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Stress, Health and Senescence in a long-lived mammal, the roe deer (*Capreolus capreolus*)

Lucas D Lalande

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**THESE de DOCTORAT DE
L'UNIVERSITE CLAUDE BERNARD LYON 1**

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**Stress, Health and Senescence in a long-lived
mammal, the roe deer (*Capreolus capreolus*)**

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Photography in the present manuscript have been used with the permission of **Gaspard Dussert** (do not hesitate to check his Instagram @gasp.in.nature, his work is amazing) and **François Débias**. Many thanks.

Table of contents

ABSTRACT	- 5 -
RESUME	- 7 -
LIST OF ARTICLES	- 9 -
LIST OF FIGURES, BOXES AND TABLES	- 10 -
LIST OF APPENDICES	- 11 -
LIST OF ORAL PRESENTATIONS AND POSTERS	- 12 -
INTRODUCTION	- 13 -
1. STRESS RESPONSE	- 14 -
<i>a) From homeostasis to allostasis</i>	- 14 -
i. Homeostasis	- 14 -
ii. Homeostasis and stress	- 15 -
iii. Allostasis	- 16 -
<i>b) The stress response</i>	- 19 -
i. Definitions	- 19 -
ii. The stress response	- 19 -
<i>c) Glucocorticoids and the stress response</i>	- 20 -
i. Physiological axes of the stress response	- 20 -
ii. Roles of glucocorticoids at baseline and stress-induced levels	- 21 -
iii. Glucocorticoids: acute and chronic stress	- 22 -
2. STRESS RESPONSE AND LIFE-HISTORY TRAITS	- 26 -
<i>a) Integrating physiology and ecology</i>	- 26 -
<i>b) The adaptive and maladaptive facets of the stress response</i>	- 28 -
<i>c) The consequences of GCs on fitness and individual performances</i>	- 29 -
i. Baseline levels	- 29 -
ii. Acute stress-induced levels	- 29 -
iii. Chronic stress-induced levels	- 30 -
<i>d) Early-life glucocorticoids and their long-term consequences</i>	- 31 -
3. IMPLICATIONS OF GCs FOR PHYSIOLOGY AND HEALTH	- 33 -
<i>a) GCs and body mass/condition</i>	- 33 -
i. The interest of studying body mass	- 33 -
ii. The implications of GCs on body mass	- 34 -
<i>b) GCs and physiological parameters</i>	- 36 -
i. Physiological parameters as proxies of body condition	- 36 -
ii. Implications of GCs for haematological parameters	- 37 -
<i>c) GCs and immunity and immunosenescence</i>	- 39 -

i. The immune system	- 39 -
ii. The immune system as a life-history trait	- 41 -
iii. Immunosenescence	- 42 -
iv. Implications of GCs for immunity and immunosenescence	- 44 -
4. SYNTHESIS AND STRUCTURE OF THE THESIS	- 48 -
a) <i>Synthesis and objectives</i>	- 48 -
b) <i>Structure of the thesis</i>	- 50 -
CHAPTER 1 – STUDY SYSTEM AND MATERIAL & METHODS	- 53 -
1. STUDY SYSTEM, THE ROE DEER	- 54 -
a) <i>General description and geographic distribution</i>	- 54 -
b) <i>Diet</i>	- 55 -
c) <i>Survival</i>	- 55 -
d) <i>Reproduction</i>	- 55 -
2. TWO CAPTURE-MARK-RECAPTURE PROGRAMS	- 57 -
a) <i>Study sites</i>	- 57 -
b) <i>Capture protocol</i>	- 58 -
3. PHYSIOLOGICAL MEASUREMENTS	- 61 -
a) <i>Measuring stress in the wild</i>	- 62 -
i. Blood measurements and other matrices	- 62 -
ii. Faecal glucocorticoid metabolites (FGMs)	- 63 -
iii. N:L ratio	- 65 -
b) <i>Physiological measurements</i>	- 67 -
c) <i>Immune measurements</i>	- 68 -
i. Innate immunity	- 68 -
ii. Adaptive immunity	- 70 -
d) <i>Parasitic loads</i>	- 71 -
4. INDIVIDUAL CHARACTERISTICS AND ENVIRONMENTAL CONDITIONS	- 72 -
CHAPTER 2 – GLUCOCORTICOIDS AND N:L RATIOS IN RELATION TO BODY MASS	- 73 -
1. GLUCOCORTICOIDS NEGATIVELY RELATE TO BODY MASS ON THE SHORT-TERM IN A FREE-RANGING UNGULATE	- 74 -
2. N:L RATIO AND BODY MASS IN A LONG-LIVED UNGULATE: A RELEVANT ALTERNATIVE?	- 89 -
CHAPTER 3 – GLUCOCORTICOIDS AND THE SENESCENCE OF PHYSIOLOGICAL CONDITION	- 108 -
CHAPTER 4 – EARLY-LIFE GLUCOCORTICOIDS AND IMMUNOSENESCENCE	- 137 -
DISCUSSION	- 173 -
1. SUMMARY OF THE RESULTS AND OUTLINE	- 174 -
2. A CONSOLIDATION OF THE PHYSIOLOGY/LIFE-HISTORY NEXUS	- 177 -
a) <i>The evolutionary ecology relevance of the stress response</i>	- 177 -
b) <i>On the importance of longitudinal studies</i>	- 178 -
3. TOWARDS A MORE COMPREHENSIVE ESTIMATION OF CHRONIC STRESS	- 180 -

<i>a) GCs do not equate to stress</i>	- 180 -
<i>b) Estimating chronic stress in the wild</i>	- 182 -
<i>c) Early-life GCs and later fitness</i>	- 183 -
<i>d) Methodological drawbacks of using FGMs</i>	- 184 -
<i>e) Measuring allostasis</i>	- 186 -
4. MECHANISMS LINKING GCs AND PHYSIOLOGICAL DETERMINANTS OF SENESCENCE	- 188 -
<i>a) Chronic stress and senescence</i>	- 190 -
<i>b) A conceptual framework linking GCs and cellular senescence</i>	- 191 -
<i>c) Exploration of the link between GCs, oxidative stress and telomere length</i>	- 193 -
i. Methods	- 193 -
ii. Results	- 194 -
iii. Interpretation, conclusion and perspectives	- 195 -
5. RECOMMENDATIONS TO DETECT ASSOCIATIONS BETWEEN CHRONIC STRESS AND FITNESS	- 198 -
<i>a) Life-history contexts and resource acquisition</i>	- 198 -
<i>b) Experimental manipulations</i>	- 199 -
<i>c) Is chronic stress a reality in the wild?</i>	- 201 -
<i>d) Fitness metrics</i>	- 203 -
CONCLUSION	- 204 -
ACKNOWLEDGEMENTS	- 205 -
REFERENCES	- 210 -
APPENDICES	- 233 -
APPENDIX A – CAPTURE FORM	- 234 -
APPENDIX B – SUPPLEMENTARY INFORMATION: GLUCOCORTICOIDS AND BODY MASS	- 237 -
APPENDIX C – SUPPLEMENTARY INFORMATION: N:L RATIOS AND BODY MASS	- 257 -
APPENDIX D – SUPPLEMENTARY INFORMATION: GLUCOCORTICOIDS AND PHYSIOLOGICAL CONDITION	- 274 -
APPENDIX E – SUPPLEMENTARY INFORMATION: GLUCOCORTICOIDS AND IMMUNOSENESCENCE	- 280 -
APPENDIX F – SIDE PROJECT: SARS-CoV-2 IN FREE-RANGING ROE DEER	- 286 -
APPENDIX G – PREVIOUS PUBLICATION: BODY MASS SENESCENCE IN ASIAN ELEPHANTS	- 303 -

Abstract

Stress, health and senescence in a long-lived mammal, the roe deer (*Capreolus capreolus*)

The stress response – a suite of physiological and behavioural adjustments – allows organisms to support heightened energy requirements associated with unpredictable environmental challenges (*i.e.* stressors). The stress response involves the activation of different physiological systems, among which the most studied is the hypothalamic-pituitary-adrenal axis leading to the enhanced production of glucocorticoids (GCs). At baseline levels, the primary role of GCs is energy mobilisation and regulation. These hormones act as mediators of allostasis, the active process of reaching differential homeostatic states according to physiological demands. Following exposure to a short-term, temporary stressor (*i.e.* acute stress), increased GC levels favour energy allocation towards functions promoting immediate survival at the expense of other non-immediately essential functions (*e.g.* growth, reproduction), and is therefore adaptive. On the other hand, individuals repeatedly exposed to stressors, or exposed to stressors that last for longer (*i.e.* days-weeks) can have elevated baseline GC levels. They enter into states of chronic stress during which resources are deviated away from physiological functions such as growth, reproduction or immunity on the long-term, with expected adverse consequences on fitness. Measuring states of chronic stress in the wild is challenging and associations between GCs and fitness-related traits are equivocal due to the influence of life-history and environmental contexts on such relationships.

