State transitions: a major mortality risk for seasonal species

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LETTER

State transitions: a major mortality risk for seasonal species

Julie Landes,[1,2] Martine Perret,[2] Isabelle Hardy,[2] Carlo Giovanni Camarda,[3] Pierre-Yves Henry[2†] and Samuel Pavard[1*†]

Abstract

Ageing results from the accumulation of multifactorial damage over time. However, the temporal distribution of this damage remains unknown. In seasonal species, transitions between seasons are critical periods of massive physiological remodelling. We hypothesised that these recurrent peaks of physiological remodelling are costly in terms of survival. We tested whether captive small primates exposed to an experimentally increased frequency of seasonal transitions die sooner than individuals living under natural seasonality. The results show that experiencing one additional season per year increases the mortality hazard by a factor of 3 to 4, whereas the expected number of seasons lived is only slightly impacted by the seasonal rhythm. These results demonstrate that physiological transitions between periods of high and low metabolic activity represent a major mortality risk for seasonal organisms, which has been ignored until now.

Keywords

Ageing, biological rhythm, life stages, mortality, photoperiod, physiological state transition, primate, seasonality, survival analyses.

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INTRODUCTION

Ageing is the deterioration of physiological functions through life that leads to an increase in mortality with age (Vaupel *et al*. 1998; López-Otín *et al*. 2013). According to the disposable soma theory, organisms invest their limited resources in reproduction and growth, at the expense of allocation to somatic maintenance and repair, which results in an accumulation of deteriorations with age (Kirkwood & Austad 2000). However, as such, the theory neglects the temporal dynamics of soma deterioration while time can be as limiting a resource as energy (or even more; Lorenzini *et al*. 2011), particularly in highly seasonal environments generating a race to reproduce on time. To comprehend the process of ageing, it is necessary to understand how and when deteriorations occur during an organism's life and what internal and environmental constraints cause these deteriorations. Damage accumulates continuously over an organism's life due to metabolic activity, as demonstrated, for example, by caloric restriction experiments (Finkel & Holbrook 2000). Another potential source of molecular, cellular and functional damage during an organism's lifetime is the intense physiological remodelling that occurs during the transition between different metabolic or activity states, when the organism shifts from low to high activity (and *vice-versa*). In complex inorganic dynamic systems, transitions between inactive and active states are known to generate an accumulation of irreparable damage and system ageing. For example, computers and cars have a high risk of breaking down when starting up or switching off, i.e., times when they are not producing the maximal workload (within the range of specifications of use). In industry, the standard ageing experimental test (to test how a device will age; Escobar & Meeker 2006) consists of counting the maximal number of transitions between the 'inactive' and 'active' states a device can endure before breaking (e.g., the number of switches for a light bulb) rather than the total time it can remain in the active state (e.g., number of hours of light for a light bulb). We propose that this transition-based component of ageing also applies to biological systems. Some damages would accumulate continuously through life (like those proportional to the mass-specific metabolic rate; Jones *et al*. 2008), but supplementary damages would specifically occur at the transition between states. The costs they entail would depend on the frequency and strength of these physiological state transitions. This discontinuous somatic deterioration through time may alter the rate of organism senescence. This hypothesis is supported by the fact that in species exhibiting complex life cycles, mortality aggregates at each transition between developmental stages (Horvitz & Tuljapurkar 2008).

Organisms living in seasonal environments typically alternate between two phenotypic states throughout the annual cycle: a fully active, reproductive state during the favourable season(s), and a resilient, hypometabolic state during the unfavourable season(s) (Gwinner 1986; Ruf *et al*. 2012). The timing of reproduction is selected such that the increase in metabolic expenditure imposed by reproduction coincides with the annual peak(s) in resource availability (Helm *et al*. 2013). Outside of the reproductive period, behaviour and physiology are modulated such that biological maintenance is secured and the chance of survival until the next reproductive opportunity is maximised. Phenotypic transitions between seasons therefore compel an extensive remodelling of the organismal biology (Ramenofsky

[1]*Eco-Anthropologie et Ethnobiologie, UMR 7206 CNRS, MNHN, Université Paris Diderot, Sorbonne Universités, Musée de l'Homme, 17 place du Trocadéro, 75016 Paris, France*

[2]*Mécanismes Adaptatifs et Evolution, UMR 7179 CNRS, MNHN, Sorbonne Universités, 1 avenue du Petit Château, 91800 Brunoy, France*

[3]*Mortalité, Santé, Épidémiologie, UR 05 INED, 133 Boulevard Davout, 75980 Paris, France*

