at a given Ta (McNab 1980), this indicates that an individual regulates the MR-Tb trade-off differently between seasons and between years. This raises the question as to what determines an individual's location along the 'MR-Tb continuum' at a given time point. Our results show that foraging costs are one such determining variable (Fig. 6), which is consistent with earlier results that show the role of fat reserves on the levels of nocturnal hypothermia (Nord et al. 2011). Age likely also plays a role, since the degree of hypothermia also increases with age (Florez-Duquet and McDonald 1998; Blatteis 2012). Thus the low within individual correlation between MR at multiple Ta shows that individuals change location along the 'MR-Tb continuum' with depending on environmental conditions, climatic variables and/or age.

In evolutionary terms, repeatabilities are relevant because time consistent differences between individuals are a minimum requirement for natural selection to act on, i.e. repeatability sets an upper limit to heritability (Falconer and Mackay 1996) under certain circumstances (Dohm 2002). That is because between individual variance arises due to genetic and to environmental sources of variation whereas heritability includes only genetic differences among individuals, while both are proportional to the total

phenotypic variance (Falconer and Mackay 1996). Our study suggests that MR as a whole (i.e. BMR and SMR) is not heritable, or as we mentioned above, should not be considered as one trait only, but rather as a composite of multiple traits, such as, BMR, body temperature regulation, insulation, that each have their heritability given certain environmental conditions. This is consistent with the view that the genetic architecture of MR is complex (Tieleman et al. 2009; Arnqvist et al. 2010).

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We thank several people that made it possible to collect the data for this manuscript, including Gerard Overkamp, Jorrick Wubs, Maite Punter and the late René Beamonte. We thank Irene Tieleman for the use of the Thermocouple and for useful discussions. Egbert Koetsier set up the experimental population, supported by an NWO Vici grant to SV.

Supplementary information to:

Individual variation in metabolic reaction norms over ambient temperature causes low correlation between basal and standard metabolic rate

Table of contents

Supplementary information S1: Distribution of number of measurements per bird

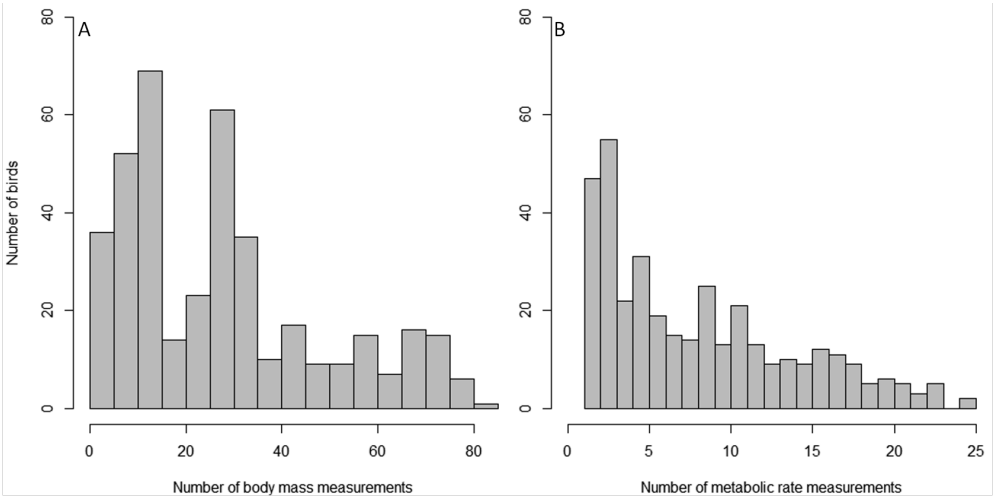

Fig. S1 Number of birds with a count of their body mass measurements (A) and metabolic rate measurements (B).

Supplementary information S2: Body mass variance components and repeatability

Table S1 Variance components and repeatability estimates (±95% credible intervals) for the body mass traits shown in Fig. 2.

Dataset	Variance		Repeatability
Whole population	**Between individual**	**Within individual**	
Body mass	1.51 (1.36-1.81)	0.70 (0.68-0.72)	0.69 (0.66-0.72)
Size corrected	1.03 (0.90-1.19)	0.70 (0.68-0.72)	0.59 (0.56-0.63)
Benign environment			
Body mass	1.70 (1.41-2.17)	0.84 (0.81-0.87)	0.66 (0.63-0.72)
Size corrected	1.11 (0.89-1.32)	0.83 (0.81-0.87)	0.56 (0.52-0.62)
Harsh environment			
Body mass	1.11 (0.90-1.35)	0.55 (0.53-0.57)	0.67 (0.62-0.71)
Size corrected	0.80 (0.62-0.94)	0.55 (0.53-0.57)	0.58 (0.53-0.63)

Supplementary information S3: Association between ambient temperature and basal metabolic rate in the thermoneutral zone

Table S2 Within the thermoneutral zone, temperature has a quadratic effect on BMR. This analysis was based on 1076 measurements with Ta ranging from 32.5°C till 39°C. Temperature was mean centered.

	coef (± SE)	F-ratio	DF	p
Intercept	0.22(0.00093)			
Temperature [+1°C]	0.0029(0.00080)	10.4	1	0.0013
Temperature^2 [+1°C]	0.00091(0.00043)	4.4	1	0.036

Supplementary information S4: Metabolic rate variance components and repeatability

Table S3 Variance components and repeatability estimates (± 95% credible intervals) for the metabolic rate traits in Fig. 3.

	between individual variance		within individual variance		repeatability		
Whole population	**BMR**	**SMR**	**BMR**	**SMR**	**BMR**	**SMR**	**BMR-SMR**
Whole organism	0.00035 (0.00029-0.00045)	0.0013 (0.0010-0.0016)	0.00036 (0.00032-0.00039)	0.0021 (0.0020-0.0022)	0.51 (0.45-0.57)	0.41 (0.33-0.44)	0.10
Mass corrected	0.00074 (0.00060-0.00096)	0.00075 (0.00058-0.00095)	0.00032 (0.00029-0.00035)	0.0018 (0.0016-0.0019)	0.27 (0.21-0.35)	0.31 (0.25-0.36)	-0.04
Spring data							
Whole organism	0.00034 (0.00025-0.00045)	0.0016 (0.0013-0.0021)	0.00030 (0.00025-0.00035)	0.0013 (0.0012-0.0015)	0.55 (0.46-0.63)	0.54 (0.48-0.61)	0.01
Mass corrected	0.000086 (0.000050-0.00014)	0.00073 (0.00056-0.00099)	0.00026 (0.00022-0.00031)	0.0011 (0.00097-0.0012)	0.23 (0.16-0.37)	0.44 (0.33-0.49)	-0.21
Autumn data							
Whole organism	0.00038 (0.00027-0.00050)	0.0011 (0.00079-0.0014)	0.00036 (0.00031-0.00044)	0.0021 (0.0019-0.0023)	0.50 (0.40-0.59)	0.33 (0.27-0.40)	0.17
Mass corrected	0.000069 ($5.5e^{-16}$-0.00010)	0.00067 (0.00046-0.00086)	0.00036 (0.00031-0.00047)	0.0019 (0.0017-0.0021)	0.16 (0.04-0.27)	0.26 (0.19-0.32)	-0.10
Benign environment							
Whole organism	0.00041 (0.00031-0.00056)	0.00096 (0.00063-0.0013)	0.00037 (0.00033-0.00043)	0.0027 (0.0019-0.0023)	0.55 (0.44-0.62)	0.25 (0.18-0.34)	0.30
Mass corrected	0.00014 (0.000079-0.00020)	0.00080 (0.00050-0.0011)	0.00032 (0.00028-0.00037)	0.0011 (0.0019-0.0023)	0.30 (0.21-0.41)	0.27 (0.19-0.35)	0.03
Harsh environment							
Whole organism	0.00017 (0.00013-0.00027)	0.00052 (0.00035-0.00073)	0.00032 (0.00027-0.00037)	0.0014 (0.0013-0.0015)	0.38 (0.27-0.46)	0.30 (0.20-0.35)	0.08
Mass corrected	0.000086 (0.0000039-0.00013)	0.00020 (0.00012-0.00037)	0.00032 (0.00028-0.00037)	0.0013 (0.0012-0.0015)	0.19 (0.10-0.30)	0.15 (0.08-0.22)	0.04
All data	**SMR12**	**SMR26**	**SMR12**	**SMR26**	**SMR12**	**SMR26**	**SMR12-SMR26**
Whole organism	0.0015 (0.0012-0.0020)	0.00091 (0.00068-0.0012)	0.0022 (0.0020-0.0025)	0.0012 (0.0010-0.0013)	0.41 (0.34-0.48)	0.45 (0.37-0.52)	-0.04
Mass corrected	0.00087 (0.00064-0.0012)	0.000054 (0.00040-0.00072)	0.0018 (0.0016-0.0019)	0.00089 (0.00078-0.0010)	0.34 (0.27-0.42)	0.37 (0.30-0.46)	-0.03

Supplementary information S5: Phenotypic correlations between metabolic traits

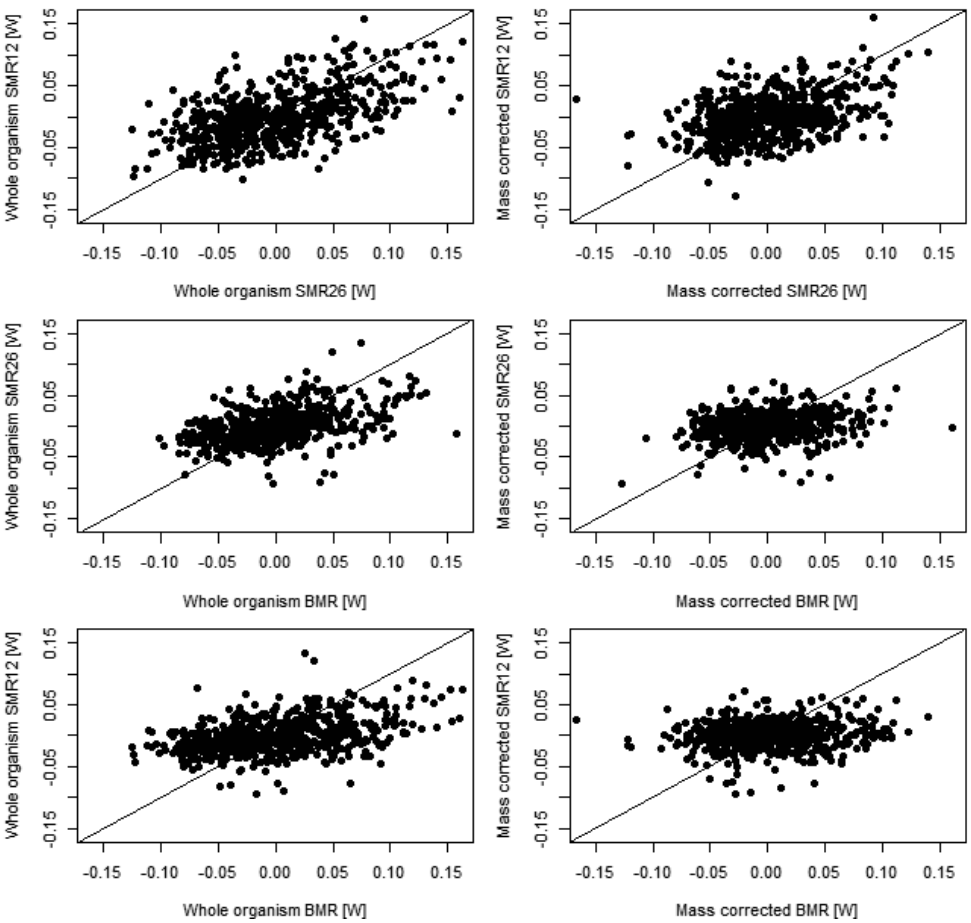

Fig. S2 Phenotypic correlations between metabolic traits. Whole organism and mass corrected metabolic rates are shown on the left and right respectively.

Supplementary information S6: Body temperature

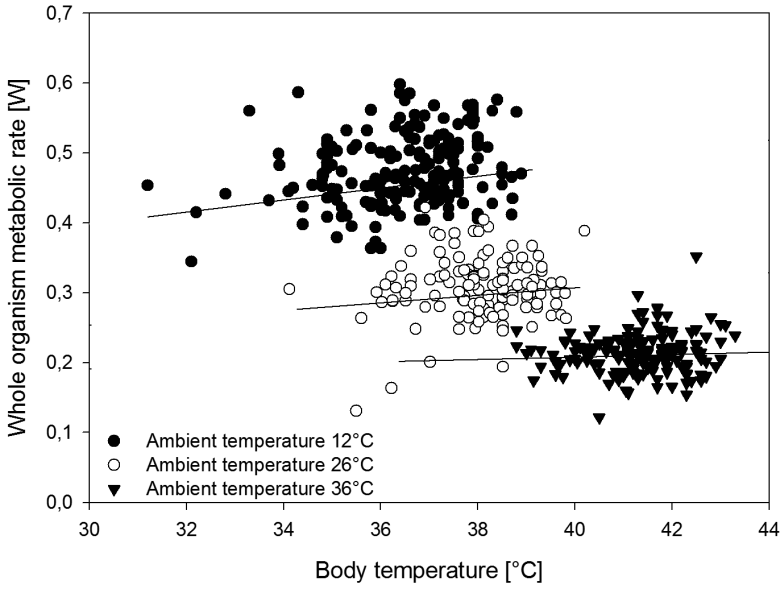

Fig. S3 Correlation between metabolic rate and body temperature for each of the ambient temperatures.

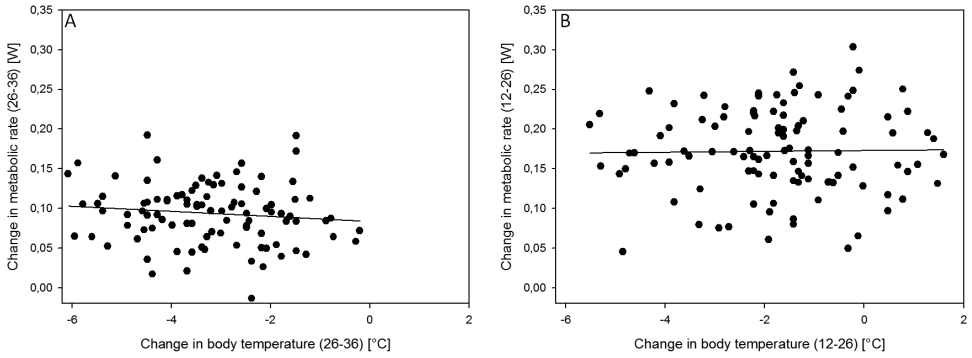

Fig. S4 Changes within individuals in metabolic rate do not correlate with changes in body temperatures when ambient temperatures decline from (A) the thermoneutral zone to 26°C or (B) 26°C to 12°C.

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Chapter 11

Mosaic aging of mass and metabolism in a passerine

Michael Briga, Blanca Jimeno & Simon Verhulst

Abstract

Lifespan varies due to numerous causes, but it remains unclear to what extent lifespan is scaled to aging, i.e. the decline in organismal functioning with age. In zebra finches, males outlive females and increased foraging costs shorten lifespan. Here we investigated to what extent these two determinants of lifespan concomitantly alter aging. We monitored 597 individuals for up to eight years and collected over 18.000 measurements on mass and metabolic rate at and below thermoneutral ambient temperatures (BMR and SMR respectively). Mass positively predicted lifespan at young age, but BMR or SMR never did. Traits differed in their age trajectories. Mass aged quadratically, but linearly for females in high foraging costs. BMR decreased linearly with age, while SMR increased until the bird's terminal year, independently of foraging costs or sex. Thus aging is not a synchronized process, but instead different traits within a single organism age at own time and pace. The association between lifespan and aging is thus molded by trait specific age trajectories, the environment and their interaction.

Introduction

Aging, the decline in organismal functioning with age resulting in declining fecundity and vitality, is common in humans, model organisms and in the wild (Nussey et al. 2013; Belsky et al. 2015; Fontana and Partridge 2015). Aging is followed by death and therefore the (implicit) assumption is often made that factors changing lifespan also alter aging. However, aging is different from lifespan in that it explicitly refers to the decline in organismal functioning preceding death, and thus can vary in duration and rate. Therefore a key question is to what extent factors that alter lifespan also affect aging (Williams 1999; Christensen et al. 2009; Kennedy et al. 2014; Bansal et al. 2015). This question is important for our fundamental understanding of the aging process and for the societal challenges faced by a rapidly aging population (Williams 1999; Christensen et al. 2009; Kennedy et al. 2014). For example, in humans, life expectancy has increased continuously since the 19th century, but to what extent this increase is accompanied by delays in aging remains unclear (Christensen et al. 2009). Studies on model organisms in laboratory environments have shown that caloric and dietary restriction extend lifespan and can delay the onset of age-related pathologies such as type 2 diabetes, cancer and neurodegenerative diseases (Fontana and Partridge 2015). However, there are various examples in these same model organisms, showing that lifespan and aging can readily be uncoupled (Burger et al. 2007; Rueppell et al. 2007; Burger et al. 2010; Bansal et al. 2015). Thus, while the environment can alter both lifespan and aging, it remains largely unknown to what extent changes in lifespan are associated with changes in aging.

Evolutionary theory predicts that traits should age in synchrony (Williams 1957; Maynard-Smith 1962). However, an organism experiences heterogeneous declines in functioning with age between traits, tissues and cells (Herndon et al. 2002; Bansal et al. 2015; Belsky et al. 2015; Hayward et al. 2015), a phenomenon coined *´mosaic aging´* (Cevenini et al. 2008; Walker and Herndon 2010). For example, declines in human fertility and survival with age are clearly not in synchrony (Lahdenperä et al. 2004). In *Drosophila*, muscular functioning shows profound declines in functioning with age, whilst the functioning of nervous system appears age-independent (Herndon et al. 2002). Furthermore traits can age following various shapes, with declines being gradual, accelerating and/or terminal, i.e. triggered shortly before death (Fig. 1). The origins of this variation remain unexplained. However this variation may be important to take into account when studying the scaling of lifespan and aging. On one hand, it is possible that factors affecting lifespan exert consistent effects on multiple traits. However, some traits age more readily or better predict lifespan than others, and thus may be more prone to factors affecting lifespan. Investigating trait specific aging trajectories can thus be essential to understand whether or when factors affecting lifespan will also affect aging.