Throughout my Ph.D., I aimed at evaluating the relationships between baseline GC levels and fitness-related traits reflecting individual health (*i.e.* body mass, physiological condition, immunity), and their senescence patterns in a long-lived mammal. This work was conducted in two roe deer (*Capreolus capreolus*) populations experiencing markedly contrasted environmental conditions, and which have been longitudinally followed at the individual-based level.

I evidenced that GCs related to health parameters on the short-term (*i.e.* when measured at the same sampling event), but that GCs did not have carry-over effects on the senescence of those traits. In a first chapter, I showed that GCs were negatively associated to body mass in adults, but not juveniles, of both populations and both sexes, supporting the idea that it is essential to take into account the life-history stage when evaluating relationships between GCs and life-history traits. I also found that the Neutrophil:Lymphocyte (N:L) ratio – another biomarker increasingly used as a proxy of the stress response – related negatively to body mass on the medium term (*i.e.* change in individual body mass between two consecutive years) during the late growth phase. This suggests that GCs and N:L ratios describe different components of the stress response and are not interchangeable. In a second part, I showed that GCs positively related to creatinine levels (*i.e.* a proxy of muscle mass and renal efficacy) on the short-term, suggesting an increased catabolic state in individuals with elevated GCs. I also found a sex-

specific association between GCs and albumin, the main blood protein, suggesting that sex hormones can modulate GC-life-history trait relationships. In a third chapter, I found that GCs measured during early-life did not modulate patterns of immunosenescence but were only positively associated to the abundance of lung parasites in adults.

In the last chapter of this thesis, I discuss the relevance of the present work for the field of ecophysiology. I have investigated the associations between GCs and various fitness-related traits at different temporal scales, using longitudinal data on a free-ranging mammal. This allowed to account for much of the sources of variations identified in the literature. I then explore and develop future research perspectives, both for the roe deer program, and for research in general, and I argue that assessments of chronic stress in wild animals should ideally be done through the construction of indices including several biomarkers reflecting the whole stress response. Furthermore, I explore the physiological mechanisms linking GCs and senescence by incorporating recent insights about the link between GCs and telomere attrition. I advocate to develop such mechanistic approaches to identify relevant biomarkers of chronic stress and to better predict its consequences. Finally, I conclude with some recommendations for researchers that aim at evaluating GC-fitness relationships.

Keywords: ecophysiology, stress response, cort-fitness hypothesis, allostasis, life history, individual performances, ungulates

Résumé

Stress, santé et sénescence chez un mammifère longévif, le chevreuil (*Capreolus capreolus*)

La réponse au stress – une série d’ajustements physiologiques et comportementaux – permet aux organismes de répondre aux exigences énergétiques accrues liées aux défis environnementaux imprévisibles (*i.e.* stressseurs). La réponse au stress implique l’activation de systèmes physiologiques, parmi lesquels le plus étudié est l’axe hypothalamo-pituitaire-surrénalien, qui entraîne une production accrue de glucocorticoïdes (GCs). A niveaux basaux, les GCs ont pour rôle principal la mobilisation et la régulation de l’énergie. Ils agissent comme médiateurs de l’allostase, le processus actif permettant l’établissement et la réalisation d’états homéostatiques en fonction des exigences physiologiques. Suite à une exposition à un stressseur temporaire et de courte durée (*i.e.* stress aigu), l’augmentation des niveaux de GCs contribue à mettre à disposition des ressources énergétiques pour des fonctions favorisant la survie immédiate au détriment d’autres fonctions non immédiatement essentielles (comme la croissance ou la reproduction), et est donc adaptatif. En revanche, les individus exposés de manière répétée à des stressseurs ou exposés à des stressseurs de longue durée (*i.e.* jours-semaines) peuvent présenter des niveaux basaux de GCs élevés et entrer dans des états de stress chronique, au cours desquels les ressources sont détournées des fonctions physiologiques telles que la croissance, la reproduction ou l’immunité sur le long terme, avec des conséquences préjudiciables attendues sur la valeur sélective. Mesurer les états de stress chronique dans la nature pose un problème complexe, et les associations entre les GCs et les traits liés à la valeur sélectives sont équivoques, notamment en raison de l’influence des contextes liés à l’histoire de vie et de l’environnement sur de telles relations.

Pendant ma thèse, j’ai cherché à évaluer les relations entre les niveaux de GCs basaux et des traits liés à la valeur sélective et reflétant la santé des individus (*i.e.* masse corporelle, condition physiologique, immunité), et la sénescence de ces traits chez un mammifère sauvage longévif. Ce travail a été mené dans deux populations de chevreuils (*Capreolus capreolus*) présentant des conditions environnementales nettement contrastées, et qui ont été suivies longitudinalement au niveau individuel.

Ce travail a permis de montrer que les GCs étaient liés aux paramètres de santé à court terme (c’est-à-dire mesurés lors de la même prise d’échantillon), mais que les GCs n’avaient pas d’effets durables sur la sénescence de ces traits. Dans un premier chapitre, j’ai démontré que les GCs étaient négativement associés à la masse corporelle chez les adultes, mais pas chez les juvéniles, des deux populations et des deux sexes, ce qui soutient l’idée que le stade de développement est essentiel à prendre en compte lors de l’évaluation des relations entre les GCs et les traits liés à l’histoire de vie. Cependant, j’ai aussi mis en évidence que le ratio Neutrophile:Lymphocyte (N:L) – un autre biomarqueur de plus en plus utilisé comme

indicateur de la réponse au stress – était négativement lié à la masse corporelle à moyen terme (*i.e.* changement de la masse corporelle individuelle entre deux années consécutives) pendant la phase de croissance tardive. Cela suggère que les GCs et les ratios N:L décrivent des aspects différents de la réponse au stress et ne sont pas interchangeables. Dans un second temps, j'ai montré que les GCs étaient positivement liés aux niveaux de créatinine (*i.e.* un indice de la masse musculaire et du fonctionnement rénal) à court terme, suggérant un état catabolique accru chez les individus présentant des valeurs élevées de GCs. J'ai également trouvé une association sexe-spécifique entre les GC et l'albumine, la principale protéine sanguine, ce qui suggère que les hormones sexuelles peuvent moduler les relations entre GCs et traits d'histoire de vie. Enfin, dans un troisième chapitre, j'ai constaté que les GCs mesurés au début de la vie ne modulaient pas les trajectoires d'immunosénescence mais étaient uniquement positivement associés à l'abondance des parasites pulmonaires chez les adultes.

Dans le dernier chapitre de cette thèse, je discute de la pertinence du présent travail pour le domaine de l'écophysiologie. J'ai étudié les associations entre les GCs et une variété de traits liés à la valeur sélective, à différentes échelles temporelles, et en utilisant des données longitudinales obtenues sur mammifère sauvage, permettant de tenir compte de nombreuses sources de variation identifiées dans la littérature. J'explore et développe également des orientations de recherche futures, tant pour ce programme de recherche sur le chevreuil que pour la recherche en général, et j'insiste sur l'importance d'évaluer le stress chronique chez les animaux sauvages par le biais de la construction d'indices incluant plusieurs biomarqueurs reflétant l'ensemble de la réponse au stress. Dans cette dernière partie, j'explore également les mécanismes physiologiques liant les GC à la sénescence en intégrant des connaissances récentes sur le lien entre GCs et raccourcissement des télomères. Je plaide en faveur du développement de telles approches mécanistiques pour identifier des biomarqueurs pertinents du stress chronique et mieux prédire les conséquences du stress. Enfin, je conclus cette thèse par quelques recommandations pour les chercheurs qui cherchent à évaluer les relations entre les GCs et la valeur sélective.

Mots clés : écophysiologie, réponse au stress, hypothèse cort-fitness, allostasie, histoire de vie, performances individuelles, ongulés

List of articles

CHAPTER 2

Lalande, L. D., Gilot-Fromont, E., Carbillet, J., Débias, F., Duhayer, J., Gaillard, J.-M., Lemaître, J.-F., Palme, R., Pardonnet, S., Pellerin, M., Rey, B. and Vuarin, P. 2023. Glucocorticoids negatively relate to body mass on the short-term in a free-ranging ungulate. *Oikos* **2023**(10): e09769.

Lalande, L. D., Carbillet, J., Débias, F., Gaillard, J.-M., Garcia, R., Lemaître, J.-F., Pellerin, M., Rey, B., Vuarin, P. and Gilot-Fromont, E. 2023. Neutrophil:Lymphocyte ratio may reflect body condition and growth rates rather than stress response in a wild ungulate. *In preparation*.