**Correspondence: E-mail: samuel.pavard@mnhn.fr*

[†]*These authors contributed equally.*

& Wingfield 2007; Ruf & Geiser 2015), with simultaneous pleiotropic activations and deactivations of major physiological functions (reproduction, metabolism, behaviour, nutrition, physical activity). We hypothesise that this adaptation to seasonal environments comes at a cost (despite a net survival benefit over winter; e.g. Liow *et al.* 2009; Turbill *et al.* 2011), and generates a source of temporal discontinuity in soma deterioration through age that has been ignored until now. The cost of seasonal physiological transitions is expected to emerge from imperfect biological regulations, which generate an accumulation of cellular (including DNA) and organismal damage (López-Otín *et al.* 2013), as well as from imperfect seasonal regulation of competing functions (e.g., reproduction *vs.* energy-saving processes; Ricklefs & Wikelski 2002) and from suboptimal phenotypic matching with environmental changes (Auld *et al.* 2009). This recurrent phenotypic remodelling is therefore expected to contribute to the ageing process in seasonal species. Under this hypothesis, damage is predicted to accumulate as a function of the frequency of these transitions. Thus, we hypothesise that seasonal transitions between markedly different metabolic states increase both mortality and the metabolic load (such as oxidative damages) that accumulates as the organism chronologically ages (i.e., the consensual main biological mechanism for ageing; Selman *et al.* 2012). In this case, we predict that increasing the number of transitions between seasonal phenotypic states per time unit, all else being equal (particularly the amount of time spent in the active state), will increase mortality and potentially accelerate ageing (i.e., the rate at which mortality increases with age).

To test our hypothesis, captive grey mouse lemurs (*Microcebus murinus*, Miller 1777) were exposed to different frequencies of seasonal transitions. In this species, the seasonal physiological changes are triggered by changes in photoperiod. Thus, only photoperiodic regimen was manipulated, with all other environmental conditions held constant across all individuals; this allowed the effect of seasonal physiological transitions to be isolated. Previous work has shown that individuals experiencing experimental reductions in the durations of the seasons, i.e., accelerated seasonal rhythms, maintain their physiological seasonality, particularly body mass variation and reproductive function activation (Perret 1997). Reductions in season duration also induce the earlier emergence of age-related phenotypes, such as morphological modifications and circadian rhythm disorders (Aujard *et al.* 2001; Cayetanot *et al.* 2005), but their effects on mortality trajectories with age have not been investigated (Perret 1997). We analysed mortality with increasing age for individuals that were artificially exposed to different paces of seasonal alternation (hereafter called seasonal rhythm), ranging from two to five seasons per year. We assessed whether experimentally increasing the frequency of seasonal transitions led to an increase in mortality and whether this increase reflects organismal ageing.

MATERIAL AND METHODS

Biological model and manipulation of seasonal rhythm

The grey mouse lemur is a small (50–130 g), nocturnal primate with a life expectancy of approximately 2–3 years in the wild and approximately 5–6 years in captivity (Kraus *et al.* 2008; Languille *et al.* 2012). It lives in a highly seasonal habitat, the dry tropical forests of Madagascar, where the environment alternates between an abundance of resources during the wet season and pronounced food and water shortages during the dry season (each season lasts approximately six months; see Supplementary Information S1). The wet season corresponds to the period of reproduction (Kraus *et al.* 2008). In contrast, during the dry season, grey mouse lemurs remain inactive in their shelters and markedly reduce their metabolic needs by entering daily torpor (Schmid 2001; Vuarin *et al.* 2015). They fatten at the end of the wet season and consume this stocked energy to survive the unfavourable caloric and hydric restrictions of the dry season (Génin & Perret 2000; Hämäläinen *et al.* 2014). These seasonal metabolic and behavioural changes are synchronised by the changes in day length between the long-day (>12 h) wet seasons and the short-day (<12 h) dry seasons (Perret & Aujard 2001). More precisely, exposure to a short day length induces inactivity and an endocrine shift to temporary, controlled hypometabolism and hypothermia as well as fat-based metabolism. In contrast, long days induce a return to permanent normothermia, regular locomotor activity, and the activation of reproductive functions (see Perret & Aujard 2001 or Languille *et al.* 2012 for a detailed review of the photoperiod-dependence of physiological functions in the grey mouse lemur). Photoperiod alternations are sufficient to trigger metabolic and behavioural changes in captive animals similar to those observed *in natura*, although food and water are provided *ad libitum* throughout the year. In females, the activation of reproductive functions is triggered by increasing day length, whereas in males, it is triggered by 4 to 5 months of exposure to short days (i.e., photorefractoriness, Perret & Aujard 2001). In captivity, the natural photoperiodic regimen of grey mouse lemurs is reproduced by alternations between 6 months of long-day photoperiod (hereafter called LD period; 14 h of light per day) and six months of short-day photoperiod (hereafter called SD period; 10 h of light per day; Perret 1997; Supplementary Information S2). These alternations also generate seasonal variation in survival. As in its natural habitat, the unfavourable season (SD season in captivity), in which energy-saving mechanisms are employed, is associated with higher survival than is the favourable season (LD season in captivity; Perret 1997; Kraus *et al.* 2008; Languille *et al.* 2012).

