

RESEARCH ARTICLE

Fat content of striped mice decreases during the breeding season but not during the food-restricted dry season

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ABSTRACT

Individuals that are capable of accumulating appropriate fat stores are assumed to have selective advantages when food becomes scarce. Similarly to species from temperate zones, some species inhabiting arid areas accumulate fat stores prior to periods of food limitation. Yet, we have little knowledge concerning seasonal variation in body composition and the relationship between fat store size and disappearance risk in species from arid habitats. Using the water dilution method, we examined the body composition of African striped mice (*Rhabdomys pumilio*) living in a seasonal habitat with a long food-restricted dry season. We tested for seasonal changes in body composition ($N=159$ measurements of 113 individuals) and whether dry season survival was related to fat mass ($N=66$ individuals). Fat stores were similar in size at the onset and the end of the dry season, but surprisingly smaller at the onset of the moist breeding season. Fat stores showed a negative relationship with food availability. Individual variation in fat stores was not associated with disappearance risk, but there was a positive association of disappearance risk with body mass. Increased disappearance risk of heavy individuals suggests elevated dispersal rates in competitive individuals. This study suggests that non-breeding philopatric striped mice do not accumulate large fat stores prior to the food-limited dry season but that they might mobilize fat stores at the onset of the breeding season to satisfy the energetic demands of reproduction and/or to decrease costs associated with larger fat stores, such as increased predation risk.

KEY WORDS: Disappearance, Energetics, Fat reserves, Lipid analysis, Method validation, Survival

INTRODUCTION

Food shortages are pervasive and re-occurring impediments encountered by most animals, especially species living in seasonal environments (Fretwell, 1972). To increase survival probability during periods of limited food availability and to fuel demands for maintenance and/or reproduction, species can rely on internal or external nutrient stores (Giannoni et al., 2001; Gutman et al., 2006; McNamara and Houston, 1990; Parker et al., 2009; Worden and Pekins, 1995; Young, 1976). For example, many species from temperate areas undergo a fattening period during

summer or autumn to sustain them during a nutritionally demanding winter (Baldwin and Kendeigh, 1938; Bednekoff and Houston, 1994; Fleharty et al., 1973). Endogenous lipid stores are the main source of energy catabolized during periods of food limitation (John, 2005; McCue, 2010, 2012; Mellanby, 1942). Quantifying energy stores and determining their influence for individual survival is of prime importance to understand how animals cope with food constraints imposed by their natural environment.

Animals have to continuously adjust physiologically to changes in their environment (called allostasis), and when energy expenditure exceeds energy acquisition, allostatic overload occurs (McEwen, 2000; McEwen and Wingfield, 2003; Romero et al., 2009). This leads to a decrease in body condition, the onset of various pathologies [e.g. reduced immune function (Tannock and Smith, 1972) and a decline in health status (Schoepf et al., 2017)] and finally, if it persists, starvation leading to death. Individuals that are capable of accumulating appropriate energy stores are assumed to have a selective advantage during energetically highly demanding periods or when energy supplies do not meet short-term needs (Lindstedt and Boyce, 1985; Young, 1976). The majority of studies that examined the relationship between fat stores and survival probability has been conducted on animals in temperate zones with a focus on their over-winter survival (Boos et al., 2002, 2005; Buskirk and Harlow, 1989; Dugan et al., 1981; Monteith et al., 2013; Murie and Boag, 1984; Piper and Wiley, 1990). For example, both mule deer *Odocoileus hemionus* (Bender et al., 2007) and subordinate great tits *Parus major* (Gosler, 1996) with larger fat stores have higher survival rates. Thus, it has been well documented that fat stores can help animals to survive the food-restricted winter in temperate zones (Bender et al., 2007; Boos et al., 2002; Murie and Boag, 1984; Waite, 1992).

Food shortages also occur in semi-deserts in the subtropics (Nagy, 1988), and some species inhabiting these areas accumulate fat stores prior to periods of food limitation. For example, several Malagasy lemurs, such as *Lemur catta*, *Eulemur* sp., *Cheirogaleus medius* and *Microcebus murinus*, as well as Australian fat-tailed dunnarts *Sminthopsis crassicaudata* have an elevated body fat content when entering periods of reduced food availability (Fietz and Dausmann, 2006; Morton, 1978; Schmid, 1999; Simmen et al., 2010). In addition to food shortages, animals from semi-arid areas also face the problem of water scarcity (Degen, 1997; Nagy, 1994). Body water plays a central role in nutrient transport, maintenance of cell volume and thermal regulation (McKinley et al., 2008; Siri, 1956) and thus, water conservation is especially important for animals from arid areas. Yet, we know little about seasonal changes in fat stores and body hydration (i.e. water conservation) from animals inhabiting semi-arid areas that simultaneously experience food scarcity and dryness. It is also important to understand the relationship between energy stores and survival of species living in such areas.

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The African striped mouse *Rhabdomys pumilio* (Sparman 1784) is an ideal species in which to examine such questions because they live in a habitat with pronounced seasonal variation in food and water availability (Schradin and Pillay, 2006). Individuals are born in spring and subsequently have to survive the food-restricted dry season during summer and autumn before they can reproduce in the following winter (moist season) (Schradin et al., 2012). Body mass of striped mice varies seasonally (Schradin and Pillay, 2005), which may indicate seasonal changes in fat mass. Approximately 70% of striped mice disappeared from our study population before reaching the end of their first year (Schradin and Pillay, 2005), while in some years, this rate is much higher at 99% (Schradin et al., 2010). Striped mice experience nutritional limitation during the dry season, which results in the mobilization of somatic reserves (Rimbach et al., 2017; Schoepf et al., 2017). This precipitates a decline in their health status, which progressively deteriorates during the dry season (Schoepf et al., 2017). Blood parameters that are indicative of nutritional state (i.e. albumin, glucose and total protein) are especially impaired in individuals that disappear during the dry season (Schoepf et al., 2017). However, we do not know to what extent striped mice store fat during the moist season to be able to cope with the coming food-restricted dry season and whether their survival is influenced by the amount of fat they store.