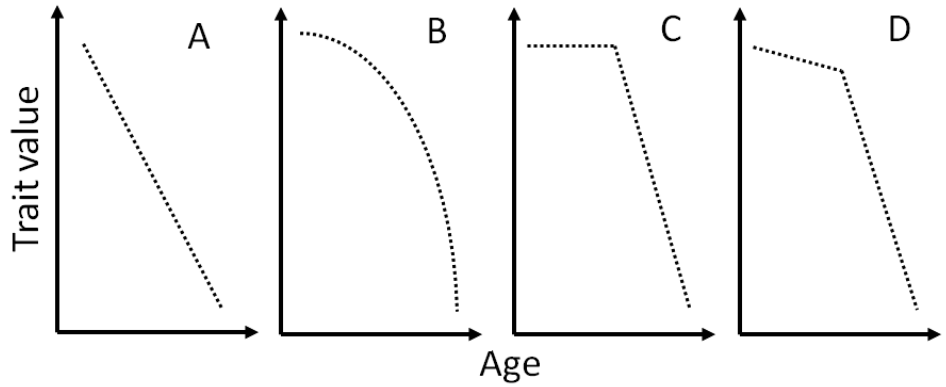

Fig. 1 Schematic representation of four age shapes tested in this manuscript. Aging may be determined by chronological age, following gradual (A) or accelerating decline (B). Alternatively aging may be age independent and better described by years before death resulting in terminal declines (C) or by a combination of age dependent and terminal declines (D). Note that this figure refers only to the aging phase, i.e. when traits decline in performance.

Here, we test whether two factors that affect zebra finch lifespan concomitantly affect aging of mass and metabolism. Our first source of variation is a sex-specific bias in lifespan. Sex biases in lifespan are common in nature and we here use this bias to investigate whether the shortest living sex shows accelerated aging (Bonduriansky et al. 2008). In our system we found that females lived shorter than males because of a steeper actuarial senescence, i.e. the increase in mortality rate with age (Chapter 3). Thus if lifespan and aging are scaled, we expect in our system females to age faster than males.

Our second source of variation in lifespan is an experimental manipulation of environmental quality. The environment can affect lifespan at all ages, but especially the development phase is thought of as a particularly important for adult lifespan and health (Lindström 1999; Hales and Barker 2001; Metcalfe and Monaghan 2001; Lummaa and Clutton-Brock 2002). In our model organism, we altered developmental conditions by cross fostering chicks to either small or large broods. Chicks growing up in large broods beg more, receive less food and have impaired growth, and thus large broods are harsh developmental conditions (de Kogel 1997; Kilner 2001; Griffith and Buchanan 2010; also in our system: Box A). However, the long term effects of developmental conditions can depend on the environmental conditions in adulthood (Gilbert 2001; Bateson et al. 2004; Monaghan 2008; Hanson and Gluckman 2014; Monaghan and Haussmann 2015). In our system, we investigated this context dependence by introducing a foraging costs manipulation during adulthood: we experimentally increased the flight costs per food

reward (Koetsier and Verhulst 2011). Birds from both developmental conditions were thus exposed to either low or high foraging costs in a 2x2 design. We thus created four experimental groups: BB, BH, HB, HH, where the first letter stands for benign (B) of harsh (H) developmental conditions (i.e. small or large brood size), and the second letter stands for benign (B) or harsh (H) foraging conditions in adulthood. We reported previously that harsh foraging conditions shorten lifespan but only for birds that grew up in harsh developmental conditions (i.e. the HH group; Chapter 3). Thus if the effects of the environmental manipulation on lifespan are associated with changes in aging, we expect the HH group to show accelerated aging compared to all other treatment combinations.

Environmental manipulations like ours alter an individual's energy balance (Wiersma and Verhulst 2005) and we therefore chose to monitor the age trajectory of traits that are associated with energy metabolism: mass, mass corrected basal metabolic rate (BMR_m), mass corrected standard metabolic rate (SMR_m) and hematocrit, i.e. blood oxygen stores which is important for metabolic activity (Petit and Vézina 2014). BMR is the minimum energy expenditure of a post-absorptive adult animal measured during the rest phase at thermoneutral temperatures. SMR in our study differs from BMR only in that metabolic rate was measured at an ambient temperature (T_a) below the thermoneutral zone and hence SMR includes energy for thermoregulation. Mass, BMR_m and SMR_m are repeatable over prolonged periods of time (Versteegh et al. 2008; White et al. 2013) but correlate poorly with each other and thus independently characterize individuals (Chapter 10). For mass, various age trajectories (Fig. 1) have been described depending on the species (Tafani et al. 2013; Hämäläinen et al. 2014; Hayward et al. 2015; Zhang et al. 2015), but to what extent variation in these trajectories has an environmental origin remains unidentified. For BMR_m, there are consistent reports of declines with age in variety of species (Elliott et al. 2015), including the zebra finch (Moe et al. 2009), but these studies had smaller sample sizes, could not test various possible age trajectories (Fig. 1) and did not quantify SMR_m. Furthermore, associations between BMR_m and lifespan are little studied in birds, but reports in mammals show mixed associations (Speakman et al. 2003; Speakman et al. 2004), which were suggested to depend on environmental quality (Burton et al. 2011). However, little is known on whether environmental quality affects (i) metabolic aging (Bouwhuis et al. 2011) and (ii) the association between lifespan and metabolism (Burton et al. 2011). We thus investigated whether environment quality affects the age trajectory and aging rate of mass, BMR_m, SMR_m and hematocrit and whether these effects are consistent with those on lifespan.

Material and Methods

Experimental setup

The birds used here grew up in either experimentally small broods (with 2 or 3 chicks) or large broods (between 5 and 8 chicks). These brood sizes are within the range observed in the wild (Zann 1996). Chicks growing up in large broods beg more, receive less food and have impaired growth (Box A). Large broods are thus a harsh developmental environment.

After nutritional independence and before the start of the foraging cost manipulation, i.e. between 35 days till approximately 120 days, young were housed in larger indoor cages with up to 40 other young of the same sex and two male and two female adults. Once adult, birds were subjected to a long-term foraging experiment (Koetsier and Verhulst 2011). Briefly, birds were housed in eight single sex outdoor aviaries (four per sex; L*H*W 310*210*150 cm) located in Groningen, the Netherlands (53° 13' 0" N / 6° 33' 0" E). Food (tropical seed mixture), water, grit and cuttlebone were provided *ad libitum*. In addition the birds received fortified canary food (''egg food'', by Bogena, Hedel, the Netherlands) in weighed portions. Each aviary contained an approximately equal number of birds and to keep densities within aviaries within a limited range, new birds were added regularly to replace those that died. The first batch was 3-24 months old when the experiment started and birds added later were three to four months old.

Data collection

Between December 2007 and December 2015, we collected 15.443 mass measurements on 597 individuals, with birds being measured between 1 and 95 times over their lifetime (Fig. S1A). Data were collected (almost) monthly from individuals covering an age range from 0.4 months till 9.4 years (Figs. S2A & S3A). Measurements were randomized across sex and experimental groups.

Between December 2007 and April 2013 we collected a total of 3.213 respirometry measurements on 407 individuals. Of those, 1233 measurements on 386 individuals were basal metabolic rate measurements (BMR, Fig. S1B), i.e. the minimum energy expenditure of a postabsorptive adult animal measured during the rest phase at thermoneutral temperatures (see McNab 1997 and references therein). In the thermoneutral zone an organism does not increase its metabolic rate in order to maintain body temperature and for the zebra finch this is at ambient temperatures between 32°C and 39°C (Calder 1964; Chapter 10). The other 1980 measurements on 372 individuals were of standard metabolic rate (SMR, Fig. S1C), which we measured in the same way

as BMR, except that the ambient temperature was below thermoneutrality, between 5°C and 32°C. BMR and SMR data were collected over an age range from 0.4 months till 7.2 years (Fig. S2A & B), in the same seasons, i.e. mostly in spring and autumn (Fig. S3B & C) and measurements were randomized across sex and experimental groups.

Metabolic rate was measured overnight using an open flow respirometer situated in a dark acclimatized room. Metabolic rate measurements started close to sunset (mean = 18:10 h; SD 01:17). Up to sixteen individuals were taken from the aviaries and randomly transferred to one of sixteen 1.5 L metabolic chambers. Neither food nor water was available for birds during the metabolic measurements. Birds were weighed before and after each measurement and we used the mean value to capture mass corrected BMR or SMR. Technical details about the equipment can be found in Bouwhuis et al. (2011). In brief, the air-flow through the metabolic chambers was controlled at 25 l h^{-1} by mass-flow controllers (5850S; Brooks, Rijswijk, the Netherlands) calibrated with a bubble flow meter. Air was dried using a molecular sieve (3 Å; Merck, Darmstadt, Germany) and analyzed by a paramagnetic oxygen analyzer (Servomex Xentra 4100, Crowborough, UK). During measurements each metabolic chamber or reference outdoor air was sampled every 8 min for 60s to stabilize measurement levels. In each sampling, we measured O_2 and CO_2 concentration and oxygen consumption was calculated using Eq. (6) of Hill (1972). An energy equivalent of 19.7 kJ l^{-1} oxygen consumed was used to calculate energy expenditure in watt (W). Metabolic rate was taken to be the minimum value of a 30 minutes running average, which included 3–6 measurements per individual. The first measurement hour was excluded to minimize potential effects of handling stress and incomplete mixture of air in the metabolic chamber. Body mass for the metabolic rate measurements was calculated as the average of the before and after measurement values.

Statistical analyses

Data for all traits were collected throughout the year. To avoid confounding age patterns with seasonal effects, we corrected for daily and seasonal variation in trait values. To this end, we first investigated for each trait how best to correct for this variation. For mass, we captured daily and seasonal variation in 3 variables: (i) daylength, (ii) photoperiod dynamics (increase vs. decrease) and (iii) time of measurement. For metabolic rates, we investigated the effects of daily and seasonal variation with: (i) daylength, (ii) photoperiod dynamics and (iii) the minimum ambient temperature (MinT). Temperature data were collected at the weather station of Eelde, approximately 7 km from the aviaries (http://www.knmi.nl/klimatologie/), where temperature was recorded 1.5 m above ground, every hour with accuracy of 0.1 °C. Temperature data at the weather station

reflect well the climate at the aviaries as shown from aviary temperature data collected during various seasons of these nine years (Briga and Verhulst 2015). The effect of MinT on trait values can last over various timescales (van de Pol and Cockburn 2011). We therefore weighed MinT over a time window as we had found earlier to affect lifespan in our study population (Briga and Verhulst 2015). This weighing function included for 77% the MinT within 24 hours before measurement and reached 100% within 5 days. A weighted approach provided a better model fit than using MinT of the day before measurement (BMR ΔAICc=-0.7; SMR ΔAICc=-1.5). We thus used these variables to correct for daily and seasonal variation in trait values (see supp. information 2).

Population level associations between trait values and age can be composed of two processes: (i) a within individual change in trait value with age and (ii) a between individual change due to selective mortality of individuals with certain trait values. We distinguished the contributions of these two processes using a within subjects centering approach (van de Pol and Verhulst 2006; van de Pol and Wright 2009). In this approach the within individual changes are captured in a Δage term, which is the age at measurement mean centered per individual. Within individual changes can also show terminal changes before death. We therefore added a terminal term as a separate variable, coded as a binomial factor for whether or not an individual had died within the year following the measurement. The between individual change is captured by the term lifespan, mean centered across our population. In this formulation, selective disappearance occurs when the coefficients differ within and between individuals. Whether this difference is significant can be tested with a model including age and lifespan (van de Pol and Wright 2009). For censored birds, i.e. those still alive (N=179) or that died an accidental death (N=16), lifespan is unknown and thus received a lifespan of zero. These birds thus contributed only to within and not to between individual trait change. Finally, to test whether within or between individual change is environment specific we included the interaction between the age terms and our experimental manipulations. Tests for context dependent developmental effects were done with three-way interactions (e.g. Δage*development*adult).

All analyses were done using a general linear mixed modeling approach with the function 'lmer' of the package 'lme4' version 1.1-10 (Bates et al. 2015) in R version 3.2.1 (R Core Team 2015). All analyses included individual as a random intercept and Δage nested within individuals as a random slope. The random slope quantifies the variation within individual in senescence (van de Pol and Verhulst 2006) and is required for the correct estimation of confidence intervals when investigating within individual changes (Schielzeth and Forstmeier 2009). Such models require considerable sample

sizes to accurately estimate fixed and random effects but our data (Fig. S1) fulfilled those requirements (van de Pol 2012). Residuals of all final models were normally distributed and without influential data points or outliers (Figs. S7 & S8). Confidence intervals of model parameters were estimated with the Wald approximation in the function 'confint'. Occasionally (e.g. to compare manipulation effects across models) we reported effect sizes, estimated as the ratio of the coefficient to the variable's standard deviation (Nakagawa and Cuthill 2007; equation 1).

To investigate the association between mass and survival we used Cox Proportional Hazard analyses (CPH) using the function 'coxph' in the package 'survival' version 2.38-1 (Therneau 2015). We used residual mass, corrected for temporal and seasonal covariates (see supp. information 2). Both experimental manipulations and their interaction were included in all CPH models. CPH analyses require predictors to be proportional with age, which was not the case for residual mass. We therefore stratified age at measurement into three groups, based on age tertiles. In this approach mass was proportional over the stratified age interval as indicated by the 'cox.zph' function (X^2=1.22, p>0.27).

To find the model best supported by the data we used Burnham and Anderson's model selection approach (Burnham and Anderson 2002; Burnham et al. 2011) based on second order Akaike Information Criterion (AICc) with the function 'dredge' of the package 'MuMIn' version 1.15.1 (Barton 2015). In brief, this is a hypothesis-based approach that generates, given a global model, subset models that best fit the data.

Results

Mass

We collected 15.443 measurements on 597 birds covering an age range from 0.4 months till 9.4 years (Figs. S1-S3). Birds reared in large broods weighed 0.56g (95%CI: 0.77, 0.35) less than birds reared in small broods (Table S2A; ΔAICc=-22.4), and birds in the harsh adult environment weighed 0.66g (95%CI: -0.87,-0.45) less than birds in the benign adult environment (ΔAICc=-32.4). The effects of both manipulations on mass were additive (developmental * adult environment ΔAICc=3.3; Fig. 2). Because mass was to a large extent determined by an individual's size (r=0.56), we investigated to what extent the manipulation effects on mass were mediated via size. Growing up in large broods resulted in smaller adult body size (N=594 individuals; t=-4.37; p=0.00001), while there was no association between the adult manipulation and size

(t=-1.41; p=0.16). When we analyzed the manipulation effects on mass including body size as a covariate, we still found that both manipulations resulted in lower mass, but the effect of the brood size manipulation on mass became smaller (ΔAICc=-1.7 vs. -22.4) while the adult manipulation effect became more significant (ΔAICc=-40.8 vs. -32.4; Fig. S4; see supp. information 3 for further details). Thus both manipulations affected mass, but the effect of the developmental manipulation occurred mostly via body size, while, as expected, the effect of the adult manipulation was size independent.

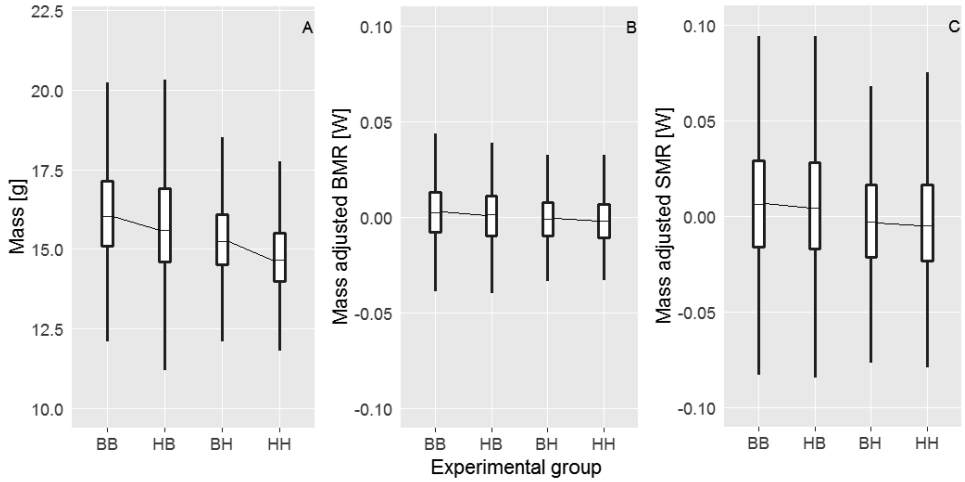

Fig. 2 Harsh environments decreased mass (A), mass adjusted basal metabolic rate (BMR_m; B) and mass adjusted standard metabolic rate (SMR_m; C). Shown are boxplots with median, quartiles and 95% CI. Statistical analysis showed the effects of developmental and adult environments to be additive for mass, while only the adult environment had a significant effect on BMR_m and SMR_m. Horizontal lines connect groups from different brood sizes in the same foraging treatment.

We investigated the age trajectory of mass within individuals, testing for the scenarios in Fig. 1, and whether this trajectory differed in response to the experimental manipulations. In the complete dataset, the age trajectory was best described by a quadratic shape (Table S3), rather than a linear shape (ΔAICc=+375.5) or a terminal effect either solely (ΔAICc=+354.5) or in combination with any of the previous shapes (+5.3<ΔAICc<+341.9). However, sexes differed in their age trajectory (Δage^2 * sex ΔAICc=-46.2) and in the environmental susceptibility of their age trajectory (Δage^2 * foraging treatment * sex ΔAICc=-37.9). Moreover, females were slightly heavier than males (ΔAICc=-50.7; Table S4). To gain better insight in these interactions, we further analyzed males and females separately.

In males, the best fitting age trajectory was quadratic (ΔAICc<-163.0; Table S3) independent of the environmental manipulations (ΔAICc>+7.1; Fig. 3C-F; Table S5). The quadratic random term varied little between individuals relative to individual as random intercept (variance explained: 1.8% vs. 81%), showing that individuals differed more in their mean mass than in their mass age trajectory. A quadratic age trajectory can reflect a variety of shapes. To better describe this trajectory, we first estimated the age at which this maximum was reached (see supp. information 6) which was at Δage = 1.0 year. We then investigated the pre- and post-peak shape. In the pre-peak phase, mass increased significantly with age (0.07 g/yr; 95%CI: 0.04, 0.11; ΔAICc=-8.0). Mass decreased post peak, albeit not significantly (-0.03 g/yr; 95%CI: -0.17, 0.09; ΔAICc=+5.3). Thus male mass showed a quadratic age trajectory with a peak at Δage=1 year followed by a short, shallow decline.