We manipulated seasonal rhythm such that individuals were exposed to two to five seasons per year throughout their lifetimes (see Methods and Supplementary Information S2 and S3). The reference individuals (N = 576 individuals, 3984 season-individuals; Reference group in Table 1) were maintained throughout their lives under a natural-like seasonal rhythm of two six-month seasons per year (LD/SD duration ratio: 1.00 ± 0.04). The accelerated individuals (N = 153 accelerated individuals, 1146 season-individuals) were maintained under seasonal rhythms ranging from three to five seasons per year (Fig. 1), with LD and SD seasons of similar duration (LD/SD: 1.12 ± 0.09). The accelerated seasonal rhythms were applied for the purpose of experiments investigating the effects of accelerated seasonal rhythm on physiological performance in captive grey mouse lemurs (Aujard *et al.* 2001;

Table 1 Description of mean life history traits for reference individuals and for the three groups of accelerated individuals (defined according to their seasonal rhythm, Figure 1): (i) the seasonal rhythm, (ii) the number of seasons lived, (iii) age at entrance in SD2 (i.e. when they enter the study), (iv) for females only (Supplementary Information S6), the number of successful reproductions (i.e., until parturition), the numbers of females that reproduced once or twice over their life, and that reproduced during their LD1 or LD2 (among those that reproduced), the number of weaned offspring over lifetime, and (v) the number of individuals that experienced natural death and censoring. Standard deviations are indicated in brackets. The mean age at entrance in SD2 for the males from Group A3 is unknown as they entered the study at age 1500 days (see Supplementary Information S3)

	Reference		Group A1		Group A2		Group A3		
	Females	Males	Females	Males	Females	Males	Females	Males	Total
Mean seasonal rhythm (seasons/year)	2.0 (± 0.02)		2.6 (± 0.07)		2.5 (± 0.21)		4.9 (± 0.38)		
Mean no. of seasons lived	6.9 (± 3.0)		7.3 (± 3.5)		9.1 (± 4.9)		24.4 (± 3.8)		
Mean age at entrance in SD2 (days)	468.3 (± 16.2)		347.4 (± 24.4)		385.0 (± 56.8)		–		
Mean no. of successful reproductions over lifetime	0.92 (± 0.75)	–	0.98 (± 0.94)	–	0.79 (± 0.98)	–	–	–	
Females reproducing once or twice	68%	–	56%	–	42%	–	–	–	
Females reproducing in LD1 or LD2	63%	–	75%	–	60%	–	–	–	
Mean no. of weaned offspring over lifetime	1.73 (± 1.78)	–	1.53 (± 1.47)	–	1.26 (± 1.76)	–	–	–	
Death from natural causes	97	70	35	29	6	12	–	9	258
Censored	201	208	22	21	13	3	–	3	471

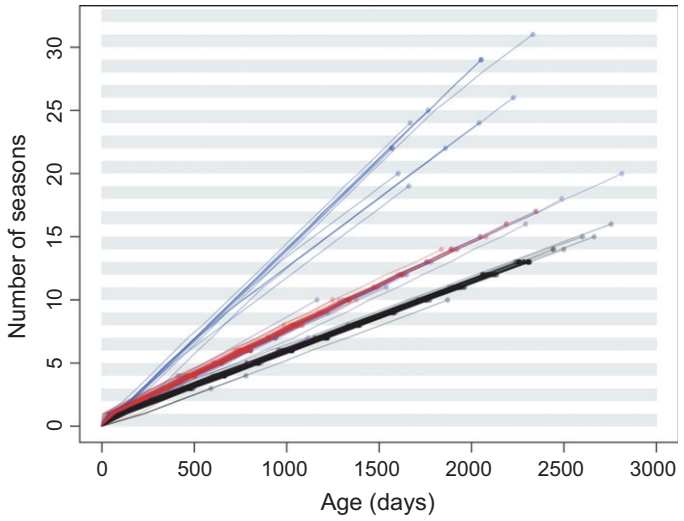

Figure 1 Association between chronological age (in number of days) and the number of seasons experienced according to the seasonal rhythm. Each line corresponds to the trajectory of a single individual across the different seasons. Each dot represents the last season lived by a given individual, regardless of whether it was dead or censored at this age (see Supplementary Information S2). The grey bands correspond to LD seasons. The biological age equals the chronological age in reference individuals (in black, two seasons per year), whereas the biological age increases faster than the chronological age in experimentally accelerated individuals (two to three seasons per year are shown in purple and red; four to five seasons per year are shown in blue; see Supplementary Information S3).