Our study aimed to: (1) describe seasonal changes in body composition (fat and water content); (2) assess in how far this relates to changes in food availability ($N=159$ measurements); and (3) to investigate whether the size of fat stores at the onset of the dry season is associated with disappearance probability until the subsequent breeding season ($N=66$ individuals). We hypothesized that; (1) throughout the year, variation in fat mass (FM) will show a positive relationship with variation in food availability; and (2) that striped mice with larger fat stores are more likely to survive the dry season. To determine FM in free-living striped mice, we validated the water dilution method by comparing estimates acquired via this indirect method with those obtained via the 'golden standard' method (i.e. biochemical analysis) of body composition. We aimed to determine the hydration coefficient of striped mice, a species living in a semi-arid habitat, because to date, the hydration coefficient of animals adapted to arid habitats has not been determined, which may cause errors in studies of animals that inhabit arid environments. This validation was important to ensure that estimates of body composition acquired via the water dilution method were accurate, especially in lean animals.

MATERIALS AND METHODS

Data collection on free-living striped mice

Study site and species

We collected data in the Succulent Karoo semi-desert of South Africa. Here, striped mice typically live in social groups, consisting of one breeding male, two to four breeding females and their adult philopatric offspring of both sexes, and groups can contain up to 30 adult individuals of both sexes during the dry season (Schradin and Pillay, 2004). Summer (December–February) and also most of the autumn (March–May) are hot and dry with low food availability (Schradin and Pillay, 2004, 2006). Striped mice typically breed in the austral winter/spring (August–November/December) when food is abundant. Striped mice can reach sexual maturity at around 6 weeks of age, but typically do not start reproducing in the breeding season in which they were born (Schradin and Pillay, 2004). Females show a higher level of natal philopatry than males, which are more likely to disperse than females (Solmsen et al., 2011). We studied individuals that were born in either August–December 2014

[$N=70$; data used to estimate daily energy expenditure in another study (Rimbach et al., 2018a)] or in September–December 2016 ($N=89$; Table 1).

Trapping and disappearance probability

We trapped the study population continuously throughout the study and continued after this study ended. Striped mice were trapped using Sherman-style live-traps ($26\times 9\times 9$ cm) baited with wheat bran flakes, raisins, sea salt and sunflower oil. We placed traps close to a group's nest in the morning and the late afternoon and checked them 30–45 min later. We weighed captured individuals using an electronic scale (± 0.1 g) and recorded their reproductive state (males: whether they were scrotal; females: whether nipples were visible and/or their vagina was perforate). For individual recognition, all mice were marked with ear tags (National Band and Tag Co., Newport, KY, USA). The use of experimental animals complied with South African animal welfare laws. Animals were captured, handled and euthanized ($N=24$ free-living mice were euthanized; 113 individuals were handled and subsequently released) following protocols approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (AESC 2012/37/2A extended until December 2019, AESC 2014/40/B, AESC 2017/01/02/B, Department of Environment and Nature Conservation Permit No. 0474/2017).

We classed an individual as 'disappeared' after it had not been trapped or observed for at least one year, which is more than the average life span of striped mice at our study site. We set the date it was last trapped as its day of disappearance. At the study site, the breeding season typically starts in July/August (Schradin and Pillay,

Table 1. Seasonal variation in food availability, and sample sizes for measurements of body composition and body condition for two birth cohorts of striped mouse

Birth cohort	Year	Month	Food availability	Body composition (estimated)	Body condition
2014	2014	October	4.31 \pm 1.47	3	3
	2014	November	2.81 \pm 0.86	6	6
	2014	December	2.33 \pm 0.88	5	5
	2015	January	1.85 \pm 0.90	3	3
	2015	February	1.50 \pm 0.84	16	16
	2015	March	1.13 \pm 0.73	19	19
	2015	April	1.25 \pm 0.57	–	–
	2015	May	1.80 \pm 0.89	2	2
	2015	June	2.94 \pm 1.04	2	2
	2015	July	5.88 \pm 1.72	8	8
	2015	August	6.60 \pm 1.78	6	3
Subtotal				70	67
2016	2017	January	2.49 \pm 0.66	23	20
	2017	February	1.31 \pm 0.88	–	–
	2017	March	1.09 \pm 0.74	–	–
	2017	April	1.19 \pm 0.77	–	–
	2017	May	1.50 \pm 0.78	28	25
	2017	June	1.50 \pm 0.54	–	–
	2017	July	2.30 \pm 0.83	–	–
	2017	August	7.10 \pm 1.04	23	18
	2017	September	6.40 \pm 1.58	–	–
	2017	October	3.38 \pm 1.72	15	12
Subtotal				89	75
Total				159	142

Light red indicates dry season onset, dark red indicates end of the dry season and blue indicates the onset of the moist season. Food availability is a measure of number of plant species in 4 m²; mean \pm s.d. Body condition index=body mass divided by body length. Body composition score was estimated using stable isotope dilution (water dilution method).

2004). Individuals can immigrate and disperse throughout the year, although a peak of dispersal occurs around June (Vuarin et al. 2019). Immigration is much lower than disappearance, and consequently, most mice that disappeared can be assumed to have died (Vuarin et al., 2019). Individuals that were still present on 1 June were regarded as having survived the dry season.

Food availability

Using the Braun–Blanquet method (Werger, 1974), we conducted plant surveys on the 1st and 15th of each month. The number of food plant species, for which the palatability was known from behavioural observations (Schradin and Pillay, 2006), was recorded in eight monitoring plots (4 m² each) within the field site. As a measure of food availability, we used the number of species averaged over all monitoring plots (Table 1). For analyses, we used food availability averaged over 4 weeks prior to the measurement of body composition.

Body condition index

We calculated an index for body condition as body mass divided by body length. Thus, the larger this index, the heavier an individual for its given body length. We measured body length as the distance from the tip of the nose to the anus to the nearest 1 mm. Body length measurements were available for 89.3% of the body composition measurements (142 out of 159; Table 1), and they were obtained within 6.4±9.9 days of injection with doubly labelled water (DLW) (i.e. when body mass was measured).