In females, the age trajectory differed between the benign and harsh foraging environment (Δage^2 * treatment ΔAICc=-16.1; Table S6). Analyzing these treatments separately revealed that females in benign foraging environment had a quadratic age trajectory (ΔAICc=-9.3; Fig. 3C & D), which was independent of brood size (ΔAICc>+6.5; Table S7A). The quadratic random term varied little between individuals relative to individual as random intercept (variance explained: 1.9% vs. 70%), showing that also female mass differed more between individuals in the mean than in the age trajectory. Females reached their maximum mass at a younger age than males, at Δage = 0.03 year. The pre-peak increase in mass was stronger than in males (0.29 g/yr; 95%CI: 0.18, 0.39; ΔAICc=-17.0), but the post peak decrease was similar to that of males (-0.08 g/yr; 95%CI: -0.17, 0.01; ΔAICc=+3.5). Thus, for females in the easy treatment, mass changed quadratically with age, characterized by a steep increase with a peak halfway through their life followed by a shallow decline.

For females in the hard treatment mass age trajectories were linear (ΔAICc=-8.1; Fig. 3E & F) and differed between birds from small and large broods (Δage * brood size ΔAICc=-10.9; Table S7B). For females from small broods, mass increased linearly with age, while females from large broods decreased their mass with age (Fig. 3E & F; Table S6B). These rates of change (in absolute value) were similar for both groups (small broods: 0.10 g/yr; 95%CI: 0.03, 0.17; large broods: -0.14 g/yr; 95%CI: -0.35, -0.13). Thus, for females in the harsh adult environment mass changed linearly with age which sign of the slope depended on the developmental conditions.

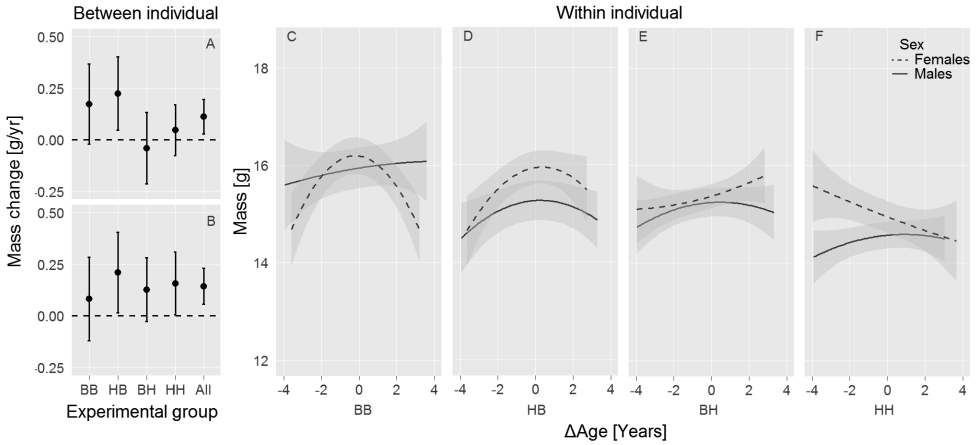

Fig. 3 Mass age trajectories are sex and environment dependent. Panels (A) and (B) show the between individual change, which is independent of the experimental manipulations in both males and females. Panels (C-F) show within individual change per experimental group. Males show a quadratic age trajectory that was independent of experimental group. In contrast, females showed a quadratic age trajectory when foraging costs were low (C & D) but when foraging costs were high the age trajectory was linear with a slope that depended on the developmental environment (E & F). For data plots, see fig. S7.

Using Cox proportional hazards (CPH) survival analysis (Cox 1972), we investigated the association between mass and lifespan and whether this association was environment dependent. To avoid temporal covariates on mortality (e.g. Briga and Verhulst 2015), we used the residual mass corrected for time and seasonal variation as discussed in supp. information 2. To avoid confounds of the experimental manipulations, we included rearing brood size, foraging treatment and their interaction in the models (Table S8). We only used one measurement (the first) per individual to avoid the problem that mass of longer living individuals will approach the population mean more due to their larger number of measurements (Verhulst et al. 2013).

Given that males and females have different age trajectories (Fig. 3), we investigated the association between mass and lifespan for the sexes separately. In males, heavier birds lived longer (ΔAICc=-2.1; Table S8A) and this was independent of the experimental manipulation (ΔAICc>+1.9). In females the association between mass and lifespan depended on brood size (ΔAICc=-0.82; Table S8B). For females reared in small broods, we found little evidence for an association between mass and lifespan (ΔAICc=+1.9; Table S8C). In contrast, among females reared in large broods the heavier birds lived longer (ΔAICc=-4.7; Table S8D). In none of the analyses did we find any evidence for a foraging treatment effect on the association between mass and lifespan (ΔAICc>+1.9; Table S8C & D). Thus heavier birds lived longer (Fig. 3A), except for females reared in small broods where there was no association between mass and lifespan.

Association between traits and lifespan can be age dependent. For example, in humans the predictive value of lifespan biomarkers, such as telomere length, body mass index or blood pressure weakens with age (Boonekamp et al. 2013). We thus investigated if the association between mass and lifespan changed with age. Indeed, we found that the association between mass and lifespan decreased with age in the treatment combinations separately ($150<N<304$ individuals; cox.zph $X^2>3.9$; $p<0.05$) and in the whole dataset (Fig. 3A; $N=597$ individuals; cox.zph $X^2=13.7$; $p=0.00022$). Thus the predictive value of mass on lifespan decreased with age.

Associations between mass and lifespan can be mediated by size. We thus repeated the above models including size as a covariate. In males, the association between size and lifespan contrasted with that of mass and lifespan because smaller males tended to live longer (ΔAICc=-0.76) and thus including size as a covariate reinforced the positive association between mass and lifespan (ΔAICc=-2.6 vs. -2.1). In females, there was no association between size and lifespan (ΔAICc>+1.1) and adding size as a covariate did not change the previously found associations between mass and lifespan. Thus in males, small birds tended to live longer, but in females there was no association between size and survival. Therefore the positive associations between mass and lifespan were not confounded by size.

Basal metabolic rate

We collected 1233 basal metabolic rate (BMR) measurements on 386 individuals over an age range from 0.4 years till 7.2 years (Fig. S1B & S2B). BMR was lower in response to both harsh environments but only the harsh adult environment provided a better model fit (development ΔAICc=+1.1; adult ΔAICc=-35.1; Table S2B). There was no evidence for context dependent developmental effects (development * adult ΔAICc=+11.6). Because BMR correlated well with mass ($r=0.60$), the environmental effects on BMR may be mediated by mass. In order to capture BMR dynamics without the confounding effects of mass, we ran all analyses below with mass as a covariate and refer to this mass adjusted BMR as 'BMR_m'. Our environmental manipulations affected BMR_m similarly as whole organism BMR, i.e. the harsh adult environment significantly decreased BMR_m while developmental conditions had no effect (development ΔAICc=+9.6; adult ΔAICc=-11.0; development * adult ΔAICc=+21.2; Table S2C). Note that, as expected, the effect of the adult environment was more pronounced on whole organism BMR than on BMR_m due to its effect on mass (-0.016W vs. -0.008W respectively). Thus, harsh adult but not developmental environments decreased energy consumption at thermoneutrality and this was in part due to lower mass. Birds facing high foraging costs thus also decrease their energy consumption per unit body tissue.

We investigated the age trajectory of BMR_m within individuals, testing for the various scenarios shown in Fig. 1, and found that the best fitting shape was a linear decline with age (ΔAICc<-12.6; Fig. S8A; Table S3) without any evidence for a terminal effect (ΔAICc=+12.4; Fig. 4; Table S3). This shape was consistent for all experimental groups (ΔAICc>+11.5; Fig. 4; Table S9). The variation between individuals in (within individual) change with age was small compared to the variation between individuals in BMR_m: variance explained of <0.1% vs. 25% for random slope and intercept respectively. Sexes did not differ in their BMR_m (ΔAICc=+7.7), in their BMR_m response to the environmental manipulations (ΔAICc>+15.3), or in their rate of BMR_m aging (ΔAICc=+17.5). Thus, BMR_m linearly decreased with age independently of environment or sex.

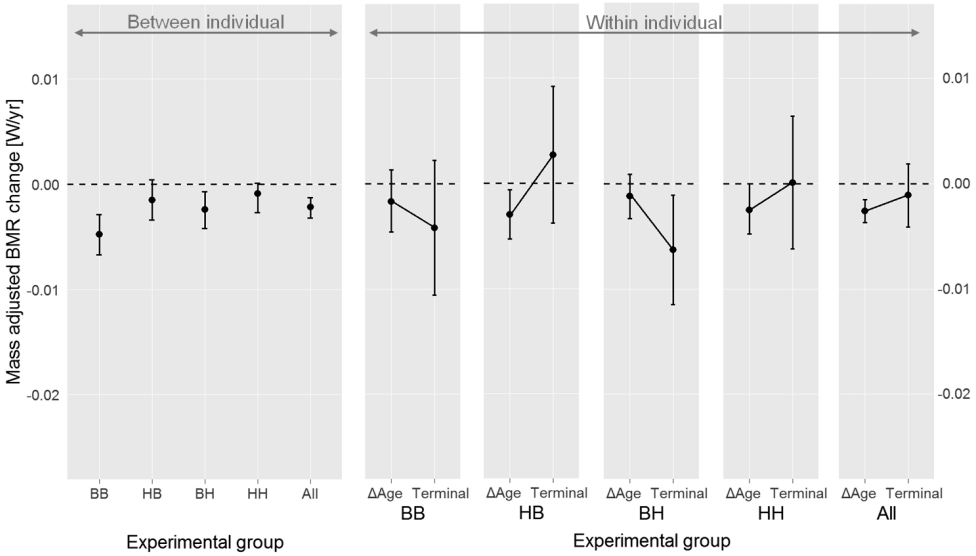

Fig. 4 BMR declines with age in all experimental groups. Shown are model coefficients ± 95%CI. Within individuals, BMR declines gradually with age, including in the last year of life (Terminal). Age trajectories are consistent across groups, except perhaps for the within individual change for the BH group, but the difference with other groups is not significant. Note that there is no evidence for selective disappearance because the coefficient of between individual change was similar to that of Δage. For a data plot of the age trajectory, see fig. S8A.

We then investigated the association between BMR_m and lifespan. The gradual decline in BMR_m with age was similar between and within individuals (slopes: -0.0021 W/Yr vs. -0.0027 W/Yr respectively; Fig. 4) and this was so for all experimental groups (ΔAICc>+11.5; Fig. 4; Table S9). This shows there was no association between BMR_m and lifespan (ΔAICc=+13.1; Fig. 6B). Fig. 6B also indicates there was no quadratic association between BMR_m and lifespan (e.g. when individuals with high and low BMR_m

have short lifespans) which was confirmed statistically (lifespan^2: ΔAICc=+12.0). Thus, we found no evidence for an association between BMR_m and lifespan.

Standard metabolic rate

We collected 1980 standard metabolic rate (SMR) measurements on 372 individuals over an age range from 0.4 years till 7.2 years (Fig. S1B, S2B). Whole organism SMR was lower in response to both harsh environments, but only convincingly so for the adult treatment (development ΔAICc=-1.1; adult ΔAICc=-103.7; Table S2D). There was no evidence for context dependent developmental effects (development * adult ΔAICc=+8.7). SMR correlated well with mass (r=0.53, after adjustment for ambient temperature at measurement) and we therefore tested whether these effects remained on SMR adjusted for mass (SMR_m). Such a model revealed no effect of developmental manipulation on SMR_m (ΔAICc=+10.5; Table S2E), but the effect of the adult manipulation remained (ΔAICc=-69.9). As for BMR, the foraging costs effect was more pronounced on whole organism SMR than on SMR_m (-0.045W vs. -0.030W respectively). Interestingly, the foraging cost effect on SMR_m was more than twice that of BMR_m (Fig. 2; effect size Cohen d = 0.67 vs. 0.28 respectively). Thus, both harsh environments decreased energy consumption, but the developmental effect was mediated via mass, while the effect of foraging costs was both, mass dependent and independent.

We then investigated the age trajectory of SMR_m within individuals, testing for the various scenarios shown in Fig. 1. In contrast to the BMR_m decrease with age, SMR_m *increased* with age (ΔAICc=-0.12; Fig. S8B), except in the last year of life (Fig. 5; Table S3). This terminal effect was not supported by the model selection approach (ΔAICc=+5.4), but it was significantly negative at -0.0072W/Yr (95%CI: -0.013, -0.0015; t=-2.6; p=0.01), and hence differed even more from the positive Δage coefficient of 0.0061W/Yr (95%CI: 0.0034, 0.0087). Thus for the last year of life the coefficient was effectively zero (0.0061 – 0.0072 = -0.0011). A discrepancy between the model selection and significance testing approaches can arise when terms are correlated. Indeed Δage and terminal year were fairly well correlated (r=-0.48). This pattern (Δage + terminal) could reflect a quadratic age trajectory, but this hypothesis was not supported by the data (Δage^2 ΔAICc=+7.1). The variation between individuals in (within individual) rate of change with age was small compared to the variation between individuals in SMR_m: variance explained of 1.5% vs. 28% for random slope and intercept respectively. The SMR_m age trajectory did not differ between the experimental groups (ΔAICc>+8.2; Fig. 5; Table S10). Sexes did not differ in their SMR_m (ΔAICc=+11.5), in their SMR_m response to the environmental manipulations (ΔAICc>+21.4), or in their rate of SMR_m aging (ΔAICc>+22.1). Thus, SMR_m increased gradually with age, except for the last year of life in which there was no further change, and this trajectory was independent of environment or sex.

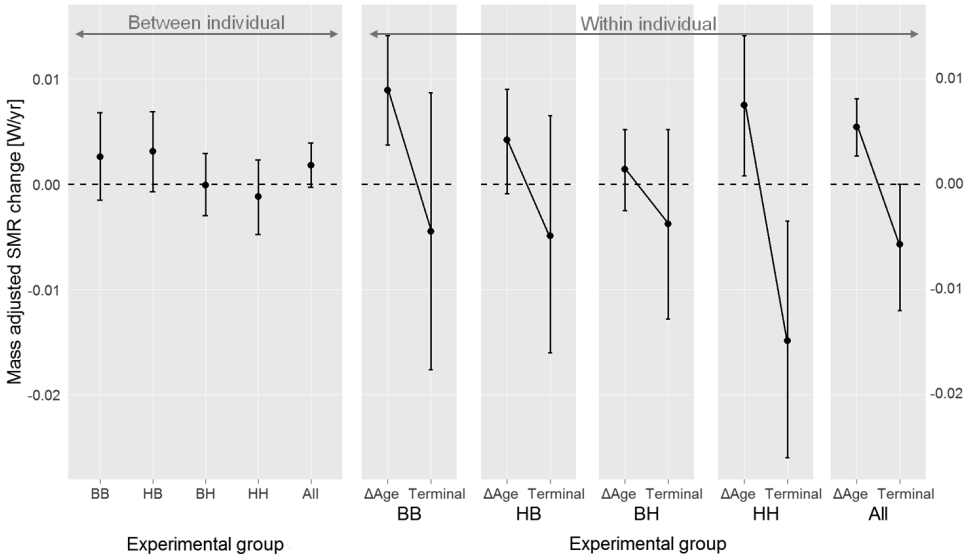

Fig. 5 Between and within individual changes with age in SMR_m are independent of experimental treatment. Shown here are model coefficients ± 95%CI. Within individuals, SMR increased gradually with age, but not in the last year of life (Terminal). Note that there is no selective disappearance because the coefficient of between individual change was similar to that of Δage. For a data plot of the age trajectory, see fig. S8B.

Finally, we investigated the association between SMR_m and lifespan. The gradual increase in SMR_m with age was similar between and within individuals (Fig. 5; 0.0022 W/Yr vs. 0.0043 W/Yr respectively). The coefficients for between and within individual change differed somewhat but including this difference in the model was not supported in the model selection (ΔAICc=+13.3). This shows that there was no selective disappearance (Fig. 6C) and this was consistent in all experimental groups (ΔAICc>+8.2; Fig. 5; Table S10). We also tested for a quadratic association with lifespan, but this was not supported by our data (lifespan^2: ΔAICc=+12.0; Fig. 6C). Thus, we found no evidence for an association between SMR_m and lifespan.

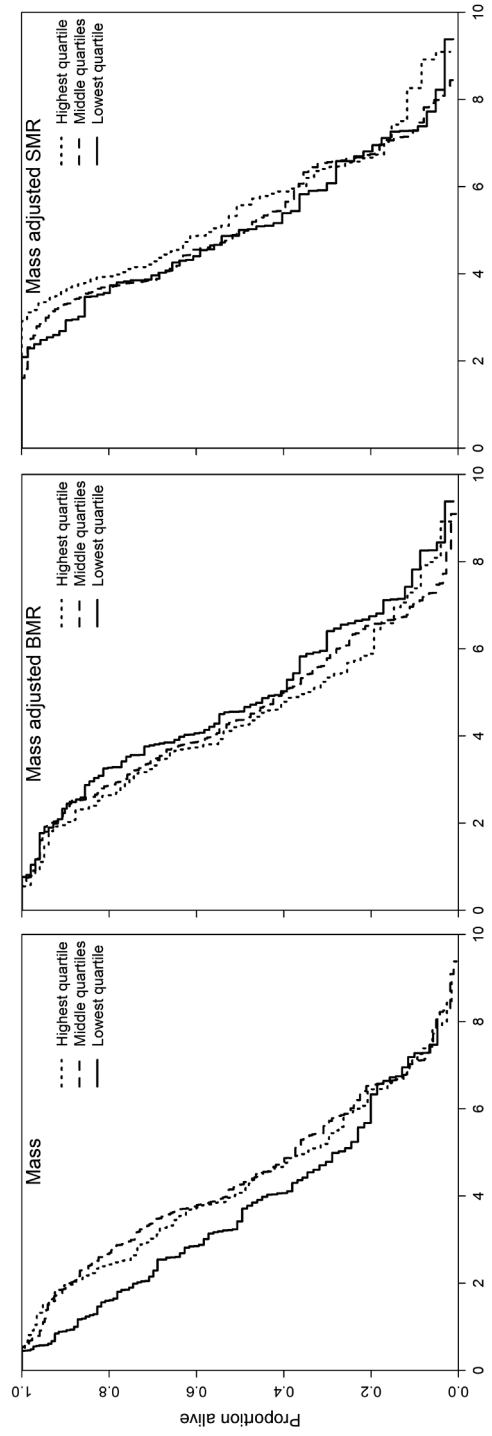

Fig. 6 Heavy individuals are longer lived, while we found no association between BMR_m or SMR_m and survival. Note that positive association between mass and survival held for males and females from large broods, but not for females from small broods (Table S8) and decreased with age. There was no evidence of selective disappearance with respect to BMR_m or SMR_m. Note that, to exclude any within-individual age effects, we only used individual's first measurement and for SMR_m excluding those individuals whose first measurement fell within the terminal year.