Perret & Aujard 2001; Cayetanot *et al*. 2005; Groups A2 and A3 in Table 1) but also as part of photoperiod manipulations aiming at increasing the frequency of reproductive events for population management purposes (Group A1 in Table 1). Therefore, rhythm of reproduction was also accelerated in these groups. However, most females reproduced only once or twice during their life for management purposes (Table 1). Because nulliparous females had systematically the opportunity to breed, these reproductions mainly occurred in early adult life (Table 1). This explains why females from all groups had a low and similar mean number of reproductive events. Thus, differences in seasonal rhythm were unlikely to lead to differences in adult mortality due to a higher investment in reproduction.

Accelerated individuals of Group A1 experienced approximately 20 weeks of LD and SD. Those of Group A2 were maintained at various seasonal rhythms ranging from 2 to 3 seasons per year. Finally, Group A3 includes males exposed to 10 or 13 weeks of LD and SD (5.2 or 4 seasons per year). Except for the photoperiodic regimen, captive conditions were the same for all individuals.

Mortality data

A 19-year dataset was obtained by monitoring the 576 reference individuals and 153 accelerated individuals and analysed. Among these individuals, 258 (35%) experienced a natural death, whereas the remaining 471 were right-censored (Table 1); i.e., they were alive at the end of the study (on 1st January 2013), had been transferred to another laboratory or had escaped at a known date, or had died from 'unnatural' causes (euthanasia for an experiment requiring sacrifice or death during anaesthesia or other experimental procedure). Available data consisted of the dates of birth and natural death or exclusion (censoring) of each individual and the dates of season change, which allowed the seasonal rhythm experienced by each individual to be determined. The accuracy of these dates is ± 2 days as the animals were monitored every day in the week but not over the weekend. The sex, monthly fluctuations in body mass, and reproductive history (for females only, the polyandrous mating system prevented us from characterising male reproductive history; Huchard *et al*. 2012) of each individual was known. Only fully grown, sexually mature individuals were considered (from their entrance in the SD2 season to death or censoring).

Survival analyses

We used event history analysis (i.e., survival analysis) to analyse the effects of seasonal rhythm (and adjusting variables) on age-specific mortality hazard. Mortality hazard (also known as the force of mortality) is a measure of the proneness to failure as a function of the age of the individual (failure being the individual's death; Lee & Go 1997). These statistical techniques allowed the use of all available data, including censored and truncated data, and accounted for the periodicity of individual mortality due to seasonality by incorporating season as a time-varying covariate.

We investigated the effect of seasonal rhythm on mortality between individuals exhibiting large differences in seasonal rhythm throughout their lives. To do so, we used the mean seasonal rhythm experienced by an individual over its past life (i.e., the age at entrance in a season divided by the number of the seasons from entry into the SD2 period to the considered season). We also controlled for potential short-term variations in seasonal rhythm that occurred within an individual's life due to daily captive population management (the date of photoperiodic change varied from a few days to two weeks; see Supplementary Information S4). We also took into account factors that are known to affect mortality in this species, i.e., sex, season and their interaction (Kraus *et al.* 2008; Languille *et al.* 2012), and adjustment variables (see Supplementary Information S4 for a detailed description): (i) year of birth and year when entering a given season, to control for potential period and cohort effects; and (ii) the identities of individuals and their mothers, which were entered as random variables to control for interindividual heterogeneity and heterogeneity between litters due to maternal effects (also called frailty in survival analyses; Vaupel *et al.* 1979), as well as consistencies within maternal lineages. Because the survival cost of seasonal rhythm may differ between the active and the inactive seasons, we also considered the interaction between seasonal rhythm and season. Males and females were analysed separately because seasonal fluctuation in mortality is known to differ between sexes (Kraus *et al.* 2008; Languille *et al.* 2012) and because complex interactions were found between sex and season and between sex and other adjustment variables (not shown, preliminary analysis).

Theoretically, the more an individual reproduces, the more it compromises its longevity by allocating resources to its reproduction at the expense of its maintenance (e.g. Boonekamp *et al.* 2014). Since seasonal acceleration also increases the absolute number of reproduction opportunity per chronological year, we needed to determine whether an effect of seasonal rhythm could be confounded by an increased frequency of reproductive events. To do so, we assessed how the coefficient estimates for the effect of seasonal rhythm were affected by the inclusion of variables describing female reproductive effort in the selected models, considering an immediate effect of current reproductive success (i.e. whether the female reproduced or not during a given reproductive season), and/or a cumulative effect of reproduction through time (number of past successful reproductions and cumulated number of weaned offspring).