CHAPTER 3

Lalande, L. D., Carbillet, J., Débias, F., Gaillard, J.-M., Garcia, R., Lemaître, J.-F., Pellerin, M., Rey, B., Palme, R., Vuarin, P. and Gilot-Fromont, E. 2023. Glucocorticoids covary positively with haematological indices of body condition, but do not modulate patterns of haematological senescence in a wild ungulate. *In preparation*.

CHAPTER 4

Lalande, L. D., Bourgoïn, G., Carbillet, J., Cheynel, L., Débias, F., Ferté, H., Gaillard, J.-M., Garcia, R., Lemaître, J.-F., Palme, R., Pellerin, M., Peroz, C., Rey, B., Vuarin, P. and Gilot-Fromont, E. 2023. Glucocorticoids and immunosenescence patterns in a wild ungulate. *In preparation*.

APPENDICES

Perez, G. & **Lalande, L. D.**, Bourgoïn G., Legros, V., Kodjo, A., Verheyden, H., Bourret, V., Pellerin, M., Lemaître, J.-F., Peroz, C., Rey, B., Débias, F., Garcia, R. and Gilot-Fromont, E. 2023. Likely circulation of a coronavirus in roe deer (*Capreolus capreolus*) populations suggested by SARS-CoV-2 serological investigation in France. *In preparation*.

Lalande, L. D., Lummaa, V., Aung, H. H., Htut, W., Nyein, U. K., Berger, V. and Briga, M. 2022. Sex-specific body mass ageing trajectories in adult Asian elephants. *Journal of Evolutionary Biology* **35**(5): 752-762.

List of figures, boxes and tables

Figure 1. Homeostasis	- 14 -
Figure 2. General Adaptation Syndrome	- 16 -
Figure 3. Allostasis	- 18 -
Figure 4. HPA axis	- 23 -
Figure 5. GC response	- 24 -
Box 1. GC-fitness hypotheses	- 25 -
Figure 6. Physiology/life-history nexus	- 27 -
Figure 7. Vertebrate immune system	- 41 -
Box 2. Eco-immunology	- 42 -
Figure 8. GCs and immunity	- 46 -
Box 3. Evolutionary theories of senescence	- 47 -
Figure 9. Roe deer geographic distribution	- 54 -
Figure 10. Sexual dimorphism in roe deer	- 56 -
Figure 11. CMR program in Chizé and Trois-Fontaines	- 58 -
Box 4. Authorisations and ethics	- 58 -
Figure 12. Manipulations	- 60 -
Figure 13. Release	- 61 -
Box 5. FGMs extraction	- 65 -
Box 6. HA-HL assays	- 69 -
Table 1. Results summary	- 176 -
Table 2. Allostatic load biomarkers	- 189 -
Figure 14. Conceptual framework linking GCs and senescence	- 193 -
Figure 15. Preliminary results: GCs, oxidative stress and telomere length	- 197 -
Figure 16. GCs and environment covariations	- 200 -

List of appendices

Appendix A – Capture form

Appendix B – Supplementary information: Glucocorticoids and body mass

Appendix C – Supplementary information: N:L ratios and body mass

Appendix D – Supplementary information: Glucocorticoids and physiological condition

Appendix E – Supplementary information: Glucocorticoids and immunosenescence

Appendix F – Side project: SARS-CoV-2 in free-ranging roe deer

Appendix G – Previous publication: Body mass senescence in Asian elephants

List of oral presentations and posters

POSTERS

Lalande, L. D., Gaillard, J. M., Lemaître, J. F., Gilot-Fromont, E., Vuarin, P. 2021. *Linking baseline stress levels and health in a long-lived ungulate, the roe deer (Capreolus capreolus).* **Young Researchers in Biology of AURA Region Congress.** Lyon, France (attended remotely).

Lalande, L. D., Gaillard, J. M., Lemaître, J. F., Pellerin, M., Gilot-Fromont, E., Vuarin, P. 2021. *The grass is always greener on the other side my deer.* **BES Ecology Across Borders 2021.** Liverpool, UK (attended remotely).

Lalande, L. D., Gaillard, J. M., Lemaître, J. F., Pellerin, M., Gilot-Fromont, E., Vuarin, P. 2022. *The grass is always greener on the other side my deer.* **Ecology & Behaviour Meetings.** Strasbourg, France.

ORAL PRESENTATIONS

Lalande, L. D., Lummaa, V., Aung, H. H., Htut, W., Nyein, U. K., Berger, V. and Briga, M. 2022. *The weight of getting old in Asian elephants: Body mass ageing trajectories differ between sexes.* **Decryp'thèse 2022.** Lyon, France.

Lalande, L. D., Gaillard, J. M., Lemaître, J. F., Pellerin, M., Bourgoïn, G., Vuarin, P., Gilot-Fromont, E. 2023. *Immunosenescence in a wild mammal: Is stress impacting the way you get old?* **EMPSEB 28.** Glasgow, Scotland.

Introduction



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1. Stress response

a) From homeostasis to allostasis

i. Homeostasis

Stability is the key to the maintenance of life. The French physiologist Claude Bernard developed the idea of internal environment (*i.e. milieu intérieur*), in opposition to the external environment. According to his view, the internal environment, composed of interstitial fluids, plasma and lymph, promotes the stability of the organs and tissues that it surrounds to ensure the organism constancy despite an external fluctuating environment (Bernard 1865). Claude Bernard's concept was then described and extended by the work of Walter B. Cannon during the 1920s, who coined the term of 'homeostasis' (from the greek *hómoios*, 'similar', and *stasis*, 'standing still'). Homeostasis describes the steady states that are essential to the maintenance of life, and the physiological processes through which internal conditions can be held almost constant (*i.e.* within narrow limits) to prevent wide oscillations that could result from variations of the external environment (Cannon 1929, [Figure 1](#)). Maintenance of internal conditions within narrow limits allows physiological systems to function normally (*e.g.* human's core body temperature is 37 ± 0.5 °C, which is the temperature range required for the optimal functioning of metabolic processes; Osilla et al. 2023) and is therefore adaptive in nature. Bernard and Cannon's idea can be summarised as maintaining 'stability through constancy' (Fink 2017).

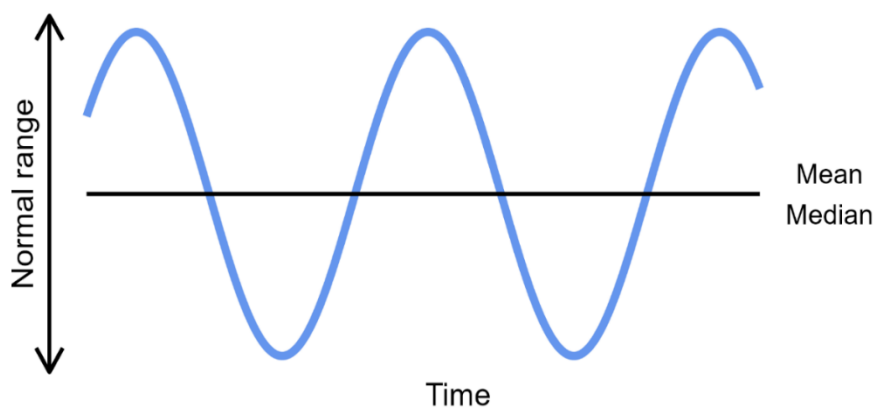


Figure 1. Schematic representation of the principle of homeostasis. On the y-axis, physiological parameters (*e.g.* blood pressure, heart rate, temperature, blood glucose) oscillate around a mean or median value within a physiological, narrow range (adapted from Davies 2016).

ii. Homeostasis and stress

A central aspect to the concept of homeostasis has however been eluded so far. Indeed, 'stress' is tightly linked to these processes but defining stress is not an easy task. In addition to homeostasis, Cannon coined the term 'fight or flight' to describe the rapid and non-specific response of an individual to a threat, and considered as a behavioural and physiological reflex governed by the sympathetic nervous system (SNS) that leads to the coordination of homeostasis: the 'stress response' (Boonstra 2013, Davies 2016, Fink 2017). While Cannon focused on the short-term, immediate response to a threat (*i.e.* acute stress), Hans H. B. Selye, considered as 'the father of stress' by physiologists, studied individual responses to threats of longer duration (*i.e.* chronic stress) giving rise to the General Adaptation Syndrome (GAS; Selye 1936), a non-specific response to negative stimuli. Divided in three parts, the GAS described an early 'general alarm reaction' to a nocuous stimulus (*i.e.* similar to the 'fight or flight' response proposed by Cannon), the 'resistance' phase, when the stimulus persists and during which organs maintain an almost normal functioning, and the 'exhaustion' phase during which adaptation to the stimulus is lost, resulting in physiological damages and eventually death (Selye 1936, Fink 2017, [Figure 2](#)). Although the GAS has lost scientific relevance because it is a consequence of extreme stress, it emphasised the role of stress in the maintenance of life and contributed to the development of the ecology of stress with the idea that chronic stress leads to pathologies in natural populations (Boonstra 2013, Fink 2017). It also permitted the emergence of 'heterostasis' (from the greek, *héteros*, 'other'), a concept suggesting that the organism will set new steady points that allow its adjustment and its resistance to stressful events (Selye 1973). This concept can be seen as the first step towards the idea of allostasis.