Estimation of body composition from body water

Using the water dilution method (Speakman, 1997), we estimated FM and fat-free mass (FFM) of 113 free-living striped mice (159 measurements; Table 1). We trapped individuals in the morning at their nest, brought them to the research station, weighed them (±0.1 g), anesthetized them with di-ethyl ether and took a first blood sample (~100 µl) from the sub-lingual vein into glass capillaries, which we then flame-sealed. After disinfecting a part of the abdomen for an injection site, we injected highly enriched DLW intraperitoneally at a dose of 3.38 g kg⁻¹ body mass (1.0 g of 97% ¹⁸O and 0.35 g of 99.9% ²H). We weighed syringes immediately before and after administration (±0.0001 g, Mettler-Toledo balance). In small vertebrates, an isotopic equilibration in body water is typically reached within 1 h (Degen et al., 1981; Poppitt et al., 1993). Thus, after 1 h, we weighed individuals (±0.1 g), anesthetized them with di-ethyl ether, took a second blood sample (~100 µl) to determine the maximum isotope enrichment. Between blood samplings, animals were kept in Sherman-style live traps (26×9×9 cm) without food and water to shield them from visual stressors. Subsequently, individuals were released at their nest. We kept sealed capillaries in a fridge at 4°C until transport to the IPHC-DEPE laboratory in Strasbourg, France for isotope analysis.

Isotope analysis was conducted as previously described in detail (Chery et al., 2015; Rimbach et al., 2018a). Blood samples were vacuum distilled for 5 min and 0.1 µl distillate was injected into an elemental analyser with thermal conversion (TC/EA, Thermo, Bremen, Germany), which was connected to a continuous flow isotope ratio mass spectrometer (IRMS-DELTA V PLUS, Thermo, Bremen, Germany). Distillates were pyrolyzed at 1400°C into H₂ and CO gas in a glass carbon tube under pure He flow at 90 ml min⁻¹. H₂ and CO were further separated at 110°C on a molecular sieve GC column before sequential analysis in the isotope ratio mass spectrometer. Results were first drift corrected and optionally a memory effect correction was applied. Results were

normalized versus the VSMOW2/SLAP2 international scale. All analyses were performed in quadruplicate. Total body water (TBW) was calculated from the ¹⁸O dilution space divided by 1.007 to correct for *in vivo* isotopic exchange (Racette et al., 1994). The average isotope dilution space ratio (*R*) was 1.029±0.018 (mean±s.d.; *N*=159). FFM was derived from TBW assuming a hydration coefficient of 73.2% (Blanc et al., 2005). FM was calculated from the difference between body mass and FFM.

Validation of the water dilution method by chemical analysis

It is challenging to accurately measure fat stores, especially in small animals and particularly in free-living individuals. To ensure our estimates obtained by the water dilution method were reliable, we compared estimates acquired via this indirect method with those obtained via a direct method of body composition measurement. This validation was important because estimates of FM were surprisingly low and we wanted to ensure that these estimates were accurate. Carcass lyophilisation is the ‘golden standard’, and a direct and accurate method for determining body reserves (Robbins, 1993), but it requires killing of the study animals in order to chemically analyse their body composition. This procedure is time consuming and the killing of animals makes it ethically undesirable, especially when working with free-living animals. In contrast, the water dilution method is indirect, less invasive and can be used repeatedly on the same individuals both in the laboratory and under free-living conditions.

For the validation study, we used a total of 54 mice (24 free-living and 30 captive individuals). We trapped 4 males and 5 females in May 2017 and 5 males and 4 females in October 2017 at Klein Goegap (Goegap Nature Reserve) that we injected with DLW (see details in the previous section ‘Estimation of body composition’). In February 2018, we also injected 8 females and 6 males from the captive colony (Garnier and Schradin, 2019) at the IPHC-DEPE in Strasbourg with DLW with the same procedure. After the second blood sample for the water dilution method was taken, animals were euthanized via cervical dislocation. In addition, we determined body composition via biochemical analysis of an extra 22 striped mice (free-living: 4 in May and 2 in October; captive: 6 females and 10 males in February 2018) for which DLW samples could not be analysed. We weighed animals and immediately placed them in a plastic bag to avoid water loss resulting from bleeding or dehydration. Carcasses were stored at -20°C until lyophilisation at the CNRS, Strasbourg, France.

For the carcass analysis, we used standard procedures applied at our laboratory (Boos et al., 2005; Mata et al., 2006). To ensure an adequate elimination of all body water, we cut the carcasses into small pieces (<1 cm³) and broke all bones. Subsequently, we freeze-dried carcasses in a freeze dryer (Christ Martin ALPHA 1-4) to constant mass for 2 weeks. We calculated TBW as the difference between fresh and dry body mass. After drying, we ground the carcasses and homogenized each one into a fine powder, which we stored at -20°C in sealed boxes until chemical analyses. Samples were lyophilized for 48 h just before analysis to eliminate any traces of water. We determined body lipid content using chloroform:methanol (2:1, v/v) solution as extraction solvent on 1 g of powdered tissue (Folch et al., 1957). Using a 100–150 mg sample, we determined the nitrogen content of the carcasses using the Kjeldahl method, and we calculated protein content as nitrogen content×6.25 (Robbins, 1993). We measured ash content on 1–2 g of sample after total combustion at 500°C for 24 h in a muffle furnace. The sum of the ash, lipid, protein, and water masses represented 98.4±1.0% of the fresh body mass. The mean *R*=1.035±0.016 (*N*=32).

Statistical analyses

We analysed all data using R v.3.5.1 (<https://www.r-project.org/>). Data are presented as means±s.d.

Body composition by direct chemical analysis

We used ANCOVAs, with body mass as a co-variate, to examine differences in FM, TBW, protein and ash between free-living and captive striped mice. We included the interaction term between condition (free-living versus captive) and body mass, and removed non-significant interaction terms.

Validation of the water dilution method

To assess whether differences in body water determinations were obtained from ^{18}O , deuterium or after lyophilisation are related to body mass, FM or FFM, we regressed delta water values (^{18}O and ^2H ; ^{18}O or ^2H and water measured after lyophilisation) with body mass, FM and FFM.