We constructed Cox models (Klein & Moeschberger 2003; Supplementary Information S5). These are flexible models that do not constrain the distribution of the baseline hazard and thereby allow mortality to be analysed without modelling the age trajectories of mortality for the studied organism. The hazard function is modelled as $h(t|y) = h_0(t).e^{\beta y}$, with $h_0(t)$ being the mortality hazard baseline of unspecified distribution, t being the age at death or censoring, and β being the parameter quantifying the association between covariate y and time at death. Cox models assume that the effect of a covariate is proportional to the baseline mortality level at all ages (i.e., they are proportional hazard models). Although this hypothesis was systematically tested, we also examined models that relaxed the proportionality hypothesis by incorporating the interaction between seasonal rhythm and age of entry to the season. Where such interaction was detected, piecewise Cox models (Klein & Moeschberger 2003) were constructed to explore potential non-linear effects of this covariate on mortality with age. Piecewise Cox models estimate mortality functions for different periods of the individual's life. They were implemented by incorporating a time-varying covariate that defined two or more time intervals (see Supplementary Information S5). We considered all possible models that incorporated the above-mentioned covariates and interactions (see also Supplementary Information S4 and S5). Interindividual heterogeneity and mother effects were entered as gamma-shaped distributions.

Model selection was based on Akaike's Information Criterion (AIC; Burnham & Anderson 1998). Considering all possible covariate combinations allowed us to calculate the AIC weight for each covariate (see details in Supplementary Information S5). AIC is not compelling for comparing among models that include random variables (Jiang *et al.* 2008). Therefore, the selected models that did not incorporate interindividual heterogeneity or maternal effects (random variables) were compared with corresponding models that included one of these covariates to assess any changes in the magnitude or significance of the effects.

Finally, parametric equivalents of the selected Cox models (in term of covariates incorporated; Supplementary Information S5) were considered to test for a functional relationship between seasonal rhythm and ageing: Proportional Hazard (PH) models test whether a variable has a proportional effect over age, and Accelerated Failure Time (AFT) models test whether this effect varies with age (see Supplementary Information S5). This procedure allowed us to assess whether the mortality patterns of accelerated individuals reflected changes in the rate of ageing.

RESULTS

The acceleration of seasonal rhythm strongly increased mortality throughout the adult life of females and during the first part of adult life in males (Fig. 2, Table 2). Model selection evidenced a large effect of seasonal rhythm on mortality independent of season (selected Cox models are presented in Table 2; model selection information and diagnostic plots are provided in Supplementary Information S5). More precisely, our best models predict that undergoing an additional season

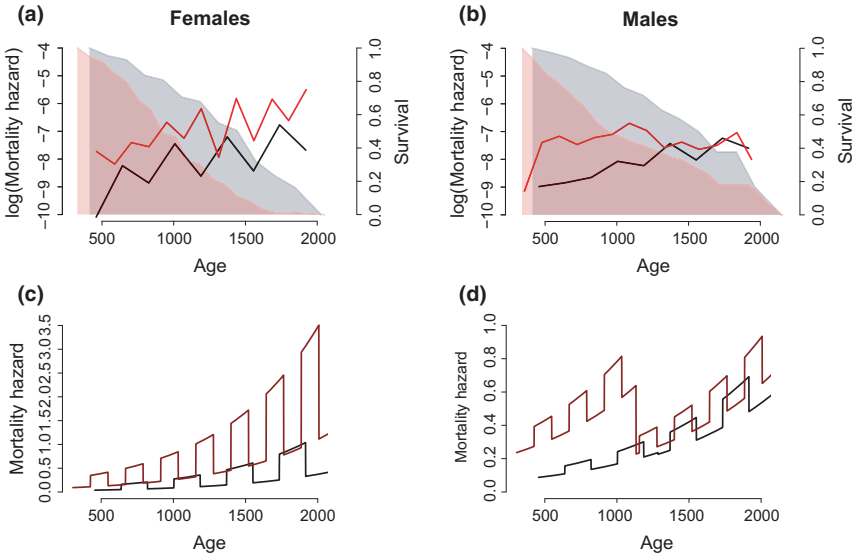

Figure 2 Effect of seasonal rhythm acceleration on the mortality trajectories of females and males with increasing age. Mortality hazard (measure of the proneness to failure as a function of the age of an individual) by age for females (a and c) and males (b and d) and for a reference individual (in black) and an accelerated individual exposed to an additional season per year (in orange). (a and b) Mortality hazard (on a logarithmic scale) and survival estimates from the respective selected Cox models (see Supplementary Information S5). Each point corresponds to the middle of a season. The corresponding survival curves are represented by the coloured areas. (c and d) Mortality hazard estimations from the selected Gompertz-shaped parametric models (see Supplementary Information S5). These models consist of parametric versions of the selected Cox models.

per year increases the risk of death by three and four times in females and males, respectively (Fig. 2). This would result in a decrease in the remaining life expectancy at entry into SD2 from 4.1 to 2.8 years for females and from 4.5 to 3.3 years for males (E_{SD2} = Σ(survival at the beginning of a season*length of the season)). The estimated number of seasons lived (E_0 = seasonal rhythm × number of seasons lived) by accelerated individuals (three seasons/year) was approximately the same as for reference individuals: 8.5 and 8.1 seasons, respectively, for females and 9.9 and 9.1 seasons, respectively, for males. This relationship between seasonal rhythm and life expectancy remains uniform until approximately 3.5 seasons per year. In males, we detected non-linearity in the effect of seasonal rhythm over time, and a piecewise Cox model was selected (Table 2; Fig. 2b). The increase in mortality associated with seasonal rhythm acceleration decreases after 3.5 years of age. At this age, accelerated males experiencing an additional season per year show a mortality hazard only 1.3 times that of references males (at younger ages, their mortality hazard is four times that of reference males; Fig. 2b and d). These results are robust to the inclusion of interindividual heterogeneity and maternal effects to the model (Supplementary Information S5).