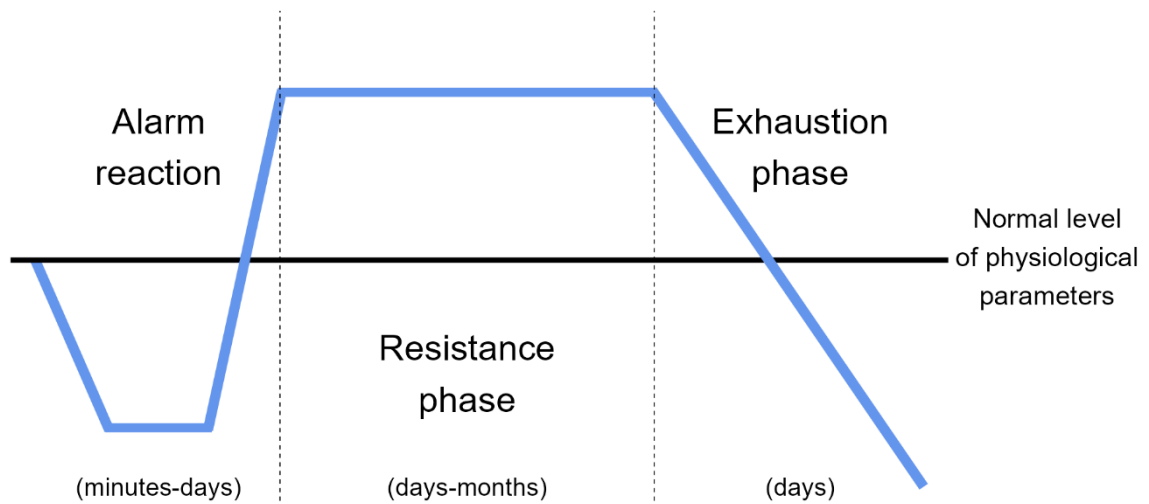


Figure 2. Schematic representation of the General Adaptation Syndrome described by Selye (1936). Exposure to a stressor impairs physiological parameters during a short alarm period, resulting in a short-term decrease of these parameter levels. If the stressor exposure persists, resistance takes place and physiological levels are maintained above normal to resist and for the organism to function despite the stressor. After several days to months, the organism is unable to maintain enhanced physiological level and physiological parameter levels decrease during the exhaustion phase, potentially leading to pathologies and death (adapted from Quintard 2001).

iii. Allostasis

From the 1980s onwards, the idea of maintaining ‘stability through constancy’ has been challenged, as evidences that all parameters of the internal environment of an organism vary within wider ranges according to demands associated with environmental fluctuations have accumulated (Sterling and Eyer 1988, McEwen 1998, McEwen and Wingfield 2003, Wingfield 2005, Romero et al. 2009). Sterling and Eyer (1988) coined the term ‘allostasis’ (from the greek *állos*, ‘other’, ‘different’) as describing the maintenance of ‘stability through change’ (Sterling and Eyer 1988, Fink 2017). Therefore, allostasis encompasses all physiological processes by which steady states are set and reached according to an individual state (*e.g.* awoken, asleep, moving, breeding, gestating, migrating) and according to the external environment (*e.g.* predation, infection, climatic events, resource availability). It means that optimal values for a given parameter might change on a daily or seasonal basis according to an individual requirements (McEwen 1998, McEwen and Wingfield 2003, 2010, Romero et al. 2009).

Allostasis thus supports homeostasis, and removed the limitations associated to the term ‘steady states’ used by Cannon (1929). These steady states are actually meant to change according to current individual and environmental states (McEwen and Wingfield 2003). These contextual setpoints define an individual ‘allostatic state’. By extension, the ‘allostatic load’ is the cumulative result of an allostatic state, and results from the energy required for an individual to survive and complete predictable daily and seasonal activities (*e.g.* breeding, migration, moulting, foraging) and face unpredictable events (*e.g.* predation, infection, starvation) (McEwen and Wingfield 2010). Accordingly, an individual can be in a state of ‘allostatic overload’ if energy acquisition is lower than the energy required to support a given allostatic state (McEwen and Wingfield 2010). Within this framework, we can describe three physiological states according to the situation of an organism (McEwen and Wingfield 2003, Landys et al. 2006, [Figure 3](#)). The physiological state A reflects an organism performing daily activities and supporting the basic requirements of life. The physiological state B describes a more demanding state during which an organism engages in predictable demanding activities according to predictable environmental or life-history changes. As these demands are predictable and can be anticipated, they are not considered as true stressors (*i.e.* distress; Koolhaas et al. 2011). Finally, the physiological state C is a facultative state triggered when unpredictable environmental-associated demands exceed the capacity of the organism in a given life-history stage to adjust. The tuning of the physiological state between these three different states, and associated morphological, behavioural or physiological consequences, are regulated by primary mediators such as elements of the immune system, neural processes or hormones, the latter being the focus in the next sections (McEwen 1998, Wingfield and Kitaysky 2002, McEwen and Wingfield 2003, 2010, Landys et al. 2006).

To sum up, allostasis is the active process of supporting homeostasis through the setting of steady points adapted to an individual current internal state and external environment

(Sterling and Eyer 1988, McEwen 1998). In other words, the perception of stressful events triggers behavioural and physiological mechanisms that permits the organism to normally function in accordance with the conditions experienced. However, stress can be an ambiguous term and it should be properly defined.