We assessed the agreement between the estimates of FM and TBW obtained via water dilution method and lyophilisation using the 95% limits of agreement (LoA) method (Altman and Bland, 1983; Bland and Altman, 2003). We used Shapiro–Wilk tests to assess whether data was normal distributed. TBW was normal distributed and we log-transformed FM to reach normal distribution which is required for the LoA (Altman and Bland, 1983). We calculated the mean difference (i.e. bias) and the LoA (bias±2 s.d.) between both methods using the function ‘bland.altman.stats’ from the package ‘BlandAltmanLeh’ (<https://cran.r-project.org/web/packages/BlandAltmanLeh/index.html>). We set *a priori* limits of agreement at 5–8% for FM and 1–2% for TBW (Schoeller, 2005). We plotted Bland–Altman plots (mean-difference plots) using the package ‘ggplot2’ (<https://cran.r-project.org/web/packages/ggplot2/index.html>).

Seasonal variation in body composition and total body water (TBW)

To assess whether FM and % TBW changed between the onset of the dry season (December 2014–March 2015, January 2017; $N=66$), the end of the dry season (May 2015 and May 2017; $N=30$) and the moist season (August 2015 and August 2017; $N=29$), we used two linear mixed models (LMM) with Gaussian error structure and identity link using the package ‘lme4’ (<https://cran.r-project.org/web/packages/lme4/index.html>). We used the square root of FM to reduce heteroscedasticity when fitting the model. We used study period (dry onset, dry end and moist) and sex as explanatory variables, and individual ID as a random factor because several individuals were sampled in more than one period. In addition, we included body mass as a covariate in the FM model. In the FM model, we included the interaction terms between sex and body mass and between body mass and study period. Moreover, we included the interaction between sex and study period in both models and removed non-significant interaction terms.

We used an additional LMM to assess the relationship between FM, food availability, body condition and sex using data collected throughout the year ($N=142$). Plotting the raw data suggested curvilinear relationships but including quadratic terms of food availability and body condition as additional fixed effects was not possible because raw data and their quadratic terms were highly correlated. A model including only the raw data and a model including only their quadratic terms produced similar AIC values and results, and thus we present the results of the model including the quadratic terms. We standardized (z -transformed) all numeric predictors for more accurate model fitting and to facilitate comparisons of model estimates (Schielzeth, 2010). We tested

interactions between all explanatory variables and removed non-significant interaction terms.

We verified models by inspecting $Q-Q$ plots and by plotting model residuals against fitted values. Prior to running each model, we checked for multi-collinearity by calculating variance inflation factors (Zuur et al., 2010) for the predictor variables using the ‘vif’ function in the car package (Fox and Weisberg, 2011). Vifs did not indicate collinearity (all vifs<2). To assess model stability, we ran diagnostics (dfbetas) that did not suggest the existence of influential cases.

Body composition and disappearance probability

We examined whether FM stored at the onset of the dry season influences disappearance probability until the subsequent breeding season using a Cox Proportional Hazards model [function ‘coxph’ of the ‘survival’ package (<https://cran.r-project.org/web/packages/survival/index.html>)]. For this analysis, we used measurements of FM collected from 66 non-breeding philopatric individuals at the onset of the dry season (December 2014–March 2015, January 2017). We used survival time (days since the measurement of body composition) as the dependent variable. The censoring date was set to the 1 June. Survival time was then calculated as the number of days from the measurement of body composition until the last trapped date or the censoring date if the individual was still present in the population and trapped later. In addition to FM, we also included body mass and sex into the model. The interactions of FM with body mass and sex were also included. We checked whether our model satisfied the proportional hazard assumption using scaled Schoenfeld residuals and assessed the significance of effects using log-likelihood ratio statistics (‘anova’ function). We assessed nonlinearity through visual inspection of Martingale residuals, which exhibited no obvious trends. We visualized significant effects of variables on hazard ratios using parametric simulations in the package ‘simPH’ (Gandrud, 2013). We used ribbons to indicate the areas containing the central 95% and 50% of 1000 simulations, reflecting an empirical confidence interval.

RESULTS

Body composition by direct chemical analysis

Carcasses of free-living striped mice (males and females combined) consisted of 3.3 g FM, 26.8 g TBW, 1.8 g ash and 7.3 g protein and carcasses of captive individuals consisted of 6.3 g FM, 32.8 g TBW, 2.0 g ash, 9.2 g protein on average (Fig. 1; see Table S1 for values as % of body mass). After controlling for body mass, captive and free-living individuals did not differ regarding FM (ANCOVA: $F=1.885$, $P=0.176$; Fig. 1A), TBW ($F=1.411$, $P=0.240$; Fig. 1B) or protein ($F=1.368$, $P=0.248$; Fig. 1D). Free-living individuals had a higher ash content ($F=5.541$, $P=0.022$; Fig. 1C). The median hydration coefficient (ratio of body water and FFM) determined via chemical analysis was $73.19\pm 1.4\%$.

Validation of the water dilution method

Delta water values (^{18}O and deuterium; ^{18}O or deuterium and water measured after lyophilisation) were not significantly related to body mass, adiposity or FFM (all $r<0.32$). When comparing measurements from chemical analysis and ^{18}O or ^2H , respectively, the mean differences for FM were 0.23 g and 0.27 g (Table 2; Fig. S1), which were equivalent to average discrepancies of 4.9% and 5.8% FM. For TBW, the mean differences were 0.65 g and 0.70 g (Table 2; Fig. S1), which were equivalent to average discrepancies of 2.1% and 2.3% TBW. Both FM and TBW were slightly underestimated by the water dilution method, but this