Discussion

In this study we investigated whether differences in lifespan, either due to sex or to the experimental manipulation of environmental quality, were associated with changes in aging of mass and two metabolic traits. Before discussing the age trajectories, we briefly discuss the experimental effects on the average values of the three traits.

Manipulation effects on mass and metabolism

Birds reared in large broods had lower mass in adulthood, in agreement with earlier studies (reviewed in Griffith and Buchanan 2010), and this effect was almost entirely due to their smaller structural size. Imposing foraging costs during adulthood also resulted in lower mass, independent of size or rearing brood size. Size independent mass variation typically reflects variation in energy reserves, and theory predicts energy reserves to increase with increasing starvation risk (reviewed in Brodin 2007). Starvation risk is higher in the high foraging cost treatment because it increases susceptibility to factors that increase energy needs (e.g. temperature) or impair foraging (e.g. illness). However, increased energy reserves also incur energetic costs (Kvist et al. 2001; Hambly et al. 2004; Schmidt-Wellenburg et al. 2008), which reduces optimal energy reserves, and this effect will also be stronger in the high foraging costs treatment because birds spend more time flying (Koetsier and Verhulst 2011). The lower mass of birds experiencing higher foraging costs suggests that the birds weighed the energetic costs of carrying extra mass more than the decrease in starvation risk. This result is consistent with the findings of experiments with captive birds and mammals in which foraging costs were increased without changing predictability, which consistently resulted in lower mass (reviewed in Wiersma and Verhulst 2005).

Whole organism and mass adjusted BMR and SMR were reduced in response to increased foraging costs. These results are consistent with earlier studies in birds and laboratory rodents (Wiersma and Verhulst 2005; Vaanholt et al. 2007; Schubert et al. 2008; Schubert et al. 2009). Physiologically, these reductions can be achieved by reducing the size of metabolic expensive organs (e.g. heart, liver and kidneys, Piersma and Lindström 1997; Piersma and van Gils 2011) and by hypothermia (reviewed in Geiser 2004; also in our population: Chapter 10). Note that the foraging cost effect on SMR_m was more than twice that on BMR_m, which indicates a strong role for a flexible energy saving mechanism (because both measurements where done randomly within few days interval), possibly larger nocturnal hypothermia.

The brood size manipulation did not affect BMR_m or SMR_m in adulthood. This is consistent with one study (Bech et al. 2004) but contrasts with other earlier studies in the same species which reported that adverse developmental conditions (large broods, lower diet quality) resulted in an elevated metabolic rate in adulthood (Verhulst et al. 2006; Criscuolo et al. 2008; Krause et al. 2009). Some of the earlier results were limited to specific conditions, depending on whether or not catch-up growth had occurred (Criscuolo et al. 2008) or whether birds were food deprived prior to measurement (Krause et al. 2009). It is possible that for a developmental effect on metabolic rate full compensatory growth needs to occur (Criscuolo et al. 2008), which was not the case in the two experiments with negative results, i.e. ours (Fig. 2) and Bech et al. (2004). Similarly in song sparrows (*Melospiza melodia*) corticosterone treatment or food restriction during development did not affect size but increased BMR_m at adulthood (Schmidt et al. 2012). Thus, it is possible that we found no effect of developmental conditions on metabolic rate in adulthood because birds did not show full compensatory growth.

The mass age trajectory and its sensitivity to environmental conditions

Mass changed with age within individual birds: we found a quadratic age trajectory for males and for females in the benign adult environment. Quadratic mass age trajectories are commonly observed in humans (reviewed in Kuk et al. 2009) and in laboratory rodents (Yu et al. 1985; Murtagh-Mark et al. 1995; Turturro et al. 1999; Miller et al. 2002) with apparent terminal declines a few weeks before death in rats (McDonald et al. 1996; Black et al. 2003). Similar quadratic associations as described here were also described in wild bighorn sheep *Ovis Canadensis* (Nussey et al. 2011). Thus for mass quadratic age trajectories were described in a variety of species.

In wild mammals however various other mass age trajectories have been described as well: accelerating declines in Roe deer *Capreolus capreolus* (Nussey et al. 2011), terminal declines in Soay sheep *Ovis aries* (Hayward et al. 2015), accelerating and terminal declines in European badgers *Meles meles* (Beirne et al. 2015) and in male Alpine marmots *Marmota marmota* (Tafani et al. 2013). In wild birds, common terns (*Sterna hirundo*) decline in mass with age, although the age trajectory was not investigated explicitly (Zhang et al. 2015). The origins of variation in age trajectories remain poorly known. In laboratory rodents it was shown that food intake is important: calorie restriction blunts the quadratic age trajectory (Yu et al. 1985; Murtagh-Mark et al. 1995; Turturro et al. 1999) and in some case causes mass to decline linearly with age (Turturro et al. 1999) but the terminal decline remained (Black et al. 2003). Together these and our results show that variation in environmental quality can profoundly alter the mass age trajectory. Note however

that caloric restriction is rarely encountered in nature. Rather, low food availability is expressed as increased foraging costs, which can be profoundly distinct from caloric restriction in that it shortens lifespan rather than prolonging it (Chapter 3).

For mass, we observed that the age trajectories differed between the sexes. There is considerable evidence for sex-specific age trajectories for a variety of traits, although this evidence comes mostly from mammals. For example, in Alpine marmots, males show gradual and terminal declines while no changes with age were found in females (Tafani et al. 2013). In European badgers, males decline in mass faster than females with both sexes also having a terminal decline (Beirne et al. 2015). No support for sex-specific mass trajectories was found in grey mouse lemurs in captivity nor in the wild (Hämäläinen et al. 2014). Previous data in captive zebra finches found that males gained weight with age, while females did not, but this was based on small sample with three measurements per individual (Moe et al. 2009). The origins of sex-specific age trajectories continue to attract interest, but in general it is believed that the shortest living sex ages fastest (Bonduriansky et al. 2008; Maklakov and Lummaa 2013), although there are many exceptions. For example, human females typically outlive males but their age associated decline in mass starts a decade earlier (reviewed in Kuk et al. 2009). In zebra finches, females live shorter than males (Chapter 3) and thus we would expect that mass ages earlier in females than in males. For the quadratic age trajectory, our results indicate an earlier start in post peak decline in females than in males, which would be consistent with this hypothesis.

The mass age trajectory was more sensitive to environmental condition in females than in males. Sex biased environmental sensitivity is well known in many species, although its causes in birds remain unclear (Jones et al. 2009). Sex-specific sensitivity has often been investigated on traits at fledging (mass, size, immunocompetence, survival, meta-analysis: Jones et al. 2009). However, sex biased environmental sensitivity with regards to age trajectories has to the best of our knowledge not previously been investigated. In zebra finches, females were described to be more sensitive to developmental conditions than males in their growth and early survival (e.g. de Kogel 1997; Martins 2004). In our study we found no evidence for sex-specific environmental sensitivity in terms of growth, size, sexual coloration or survival (Chapter 3; Simons et al. 2016) Thus the female biased environmental sensitivity of the mass age trajectory is the first evidence for sex-specific environmental sensitivity and is consistent with the female biased sensitivity found in other zebra finch studies (de Kogel 1997; Martins 2004).

The body composition changes underlying the mass age trajectory can be complex. A well known change is the loss of skeletal muscle (sarcopenia), as was observed for humans (reviewed in Mitchell et al. 2012; Ballak et al. 2014), in captive rhesus monkeys *Macaca mulatta* (Colman et al. 2008) and in laboratory rodents (Ballak et al. 2014; van Norren et al. 2015). Interestingly, in these species, sarcopenia is amenable by the environment in adulthood, e.g. it is attenuated by (voluntary) exercise and by caloric restriction (Speakman and Mitchell 2011; Mercken et al. 2012). Also fat content changes with age, with a quadratic age trajectory in humans (Kuk et al. 2009), but rather linear associations in laboratory rodents which can be modulated by caloric restriction (McCarter and Palmer 1992). Thus the physiology underlying mass age trajectories and their environment specificity may be due to changes in both muscle and fat content, but how body composition changes with age in birds remains to be investigated.

Metabolic aging

BMR_m decreased with age, but SMR_m increased with age till terminal year. The decline in BMR_m is consistent with results from earlier studies in zebra finches (Moe et al. 2009) and in other species including humans (meta-analysis: Elliott et al. 2015). In contrast, the age trajectory of SMR_m is new and this is to our best knowledge the first study to investigate the age trajectory of SMR_m. The discrepancy in age trajectory between BMR_m and SMR_m is important because it indicates that these two energetic traits age independently. Indeed, the correlations between BMR_m and SMR_m within individual were low ($0.04<r<0.22$; Chapter 10). These results thus confirm that BMR_m and SMR_m are independent traits.

The mechanism underlying the change in BMR_m with age is likely the reduction in the size of metabolic expensive organs, such as heart, liver, kidneys and muscle, as was shown for humans and laboratory rodents (Roberts and Rosenberg 2006). In addition there is evidence for a small age associated decline in metabolism per unit of tissue (Roberts and Rosenberg 2006) and for reductions in body temperature (Florez-Duquet and McDonald 1998; Weinert 2010; Blatteis 2012). We note however that all the above examples are in mammals and that studies in birds on body composition and hypothermia in the context of aging are yet lacking. Individual variation in metabolic rate in birds was shown to be associated with similar changes in body composition as in laboratory rodents (Piersma and Lindström 1997; Piersma and van Gils 2011) and nighttime reductions in body temperature are also common in birds (Geiser 2004). We thus hypothesize that reductions in organ size and in hypothermia are two mechanisms that reduce energy consumption with age in birds.

The difference in age trajectory between BMR_m and SMR_m indicates that there is aging of thermoregulation in response to cold ambient temperatures. We suggest two explanations for the increase in SMR_m with age. One possibility is that birds need more energy to maintain body temperature as they age. Secondly, birds might have lower tolerance for low body temperature as they age. This might occur because the ability to warm up might decrease with age. For example, in laboratory rodents, the ability to reduce heat loss in response to cold and the efficiency of metabolic heat production decreases with age (Florez-Duquet and McDonald 1998). Note though that birds differ from laboratory rodents in that thermoregulatory responses are regulated by skeletal muscles while mammals use brown adipose tissue (Dawson and O'Connor 1996; Mezentseva et al. 2008). We thus cautiously suggest that the mechanisms underlying the different age trajectories between BMR_m and SMR_m likely reflect changes in insulation and efficiency of heat production.

Mosaic aging and the association with lifespan

Evolutionary theory (Williams 1957; Maynard-Smith 1962) suggests that traits would evolve to age in synchrony, because *"natural selection will always be in greatest opposition to the decline of the most senescence-prone system"* (Williams 1957). Our findings run counter to this prediction because mass, BMR_m and SMR_m showed different age trajectories. The age trajectory of mass varied with foraging costs in females, but not in males, while BMR_m decreased linearly and SMR_m increased linearly with age until it plateaued in the terminal year (Fig. 7). This variation in age trajectories is further broadened by the age trajectories of hematocrit and bill color (Fig. 7). For hematocrit we found no evidence for aging, despite a high individual repeatability over lifetime of 0.61 (N=448, see supp. information 5). Bill color, a sexual signal, remained constant through life until a decline in the terminal year (Simons et al. 2016). Our combined results thus show that traits age asynchronously in zebra finches, which remains to be explained by evolutionary theory.

Aging in terms of declining fecundity and survival is an organismal level processes that can be seen as emergent property of the aging of individual traits. In our study, there were trait-specific aging responses to environmental change. Therefore, (environmental) factors affecting lifespan should be considered distinct from those affecting aging, similar to what was suggested for genetic factors (Burger and Promislow 2006). Predicting when or whether an environmental variable that alters lifespan will also affect the aging of some traits is currently difficult. One determinant factor is a trait's aging trajectory. For traits showing terminal declines, an environmental variable that shortens lifespan will likely accelerate aging, as we found for SMR and for bill

coloration. This however assumes that the environmental change does not alter a trait's age trajectory. Unfortunately, little is known about the extent to which age trajectories are flexible or environment-specific. Our experiment shows that the age trajectories of two metabolic traits (BMR_m and SMR_m) and of one sexual signal (bill color: Simons et al. 2016) are independent of foraging environment. In contrast, the age trajectory of mass is environment dependent, possibly because the underlying physiological changes with age are environment specific. Therefore, the association between lifespan and aging is trait specific and depends on a trait's age trajectory, the environment and their interaction.

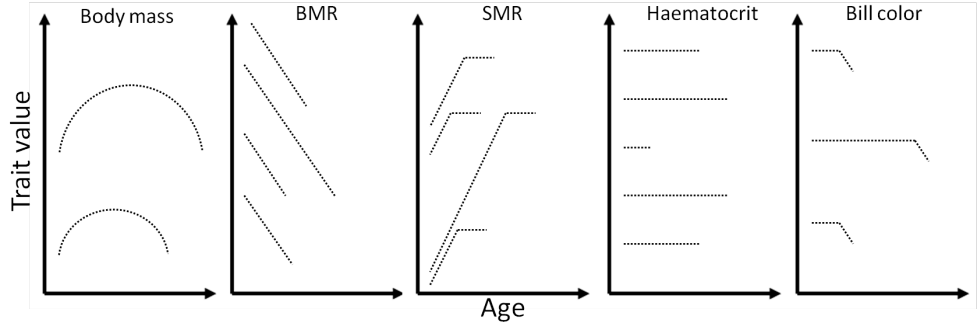

Fig. 7 Schematic representation of the age trajectories for five traits studied in zebra finches. Individuals with high mass live longer than those with low mass, and within individual showed mostly a quadratic association with age. For BMR and SMR there is no selective disappearance. Within individual, BMR linearly declines with age, while SMR increases until the final year. For hematocrit, we did not find any evidence for age associated changes or selective disappearance (see SI 5). Bill color shows stabilizing survival selection before the terminal decline in the final year (Simons et al. 2016). Note that for females the mass age trajectory differed between experimental groups (Fig. 4 C-J).

Supplementary information to:

Mosaic aging of mass and metabolism in a passerine

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Supplementary information S1: Data distributions across individuals, age and time

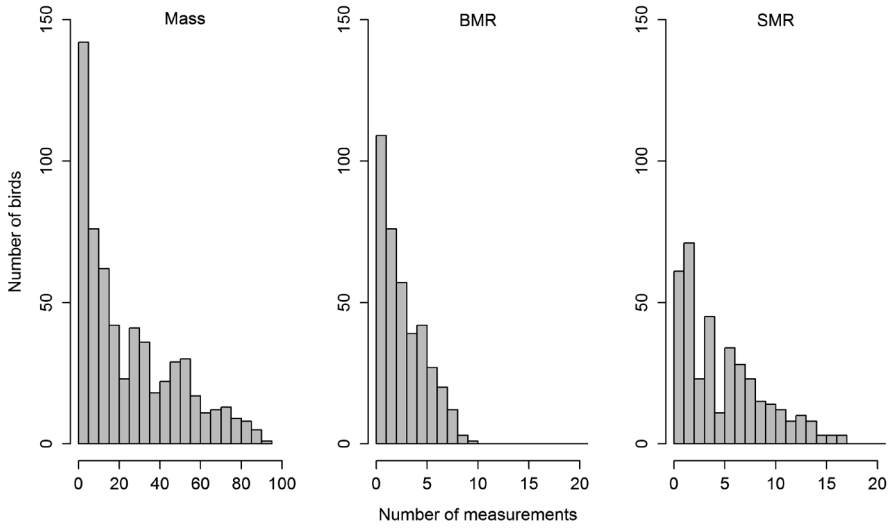

Fig. S1 Distribution of number of birds with their number of measurements for mass, BMR and SMR.

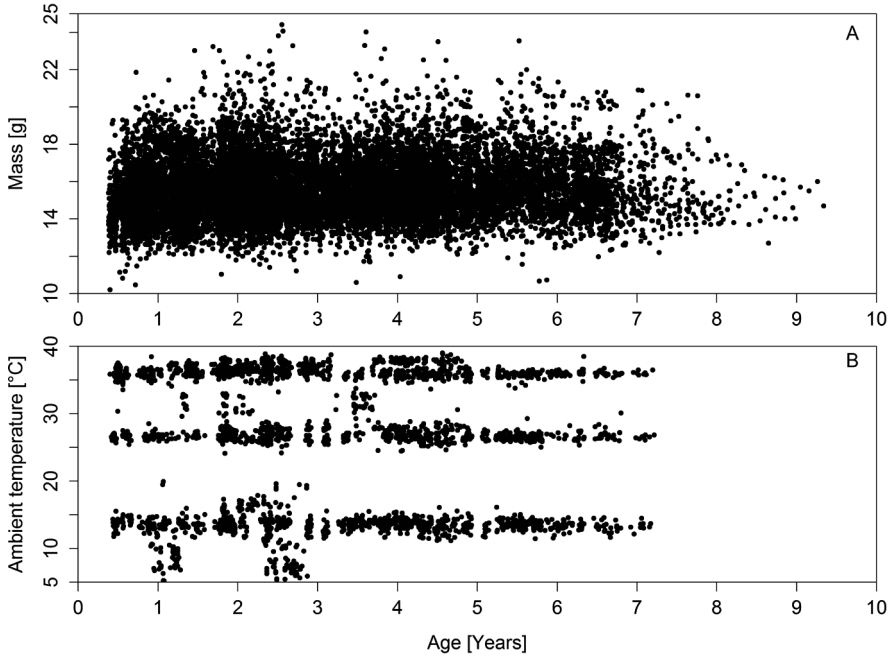

Fig. S2 Distribution of measurements as a function of age for (A) mass and (B) metabolic rate, with for BMR ambient temperatures (T_A) between 32°C< T_A <39°C and for SMR T_A <32°C.