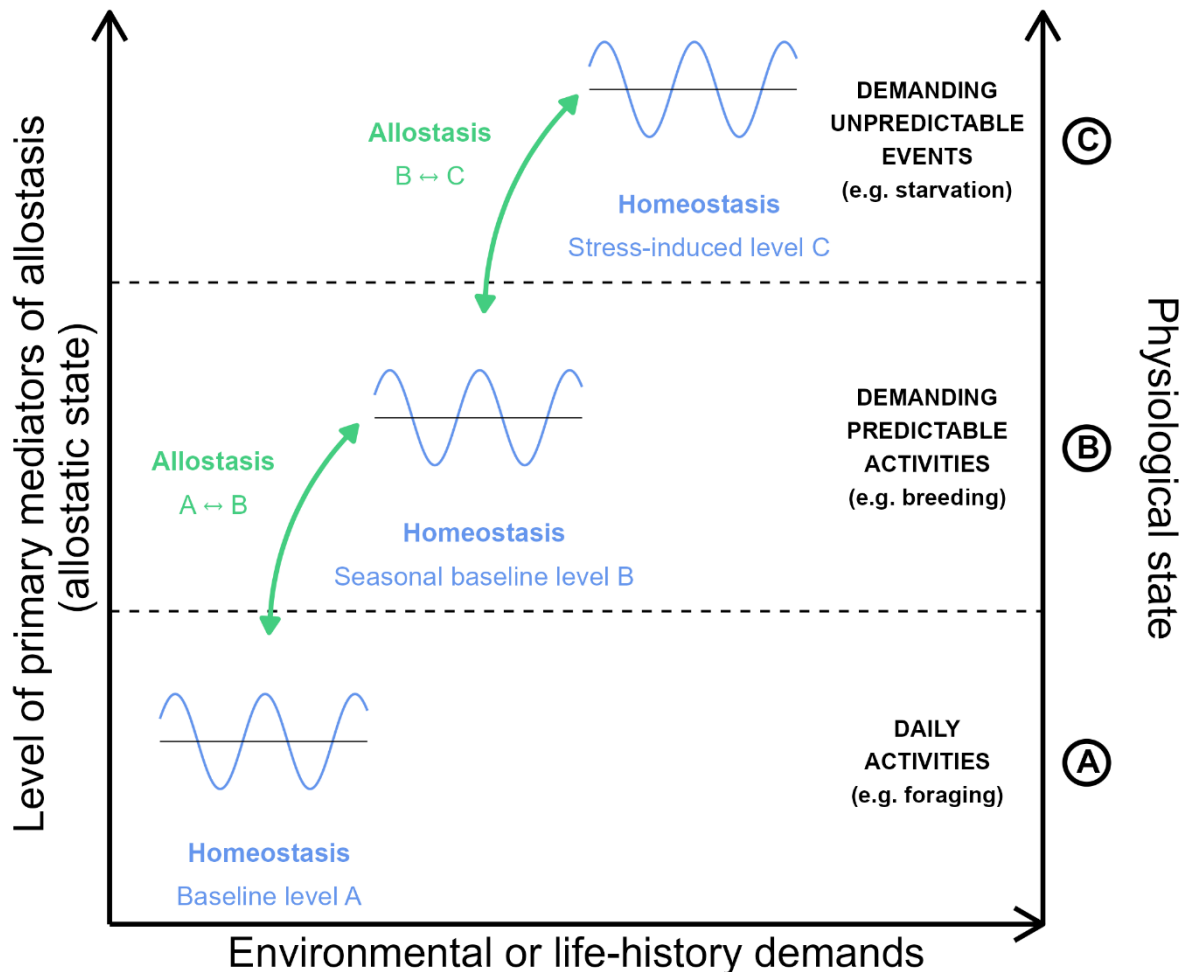


Figure 3. Graphical representation of the concept of allostasis. Homeostasis is the process of maintaining physiological parameters around a steady point. This steady point varies according to environmental conditions or life-history demands to meet the current requirements an organism is facing. Allostasis supports homeostasis by permitting new homeostatic steady points to be reached. This is done through primary mediators such as hormones, elements of the immune system or neural processes, defining an organism allostatic state. The physiological state A describes individual achieving basic physiological processes on a daily basis. During times of predictable but demanding environmental or life-history events, such as moulting, breeding, migration or seasonal food shortage (physiological state B), physiological activities are enhanced to face these demanding periods. In the face of unpredictable and demanding events, during the physiological state C, physiological processes are highly heightened to permit facultative responses such as the emergency life-history stage (Wingfield et al. 1998). Adapted from Landys et al. 2006.

b) The stress response

i. Definitions

Selye defined stress as a ‘non-specific response of the body to any demand’ (Fink 2017), because, as he noted when developing the GAS, the response of the organism to agents and drugs of diverse natures and types was similar (Selye 1936). However, this view has proven false as a vast heterogeneity in the stress response could be observed in relation to a variety of stressful events (*e.g.* Pacák and Palkovits 2001), as well as according to environmental conditions, a species life history and individual characteristics (Reeder and Kramer 2005, Boonstra 2013). Nowadays, modern stress theory defines ‘stress’ as a state during which either a real or perceived event threatens homeostasis (Chrousos 1998, Pacák and Palkovits 2001, Schneiderman et al. 2005, Reeder and Kramer 2005). Events threatening homeostasis are called ‘stressors’ and can be physical – internal (*e.g.* hypoglycemia) or external (*e.g.* climate, infections, injuries, chemical exposure) – or emotional/psychological (*e.g.* fear or anxiety due to predation) (Reeder and Kramer 2005). Stressors elicit a ‘stress response’ which is a suite of physiological and behavioural responses that aim at reaching homeostasis back – although homeostatic steady points can change according to individual internal and external contexts –, meaning that the stress response is part of the allostasis process (Sterling and Eyer 1988, Chrousos 1998, Pacák and Palkovits 2001, McEwen and Wingfield 2003, Reeder and Kramer 2005).

ii. The stress response

Physiological or behavioural mechanisms, similar to those mentioned above, also take place during the normal life cycle of an individual, without being considered a stress response (*e.g.* increased hormone levels during reproduction; Koolhaas et al. 2011). Indeed, animals have evolved and adapted to live in environments that fluctuate. Most of these fluctuations are predictable and individuals can rely on cues to anticipate future conditions, and consequently

adjust to maximise fitness (Wingfield 2003, 2008). On the other hand, environmental conditions can fluctuate in an unpredictable manner and/or get out of the ‘reactive scope’ or ‘regulatory range’ of an individual (Romero et al. 2009, Koolhaas et al. 2011). When fluctuations are both unpredictable and large enough to disrupt normal life cycles, they trigger a stress response that happens in addition to the physiological and behavioural adjustments that occur as part of the normal life cycle of an individual (McEwen and Wingfield 2003). This typically results in allostatic overload and puts the individual in an ‘emergency life-history stage’ in order to regain a manageable allostatic state (Wingfield et al. 1998, McEwen and Wingfield 2003, 2010, Wingfield 2003). The emergency life-history stage encompasses behavioural and physiological adjustments, leading some behaviours or physiological mechanisms to be prioritised over others that are therefore postponed (Wingfield et al. 1998). In response to a stressor, behavioural adjustments such as ‘fight or flight’ responses occur (*e.g.* Redondo-Gómez et al. 2023), spreading along a proactive-reactive gradient of behaviours (Koolhaas et al. 1999), including vigilance (*e.g.* Laundré et al. 2001) and dispersal (*e.g.* Silverin 1997). Physiological responses to stressors involve increased cardiovascular functions and energy mobilisation that promote locomotor activities or immune activation, all at the expense of other non-essential functions for immediate survival, such as reproduction or growth (Sapolsky et al. 2000).

c) Glucocorticoids and the stress response

i. Physiological axes of the stress response

Physiological responses to cope with stressors take place *via* two major axes by the mean of different hormones and neuropeptides, such as endorphins that mitigate the perception of pain, or catecholamines and glucocorticoids (GCs) that are involved in the regulation and mobilisation of an organism energy resources (Sapolsky et al. 2000, Reeder and Kramer 2005, Hau et al. 2016). First, within milliseconds after the perception of a stressor, the SNS activates and produces some catecholamines (*i.e.* norepinephrine and adrenaline) that fuel the organism

with energy by promoting glycogenolysis and lipolysis to support the ‘fight or flight’ behavioural response (Sapolsky et al. 2000, Reeder and Kramer 2005). In parallel to the activation of the SNS, the hypothalamic-pituitary-adrenal (HPA) axis – in mammals, birds and reptiles or hypothalamic-pituitary-interrenal (HPI) system in fish and amphibians – activates, resulting in the secretion of GCs within a few minutes following exposure to a stressor, and which can last up to several hours (Wingfield and Romero 2001, Romero and Reed 2005). A perceived stressor stimulates the paraventricular nucleus (PVN) of the hypothalamus ending up in the secretion of corticotrophin-releasing hormone (CRH) and arginine-vasopressin (AVP). These hormones reach and stimulate the anterior pituitary to produce adrenocorticotropin hormone (ACTH), released in the blood to stimulate the adrenal cortex to secrete GCs (De Kloet et al. 1988, Chrousos 1998, Sapolsky et al. 2000, Reeder and Kramer 2005, Sheriff et al. 2011, [Figure 4](#)). Under normal conditions, the activity of the HPA axis is inhibited by the hippocampus that permits the HPA axis to produce GCs at baseline levels (Cole et al. 2022). Because the production of catecholamines by the SNS ceases within minutes, and that these hormones disappear quickly, it is challenging to measure them in wild animals (Gormally and Romero 2020). This is why the study of the ecology of stress in free-ranging animals focused mainly on measuring GCs and their metabolites (Sheriff et al. 2011, Palme 2019). In rodents, birds and reptiles, GC hormones are mainly corticosterone, while in fish and most mammals, they are mainly cortisol.

ii. Roles of glucocorticoids at baseline and stress-induced levels

Once released, GCs are transported *via* the blood-stream to GC-sensitive cells, on which GCs bind through two types of receptors: the mineralocorticoid receptors (type I receptors, MR), and the glucocorticoid receptors (type II receptors, GR). At baseline levels, GCs preferentially bind to MR because of their higher affinity compared to GR (Hau et al. 2016). At baseline levels, GCs play a key role in fundamental functions linked to energy metabolism and

daily behaviours. They regulate the supply of glucose in different tissues, contribute to the maintenance of body mass, and promote locomotor and foraging activities (Landys et al. 2006). Baseline GC levels are regulated on a daily and seasonal basis, as part of the normal (*i.e.* predictable) life cycle of an individual, through negative feedback loops (Sapolsky et al. 2000, Landys et al. 2006, Busch and Hayward 2009) and, potentially, through changes in the relative concentrations of MR and GR (Romero 2002). For example, baseline GC levels peak just before an individual's peak of activity to support increased locomotor and foraging activities, and are the lowest during resting periods (Landys et al. 2006). On the other hand, GCs at moderate or high stress-induced levels start binding to GR, due to MR saturation (Hau et al. 2016). This change in how GCs are received by either the high-affinity receptors only, or both high- and low-affinity ones can explain the different actions of GCs at baseline and stress-induced levels (Landys et al. 2006, Busch and Hayward 2009). At stress-induced levels ('acute' or 'chronic', see below), GC hormones participate in organising the stress response supporting the emergency life-history stage, in order to restore allostasis, for example through the mobilisation of stored energy (Wingfield et al. 1998, McEwen and Wingfield 2003, Landys et al. 2006, Hau et al. 2016, Palme 2019).

iii. Glucocorticoids: acute and chronic stress

When facing short-term challenging situations, or 'acute stressors', an increase in GC concentration is beneficial as it facilitates processes that enhance survival ('Cort-Tradeoff' hypothesis, [Box 1](#)). Indeed, GCs elicit a reallocation of stored energy away from non-immediately essential functions such as reproduction (*e.g.* Pankhurst and Van Der Kraak 2000, Deviche et al. 2012), towards crucial activities like cardiovascular functions and locomotion (Sapolsky et al. 2000, Wingfield and Romero 2001). Following a temporary disturbance, GC concentrations quickly return to baseline (within hours) through their own negative feedbacks