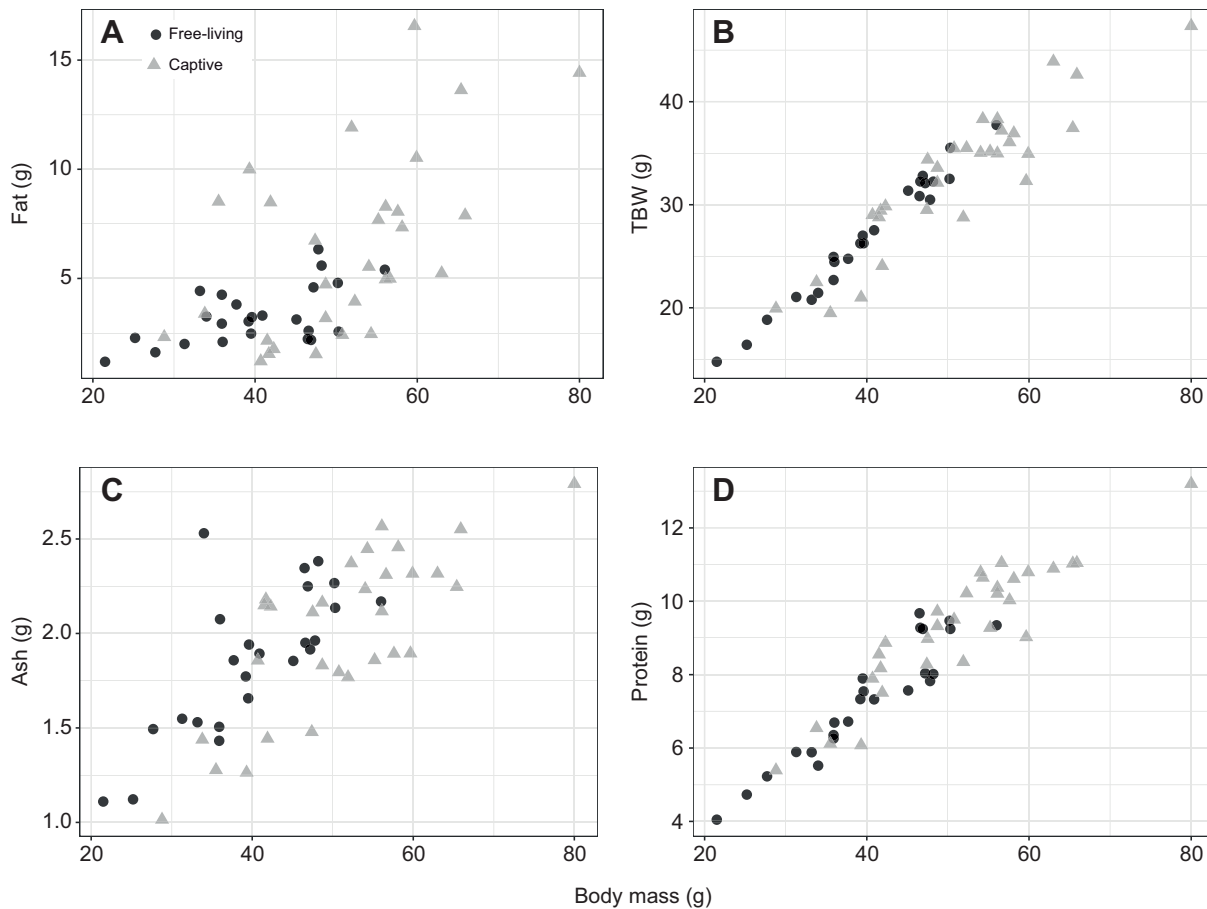


Fig. 1. Body composition of free-living and captive African striped mice (*Rhabdomys pumilio*). Comparison of (A) fat mass, (B) total body water (TBW), (C) ash and (D) protein of free-living ($N=24$, black circles) and captive ($N=30$, grey triangles) striped mice.

underestimation was within the precision limits of the water dilution method.

Seasonal variation in body composition

During the study period, average FM of striped mice was 3.1 g (0.2–10.6 g), which equalled 8.0% of striped mouse body mass (range: 0.6–26.3%). FM was similar at the onset (3.2 ± 1.9 g) and the end of the dry season (3.4 ± 1.2 g; LMM, Estimate: 0.041 ± 0.106 , $t=0.390$, $P=0.69$; Fig. 2). FM was lower at the beginning of the moist season (2.8 ± 1.6 g) than at the onset and at the end of the dry season (onset versus moist, Estimate: -0.360 ± 0.126 , $t=-2.841$, $P=0.005$; end versus moist, Estimate: -0.401 ± 0.133 , $t=-3.004$, $P=0.003$; Fig. 2). There was no difference in FM between the sexes (Estimate: -0.045 ± 0.096 , $t=-0.471$, $P=0.63$). FM increased with increasing body mass

(Estimate: 0.026 ± 0.007 , $t=3.377$, $P=0.001$; Fig. 2B). Percentage TBW was similar the onset of the dry season ($66.7\pm 3.5\%$) and the end of the dry season ($67.1\pm 2.3\%$; LMM, Estimate: 0.323 ± 0.684 , $t=0.473$, $P=0.63$). Percentage TBW was higher at the beginning of the moist season ($69.0\pm 2.4\%$) than at the onset and at the end of the dry season (onset versus moist, Estimate: 2.278 ± 0.695 , $t=3.275$, $P=0.001$; end versus moist, Estimate: 1.954 ± 0.810 , $t=2.411$, $P=0.017$). There was no difference in % TBW between the sexes (Estimate: -0.076 ± 0.559 , $t=-0.136$, $P=0.89$).

FM showed a negative relationship with food availability (Estimate: -0.083 ± 0.042 , $t=-1.976$, $P=0.051$; Fig. 3A) and a quadratic relationship with body condition (Estimate: 0.121 ± 0.044 , $t=-2.695$, $P=0.008$; Fig. 3B), where individuals with a low and a high body condition had lower FM than individuals with a medium body condition. Sex (Estimate: 0.054 ± 0.089 , $t=0.608$, $P=0.54$) did not influence FM.

Body composition and disappearance probability

During the dry season, 37.9% of the individuals disappeared and 62.1% remained in the population. FM and its interactions with body mass and sex were not associated with disappearance probability (Table 3). Body mass was positively associated with disappearance probability (Table 3, Fig. 4).

DISCUSSION

In our study, fat stores of striped mice did not change from the onset of the dry season until its end. Instead, fat stores subsequently

Table 2. Bias, critical difference and 95% confidence intervals of the bias between measurements of fat mass and total body water by chemical analysis and stable isotope dilution (water dilution method)

Comparison	Bias*	Critical difference†	95% CI of the bias
FM (L versus O)	0.2736	1.0674	0.0703–0.4770
FM (L versus H)	0.2306	0.8342	0.0717–0.3895
TBW (L versus O)	0.6528	1.76501	0.3281–0.9774
TBW (L versus H)	0.7028	1.80661	0.3704–1.0351

FM, fat mass (log-transformed; in g); TBW, total body water (in g). L, lyophilisation; O, determined via stable isotope ^{18}O ; H, determined via stable isotope ^2H . *Mean difference. †Two times s.d. of differences, equals half the difference of lower CI limit and upper CI limit.