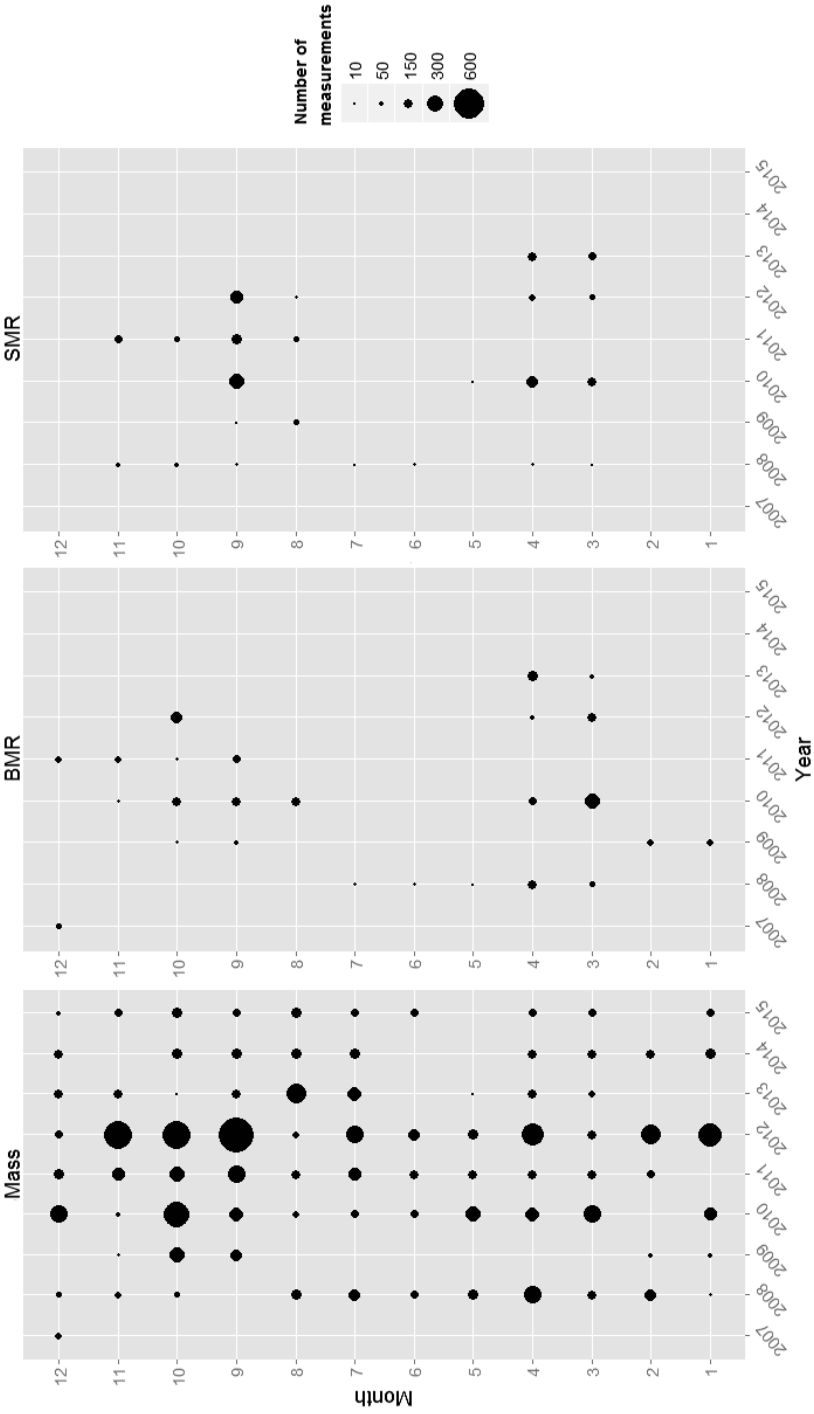

Fig. S3 Monthly distribution of number of measurements for mass, BMR and SMR.

Supplementary information S2: Time and seasonal effects on mass, BMR_m and SMR_m

Mass

To avoid confounding daily and seasonal variation in mass with age effects, we first investigated which daily and seasonal covariates affected mass (Table S1A). We found that birds get heavier within the day (time ΔAICc=-2747.1). The rate of weight gain was faster on shorter days with birds being lighter in the morning but ending up heavier at the end of the day (time * nightlength ΔAICc=-199.9). In addition, there was a biseasonal effect on daily weight gain, with birds gaining weight faster in the first half of the year relative to the second (time * photoperiod ΔAICc=-23.1). Both seasonal effects combined, birds were a little lighter in winter, a result inconsistent with most theoretical models (reviewed in Brodin 2007) and data (e.g. Krams et al. 2010; Rogers 2015) on winter fattening strategies in wild birds. These results are however consistent with what was found earlier in captive zebra finches (Meijer et al. 1996). Thus, birds showed daily mass gain, which rate was season specific. We thus included these daily and seasonal covariates and their interactions in all analyses.

Basal metabolic rate

Basal metabolic rate (BMR) was collected from 2008 till 2013 mostly during spring and autumn (Fig. S3). To avoid the confounding effect of mass, we included mass as a covariate in all analyses and hence report mass adjusted BMR (BMR_m). BMR_m was higher in spring relative to autumn (photoperiod ΔAICc=-53.6, Table S1B) and increased as days shorten, albeit not significantly (nightlength ΔAICc=+3.1). We found no evidence for season specific nightlength effects (photoperiod * nightlength ΔAICc=+9.8). BMR_m is known to increase in response to colder ambient temperatures on days of or previous to measurement (Bouwhuis et al. 2011). In our dataset, BMR_m indeed increased with colder ambient MinT (Table S1B), but adding MinT to the model yielded worse model fits both, in addition to or in replacement of nightlength and/or season (ΔAICc>+11.9). Thus, BMR_m increased with shorter and colder days, but, in our dataset, seasonal variation in BMR_m was best captured by variables coding for season and, to a lesser extent, nightlength. We thus included these covariates in all analyses.

Standard metabolic rate

Standard metabolic rate (SMR) was collected just as for BMR, from 2008 till 2013 mostly during spring and autumn (Fig. S3). We here report all mass adjusted SMR (SMR_m). Just as for BMR_m, SMR_m was higher in spring than in autumn (photoperiod ΔAICc=-32.3, Table S1C) and increased as days shortened (nightlength ΔAICc=+1.7) without evidence for season specific nightlength effects (photoperiod * nightlength

ΔAICc=+15.0). SMR also increased on colder days, but, differently from BMR_m, the effect of minimum temperature (MinT) on SMR was important (ΔAICc=-8.7). MinT and nightlength are correlated and a model with MinT fitted the data better than a model with nightlength (ΔAICc=-8.8, Table S1C). Thus, SMR_m increased with shorter and colder days and seasonal variation in SMR_m was best captured by season and MinT. We thus included these covariates in all analyses.

Table S1 Model selection results for time and seasonal effects on mass. BMR_m and SMR_m. Photoperiod (Photo) is a dichotomous variable coding for whether nightlength (Night) was increasing (0) or decreasing (1). MinT = Minimum temperature up to 5 days before measurement (see methods). Models are ordered by increasing AICc.

(A) Mass Model	Photo	Night	Time	Photo * Time	Night * Time	df	AICc	ΔAICc	weight
1	-0.38	-5.03	-1.48	0.59	7.51	8	37455.8	0	1.00
2		-4.41	-0.66		6.42	6	37478.9	23.1	0.00
3	-0.03	-4.38	-0.62		6.35	7	37483.5	27.8	0.00
4	-0.04	-0.77	2.54			6	37655.7	199.9	0.00
5		-0.75	2.53			5	37655.8	200.0	0.00
6	-0.04	-0.77	2.54	0.01		7	37660.5	204.7	0.00
7			2.58			4	37844.4	388.6	0.00
8	-0.02		2.59			5	37851.6	395.8	0.00
9	-0.04		2.57	0.04		6	37856.3	400.5	0.00
10		-0.96				4	40202.9	2747.1	0.00
11	0.01	-0.96				5	40211.4	2755.6	0.00
12						3	40466.8	3011.0	0.00
13	0.03					4	40469.3	3013.6	0.00

(B) BMR_m Model	Photo	Night	Photo * Night	MinT	Mass	df	AICc	ΔAICc	weight
1	0.01				0.01	5	-6110.9	0	0.82
2	0.01	0.02			0.01	6	-6107.8	3.1	0.17
3	0.02	0.03	-0.02		0.01	7	-6101.1	9.8	0.01
4	0.01			-0.0003	0.01	6	-6099.0	11.9	0.00
5	0.01	0.01		-0.0002	0.01	7	-6091.8	19.1	0.00
6	0.02	0.02	-0.02	-0.0002	0.01	8	-6084.5	26.5	0.00
7		-0.03		-0.001	0.01	6	-6057.4	53.6	0.00
8				-0.001	0.01	5	-6053.4	57.5	0.00
9					0.01	4	-6020.8	90.1	0.00
10		-0.02			0.01	5	-6017.1	93.8	0.00

(C) SMR_m Model	Photo	Night	Photo * Night	MinT	Mass	Measm Temp	df	AICc	ΔAICc	weight
1	0.02			-0.001	0.02	-0.01	7	-6618.1	0	0.96
2	0.02	-0.004		-0.001	0.02	-0.01	8	-6610.1	8.1	0.02
3	0.02				0.02	-0.01	6	-6609.5	8.7	0.01
4	0.03	0.04			0.02	-0.01	7	-6607.8	10.3	0.01
5	0.02	-0.001	-0.01	-0.001	0.02	-0.01	9	-6603.1	15.0	0.00
6	0.04	0.05	-0.02		0.02	-0.01	8	-6601.2	17.0	0.00
7		-0.09		-0.002	0.02	-0.01	7	-6585.8	32.3	0.00
8				-0.002	0.02	-0.01	6	-6538.6	79.5	0.00
9		-0.08			0.02	-0.01	6	-6497.8	120.3	0.00
10					0.02	-0.01	5	-6470.7	147.4	0.00

Supplementary information S3: Size and size independent manipulation effects on mass

The effect of our experimental manipulations on mass can reflect a variety of organismal changes. Here we examine in further detail to what extent the effects on mass arise due to size. As discussed before, in our dataset mass was to a large extent determined by an individual's size (r=0.56) and growing up in large broods resulted in a smaller size at adulthood (measured at the age of 120 days, N=594 individuals, t=-4.37, p=0.000014). Birds were randomly allocated to the foraging cost treatment with respect to size (N=594 individuals, t=-1.41, p=0.16) and thus, birds did not differ in size between foraging costs treatments. When analyzing the effects of the experimental manipulation on mass including structural body size as a covariate in the model, we still found that both manipulations resulted in lower mass (developmental: ΔAICc=-1.7; adult: ΔAICc=-40.8). However, the effect of the brood size manipulation on size corrected mass was considerably smaller than that on whole organism mass (ΔAICc=-1.7 vs. -22.4, Fig. S4). In contrast, the effect of foraging cost manipulation became even more significant (ΔAICc=-40.8 vs. -32.4, Fig. S4). The effect of both manipulations remained additive (developmental * adult environment ΔAICc=3.6). Thus both our harsh manipulations negatively affected mass, but the effect of the developmental manipulation occurred mostly via body size, while the effect of the adult manipulation was size independent.

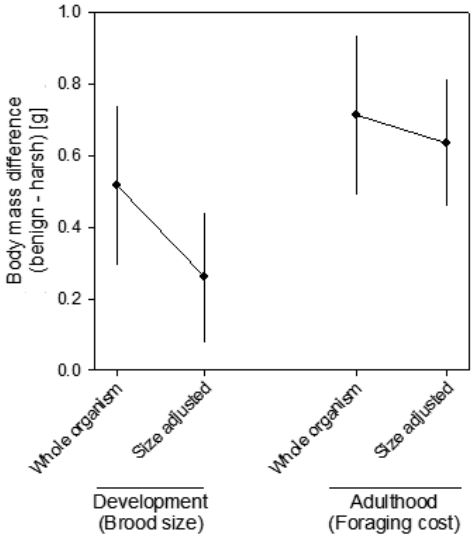

Fig. S4 The effect of the brood size manipulation on mass is mostly mediated via size, while the foraging cost manipulation during adulthood affects mass irrespective of size. Shown is the mass difference between benign and harsh environment ±95CI.

Supplementary information S4: age trajectories of mass, BMR and SMR are independent of lifespan variation within experimental groups

In the main text, we investigated whether our experimental manipulations changed the within individual age trajectory of mass, BMR_m and SMR_m. Because population composition can change with age, we used a within individual centering approach to decompose between and within individual change (van de Pol and Verhulst 2006; see methods for further details). This approach implicitly implies takes an average of the age trajectory of all individuals from a given group. This may not be true, for example it is known in various species that individuals with different lifespan vary in their behavioral and reproductive age trajectories (Bouwhuis et al. 2009; Maklakov et al. 2009). Here we investigated this by testing the interaction between lifespan and within individual age terms (Δage, Δage^2 and terminal year) and comparing the fit of the new model with each trait's best fitting model as described in the results section. For mass, adding any of interactions between Δage or Δage^2 with lifespan to the best fitting model in table S5 did not improve the model fit in males (ΔAICc>+4.8, Fig. S5A-D). The same conclusion held for female mass when adding the above terms to the best fitting models in tables S6-S7 (ΔAICc>+6.7, Fig. S5E-H). For BMR_m the linear decline with age was independent of lifespan (Δage * lifespan: ΔAICc=+7.7, added to the best fitting model in table S9A). For SMR_m the linear increase with age and the leveling off in the terminal year were both independent of lifespan (Δage * lifespan: ΔAICc=+14.5; terminal year * lifespan ΔAICc=+18.9 added to model 2 in table S10B). Thus we did not detect any differences in age trajectory between individuals with different lifespans in a given experimental group.

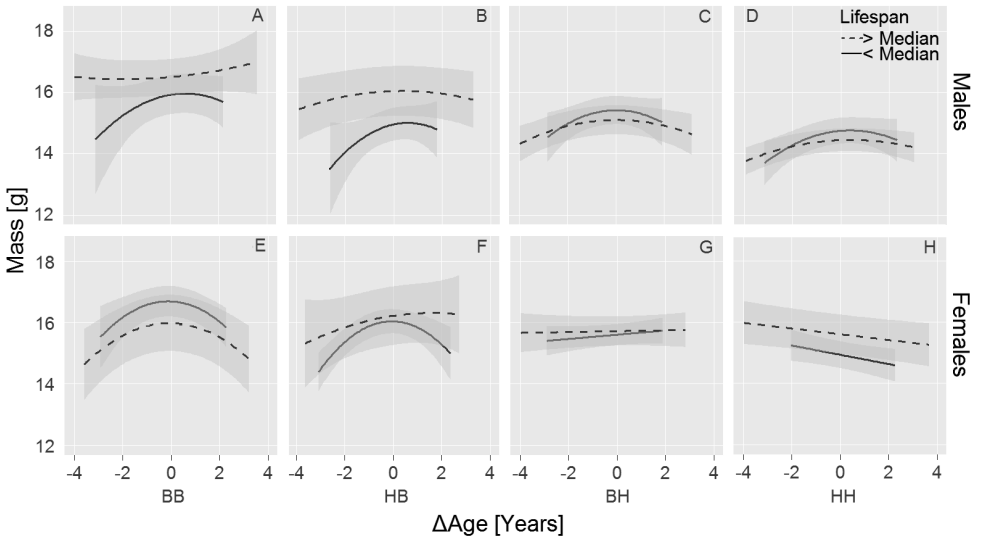

Fig. S5 Within individual age trajectories for mass are independent of lifespan variation within experimental groups. Shown are age trajectories for individuals living longer and shorter than median lifespan per experimental group.

Supplementary information S5: Hematocrit shows no evidence for age associated changes

Hematocrit is the proportion of red blood cells in total blood. We took a blood sample by puncturing the brachial vein and volume approximately 120 μl in heparanized microcapillary tubes. Within maximum an hour after sampling we centrifuged (8 min at 8,000 revolutions min^{-1}) and total blood volume and volume of red blood cells were measured immediately after.

We collected 447 hematocrit samples on 264 individuals covering an age range from 7 months till 7.2 years. We collected 117 samples in 2009, 67 in 2010, 175 in 2012, and 88 in 2013. Age was equally distributed across year of measurement, with the average age at measurement of 2.6 years in 2009, 3.1 years in 2010, and 2.8 years in 2012 and 2013. Birds were measured between 1 and 6 times (Fig. S6A) and the repeatability of hematocrit over lifetime in the subset data with individuals measured at least twice was high (r=0.58). Samples were collected mostly in March and April, but the 2009 and 2013 years also contained samples collected between September and November. We found no evidence for seasonal fluctuations in hematocrit levels (ΔAICc>+3.8). We found a weak decrease in hematocrit levels with increasing daytime (0.3% $hour^{-1}$, ΔAICc=-2.0). We thus included sampling time in all further analyses.

We first investigated whether the experimental manipulations affected mean hematocrit levels. Hematocrit levels ranged between 0.36 and 0.64 (Fig. S6C), but we found no evidence for an effect of the brood size manipulation (ΔAICc=+10.4, Fig. S6B), the foraging cost manipulation (ΔAICc=11.1, Fig. S6B) or an interaction between both manipulations (ΔAICc=+29.8, Fig. S5B). Thus our manipulations did not affect an individual's mean hematocrit values.

We then investigated whether hematocrit levels changed with age. To this end we first fitted the various age trajectories as described in Fig. 1. The best fitting model did not include any changes with age, neither within individuals (ΔAICc>+18.8, Fig. S6C) nor between individuals (lifespan: ΔAICc=+1.7). These negative results may arise because hematocrit age trajectories differ between experimental groups. However, we found no evidence that age trajectories differed between experimental groups (Δage: ΔAICc>+19.7; Δage^2: ΔAICc>+15.6; terminal year: ΔAICc>+17.1; lifespan ΔAICc>+23.8). Thus we found no evidence for aging or environment dependent age trajectories of hematocrit.