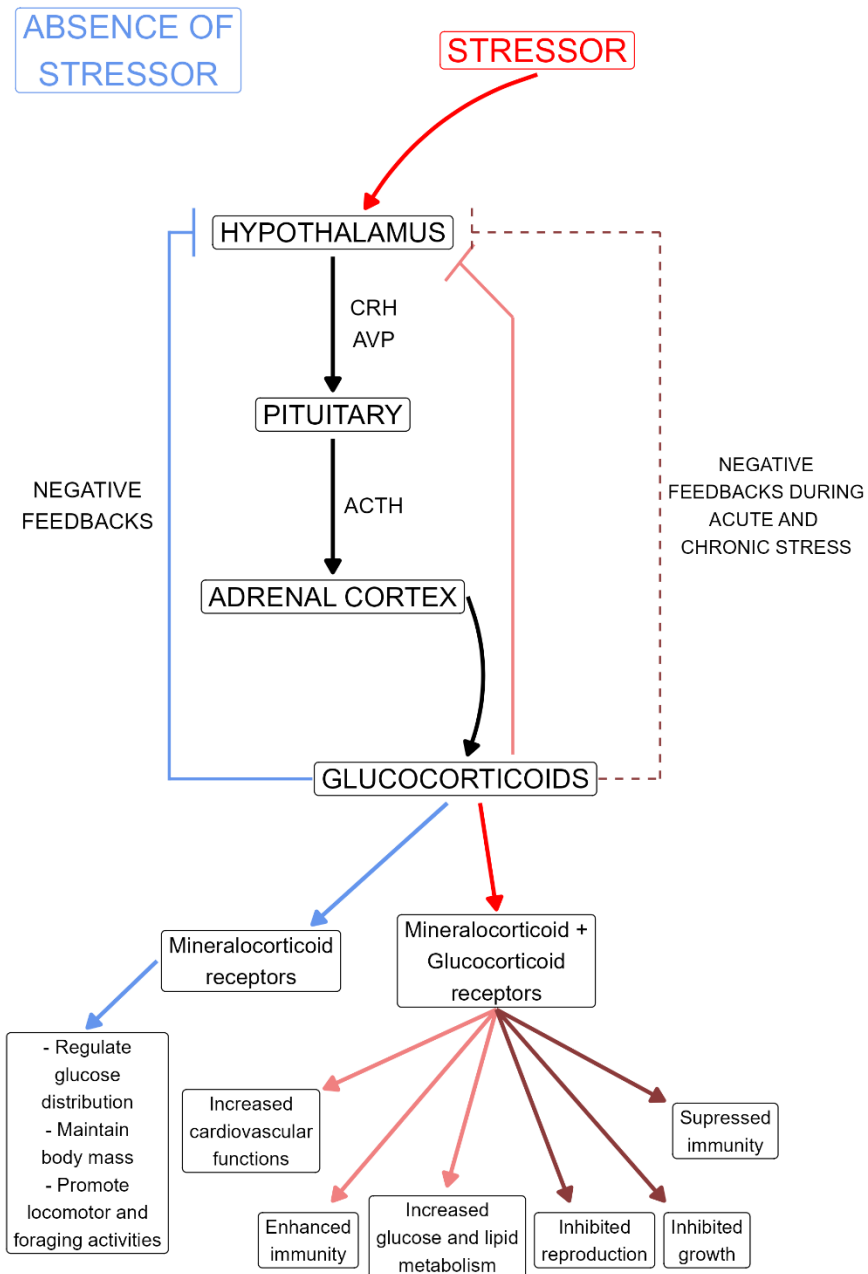


Figure 4. Schematic representation of the hypothalamic-pituitary-adrenal (HPA) axis functioning at baseline level (in absence of stressor, left part of the graphic, blue) and following exposure to a stressor (right part, red). Stress-induced activation of the HPA axis and its consequences are separated according to whether the stress is acute (*i.e.* on the short-term, light red) or chronic (*i.e.* sustained on the long-term, dark red). The hypothalamus produces corticotropin-releasing hormones (CRH) and arginine-vasopressin (AVP) that stimulate the pituitary to release adrenocorticotropin hormones (ACTH) in the blood stream that reach the adrenal cortex. The latter then produces glucocorticoids (GCs). At baseline and during acute-stress functioning, GCs regulate their own production (through negative feedbacks). During acute stress, consequences of the production of the GCs are temporary. During chronic stress, however, negative feedbacks are dysregulated leading to continuous production of GCs and consequences can be long-lasting.

(Reeder and Kramer 2005, [Figure 5](#)). However, exposure to repeated or prolonged perturbations, *i.e.* ‘chronic stressors’, such as inclement weather or food shortage, can result in persistently elevated GC concentrations and heightened baseline GC levels on the long-term (days to weeks) in part due to a dysregulation of their negative feedback effects (Sapolsky et al. 2000, Romero 2004, McEwen and Wingfield 2010, Hau et al. 2016, [Figure 5](#)). Prolonged elevation of GC concentrations in the long term can have adverse effects on individual performances as it can disrupt immune function (Martin 2009, Dhabhar 2014), inhibit growth, and lead to decreased body condition (Sapolsky et al. 2000, Reeder and Kramer 2005, [Figure 4](#)). In fact, long-term GC elevation can lead to allostatic overload (McEwen and Wingfield 2003, 2010) and the severity of these adverse effects varies among species (Boonstra 2013). Long-term GC elevations can result in higher baseline GC levels (compared to a normal life cycle level) and during longitudinal survey of wild animals, it is usually these baseline levels that are measured in order to assess individual and population health and performances (Walker et al. 2005, Wikelski and Cooke 2006, Bonier et al. 2009a).

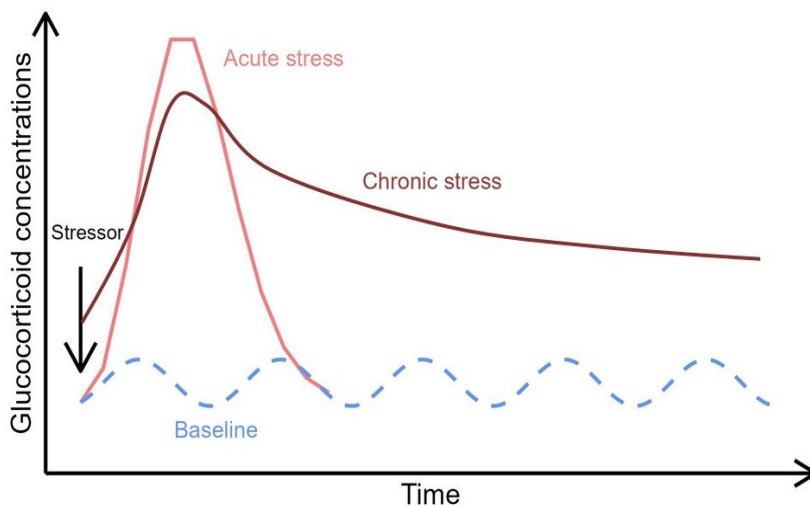


Figure 5. Glucocorticoid (GC) responses to stressors in animals according to time. Under normal conditions, in absence of stressors, GCs are secreted at baseline levels to support daily and seasonal predictable demands (dashed blue line). GC baseline concentrations can vary to some extent according to environmental and life-history demands.

Following exposure to a transient, acute stressor, GC production quickly increases and peaks (minutes-hours) before returning to baseline concentrations once the stressor stops (within hours, solid light red line). If the stressor persists over longer periods of time (hours-days, solid dark red line), disruption of negative feedback mechanisms occurs and causes GC concentrations to remain elevated for longer periods of time (hours-days). Following repeated or chronic exposure to stressors, negative feedback can be disrupted on the long-term and baseline GC concentrations can be heightened compared to other individuals as shown with the level of the dark red line when the stressor initiates. Adapted from Romero 2004.

Box 1. The role of GCs in fitness

Many hypotheses aiming at clarifying the role of GCs in relation to fitness have been developed across the history of stress physiology and range from Hans H. B. Selye’s ‘non-adaptive stress’ back in 1955, to the ‘speed of flexibility’ hypothesis proposed by Taff and Vitousek (2016). These hypotheses are reviewed in Schoenle et al. (2018), but three main competing hypotheses with testable predictions have predominated (**Table a**).

- The **Cort¹-Fitness hypothesis** (Bonier et al. 2009a) states that all fitness metrics (*i.e.* related to survival and reproductive success) should decline with increased challenges/stress. This hypothesis comes from the idea that GCs increase with environmental challenges leading to higher GC levels in challenged individuals compared to non-challenged individuals. As fitness prospects are expected to decrease with environmental challenges, an increase in GCs should reflect a fitness decline.