Considering female reproduction in the analyses did not change our results, nor the magnitude of the effect of seasonal rhythm on mortality (Table 2; Supplementary Information S6). In our captive population, an elevated reproductive effort was indeed associated with lower (instead of higher) mortality in females, and this effect was independent of seasonal rhythm (no support for interaction terms). The parametric models showed similar results to those of the Cox models in terms of the magnitudes of the effects (Fig. 2; Table 2; Supplementary Information S5). They favour the PH model for females, whereas in males, both the PH and AFT models capture the decline with age of the effect of seasonal rhythm (as observed in the Cox piecewise models).

DISCUSSION

Experimentally accelerating the frequency of seasonal transitions in a captive primate revealed that seasonal transitions are costly in terms of survival. Seasonal rhythm acceleration increased mortality throughout the adult life in females and during the first part of the adult life in males. These results are adjusted for cohort and period effects, interindividual heterogeneity, maternal effects and maternal lineage. Strikingly, individuals experiencing two and three seasons per year lived the same number of seasons rather than the same number of years. This supports previous results indicating that the number of seasons experienced is a predictor of mortality hazard that is as important as chronological age (Perret 1997).

Is the deleterious effect of accelerated seasonal rhythm on mortality actually due to the increased frequency of seasonal physiological transitions? One alternative interpretation could be that this mortality increase arose due to the increased frequency of, supposedly costly, reproductive events in accelerated individuals (Williams 1957; Reznick 1985; Boonekamp *et al.* 2014). Such a confounding effect of the cost of reproduction was unlikely in the present study. First, most reference and accelerated females reproduced only once or twice, and mainly early in their reproductive life. Second, females with higher reproductive effort showed higher - rather than lower - survival. This absence of survival cost of reproduction could be expected for a captive population (e.g. Ricklefs & Cadena 2007; Kengeri *et al.* 2013). And, third, the effect of seasonal rhythm did not depend on reproductive success.

Table 2 Estimated coefficients (and standard errors) for all variables retained in the selected models: for females, Cox and PH Gompertz models; for males, Cox piecewise (with two time intervals corresponding to adult life before and after 3.5 years of age) and parametric piecewise PH Gompertz models. Models that account for female reproduction are also reported, i.e. the previously selected Cox model with either *CumR* (the number of past successful reproductions) or *CumOff* (the cumulated number of weaned offspring). Standard errors are indicated in brackets. Significance symbols: 0–0.001'***'; 0.001–0.01'**'; 0.01–0.05'*'; 0.05–0.1'.'. *SeasonAge* is the age at entry into a season. Reference levels are 'LD' for *Season* and 'Medium years' cluster for *YearBirth* and *YearObs*. *G* and *B* stand for 'Good years' and 'Bad years' clusters, respectively (in terms of their effect on mortality; see Supplementary Information S4 for details on year clustering)

	Covariates								
	Season	*Mean.SR*	*Mean.SR: SeasonAge*	*> 3.5 years*	*Mean.SR (> 3.5 years)*	*CumR*	*CumOff*	*YearBirth*	*YearObs*	AIC
Females										
Cox	−1.03*** (± 0.26)	1.17** (± 0.39)						*G* −13.39 (± 3.9e^3) *B* 0.06 (± 0.22)	*G* −14.88 (± 3.8e^3) *B* 0.67** (± 0.23)	1247.00
Cox CumR	−1.04*** (0.26)	1.29*** (0.39)				−0.24* (0.12)		*G* −13.45 (3.8e^3) *B* 0.12 (0.23)	*G* −14.96 (3.8e^3) *B* 0.69** (0.23)	1244.99
Cox CumOff	−1.04*** (0.26)	1.24** (0.39)					−0.18** (0.06)	*G* −13.43 (3.9e^3) *B* 0.12 (0.23)	*G* −14.89 (3.8e^3) *B* 0.66** (0.23)	1240.41
PH parametric	−1.15*** (± 0.20)	1.08** (± 0.38)						*G* −10.39 (± 8.4e^3) *B* 0.065 (± 0.22)	*G* −11.88 (± 6.3e^3) *B* 0.65** (± 0.22)	2244.64
Males										
Cox piecewise	−0.18 (± 0.23)	1.46*** (± 0.35)	−1.22*** (± 0.33)					*G* NA *B* 0.88*** (± 0.25)	*G* −15.51 (± 1.8e^3) *B* 0.46. (± 0.25)	1088.75
PH parametric piecewise	−0.36. (± 0.19)	1.18* (± 0.53)		1.93 (± 1.20)	−0.99. (± 0.53)			*G* NA *B* 0.83** (± 0.27)	*G* −13.56 (± 6.7e^3) *B* 0.47. (± 0.25)	2023.64