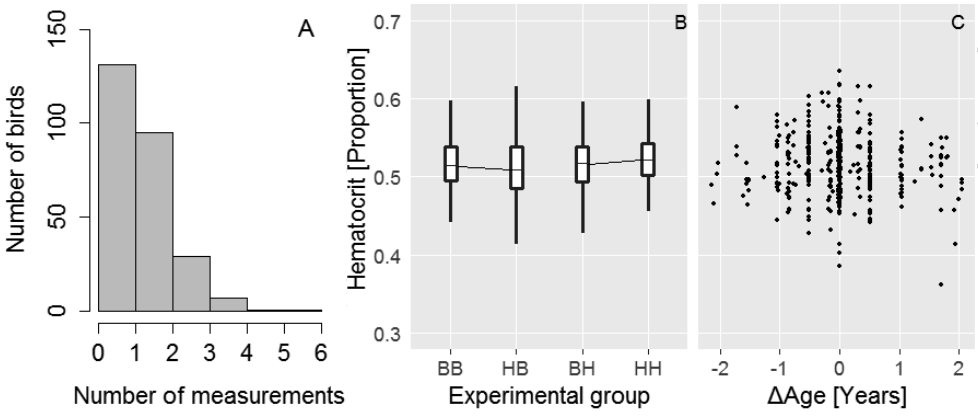

Fig. S6 Overview of hematocrit data and results. (A) Distribution of number of birds with their number of measurements. (B) No evidence for an effect of the experimental manipulations on bird hematocrit levels. Shown are boxplots with median, quartiles and 95% CI per experimental group. Horizontal lines connect groups from different brood sizes in the foraging treatment. (C) No evidence for within individual change in hematocrit levels with age.

Supplementary information S6: Age at which maximum body mass is reached

In our statistical models, age at measurement is mean centered per individual and described as Δage. In these models, the association between body mass m and Δage x is described by a quadratic function of the form:

$$m = C + Ax + Bx^2 \quad (1)$$

In equation (1) C is a constant described by all model predictors that do not include Δage, and A and B are the coefficients for Δage and Δage^2 respectively.

When maximum body mass is reached, then the following holds:

$$dm/dx=0 \quad (2)$$

Substituting equation (1) in equation (2) gives that maximum body mass is reached at age x_{max}:

$$x_{max}=-A/2B \quad (3)$$

For males A=0.041 and B=-0.021 (Table S4). Replacing those coefficients in (3) gives x_{max}=1 year.

For females in the easy treatment A=0.007 and B=-0.105 (Table S6). Replacing those coefficients in (3) gives x_{max}=0.03 years.

Supplementary information S7: Data distribution plots over Δage

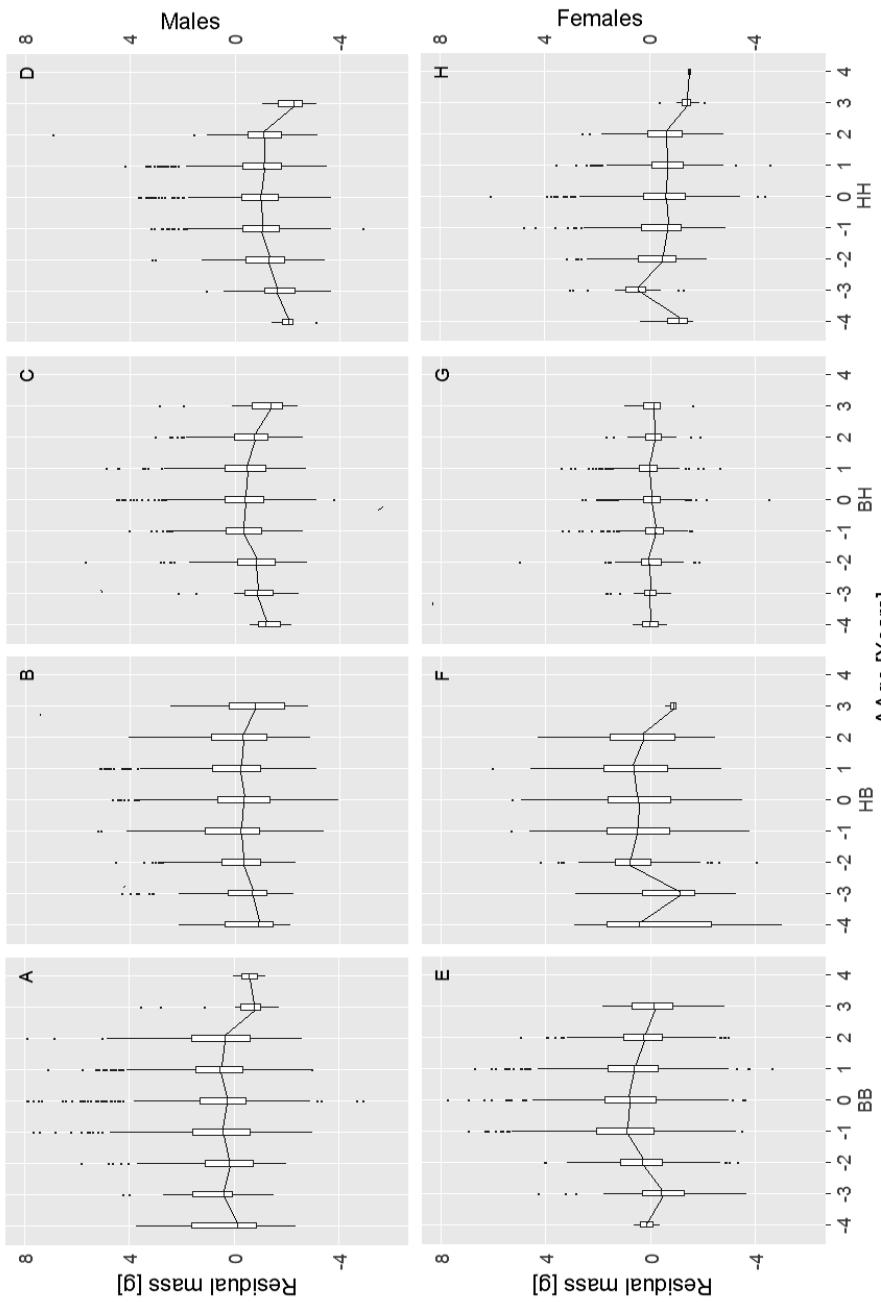

Fig. S7 Data distribution plot of mass age trajectories with Δage per experimental group. Most trajectories are quadratic, except for females in the hard treatment which are linear. Boxplots show median, quartiles and 95% CI. Horizontal lines connect medians. Residuals correct for time, seasonal variation and selective disappearance of light individuals where necessary.

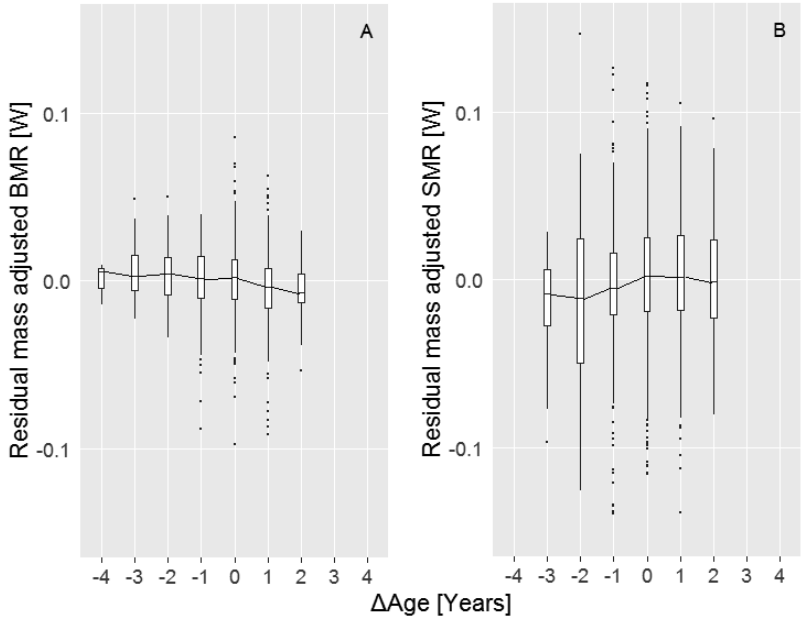

Fig. S8 BMR_m decreased with age (A), while SMR_m increased (B). Shown are boxplots with medians, quartiles and 95% CI. Horizontal lines connect medians. Residuals correct for mass, seasonal variation and manipulation effects.

Supplementary information S8: Model selection tables

Table S2 Manipulation effects on Mass (g), BMR, BMR_m, SMR and SMR_m. Models are ordered by increasing AICc.

(A) Mass	Experimental manipulations			Age covariates			Time and seasonal covariates					Model Fit			
Model	Brood size	Treat	Brood size * Treat	ΔAge	ΔAge²	Lifespan	Time	Night	Time * Night	Photo	Time * Photo	df	AICc	ΔAICc	weight
1	-0.56	-0.66		0.03	-0.03	0.12	-2.00	-5.54	8.38	-0.49	0.80	16	36402.3	0	0.84
2	-0.56	-0.65	-0.01	0.03	-0.03	0.12	-2.00	-5.54	8.38	-0.49	0.80	17	36405.5	3.3	0.17
3		-0.66		0.03	-0.03	0.14	-2.01	-5.55	8.39	-0.49	0.80	15	36424.7	22.4	0.00
4	-0.56			0.03	-0.03	0.12	-2.00	-5.54	8.38	-0.49	0.80	15	36434.7	32.4	0.00
5				0.03	-0.03	0.14	-2.01	-5.55	8.38	-0.49	0.81	14	36455.7	53.4	0.00
(B) BMR	Experimental manipulations			Age covariates		Seasonal covariates		Mass	Model Fit						
Model	Brood size	Treat	Brood size * Treat	ΔAge	Lifespan	Photo	Night		df	AICc	ΔAICc	weight			
1		-0.016		-0.001	-0.001	0.009	0.003	NA	9	-5811.0	0	0.63			
2	-0.007	-0.016		-0.001	-0.001	0.009	0.002	NA	10	-5809.9	1.1	0.36			
3	-0.006	-0.014	-0.003	-0.001	-0.001	0.009	0.002	NA	11	-5799.4	11.6	0.00			
4				-0.001	-0.001	0.009	0.004	NA	8	-5775.9	35.1	0.00			
5	-0.007			-0.001	-0.001	0.009	0.004	NA	9	-5772.5	38.5	0.00			
(C) BMR_m															
1		-0.008		-0.003	-0.002	0.010	0.010	0.011	10	-6134.2	0	0.99			
2	-0.003	-0.008		-0.003	-0.002	0.010	0.010	0.011	11	-6124.6	9.6	0.01			
3				-0.003	-0.002	0.011	0.012	0.011	9	-6123.2	11.0	0.00			
4	-0.002	-0.007	-0.001	-0.003	-0.002	0.010	0.010	0.011	12	-6113.0	21.2	0.00			
5	-0.002			-0.003	-0.002	0.010	0.011	0.011	10	-6112.4	21.8	0.00			
(D) SMR	Experimental manipulations			Age covariates			Seasonal covariates		Other covariates			Model Fit			
Model	Brood size	Treat	Brood size * Treat	ΔAge	Lifespan	Termin Year	Photo	MinT	Measm Temp	Mass		df	AICc	ΔAICc	weight
1	-0.013	-0.045		0.009	0.002	-0.012	0.026	-0.001	-0.012	NA		12	-6324.3	0	0.63
2		-0.044		0.009	0.003	-0.012	0.026	-0.001	-0.012	NA		11	-6323.1	1.1	0.36
3	-0.009	-0.041	-0.008	0.009	0.002	-0.012	0.026	-0.001	-0.012	NA		13	-6315.4	8.9	0.01
4				0.009	0.003	-0.011	0.025	-0.001	-0.012	NA		10	-6217.0	107.2	0.00
5	-0.012			0.009	0.003	-0.011	0.025	-0.001	-0.012	NA		11	-6213.0	111.2	0.00
(E) SMR_m															
1		-0.030		0.006	0.001	-0.007	0.022	-0.001	-0.013	0.019		12	-6676.6	0	1.00
2	-0.003	-0.031		0.006	0.001	-0.007	0.022	-0.001	-0.013	0.019		13	-6666.1	10.5	0.01
3	0.000	-0.028	-0.006	0.006	0.001	-0.007	0.022	-0.001	-0.013	0.019		14	-6656.7	19.8	0.00
4				0.005	0.002	-0.006	0.021	-0.001	-0.013	0.021		11	-6604.3	72.3	0.00
5	-0.001			0.005	0.002	-0.006	0.021	-0.001	-0.013	0.021		12	-6592.8	83.7	0.00

Table S3 Age trajectory fits for mass, BMR_m and SMR_m. Best fitting trajectories (bold) are quadratic for mass, linear for BMR_m and linear with terminal decline for SMR_m.

Trait	Age trajectory (within individual change)	df	AICc	ΔAICc	Remarks
All Mass	none	11	37394.1	991.8	
	Δage	13	36777.8	375.5	
	terminal year	12	37402.4	1000.1	
	Δage + terminal year	14	36744.1	341.9	
	Δage + Δage²	**16**	**36402.3**	**0**	Sex specific trajectories (Δage² *Sex: ΔAICc=-46.1)
Male Mass	none	11	17258.7	515.3	
	Δage	13	16905.2	161.8	
	terminal year	12	17259.4	516.0	
	Δage + terminal year	14	16906.4	163.0	
	Δage + Δage²	**16**	**16743.4**	**0**	
Female Mass	none	11	18996.0	508.7	
	Δage	13	18704.9	217.6	
	terminal year	12	18999.7	512.4	
	Δage + terminal year	14	18677.9	190.6	
	Δage + Δage²	**16**	**18487.3**	**0**	Manipulation specific trajectories (Δage² * Treat: ΔAICc=-14.9)
BMR	None	10	-6117.7	6.59	
	Δage	**12**	**-6124.3**	**0**	
	terminal year	11	-6114.7	9.53	
	Δage + terminal year	13	-6112.0	12.30	
	Δage + Δage²	15	-6104.4	19.82	
SMR	None	10	-6667.7	4.21	Adding terminal year worsens model fit due to negative correlation
	Δage	12	-6671.9	0	between terminal year and Δage (r=-0.48). However, this correlation
	terminal year	12	-6655.6	16.32	makes biological sense (individuals with more increase over Δage
	Δage + terminal year	**13**	**-6666.1**	**5.81**	have steeper terminal declines), terminal year is significantly nega-
	Δage + Δage²	15	-6664.0	7.85	tive (t=-2.6, p=0.01) and thus differs even more from the positive Δage coefficient (see results).

Table S4 Mass age trajectory and its response to environmental manipulations are sex-specific. Models are ordered by increasing AICc. Note that only the 35 better fitting models are shown (ΔAICc<51). Note the strong evidence for sex-specific age trajectories (ΔAge^2 * Sex ΔAICc=-46.2) with treatment specific age trajectory in females (Treat * Sex ΔAICc=-37.9) but not in males (ΔAge^2 * Treat * Sex ΔAICc=-37.9).