- The **Cort-Adaptation hypothesis** (Bonier et al. 2009a, b) predicts that high GC levels should increase reproductive effort. It is a revision of the Cort-Fitness hypothesis whose tests provided equivocal results. The Cort-Adaptation hypothesis states that during environmental challenges affecting reproduction, associated GC elevations feedback to reallocate resources towards reproductive effort, so that a positive relationship between GCs and reproductive success is expected.

- The **Cort-Tradeoff hypothesis** (Patterson et al. 2014) is based on the historic view that GCs are increased in response to stressors to promote survival over other functions such as reproduction (Wingfield et al. 1998, Wingfield and Sapolsky 2003). Because survival and reproduction are subjected to trade-offs, this hypothesis posits that GCs shift resource allocation towards survival rather than reproduction.

Table a. Expected relationship between glucocorticoid (GC) levels and fitness (survival and reproductive success) according to the three main hypotheses linking GCs and fitness.

Hypothesis	Survival	Reproduction
Cort-Fitness	↘	↘
Cort-Adaptation	—	↗
Cort-Tradeoff	↗	↘

¹ Cort stands for ‘corticosterone’ (*i.e.* the main GC hormone in reptiles, birds and rodents) and ‘cortisol’ (*i.e.* the main GC hormone in fish and non-rodent mammal species).

2. Stress response and life-history traits

a) Integrating physiology and ecology

Given the increase in the occurrence and frequency of unpredictable events across the last few decades (mainly due to human activities and global change; Seneviratne et al. 2012, Hooke and Martín-Duque 2012, Venter et al. 2016), one of the main challenge in modern ecology is to understand how wild animals respond to such events, and how the resulting alterations of the life cycle of an individual and life histories are linked (Sih et al. 2011, Crespi et al. 2013, Monaghan and Spencer 2014). Life history includes the set of behavioural, physiological and anatomical strategies that directly influence survival and reproductive success to a certain degree, and which are adaptive responses to the environment (Ricklefs and Wikelski 2002). In that sense, life-history traits, resulting from adaptations, explain a part of the variation observed in population life tables (*i.e.* birth rates, death rates), although physiological mechanisms are likely to explain more of this variation by providing a link between life history (and life tables) and the environment ([Figure 6](#)). Indeed, as described above, physiological mechanisms, including endocrine modulations in response to stressors, allow individuals to adjust and cope with a challenging environment. Whether adjustments to environmental challenges are adaptive or not, they are constrained by energy allocation trade-offs (van Noordwijk and de Jong 1986, Stearns 1989) that are regulated by physiological mechanisms (Ricklefs and Wikelski 2002). Endocrine mechanisms, with a focus on GCs and the stress response here, provide a valuable proximate mechanism that is often discarded by evolutionary biologists and ecologists to explain, at least partially, how life-history traits can be shaped by selection forces (Ricklefs and Wikelski 2002, Wingfield et al. 2008). On the other hand, physiologists often neglected the demographic or fitness-related consequences of the mechanisms they are studying (Wingfield et al. 2008). Therefore, there is a need to integrate physiology and ecology as physiological mechanisms are tuned by selection forces, and since

both adaptation and phenotypic plasticity are permitted, and constrained, by physiological adjustments, ultimately influencing population dynamics (Ricklefs and Wikelski 2002).

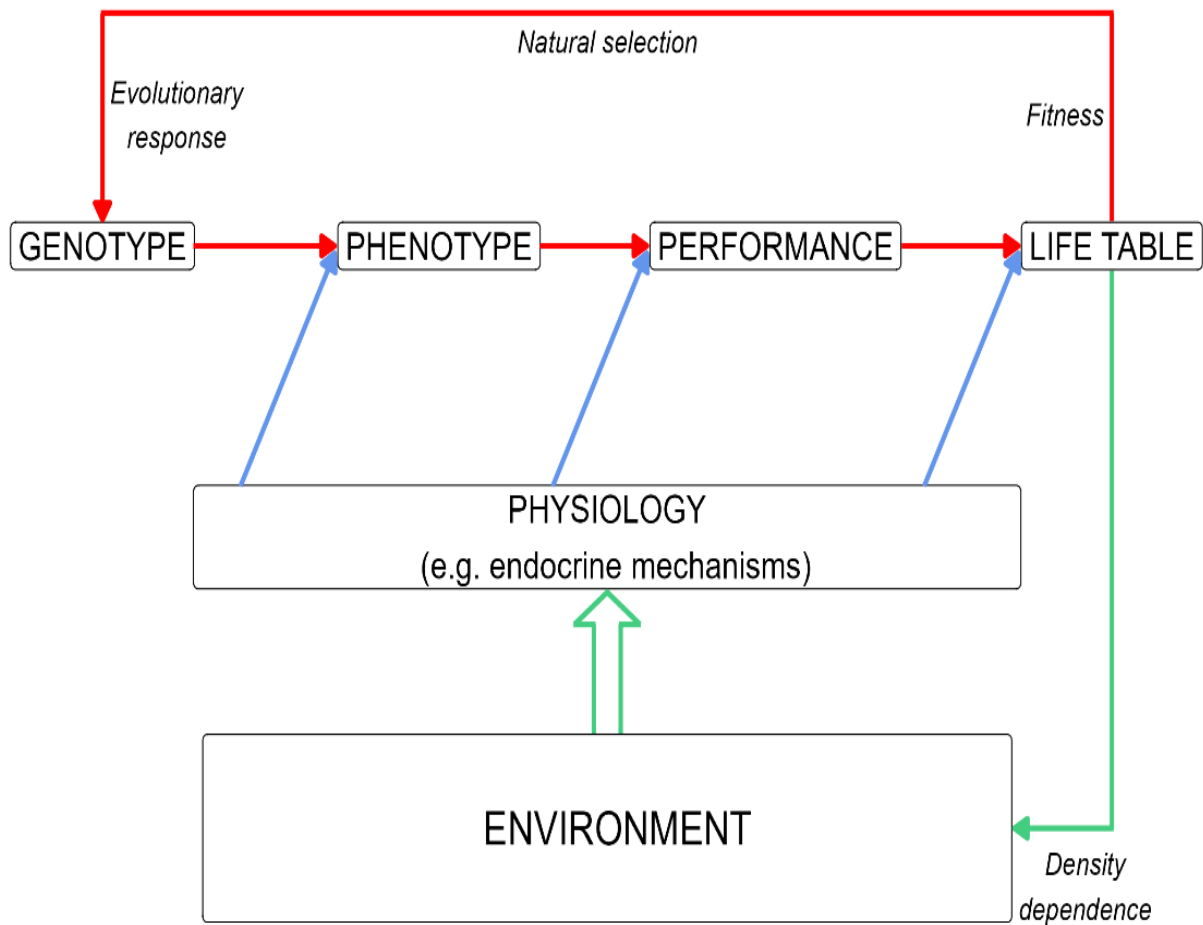


Figure 6. Graphical representation of the physiology/life-history nexus (Ricklefs and Wikelski 2002). An individual genotype produces a phenotype that allows the individual to eventually survive and reproduce in a given environment, describing the life history of this individual. The environment influences each stage of the life history of an individual through diverse physiological responses, such as endocrine mechanisms and the stress response. The physiology creates a link between an individual performance, the population life table and the environment. Finally, the genetic composition of the population can ultimately change across generations through natural selection due to inter-individual variations in survival and reproductive success among different genotypes. Organisms and populations, and associated density-dependence mechanisms, also exert pressure onto their environment which will in turn imposes individuals to adjust to the modified environment (adapted from Ricklefs and Wikelski 2002).

b) The adaptive and maladaptive facets of the stress response

The stress response has two sides, an adaptive and a maladaptive one that have been termed ‘eustress’ and ‘distress’, respectively. The acute stress response is adaptive. Indeed, it allows an individual to mount a suite of behavioural and physiological responses in order to escape a threatening situation, and survive (Sapolsky et al. 2000). However, the maladaptive facet of the stress response occurs when individuals are chronically or repeatedly stressed. In that case, the stress response is maintained on a longer time scale and energy allocation trade-offs, mediated by GCs, result in reproduction, somatic maintenance, growth or immunity to be suppressed, leading to pathology, reduced condition, and potentially death (Romero 2004). High baseline GC levels are expected to be maladaptive, partially because of their physiological effects, and on another part because they are positively linked to environmental challenges, which in turn negatively relates to fitness and individual performances, and eventually population dynamics, describing the ‘Cort-Fitness’ hypothesis (Wingfield and Sapolsky 2003, Romero et al. 2009, Bonier et al. 2009, 2010, [Box 1](#)). However, reviews show equivocal relationships between GC levels and reproductive success/survival with either positive, negative or null relationships between them (Bonier et al. 2009a, Crespi et al. 2013, Dantzer et al. 2014, Newediuk and Bath 2023). This absence of a universal negative effect of chronically elevated GC levels on fitness can partially be explained by the influence of internal (*e.g.* sex, age, life-history stage, condition) and external factors (*e.g.* parasite exposure, predation, resource availability and quality) on GC-fitness relationships (Crespi et al. 2013, Dantzer et al. 2014, Wey et al. 2015, Henderson et al. 2017, Newediuk and Bath 2023). These two facets of the stress response (*i.e.* adaptive and maladaptive) is complicated to include in research and often leads to biased interpretations towards one side or the other (Koolhaas et al. 2011).