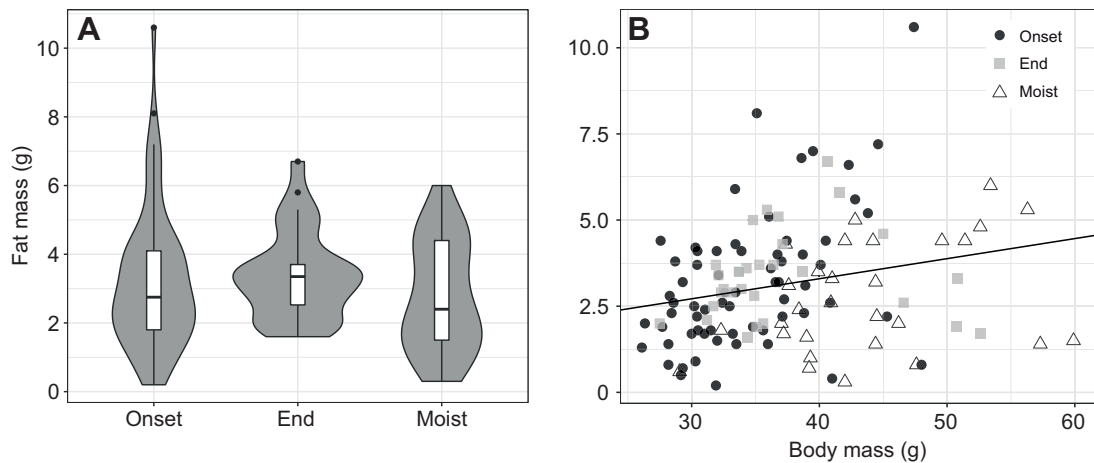


Fig. 2. Seasonal analysis of body fat in striped mice. (A) Violin plots of fat mass of striped mice at the onset ($N=66$) and end ($N=29$) of the dry season and the beginning of the moist season ($N=29$); the shaded area represents the kernel density plot, white boxplots show median values as well as 1st and 3rd quartiles and filled dots indicate outliers. (B) The relationship between body mass and fat mass ($N=125$); circles indicate data from the onset of the dry season, grey squares data from the end of the moist season and triangles data from the moist season. Linear regression line is shown ($y=0.96727+0.05825x+1.71$; $R^2=0.05$, $P=0.008$).

decreased until the beginning of the moist season. Striped mice breed in the moist season, during which they show increases in behavioural activity and in resting metabolic rate, explaining their overall higher daily energy expenditure when compared with levels in mice in the dry season (Rimbach et al., 2016, 2018a,b). Our findings suggest that they mobilize their fat stores to satisfy these energetic demands of increased maintenance costs and reproduction. Such reductions in fat stores during times when food is plentiful and the risk of starvation is low can also decrease costs associated with carrying extra fat mass, such as predation risk. Individual variation in fat content at the onset of the dry season was not associated with disappearance risk of striped mice. Together, these results suggest that striped mice do not rely on fat storage for dry season survival.

Validation

It is challenging to accurately measure fat stores, especially in small animals and particularly in free-living individuals. Our study found

that striped mice living in a semi-arid habitat have a hydration coefficient of FFM of 0.7319. This value is nearly identical to the theoretical value of 0.732. Such validation is important to accurately estimate body condition using the water dilution method in wild species living in semi-arid habitats, especially when the obtained estimates are surprisingly low. Furthermore, estimates of body water were not related to body mass, adiposity or FFM. These findings strengthen the validity of the FFM calculations and thereafter adiposity. Our validation study on striped mice showed that the mean differences ('bias') between both methods (5.3% for FM and 2.2% for TBW) lie within the *a priori* limits of agreement we expected at 5–8% for FM and 1–2% for TBW. This indicates that estimates of body composition obtained with the indirect water dilution method reflect estimates from the biochemical analysis.

Seasonal variation in body composition

Free-living striped mice showed large individual variation in adiposity, ranging from 0.6 to 23.1% of total body mass (0.2–

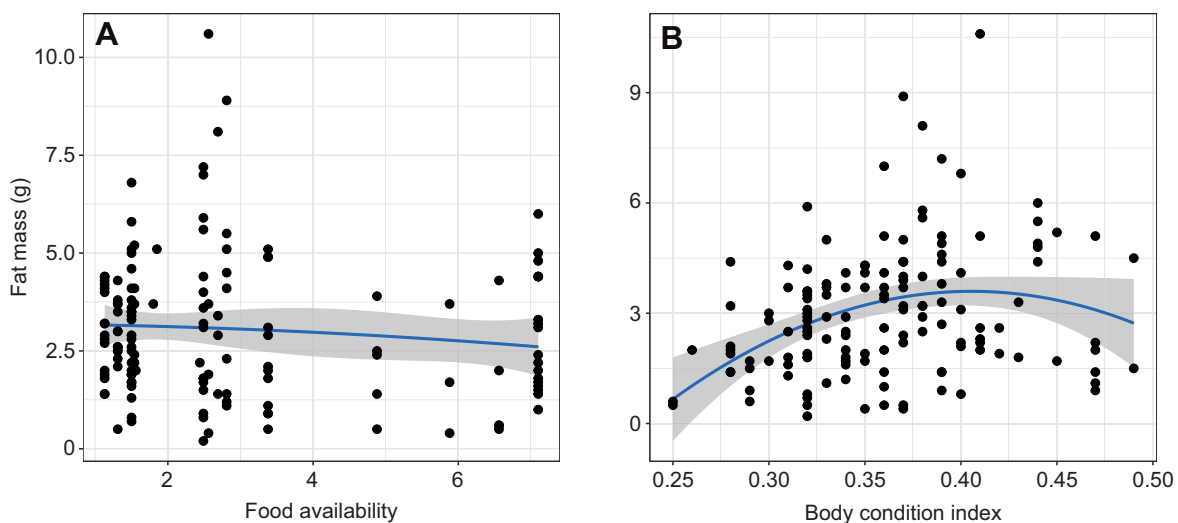


Fig. 3. Relationship of food availability and body condition with fat mass in striped mice. (A) Relationship between fat mass and food availability (mean number of food plant species per 4 m² averaged over 4 weeks prior to the measurement of fat mass). (B) Relationship between fat mass and body condition index. Blue lines indicate the quadratic regression lines and the shaded areas show the 95% confidence intervals.