All	Experimental manipulations		Age and Age * Experiment interactions					Sex and Sex * Age interactions						Time and seasonal covariates					Model Fit			
Mass Model	Brood size	Treat	ΔAge	ΔAge^2 * ΔAge	Treat * ΔAge^2	Lifespan	Sex	Brood size * Sex	Treat * Sex	ΔAge * Sex	ΔAge^2 * Sex	ΔAge * Treat * Sex	ΔAge^2 * Treat * Sex	Time	Night	Time * Night	Photo	Time *Photo	df	AICc	ΔAICc	weight
1	-0.36	-0.88	0.02	-0.12		0.10	0.13	-0.36	-0.38	0.41		0.08		-0.10	-1.97	-5.52	8.35	-0.46	0.75	20	36556.4	0	0.42
2	-0.56	-0.88	0.02	-0.12		0.10	0.13	-0.55		0.41		0.08		-0.10	-1.97	-5.52	8.35	-0.46	0.75	19	36556.4	0.1	0.41
3	-0.36	-0.88	-0.01	-0.12		0.10	0.13	-0.36	-0.38	0.41	0.05	0.08		-0.10	-1.97	-5.52	8.35	-0.46	0.75	21	36559.9	3.5	0.07
4	-0.56	-0.88	-0.01	-0.12		0.10	0.13	-0.55		0.41	0.05	0.08		-0.10	-1.97	-5.52	8.35	-0.46	0.75	20	36559.9	3.5	0.07
5	-0.36	-0.88	0.02	-0.12	0.01	0.10	0.13	-0.36	-0.38	0.41		0.08		-0.10	-1.97	-5.52	8.35	-0.46	0.75	21	36563.8	7.4	0.01
6	-0.56	-0.88	0.02	-0.12	0.01	0.10	0.13	-0.55		0.41		0.08		-0.10	-1.97	-5.52	8.35	-0.46	0.75	20	36563.8	7.5	0.01
7	-0.36	-0.88	-0.01	-0.12	0.01	0.10	0.13	-0.36	-0.38	0.41	0.05	0.08		-0.10	-1.97	-5.52	8.35	-0.46	0.75	22	36567.3	10.9	0.00
8	-0.56	-0.88	-0.01	-0.12	0.01	0.10	0.13	-0.55		0.41	0.05	0.08		-0.10	-1.97	-5.52	8.35	-0.46	0.75	21	36567.3	11.0	0.00
9	-0.36	-0.88	0.00	-0.12	-0.02	0.10	0.13	-0.36	-0.38	0.41	0.03	0.08	0.04	-0.10	-1.97	-5.52	8.35	-0.46	0.75	23	36572.7	16.4	0.00
10	-0.56	-0.88	0.00	-0.12	-0.01	0.10	0.13	-0.55		0.41	0.03	0.08	0.04	-0.10	-1.97	-5.52	8.35	-0.46	0.75	22	36572.8	16.4	0.00
11	-0.36	-0.66	0.02	-0.08		0.03	0.13	-0.15	-0.38			0.03			-1.97	-5.52	8.34	-0.46	0.76	18	36594.3	37.9	0.00
12	-0.56	-0.67	0.02	-0.08		0.03	0.13	-0.34				0.03			-1.97	-5.52	8.34	-0.46	0.76	17	36594.3	37.9	0.00
13	-0.36	-0.84	0.02	-0.08		0.03	0.13	-0.32	-0.38	0.34		0.03			-1.97	-5.52	8.34	-0.46	0.76	19	36595.0	38.6	0.00
14	-0.56	-0.84	0.02	-0.08		0.03	0.13	-0.51		0.34		0.03			-1.97	-5.52	8.34	-0.46	0.76	18	36595.0	38.7	0.00
15	-0.36	-0.66	-0.01	-0.08		0.03	0.13	-0.15	-0.38		0.05	0.03			-1.97	-5.52	8.33	-0.46	0.76	19	36597.7	41.3	0.00
16	-0.56	-0.67	-0.01	-0.08		0.03	0.13	-0.34			0.05	0.03			-1.97	-5.52	8.34	-0.46	0.76	18	36597.7	41.3	0.00
17	-0.37	-0.84	-0.01	-0.08		0.03	0.13	-0.32	-0.38	0.34	0.05	0.03			-1.97	-5.52	8.33	-0.46	0.76	20	36598.4	42.0	0.00
18	-0.56	-0.84	-0.01	-0.08		0.03	0.13	-0.51		0.34	0.05	0.03			-1.97	-5.52	8.34	-0.46	0.76	19	36598.4	42.0	0.00
19	-0.36	-0.66	0.02	-0.08	0.00	0.03	0.13	-0.15	-0.38			0.03			-1.97	-5.52	8.34	-0.46	0.76	19	36601.7	45.3	0.00
20	-0.56	-0.67	0.02	-0.08	0.00	0.03	0.13	-0.34				0.03			-1.97	-5.52	8.34	-0.46	0.76	18	36601.7	45.4	0.00
21	-0.36	-0.84	0.02	-0.08	0.00	0.03	0.13	-0.32	-0.38	0.34		0.03			-1.97	-5.52	8.34	-0.46	0.76	20	36602.4	46.0	0.00
22	-0.56	-0.84	0.02	-0.08	0.00	0.03	0.13	-0.51		0.34		0.03			-1.97	-5.52	8.34	-0.46	0.76	19	36602.5	46.1	0.00
23	-0.36	-0.66	0.02	-0.06		0.03	0.13	-0.12	-0.38						-1.96	-5.51	8.32	-0.46	0.76	17	36602.6	46.2	0.00
24	-0.56	-0.67	0.02	-0.06		0.03	0.13	-0.32							-1.96	-5.51	8.32	-0.46	0.76	16	36602.6	46.3	0.00
25	-0.36	-0.83	0.02	-0.06		0.03	0.13	-0.30	-0.38	0.33					-1.96	-5.51	8.32	-0.46	0.76	18	36603.3	46.9	0.00
26	-0.56	-0.84	0.02	-0.06		0.03	0.13	-0.49		0.33					-1.96	-5.51	8.32	-0.46	0.76	17	36603.4	47.0	0.00
27	-0.36	-0.66	-0.01	-0.08	0.00	0.03	0.13	-0.15	-0.38		0.05	0.03			-1.97	-5.52	8.33	-0.46	0.76	20	36605.1	48.7	0.00
28	-0.56	-0.67	-0.01	-0.08	0.00	0.03	0.13	-0.34			0.05	0.03			-1.97	-5.52	8.34	-0.46	0.76	19	36605.1	48.7	0.00
29	-0.37	-0.83	-0.01	-0.08	0.00	0.03	0.13	-0.32	-0.38	0.34	0.05	0.03			-1.97	-5.52	8.33	-0.46	0.76	21	36605.8	49.4	0.00
30	-0.56	-0.84	-0.01	-0.08	0.00	0.03	0.13	-0.51		0.34	0.05	0.03			-1.97	-5.52	8.34	-0.46	0.76	20	36605.8	49.5	0.00
31	-0.36	-0.64	0.02	-0.06			0.13	-0.15	-0.38			0.03			-1.97	-5.52	8.35	-0.47	0.76	17	36606.6	50.2	0.00
32	-0.56	-0.64	0.02	-0.06			0.13	-0.34				0.03			-1.97	-5.52	8.35	-0.47	0.76	16	36606.6	50.2	0.00
33	-0.36	-0.66	-0.01	-0.06		0.03	0.13	-0.12	-0.38		0.05				-1.96	-5.51	8.31	-0.47	0.76	18	36606.9	50.6	0.00
34	-0.56	-0.67	-0.01	-0.06		0.03	0.13	-0.32			0.05				-1.96	-5.51	8.32	-0.47	0.76	17	36607.0	50.6	0.00
35	-0.56	-0.66	0.02	-0.06		0.03	0.12								-1.96	-5.51	8.32	-0.47	0.76	15	36607.1	50.7	0.00

Table S5 Mass age trajectory of males is independent of experimental manipulations. Note that only the 20 better fitting models are shown (ΔAICc<17.5).

Male Mass	Experimental manipulations			Age terms and interactions								Time and seasonal covariates						Model Fit			
										Brood size	Brood size										
			Brood size			Brood size	Brood size	Treat	Treat	* Treat	* Treat				Time		Time				
Model	Brood size	Treat	* Treat	ΔAge	ΔAge²	* ΔAge	* ΔAge²	* ΔAge	* ΔAge²	* ΔAge	* ΔAge²	Lifespan	Time	Night	* Night	Photo	*Photo	df	AICc	ΔAICc	weight
1	-0.74	-0.46		0.04	-0.02							0.11	-1.43	-3.62	7.19	-0.38	0.52	16	16743.4	0	0.70
2	-0.79	-0.50	0.08	0.04	-0.02							0.11	-1.43	-3.62	7.19	-0.38	0.52	17	16745.9	2.5	0.21
3	-0.74	-0.46		0.04	-0.02	-0.003						0.11	-1.43	-3.62	7.19	-0.38	0.53	17	16750.5	7.1	0.02
4	-0.74	-0.46		0.04	-0.02			0.002				0.11	-1.43	-3.62	7.19	-0.38	0.53	17	16750.5	7.1	0.02
5	-0.74	-0.46		0.04	-0.02		-0.009					0.11	-1.43	-3.62	7.19	-0.38	0.53	17	16751.3	7.8	0.01
6	-0.74	-0.45		0.04	-0.02				-0.004			0.11	-1.43	-3.62	7.19	-0.38	0.52	17	16751.4	8.0	0.01
7	-0.79	-0.50	0.08	0.04	-0.02	-0.003						0.11	-1.43	-3.62	7.19	-0.38	0.53	18	16753.0	9.5	0.01
8	-0.79	-0.50	0.08	0.04	-0.02			0.002				0.11	-1.43	-3.62	7.19	-0.38	0.53	18	16753.0	9.5	0.01
9	-0.78	-0.50	0.08	0.04	-0.02		-0.009					0.11	-1.43	-3.62	7.19	-0.38	0.53	18	16753.7	10.3	0.00
10	-0.79	-0.50	0.08	0.04	-0.02				-0.004			0.11	-1.43	-3.62	7.19	-0.38	0.52	18	16753.9	10.4	0.00
11	-0.74	-0.46		0.04	-0.02	-0.003		0.002				0.11	-1.43	-3.62	7.19	-0.38	0.53	18	16757.6	14.1	0.00
12	-0.74	-0.46		0.04	-0.02	-0.005	-0.009					0.11	-1.43	-3.62	7.19	-0.39	0.53	18	16758.3	14.8	0.00
13	-0.74	-0.46		0.04	-0.02		-0.009	0.002				0.11	-1.43	-3.62	7.19	-0.39	0.53	18	16758.3	14.9	0.00
14	-0.74	-0.45		0.04	-0.02			0.001	-0.003			0.11	-1.43	-3.62	7.19	-0.38	0.53	18	16758.5	15.0	0.00
15	-0.74	-0.45		0.04	-0.02	-0.003			-0.004			0.11	-1.43	-3.62	7.19	-0.38	0.53	18	16758.5	15.0	0.00
16	-0.74	-0.45		0.04	-0.01		-0.009		-0.003			0.11	-1.43	-3.62	7.19	-0.39	0.53	18	16759.2	15.8	0.00
17	-0.79	-0.50	0.08	0.04	-0.02	-0.003		0.002				0.11	-1.43	-3.62	7.19	-0.38	0.53	19	16760.0	16.6	0.00
18	-0.78	-0.50	0.08	0.04	-0.02	-0.005	-0.009					0.11	-1.43	-3.62	7.19	-0.39	0.53	19	16760.7	17.3	0.00
19	-0.78	-0.50	0.08	0.04	-0.02		-0.009	0.002				0.11	-1.43	-3.62	7.19	-0.39	0.53	19	16760.8	17.3	0.00
20	-0.79	-0.50	0.08	0.04	-0.02			0.001	-0.003			0.11	-1.43	-3.62	7.19	-0.38	0.53	19	16760.9	17.5	0.00

Table S6 Mass age trajectory of females differs between benign and harsh foraging treatment (Treat * ΔAge² ΔAICc=-16.1). Note that only the 20 better fitting models are shown (ΔAICc<16.1).

Female Mass	Experimental manipulations			Age terms and interactions								Time and seasonal covariates						Model Fit			
Model	Brood size	Treat	Brood size * Treat	ΔAge	ΔAge²	Brood size * ΔAge	Brood size * ΔAge²	Treat * ΔAge	Treat * ΔAge²	Brood size * Treat * ΔAge	Brood size * Treat * ΔAge²	Lifespan	Time	Night	Time * Night	Photo	Time * Photo	df	AICc	ΔAICc	weight
1	-0.37	-0.89		0.01	-0.11				0.12			0.14	-2.98	-8.12	10.45	-0.60	1.09	17	18471.2	0	0.41
2	-0.35	-0.87	-0.05	-0.02	-0.11	0.07		0.14	0.13	-0.30		0.14	-2.98	-8.11	10.43	-0.61	1.10	21	18472.9	1.7	0.18
3	-0.37	-0.89		0.06	-0.11	-0.09			0.13			0.14	-2.98	-8.11	10.43	-0.60	1.09	18	18473.1	1.9	0.16
4	-0.35	-0.87	-0.03	0.01	-0.11				0.12			0.14	-2.98	-8.12	10.45	-0.60	1.09	18	18473.9	2.6	0.11
5	-0.36	-0.87	-0.03	0.06	-0.11	-0.09			0.13			0.14	-2.98	-8.11	10.43	-0.60	1.09	19	18475.8	4.6	0.04
6	-0.38	-0.89		0.01	-0.13		0.04		0.13			0.14	-2.98	-8.12	10.45	-0.61	1.10	18	18476.0	4.8	0.04
7	-0.37	-0.89		0.01	-0.11			0.003	0.12			0.14	-2.98	-8.12	10.45	-0.60	1.09	18	18477.7	6.4	0.02
8	-0.37	-0.87	-0.03	0.01	-0.13		0.04		0.13			0.14	-2.98	-8.12	10.45	-0.61	1.10	19	18478.7	7.4	0.01
9	-0.36	-0.87	-0.05	-0.03	-0.12	0.08	0.03	0.14	0.13	-0.30		0.14	-2.98	-8.11	10.43	-0.61	1.10	22	18479.4	8.2	0.01
10	-0.37	-0.89		0.06	-0.11	-0.09		-0.002	0.12			0.14	-2.98	-8.11	10.43	-0.60	1.09	19	18479.6	8.4	0.01
11	-0.38	-0.89		0.05	-0.12	-0.07	0.03		0.13			0.14	-2.98	-8.11	10.44	-0.61	1.10	19	18479.7	8.4	0.01
12	-0.35	-0.87	-0.03	0.01	-0.11			0.003	0.12			0.14	-2.98	-8.12	10.45	-0.60	1.09	19	18480.3	9.1	0.00
13	-0.36	-0.87	-0.03	0.06	-0.11	-0.09		-0.002	0.12			0.14	-2.98	-8.11	10.43	-0.60	1.09	20	18482.2	11.0	0.00
14	-0.36	-0.87	-0.03	0.05	-0.12	-0.07	0.03		0.13			0.14	-2.98	-8.11	10.44	-0.61	1.10	20	18482.3	11.1	0.00
15	-0.38	-0.89		0.01	-0.13		0.04	0.002	0.13			0.14	-2.98	-8.12	10.45	-0.61	1.10	19	18482.5	11.2	0.00
16	-0.36	-0.87	-0.04	0.01	-0.12		0.03		0.11		0.03	0.14	-2.98	-8.12	10.45	-0.61	1.10	20	18484.6	13.4	0.00
17	-0.36	-0.87	-0.03	-0.04	-0.13	0.09	0.05	0.16	0.15	-0.33	-0.05	0.14	-2.97	-8.11	10.42	-0.61	1.10	23	18484.7	13.5	0.00
18	-0.37	-0.87	-0.03	0.01	-0.13		0.04	0.002	0.13			0.14	-2.98	-8.12	10.45	-0.61	1.10	20	18485.1	13.9	0.00
19	-0.38	-0.89		0.05	-0.12	-0.07	0.03	-0.002	0.13			0.14	-2.98	-8.11	10.44	-0.61	1.10	20	18486.1	14.9	0.00
20	-0.37	-0.85		0.01	-0.05							0.14	-2.99	-8.13	10.46	-0.62	1.11	16	18487.3	16.1	0.00

Table S7 Mass age trajectory depends on developmental conditions (brood size) for females in the harsh (B) but not in the benign (A) foraging treatment.

(A) Mass females in benign treatment															
	Age terms and interactions						Time and seasonal covariates						Model Fit		
Model	Brood size	ΔAge	ΔAge²	Brood size * ΔAge	Brood size * ΔAge²	Lifespan	Time	Night	Time * Night	Photo	Time * Photo	df	AICc	ΔAICc	weight
1	-0.36	0.01	-0.11			0.15	-2.31	-8.45	8.97	-0.62	1.26	15	9854.5	0	0.85
2	-0.36	-0.03	-0.11	0.07		0.14	-2.32	-8.46	8.98	-0.62	1.26	16	9858.6	4.1	0.11
3	-0.37	0.01	-0.12		0.02	0.15	-2.31	-8.44	8.96	-0.62	1.26	16	9861.0	6.5	0.03
4	-0.37	-0.04	-0.13	0.10	0.05	0.15	-2.31	-8.44	8.96	-0.62	1.26	17	9863.8	9.3	0.01
5	-0.37	0.07				0.14	-2.29	-8.41	8.88	-0.65	1.31	14	9869.5	15.0	0.00
6	-0.37	0.03		0.08		0.14	-2.30	-8.41	8.89	-0.65	1.31	15	9873.4	18.9	0.00
(B) Mass females in harsh treatment															
1	-0.40	0.10		-0.24		0.14	-3.31	-7.40	11.26	-0.55	0.86	15	8175.3	0	0.98
2	-0.40	0.10	0.01	-0.24		0.14	-3.31	-7.39	11.24	-0.55	0.86	16	8183.4	8.2	0.02
3	-0.39	-0.01				0.14	-3.31	-7.40	11.27	-0.55	0.85	14	8186.2	10.9	0.00
4	-0.40	0.10	0.01	-0.24	-0.0005	0.14	-3.30	-7.39	11.24	-0.55	0.86	17	8190.4	15.1	0.00
5	-0.39	0.00	0.01			0.14	-3.31	-7.40	11.26	-0.55	0.85	15	8194.4	19.1	0.00
6	-0.40	0.00	-0.02		0.05	0.14	-3.32	-7.41	11.28	-0.55	0.86	16	8199.3	24.1	0.00

Table S8 Heavy birds live longer for males and females from large broods. For females from small broods, there is no association between mass and lifespan. Coefficients are results from Cox Proportional Hazards (CPH) models and are hazard ratios: a hazard of 1 implies no effect and, a hazard ratio of 0.89 for ´MassR´ means that the hazard rate decreases with 11% per gram weight gained. Note that the mass-lifespan association decreases with age and therefore models are stratified for age. MassR = residual mass corrected for time and seasonal covariates as explained in SI 2; Treat = Foraging treatment.

(A) Males (N=304 individuals of which 198 died)							Brood Size				
Model	Brood size	Treat	Brood size * Treat	MassR	Brood size * MassR	Treat * MassR	* Treat * MassR	df	AICc	ΔAICc	weight
1	0.78	0.81	1.43	0.89				4	1476.3	0	0.34
2	0.77	0.81	1.49	0.88		1.06		5	1478.2	1.9	0.13
3	0.78	0.81	1.43	0.89	1.01			5	1478.4	2.1	0.12
4	0.87	0.88	1.39					3	1478.4	2.1	0.12
5	0.77	0.81	1.49	0.88	1.00	1.06		6	1480.3	3.9	0.05
6	0.78	0.82	1.37	0.85	1.07	1.16	0.83	7	1481.7	5.4	0.02
(B) Females (N=293 individuals of which 204 died)							Brood Size				
Model	Brood size	Treat	Brood size * Treat	MassR	Brood size * MassR	Treat * MassR	* Treat * MassR	df	AICc	ΔAICc	weight
1	1.18	0.95	0.97	0.99	0.83			5	1484.0	0	0.26
2	1.11	0.89	1.12	0.89				4	1484.9	0.8	0.17
3	1.18	0.95	0.96	0.99	0.83	0.99		6	1486.1	2.1	0.09
4	1.11	0.89	1.13	0.88		1.02		5	1486.9	2.9	0.06
5	1.13	0.96	1.18					3	1487.5	3.4	0.05
6	1.15	0.93	0.93	0.95	0.89	1.08	0.84	7	1487.6	3.6	0.04
(C) Females small broods (N=143 individuals of which 94 died)											
Model	MassR	Treat	MassR * Treat	df	AICc	ΔAICc	weight				
1		1.03		1	554.1	0	0.65				
2	0.97	1.01		2	556.0	1.9	0.25				
3	0.92	0.99	1.11	3	557.7	3.6	0.11				
(D) Females large broods (N=150 individuals of which 110 died)											
Model	MassR	Treat	MassR * Treat	df	AICc	ΔAICc	weight				
1	0.83	0.95		2	657.6	0	0.69				
2	0.84	0.93	0.97	3	659.6	2.0	0.25				
3		1.16		1	662.3	4.7	0.07				

Table S9 BMR_m shows no evidence for environment specific Δage (A) or for terminal year (B) effects. Models are ordered by increasing AICc.