Another alternative interpretation could be that the observed increase in mortality with accelerated seasonal rhythm might have arisen from a malfunction of the organism under seasonal conditions that overly differ from those under which the species has evolved (Madagascar). Under this scenario, increased mortality would not result from an increased frequency of physiologically 'normal' seasonal transitions but of 'abnormal' seasonal transitions. For example, mouse lemurs maintained two years under the same season do not undergo seasonal body mass cycles, and only females maintain some reproductive cycling (Perret & Aujard 2001). This is not the case when the seasonal rhythm is accelerated, at least within the range used in the present experiment, as the physiological and behavioural changes remained synchronised to the photoperiodic regimen. The most visible seasonal physiological change is a large change in body mass, which nearly doubles between the LD and SD seasons (Perret 1997; Schmid 1999). Such a massive fall fattening is common in small heterotherms (Ruf & Geiser 2015). The acceleration of seasonal rhythm accelerates these changes in body mass (see Supplementary Information S7) although the magnitude of these changes is lower in accelerated individuals (likely because individuals have less time to fatten during the SD season). Furthermore, the ability of male lemurs to fatten during the first half of the SD period declines with age (Hämäläinen *et al.* 2014), and this decline was markedly more rapid in the accelerated males than in the reference males (see Supplementary Information S7). The reduced seasonal body mass variation in accelerated males older than 3.5 years strikingly coincides with a reduction in the sensitivity of mortality to seasonal acceleration (Fig. 2b and d). It could be argued that this decline in mortality in older accelerated males might have resulted from hidden heterogeneity in individual quality (Vaupel *et al.* 1979); however, the models controlled for such intermale heterogeneity. In contrast, the body mass of females continued to fluctuate seasonally throughout the female lifetime, and the mortality of accelerated females continued to increase proportionally to the number of seasons lived per year. These sex differences in life-time patterns of mortality and in individual physiological responsiveness to seasonality (i.e., seasonal body mass variation) further support our hypothesis of costly seasonal transitions shaping mortality trajectories. Indeed, old males underwent smaller physiological (mass) transitions between seasons than younger males, and their mortality was correspondingly less impacted by seasonal acceleration. Overall, we are confident that the increased mortality of accelerated individuals was not attributable to changes in reproductive costs or to abnormal physiological responses to seasonal acceleration.

Accelerated mouse lemurs exhibit an acceleration of physiological transitions associated with increased mortality. However, do they age faster? The accelerated individuals exhibited an early emergence of phenotypes linked to ageing. Melatonin production and the suprachiasmatic nucleus response to light have been found to decrease more rapidly with age in accelerated mouse lemurs (Aujard *et al.* 2001). As for mortality, these declines depend on the number of seasons experienced rather than chronological age. Another study showed that accelerated mouse lemurs show disruptions of their circadian

rhythmicity similar that those observed in aged reference individuals (Cayetanot *et al.* 2005). Here, we demonstrate that accelerated seasonal rhythm leads to an increase in mortality but not a clear increase in ageing rate (i.e., an increase in the increase of mortality with age; always true in AFT models but not in PH models). The implications of PH models in terms of ageing are more ambiguous and continue to be debated (e.g., Kraus *et al.* 2013). However, the fact that accelerated seasonal rhythm leads to the early appearance of phenotypes associated with ageing is consistent with the possibility that the observed proportional increase in mortality with age in accelerated individuals reflects more rapid ageing. If true, this would support our hypothesis that increased frequency of costly state transitions may translate into accelerated ageing.