Table 3. Analysis of the deviance table for the effects of fat mass at the onset of the dry season, body mass and sex, and the interactions of fat mass with each of the other covariates, on striped mice disappearance before the breeding season (Cox proportional hazards model, N=66)

Variable	χ^2	d.f.	P
Fat mass	0.7728	1	0.3793
Body mass	6.4161	1	0.0113
Sex	0.0019	1	0.9656
Fat mass×Body mass	1.5053	1	0.2199
Fat mass×Sex	1.1478	1	0.284

10.6 g) over the course of the entire study period. Average adiposity was 9% at the onset and at the end of the dry season, indicating that fat stores were not reduced throughout the food-restricted dry season. Many small rodents from temperate zones accumulate fat stores before winter, which often resembles a nutritionally challenging period, and lose fat during winter (Table 4). Fat content of *R. dilectus*, a sister species of *R. pumilio*, living in the Eastern Cape, South Africa varies between 21 and 31% of dry body mass with no clear seasonal changes, and fat content was similar to dry fat mass in our study (28.7% of dry body mass in *R. dilectus*, 24.5% in *R. pumilio*) (Perrin, 1981). The reason for the difference in seasonal variation in fat content between the two sister species remains to be determined.

Unexpectedly, we found that fat content dropped from 9% in the dry season to 6% in the moist season, even though food availability increased. Striped mice have a 43% higher energy expenditure in the moist season compared to the dry season (Rimbach et al., 2018a), and they have a higher metabolic rate and spent more time active (Rimbach et al., 2016, 2018b). There are two possible explanations for a decrease in fat content in the moist season. Firstly, striped mice might mobilize their fat stores in the moist season to satisfy the energetic demands of increased maintenance costs and reproduction (Rimbach et al., 2016, 2018a,b) as in other rodents such as muskrats *Ondam zibethicus* (Virgl and Messier, 1992), house mice *Mus domesticus* (Mutze, 1990) and old-field mice *Peromyscus polionotus* (Caldwell and Connell, 1968). Secondly, they might reduce costs

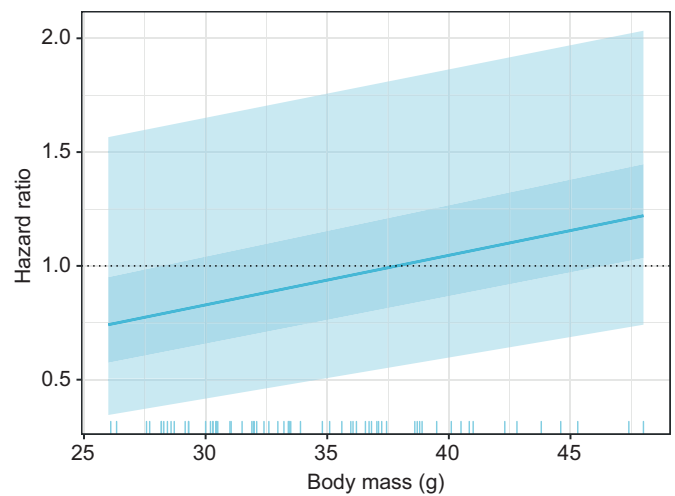


Fig. 4. Relationship between dry season disappearance risk and body mass (Cox proportional hazards model, N=66). The simulated hazard ratio (line), 50% (dark blue shaded area) and 95% (light blue shaded area) confidence intervals are shown.

associated with carrying extra fat, such as predation risk (Houston et al., 1993; Lima, 1986), in the season when food is abundant. These two explanations are not mutually exclusive because reduced fat stores due to higher maintenance costs will also decrease costs associated with larger fat stores. We found that striped mice with a low or a high body condition index (i.e. that are light or heavy for their body length, respectively) have smaller fat stores than individuals with an intermediate body condition. In sum, while average fat content of striped mice is comparable to that of small rodents inhabiting temperate areas, we found no indication that they store fat in spring to cope with the food-restricted summer dry season.

Knowledge about seasonal changes in body composition of free-living animals from the subtropics is limited. Here, we showed that fat stores of striped mice remained unchanged during the dry season, and decreased in the moist season when breeding started. These results indicate that striped mice, which live in an arid habitat with

Table 4. Overview of seasonal changes in fat content in small mammals from temperate and subtropical areas

Study species	Changes in fat content	Zone	Reference
Yellow-necked field mouse <i>Apodemus flavicollis</i>	14%* in autumn, 21% in winter, 12% in spring and summer	Temperate	Sawicka-Kapusta, 1968
Cotton rat <i>Sigmodon hispidus</i>	10% [‡] in winter, 4% at the end of spring	Temperate	Fleaharty et al., 1973
Muskrat <i>Ondam zibethicus</i>	Accumulate fat in summer and autumn, peak of 16% [§] in winter, 2% in summer	Temperate	Virgl and Messier, 1992
Harvest mouse <i>Reithrodontomys megalotis</i>	7% [‡] in winter, 6% in summer	Temperate	Fleaharty et al., 1973
Deer mouse <i>Peromyscus maniculatus</i>	6.5% [‡] in winter, 5% in summer	Temperate	Fleaharty et al., 1973
Prairie vole <i>Microtus ochrogaster</i>	No seasonal changes in fat content (4% [‡])	Temperate	Fleaharty et al., 1973
European rabbit <i>Oryctolagus cuniculus</i>	No seasonal changes in fat content (3.6% [‡])	Temperate	Boos et al., 2005
House mouse <i>Mus domesticus</i>	Accumulate fat between late autumn and early winter (8–11% [‡]), 3% in spring	Subtropical	Mutze, 1990
Ring-tailed lemur <i>Lemur catta</i>	18.6% [‡] at the end of the rainy season, 5.6% in dry season	Subtropical	Simmen et al., 2010
Fat-tailed dunnart <i>Sminthopsis crassicaudata</i>	Little caudal fat in the breeding season, caudal fat is stored in autumn	Subtropical	Morton, 1978
Striped mouse <i>Rhabdomys dilectus</i>	Variable, no clear peak in winter (average 28.7%*)	Subtropical	Perrin, 1981
Striped mouse <i>Rhabdomys pumilio</i>	9% [‡] at the onset and the end of the dry season, 6% in the moist season (24.7%* at the end of the dry season)	Subtropical	Present study