(A) BMR_m: No evidence for environment specific delta age effects

	Experimental manipulations			Age terms and interactions				Other covariates				Model Fit			
Model	Brood size	Treat	Brood size * Treat	ΔAge	Brood size * ΔAge	Treat * ΔAge	Brood size * Treat * ΔAge	Lifespan	Photo	Night	Mass	df	AICc	ΔAICc	weight
1		-0.008		-0.003				-0.002	0.010	0.010	0.011	10	-6134.2	0	0.99
2	-0.003	-0.008		-0.003				-0.002	0.010	0.010	0.011	11	-6124.6	9.56	0.01
3				-0.003				-0.002	0.011	0.012	0.011	9	-6123.2	11.02	0.00
4		-0.008		-0.002		-0.0004		-0.002	0.010	0.010	0.011	11	-6120.2	13.94	0.00
5	-0.002	-0.007	-0.001	-0.003				-0.002	0.010	0.010	0.011	12	-6113.0	21.2	0.00
6	-0.002			-0.003				-0.002	0.010	0.011	0.011	10	-6112.4	21.8	0.00
7	-0.003	-0.008		-0.002		-0.0004		-0.002	0.010	0.010	0.011	12	-6110.7	23.51	0.00
8	-0.003	-0.008		-0.003	0.0004			-0.002	0.010	0.010	0.011	12	-6110.6	23.54	0.00
9	-0.002	-0.007	-0.001	-0.002		-0.0004		-0.002	0.010	0.010	0.011	13	-6099.0	35.14	0.00
10	-0.002	-0.007	-0.001	-0.003	0.0004			-0.002	0.010	0.010	0.011	13	-6099.0	35.18	0.00
11	-0.002			-0.003	0.0004			-0.002	0.010	0.011	0.011	11	-6098.4	35.76	0.00
12	-0.003	-0.008		-0.003	0.0003	-0.0004		-0.002	0.010	0.010	0.011	13	-6096.7	37.51	0.00
13	-0.002	-0.007	-0.001	-0.003	0.0004	-0.0004		-0.002	0.010	0.010	0.011	14	-6085.0	49.15	0.00
14	-0.002	-0.007	-0.001	-0.003	0.0011	0.0003	-0.002	-0.002	0.010	0.010	0.011	15	-6072.9	61.25	0.00

(B) BMR_m: No evidence for terminal year effects

	Experimental manipulations			Age terms and interactions				Other covariates				Model Fit			
Model	Brood size	Treat	Brood size * Treat	ΔAge	Termin Year	Brood size * Termin Year	Treat * Termin Year	Brood size * Treat * Termin Year	Lifespan	Photo	Night	Mass	df	AICc	ΔAICc	weight
1		-0.008		-0.003					-0.002	0.010	0.010	0.011	10	-6134.2	0	0.99
2	-0.003	-0.008		-0.003					-0.002	0.010	0.010	0.011	11	-6124.6	9.56	0.01
3				-0.003					-0.002	0.011	0.012	0.011	9	-6123.2	11.02	0.00
4		-0.008		-0.002	-0.001				-0.002	0.010	0.010	0.011	11	-6121.8	12.42	0.00
5	-0.002	-0.007	-0.001	-0.003					-0.002	0.010	0.010	0.011	12	-6113.0	21.20	0.00
6		-0.006		-0.002	0.001		-0.004		-0.002	0.010	0.011	0.011	12	-6112.5	21.63	0.00
7	-0.002			-0.003					-0.002	0.010	0.011	0.011	10	-6112.4	21.80	0.00
8	-0.003	-0.008		-0.002	-0.001				-0.002	0.010	0.010	0.011	12	-6112.2	22.00	0.00
9				-0.003	-0.001				-0.002	0.011	0.012	0.011	10	-6110.5	23.65	0.00
10	-0.003	-0.007		-0.002	0.001		-0.004		-0.002	0.010	0.011	0.011	13	-6102.9	31.30	0.00
11	-0.003	-0.008		-0.002	-0.002	0.002			-0.002	0.010	0.010	0.011	13	-6100.6	33.55	0.00
12	-0.002	-0.007	-0.001	-0.002	-0.001				-0.002	0.010	0.010	0.011	13	-6100.5	33.63	0.00
13	-0.002			-0.003	-0.001				-0.002	0.011	0.012	0.011	11	-6099.7	34.45	0.00
14	-0.002	-0.006	-0.001	-0.002	0.001		-0.004		-0.002	0.010	0.011	0.011	14	-6091.2	42.96	0.00
15	-0.003	-0.007		-0.002	0.000	0.002	-0.004		-0.002	0.010	0.011	0.011	14	-6091.2	43.02	0.00
16	-0.003	-0.007	-0.001	-0.002	-0.002	0.002			-0.002	0.010	0.010	0.011	14	-6089.0	45.20	0.00
17	-0.003			-0.003	-0.002	0.002			-0.002	0.010	0.012	0.011	12	-6088.2	45.98	0.00
18	-0.003	-0.006	-0.001	-0.002	0.000	0.002	-0.004		-0.002	0.010	0.011	0.011	15	-6079.5	54.69	0.00
19	-0.003	-0.006	-0.002	-0.002	0.000	0.001	-0.005	0.002	-0.002	0.010	0.011	0.011	16	-6068.8	65.38	0.00

Table S10 SMR_m shows no evidence for environment specific Δage (A) or terminal year (B) effects. Models are ordered by increasing AICc.

(A) SMR_m: No evidence for environment specific delta age effects

	Experimental manipulations			Age terms and interactions				Other covariates					Model Fit			
Model	Brood size	Treat	Brood size * Treat	ΔAge	Brood size * ΔAge	Treat * ΔAge	Brood size * Treat * ΔAge	Lifespan	Photo	MinT	Measm Temp	Mass	df	AICc	ΔAICc	weight
1		-0.030		0.004				0.002	0.022	-0.001	-0.013	0.019	11	-6682.4	0	0.98
2		-0.030		0.007		-0.005		0.002	0.022	-0.001	-0.013	0.019	12	-6674.2	8.18	0.02
3	-0.003	-0.030		0.004				0.002	0.023	-0.001	-0.013	0.019	12	-6671.9	10.53	0.01
4	-0.003	-0.030		0.007		-0.005		0.002	0.022	-0.001	-0.013	0.019	13	-6663.7	18.71	0.00
5	0.000	-0.027	-0.007	0.004				0.002	0.023	-0.001	-0.013	0.019	13	-6662.7	19.72	0.00
6	-0.003	-0.030		0.005	-0.001			0.002	0.023	-0.001	-0.013	0.019	13	-6659.6	22.82	0.00
7	-0.0001	-0.027	-0.007	0.007		-0.005		0.002	0.022	-0.001	-0.013	0.019	14	-6654.5	27.90	0.00
8	-0.003	-0.030		0.007	-0.001	-0.005		0.002	0.022	-0.001	-0.013	0.019	14	-6651.5	30.87	0.00
9	0.0000	-0.027	-0.007	0.005	-0.001			0.002	0.023	-0.001	-0.013	0.019	14	-6650.4	32.00	0.00
10	0.0000	-0.027	-0.007	0.007	-0.001	-0.005		0.002	0.022	-0.001	-0.013	0.019	15	-6642.4	40.06	0.00
11	0.0004	-0.026	-0.007	0.009	-0.005	-0.008	0.009	0.002	0.022	-0.001	-0.013	0.019	16	-6635.0	47.39	0.00
12				0.004				0.002	0.021	-0.001	-0.013	0.022	10	-6612.5	69.89	0.00
13	-0.001			0.004				0.002	0.021	-0.001	-0.013	0.022	11	-6601.1	81.29	0.00
14	-0.001			0.004	-0.001			0.002	0.021	-0.001	-0.013	0.022	12	-6588.8	93.57	0.00

(B) SMR_m: No evidence for environment specific terminal year effects

	Experimental manipulations			Age terms and interactions					Other covariates					Model Fit			
Model	Brood size	Treat	Brood size * Treat	ΔAge	Termin Year	Brood size * Termin Year	Treat * Termin Year	Brood size * Treat * Termin Year	Lifespan	Photo	MinT	Measm Temp	Mass	df	AICc	ΔAICc	weight
1		-0.030		0.004					0.002	0.022	-0.001	-0.013	0.019	11	-6682.4	0	0.94
2		-0.030		0.006	-0.007				0.001	0.022	-0.001	-0.013	0.019	12	-6676.6	5.84	0.05
3	-0.003	-0.030		0.004					0.002	0.023	-0.001	-0.013	0.019	12	-6671.9	10.53	0.01
4		-0.029		0.006	-0.005		-0.004		0.001	0.022	-0.001	-0.013	0.019	13	-6666.3	16.07	0.00
5	-0.003	-0.031		0.006	-0.007				0.001	0.022	-0.001	-0.013	0.019	13	-6666.1	16.34	0.00
6	-0.0001	-0.027	-0.007	0.004					0.002	0.023	-0.001	-0.013	0.019	13	-6662.7	19.72	0.00
7	-0.0003	-0.028	-0.006	0.006	-0.007				0.001	0.022	-0.001	-0.013	0.019	14	-6656.7	25.68	0.00
8	-0.003	-0.029		0.006	-0.005		-0.004		0.001	0.022	-0.001	-0.013	0.019	14	-6655.8	26.65	0.00
9	-0.002	-0.031		0.006	-0.006	-0.003			0.001	0.022	-0.001	-0.013	0.019	14	-6655.6	26.83	0.00
10	-0.0003	-0.026	-0.006	0.006	-0.005		-0.003		0.001	0.022	-0.001	-0.013	0.019	15	-6646.4	36.03	0.00
11	0.001	-0.027	-0.006	0.006	-0.006	-0.003			0.001	0.022	-0.001	-0.013	0.019	15	-6646.3	36.16	0.00
12	-0.002	-0.029		0.006	-0.004	-0.003	-0.004		0.001	0.022	-0.001	-0.013	0.019	15	-6645.3	37.08	0.00
13	0.001	-0.026	-0.006	0.006	-0.004	-0.003	-0.004		0.001	0.022	-0.001	-0.013	0.019	16	-6636.0	46.45	0.00
14	0.002	-0.025	-0.009	0.006	-0.002	-0.007	-0.007	0.007	0.001	0.022	-0.001	-0.013	0.019	17	-6627.0	55.38	0.00
15				0.004					0.002	0.021	-0.001	-0.013	0.022	10	-6612.5	69.89	0.00
16				0.005	-0.006				0.002	0.021	-0.001	-0.013	0.021	11	-6604.3	78.16	0.00
17	-0.001			0.004					0.002	0.021	-0.001	-0.013	0.022	11	-6601.1	81.29	0.00
18	-0.001			0.005	-0.006				0.002	0.021	-0.001	-0.013	0.021	12	-6592.8	89.56	0.00
19	0.0001			0.005	-0.004	-0.004			0.002	0.021	-0.001	-0.013	0.021	13	-6582.6	99.84	0.00

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Summary

Individuals of the same species can differ up to tenfold in adult lifespan. Environmental conditions during development are thought to play an important role in generating this variation. It was hypothesised however that the effects of developmental conditions on lifespan, and more generally adult health, may depend on the environmental conditions encountered during adulthood. More specifically, individuals that grew in harsh environments may be better able to cope with similar challenges when adult. Experimental studies of this in vertebrates remain scarce. Here, we subjected over 500 zebra finches to an independent experimental manipulation of foraging costs during development (brood size) and adulthood (flight costs per food reward) in a 2x2 design and monitored them in these conditions till natural death. We found that individuals that had faced harsh environmental conditions during development lived shorter, but only when facing harsh environments during adulthood. Thus, the effect of developmental conditions on lifespan depend on the environmental conditions during adulthood, but we found no evidence that growing up in harsh environments better prepared individuals to face foraging costs during adulthood.

Aging is the decline in organismal functioning with age resulting in declining fecundity and survival probability. Aging is often regarded as a process in synchrony with lifespan: manipulations that shorten lifespan are also expected to accelerate aging and *vice-versa*. Here, we investigated whether this was case in the zebra finches subject to the aforementioned environmental manipulations. We monitored a variety of traits associated with energetics and ´state´, including mass, bill coloration (a sexual signal indicator of ´quality´) and several independent measures of metabolism. We found that different traits within a single organism age at own time and pace: some age fast in the last year of life (e.g. bill coloration), while others decline gradually all through adulthood (e.g. metabolism). The aforementioned environmental manipulations affected the aging of some traits (mass, bill coloration), but not all (metabolism). This shows that aging within an organism is an asynchronous process. Hence aging is uncoupled from lifespan.

Samenvatting

Volwassen individuen binnen één soort kunnen wel tot tienvoud verschillen in levensduur. De omgevingskwaliteit tijdens de ontwikkeling of groei kan een belangrijke rol spelen in het genereren van deze variatie. Echter, de langetermijneffecten van de groeiomstandigheden op levensduur en, meer algemeen gezondheid, kunnen afhangen van de omgevingskwaliteit waarin volwassen individuen leven. Eén hypothese stelt dat opgroeien in ongunstige omstandigheden zelfs voordeel kan opleveren wanneer volwassenen moeten omgaan met gelijkaardige ongunstige omstandigheden. Dit werd tot nu toe zelden experimenteel onderzocht in een gewervelde soort. Hier hebben we meer dan 500 zebravinken een foerageerkosten manipulatie laten ondergaan tijdens de groei (broedselgrootte) en als volwassenen (vliegkosten) in een 2x2 design en hebben we hun overleving jarenlang gevolgd tot hun natuurlijke dood. Individuen die waren opgegroeid in ongunstige omstandigheden leefden korter, maar alleen wanneer ze ook hoge foerageerkosten hadden ervaren als volwassenen. Dus, het effect van opgroei omstandigheden op levensduur hangt af van de omgevingskwaliteit die volwassenen ervaren, maar we hebben geen bewijs gevonden dat opgroeien in slechte omstandigheden individuen beter voorbereidt op hoge foerageerkosten als volwassenen.

Veroudering is de aftakeling van het lichaam met leeftijd die de kans op voortplanting en overleving vermindert. In het algemeen wordt veroudering beschouwd als een proces dat hand in hand gaat met levensduur: van manipulaties die levensduur verkorten wordt verwacht dat ze ook veroudering versnellen en omgekeerd. Hier hebben we onderzocht als dat zo is bij de zebravinken in de boven genoemde experimenten. We hebben de werking gevolgd van meerdere kenmerken van het organisme die te maken hebben met energetica en ´kwaliteit´: gewicht, snavelkleur (een partnerkeuze signaal voor ´kwaliteit´) en meerdere onafhankelijke variabelen van energieverbruik. We hebben gevonden dat elk van de bestudeerde kenmerken veroudert op zijn of haar eigen ritme: bepaalde kenmerken verouderen snel in het laatste levensjaar (bijv. snavelkleur) langzaam, terwijl andere kenmerken langzaam verminderen gedurende vele jaren (bijv. energieverbruik). De veroudering van sommige kenmerken was in zekere mate gevoelig voor de omgevingsmanipulaties (gewicht, snavelkleur), maar anderen waren dat nauwelijks (energieverbruik). Deze resultaten tonen aan dat de veroudering binnen een lichaam geen synchroon proces is en dat veroudering onafhankelijk is van levensduur.

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Statements associated with the PhD thesis

Growing up and growing old

A longitudinal study on aging in zebra finches

Michael Briga

1. Aging starts young[1,2].
   [1] *Chapters 3 & 11*
   [2] *Belsky D et al. 2015. Quantification of biological aging in young adults. Proceedings of the National Academy of Sciences 112:E4104–E4110.*

2. While aging at the population level is of ecological relevance[1,2], it helps little to our understanding of organismal aging[3].
   [1] *Chapter 3*
   [2] *Robert A et al. 2015. Actuarial senescence can increase the risk of extinction of mammal populations. Ecological Applications 25:116–124.*
   [3] *Chapter 11*

3. Aging of an organism ´emerges´ from the asynchronous aging of its parts.
   Chapter 11

4. It is often assumed that aging and lifespan are associated and hence identifying factors affecting lifespan may suffice to also understand aging[1]. Since lifespan and aging can be disconnected[2,3], aging research needs to quantify both, lifespan and aging.
   [1] *Williams GC. 1999. The tithonus error in modern gerontology. The Quarterly Review of Biology 74:405–415.*
   [2] *Chapters 3 & 11*
   [3] *Bansal A et al. 2015. Uncoupling lifespan and healthspan in* Caenorhabditis elegans *longevity mutants. Proceedings of the National Academy of Sciences 112:E277–E286.*

5. There is a need for studying lifespan and aging beyond a laboratory environment[1,2,3].
   [1] *Chapters 2, 3, 6 & 11*
   [2] *Harper JM et al. 2006. Does caloric restriction extend life in wild mice? Aging Cell 5:441–449.*
   [3] *Partridge L & D Gems. 2007. Benchmarks for aging studies. Nature 450: 165–167.*

6. Variability is a parameter of interest in biological processes independently of mean values[1,2].
   [1] *Chapters 3 & 6*
   [2] *Seligman B et al. 2016. Equity and length of lifespan are not the same. Proceedings of the National Academy of Sciences 113:8420–8423.*

7. The development of new and flexible quantitative tools[1,2,3,4] paves the way for scientific discovery.
   [1] *Kruuk LEB. 2004. Estimating genetic parameters in natural populations using the "animal model." Philosophical Transactions of the Royal Society of London B: Biological Sciences 359:873–890.*
   [2] *Hadfield JD. 2010. MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. Journal of Statistical Software 33:1–22.*
   [3] *Colchero F et al. 2012. BaSTA: an R package for Bayesian estimation of age-specific survival from incomplete mark-recapture/recovery data with covariates. Methods in Ecology and Evolution 3:466–470.*
   [4] *van de Pol M et al. 2016. Identifying the best climatic predictors in ecology and evolution. Methods in Ecology and Evolution 7:1246–1257.*

8. Societal issues stimulate both fundamental and applied research questions[1,2].
   [1] *On the issue of human lifespan variation: p. 31 in WHO. 2000. The World Health Report 2000. Geneva, Switzerland: World Health Organization.*
   [2] *On the issue of climate change and diurnal temperature variation: p. 204, 633 and 634 in Stocker TF et al. 2013. The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.*

9. Correlation does not imply causation, but a yet unresolved causal association.
   Chapter 6

10. The art of communication is as an essential tool in science as it is in many other aspects of society.