To further support our hypothesis, future studies will have to demonstrate that (i) our results can be extrapolated to a slow-down of seasonal rhythm; (ii) the increase in mortality is proportional to the strength and/or complexity of physiological remodelling at seasonal transitions, with potentially no cost in poorly seasonal species; and (iii) seasonal physiological remodelling induces proximate, physiological costs that compromise organismal functionality over time (these costs will need to be identified). To assess the (i) generality of our results, the experimental slowing of seasonal rhythm should be performed. Reducing the frequency of seasonal transitions should reduce mortality (instead of increasing it, as observed in our seasonal acceleration experiment). Unfortunately, to our current knowledge, no experiment aiming at studying the effect of slowed seasonal rhythm on mortality has yet been conducted. To assess (ii) the transferability of our results, our seasonal acceleration experimental paradigm should be applied to other species with a similar magnitude of physiological remodelling at seasonal transitions. Mortality should increase as seasonal rhythm increases. The manipulations of photoperiod that are most similar to those of the present study are the artificial reductions of the season length to shorten the interbirth interval and thus increase production in zootechnics. Reducing season length is likely to impact survival; however, these photoperiodic manipulations are aimed at increasing the reproductive effort per year, and the deleterious effect on survival due to seasonal acceleration is confounded with the survival costs of increased reproductive effort (Pelletier *et al.* 2009). Seasonal rhythm acceleration experiments should also be implemented on virtually non-seasonal organisms. We predict that modifying the seasonal rhythm of such organisms should not affect their mortality because photoperiodic changes induce only minor physiological changes, with negligible costs. The effect of the frequency of seasonal transition on mortality should increase in strength with the extent of physiological transitions. Such seasonal rhythm acceleration experiments are impossible to conduct on wild animals. And even if poorly seasonal systems exist, natural responses would still be confounded by covariation with reproductive costs, and exposure to competition, extrinsic mortality (Liow *et al.* 2009; Turbill *et al.* 2011; Tidière *et al.* 2016), or hypometabolism-based reduction of physiological ageing (Turbill *et al.* 2012). We propose that the observed latitudinal gradient in pace of life, with low-latitude organisms exhibiting longer lifespans, may be attributable in part to latitudinal differences in seasonal rhythm. The strength of seasonality mainly varies according to latitude (Ricklefs & Wikelski 2002; Hut *et al.* 2013). At high latitudes, individuals are constrained to reproduce during a very short time period (approximately three-month duration), whereas individuals from tropical latitudes can spread their reproductive effort over a longer time period (more than six months) or even continuously throughout the year (Tieleman *et al.* 2006). In birds, individuals from the tropics live longer than do temperate conspecifics and congeners (Møller 2007; Wiersma *et al.* 2007). The current explanation for this tropical-temperate difference in the pace-of-life syndrome focuses on the reduced cost of reproduction in the tropics (Ricklefs & Wikelski 2002). However, our hypothesis of costly transitions offers an alternative explanation: the relaxed seasonal rhythm in the tropics imposes weaker seasonal physiological transitions and weaker temporal constraints on the timing of annual life history stages. Weaker transitions would result in the reduced accumulation of damage during transitions between the resilient state and the reproductive state, whereas the relaxed seasonality would allow the adjustment of seasonal state alternations on an individual basis, with some individuals reproducing less frequently than others (i.e., slowing their seasonal rhythm) and thereby maintaining low survival costs. Eventually, (iii) future studies must demonstrate that seasonal transitions are indeed damageing for the organism. We evidenced a cost of frequent seasonal transitions, but the proximate (e.g., oxidative stress; Monaghan *et al.* 2009) and integrative mechanisms (e.g., metabolic deregulations, function antagonisms, phenotype-environment mismatches) are yet to be demonstrated. The costs of seasonal transitions may arise at the cellular and organismal level. The inactive-to-active state transition is likely to induce more damages than the reverse transition, as observed in mechanical systems where dysfunctions often occur when devices are switched on - not when they are switched off. Whether mortality aggregates at seasonal transitions or accumulates progressively over time also remains unclear. To evaluate this issue using our data, we would need to conduct survival analyses that are able to estimate bursts of mortality as a function of the frequency of these bursts. However, to our knowledge, no such model has yet been developed.

To conclude, in grey mouse lemurs, biological age depends on the number of seasonal transitions they experience, which supports our hypothesis of costly state transitions. The adaptation to seasonal fluctuations would entail this cost. Our results provide the first demonstration of the fundamental role that transitions between metabolic states play in shaping mortality and indicate that organisms not only age gradually over time but also age as they seasonally change their physiological states. In the present study, these state changes were seasonal, but such state changes can also be ontogenic stage transitions (Horvitz & Tuljapurkar 2008). Our hypothesis and findings suggest to view somatic ageing as the consequence of both continuous *and* discontinuous accumulations of damage through time, respectively, driven by the pace of continuous resource allocation to fuel life, and the frequency and strength of state-specific drastic, resource reallocation between competing functions. Our findings emphasise the role of temporal

physiological transitions as a major mediator between the biological and chronological ages of an organism. At the same age, individuals that undergo large metabolic fluctuations are biologically older than individuals with stable, regular metabolic output. The actual proximate (physiological) mechanisms responsible for this cost in terms of mortality due to seasonal transitions remain to be identified.

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AUTHORSHIP

MP, SP and P-YH jointly conceived the original study. MP supervised the data collection process and captive population management. IH created and updated the database. SP, CGC and JL developed the statistical models, and JL and SP conducted the analyses. JL, SP and P-YH interpreted the results and wrote the manuscript.

DATA ACCESSIBILITY

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.km2s0.

DISCLOSURES

The authors have no conflicts of interest to declare.

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