*% of dry body mass; [‡]% of wet body mass; [§]% of ingesta-free body mass.

large seasonal variation in food abundance, are well adapted to this environment. Although some species from arid habitats and many species in temperate zones increase fat stores prior to periods of food limitation (Table 4), we did not find this pattern in striped mice. Philopatric striped mice reduce their energy expenditure in the dry season by reducing their resting metabolic rate and physical activity level (Rimbach et al., 2018a). Estimates of their physical activity level are lower than predicted by allometric equations, suggesting that they display lower levels of physical activity than small mammals living in other habitat types (Rimbach et al., 2018a). Low energy expenditure may explain why philopatric striped mice did not rely on fat storage for dry season survival, at least in the two years of our study. However, we did not track changes in body composition and energy expenditure continuously throughout the dry season. Thus, it is possible that compensatory effects of energy expenditure and behavioural activity influence body composition, as has been reported in the Svalbard rock ptarmigan *Lagopus mutus hyperboreus* (Stokkan et al., 1986). Changes in the substrate type catabolized and associated production of metabolic water (i.e. from fat to carbohydrates; Frank, 1987; Takei et al., 2012) in the course of the dry season might explain why we did not find changes in fat mass and body water during the dry season. Together with previous studies (Rimbach et al., 2016; Rimbach et al., 2019), our results suggest that philopatric striped mice do not invest in fat storage but rather into somatic growth. Reaching a large body mass increases the competitive ability of striped mice (especially males) and influences their reproductive tactic (Hill et al., 2015; Schradin et al., 2009) and ultimately their fitness (Schradin and Lindholm, 2011).

Animals from semi-arid areas are faced with the problem of water scarcity (Degen, 1997; Nagy, 1994). If a lack of water hampers body water homeostasis, important physiological processes, such as nutrient transport, maintenance of cell volume and thermal regulation, can be disturbed (McKinley et al., 2008; Siri, 1956). Body water of striped mice remained unchanged during the dry season and increased in the moist season, when the rate of daily water turnover is also higher than during the dry season (Rimbach et al., 2018a). The daily water turnover rate describes the replacement of body water in a day, and is used to assess body water homeostasis. However, we were unable to estimate this rate in the current study because its measurement requires the collection of an additional blood sampling 24 h after injection with DLW. Our study shows that striped mice have a higher percentage of body water in the moist season, when most annual rain falls and fresh water-rich vegetation grows.

Body composition and disappearance probability

We did not find that individuals with smaller fat stores at the onset of the dry season were more likely to disappear than individuals with larger fat stores. This indicates that in the two study years, the survival of individuals did not depend on their fat stores. Thirty-eight per cent of the individuals disappeared up to the start of the next breeding season. The two dry seasons included in this study were harsher than the average dry season at the study site. The dry seasons of 2015 and 2017 were characterized by lower food availability and higher maximum ambient temperatures than dry seasons in the previous or subsequent years (Table S2). It is possible that under yet harsher conditions, variation in fat stores would be more predictive of the disappearance risk of individuals. For example, the harshest dry season in our long-term study occurred in 2003, when 99% of striped mice disappeared (Schradin et al., 2006, 2010), the survivors being the ancestors of our study population. It is possible that under such extreme conditions, fat stores are more important. Thus, it is likely that proximate and ultimate factors we

did not consider here played a more important role for the disappearance risk of striped mice than the size of fat stores during the two years included in the current study.

Body mass influenced disappearance risk, where heavier individuals were at a higher risk than lighter ones. Reproductive tactics of male and female striped mice are body mass dependent (Hill et al., 2015; Schradin et al., 2009). The most competitive (i.e. heaviest) males typically disperse into neighbouring territories where they become breeders (Schradin and Lindholm, 2011), and individuals of intermediate size with lower competitive ability are more likely to disperse over larger distances compared with heavy individuals, whereas small individuals do not disperse (Solmsen et al., 2011). The majority (85%) of individuals weighing <30 g remained in the population, whereas 42% weighing 30–45 g and 66% of individuals >45 g disappeared. Thus, light individuals remained in the population, and individuals of intermediate and large size disappeared either via dispersal or predation. Storing large amounts of fat increases an individual's body mass. This can incur a cost in terms of an increased mortality risk because of a higher vulnerability of predation (Houston et al., 1993; Lima, 1986), which some species avoid by decreasing their body mass in the presence of a predator (Carlsen et al., 1999; Gosler et al., 1995; Zimmer et al., 2010, 2011). Taken together, our study suggests that both dispersal patterns and predation risk might explain the observed relationship between disappearance risk and body mass.

Conclusions

Our study contributes information about seasonal variation in body composition and its dependence on environmental factors in species from arid habitats. In our two study years, larger fat stores at the onset of the dry season did not generate a selective benefit in the form of a reduced disappearance risk, nor did they decline during the dry season. Animals from arid habitats may be restricted in the amount of fat they can store because large amounts of subcutaneous fat could interfere with temperature regulation via the prevention of heat loss (Young, 1976), explaining the evolution of fat-tailed species. It is possible that under extremely harsh conditions, variation in fat stores would be more predictive of the disappearance risk of striped mice. This study suggests that philopatric striped mice do not invest into fat storage but rather into somatic growth, which can directly influence their reproductive tactic and thus fitness. Accordingly, fat stores were reduced in the moist season, which is likely to satisfy the high energetic demands of reproduction.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: R.R., S.B., J.-P.R., N.P., C.S.; Methodology: R.R., S.B., A.Z., J.-P.R., C.S.; Validation: S.B., J.-P.R.; Formal analysis: A.Z., J.-P.R.; Investigation: R.R.; Resources: S.B., A.Z., J.-P.R., N.P., C.S.; Data curation: R.R.; Writing - original draft: R.R.; Writing - review & editing: R.R., S.B., A.Z., J.-P.R., N.P., C.S.; Visualization: R.R.; Supervision: N.P., C.S.; Project administration: N.P., C.S.; Funding acquisition: S.B., J.-P.R., N.P., C.S.

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Supplementary information

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