c) The consequences of GCs on fitness and individual performances

i. Baseline levels

At baseline GC levels (*i.e.* during the normal life cycle), GCs promote locomotor and foraging activities according to daily and seasonal variations in food abundance and activity patterns. Baseline GC levels are heightened in preparation to demanding life-history stages such as reproduction (Love et al. 2014, Casagrande et al. 2018) or migration (*e.g.* Holberton 1999, Crossin et al. 2012, Hennin et al. 2016), by promoting an increased locomotor activity and body mass gain for example. Such activities are essential part of the life cycle of an organism and which have direct repercussions on fitness. Thus, GCs at baseline levels play a key role in fitness prospects by regulating energy regulation which allows the realisation of essential life cycle events.

ii. Acute stress-induced levels

At acute stress-induced levels, GCs negatively affect body mass, as largely demonstrated in laboratory conditions (*e.g.* Strack et al. 1995, Dallman et al. 1999) through decreased food intake, increased gluconeogenesis, as well as protein and lipid catabolism, resulting in muscle tissue breakdown and decreased body condition (Dallman et al. 1999, Reeder and Kramer 2005, Landys et al. 2006). Only high levels of GCs such as during acute stress affect body mass because GCs effects on body mass are likely to be mediated by low-affinity receptors, GR (Landys et al. 2006).

Similarly, the role of GCs in supporting homeostasis through allostasis during acute stress is done at the expense of investment towards current reproduction (Wingfield and Sapolsky 2003, Breuner et al. 2008). Indeed, physiological and behavioural adjustments taking place during emergency life-history stages are incompatible with reproductive activities, so that reproductive behaviours are inhibited (Wingfield and Romero 2001). Moreover, the HPA axis interact with the reproductive (hypothalamic-pituitary-gonadal, HPG) axis, in a way that the

former releases hormones that disrupt the functioning of the latter (Sapolsky et al. 2000, Wingfield and Romero 2001, Wingfield and Sapolsky 2003). Note however, that depending on species-specific reproductive tactics, certain species have evolved reproductive resistance to stress when future breeding opportunities are poor, such as semelparous species or seasonal breeders with short breeding periods (Wingfield and Sapolsky 2003).

Nevertheless, increased metabolic states promoted by GCs provide the organism with energy resources to face adverse situations of heightened requirements. Therefore, following exposure to a stressor, the immediate consequences of GCs are the promotion of survival by re-establishing homeostasis and through ‘fight or flight’ responses to escape a predator for example (Sapolsky et al. 2000). For instance, wild boars (*Sus scrofa*) exposed to carcasses of grey wolves (*Canis lupus*), their main predator, responded by displaying ‘fight or flight’ behaviours (although this was not the case when exposed to carcasses of a carnivore that rarely predate them; Redondo-Gómez et al. 2023). Acute stress GC levels also have immune enhancing effects that promote wound healing and infection resistance (Martin 2009, Dhabhar 2014). As immunity plays a major role in regulating host survival (Lochmiller and Deerenberg 2000, Shanley et al. 2009), this immune enhancement has been hypothesised to be part of the ‘fight or flight’ response to prepare individuals facing events with high possibilities of getting wounded or infected (Dhabhar 2002, 2009).

iii. Chronic stress-induced levels

When chronically stressed, however, the emergency life-history stage deviates resource allocation away from reproduction (but see the ‘Cort-Adaptation’ hypothesis; Bonier et al. 2009a, b, [Box 1](#)), somatic maintenance or the immune system. Long-term GC elevations indeed lead to immunosuppression because GCs have immunosuppressive effects that decrease the production, activity and functioning of immunoprotective cells and can result into inflammaging (*i.e.* a chronic low-grade inflammatory state favouring susceptibility to age-

related diseases; Franceschi et al. 2000, 2007) and immune dysregulation (Dhabhar 2014). On the long-term, stress-induced GC elevations (*i.e.* chronic stress) are hypothesised to negatively relate to survival and reproductive success. This is because GC levels are expected to increase with environmental challenges, which is detrimental for fitness (Cort-Fitness hypothesis; Bonier et al. 2009, 2010, [Box 1](#)), and because of the organismal damages of allostatic overload (McEwen and Wingfield 2003, 2010). However, tests of the Cort-Fitness hypothesis led to equivocal results (*e.g.* Ethan Pride 2005, Cabezas et al. 2007, Rogovin et al. 2008, Wey et al. 2015, Pinho et al. 2019) and future works attempting to link fitness and GC levels should pay attention to the appropriateness of fitness metrics, account for both an individual and its environmental contexts, rely on longitudinal studies for one to be able to detect states of chronic stress and consider population stress exposure history (Bonier et al. 2009a, Crespi et al. 2013, Dantzer et al. 2014, Fourie et al. 2016, Newediuk and Bath 2023).

d) Early-life glucocorticoids and their long-term consequences

The phenotype of an individual is determined in part by the developmental limits imposed by its genotype, but also by environmental factors. The concept of ‘reaction norm’ encompasses the set of phenotypes an organism is able to produce according to a set of environmental conditions (Stearns and Koella 1986, Nylin and Gotthard 1998). While it is well recognised that adverse environmental conditions can have detrimental consequences on immediate fitness and individual performances, the carry-over fitness consequences of environmental conditions during early-life have only been described since a few decades (Lindström 1999). Indeed, during early development (from conception to maturity), environmental conditions can shape current and subsequent growth, morphology, immunity, behaviour and reproduction (Lindström 1999, Metcalfe and Monaghan 2001). Environmental influences can be direct (*e.g.* food shortage, climate, pathogens) or indirect and generally mediated through maternal (or paternal in some instances) effects (Lindström 1999).

Environments can influence parents' physiology and behaviour, which will in turn affect offspring development. Stress exposure during early development, either directly or *via* parental hormones for instance, thus affect development and can have long-term – adaptive or adverse – consequences on individual performances, and ultimately on population dynamics (Monaghan and Haussmann 2015). For example, exposure to high conspecific density during gestation in American red squirrels (*Tamiasciurus hudsonicus*), triggering an increase in maternal GC production, led offspring to have adaptively increased growth rate, and it is very likely that this is GC-mediated. These offspring had a better juvenile survival during their first winter and higher recruitment rates during years of high density, which positively correlates with lifetime fitness (Dantzer et al. 2013). However, these offspring also had shorter longevity, a possible consequence of accelerated growth rate (Metcalf and Monaghan 2001, Dantzer et al. 2013). Experimental increase in GC levels during post-natal development in zebra finches (*Taeniopygia guttata*) effectively led to accelerated ageing rates and reduced lifespan compared to control individuals, but did not affect their survival or reproductive performances (Spencer et al. 2009).

Therefore, exposure to GCs during early-life can have both adaptive and maladaptive consequences on individual performances, and the direction of these consequences are likely to be trait-dependent and to depend on the duration and intensity of the stress exposure during early-life. Indeed, exposure to stressors early in life could provide cues for individual to adjust to potentially harsh future environmental conditions (*i.e.* predictive adaptive response; Gluckman and Hanson 2004, Gluckman et al. 2005). Monaghan and Haussmann (2015) also noted the hormetic effects of mild levels of stress exposure during development. Individuals exposed to stressors during early-life displayed reduced oxidative damages during adulthood compared to control individuals, not exposed to stressors. Still, the former individuals showed lower longevity than the latter. Whether the detrimental carry-over consequences of stress

exposure during development is the result of a ‘compensation for a bad start’ or are direct consequences of GC levels out of the coping capacities of an individual remains unclear.

Finally, stress-exposure during development has direct consequences on the HPA axis. Early-life stress exposure leads to an increased HPA axis responsiveness later in life, associated with higher-than-expected circulation of GCs, leading to an inappropriate stress responsiveness (*i.e.* hyper-responsiveness), disrupting metabolic activities and predisposing individuals to adult diseases (Harris and Seckl 2011, Maniam et al. 2014). The hormetic effects of mild GC exposure during development could actually prepare the HPA axis to respond proportionally to stressors, preventing it from hyper-responsiveness (Monaghan and Hausmann 2015).

3. Implications of GCs for physiology and health

a) GCs and body mass/condition

i. The interest of studying body mass

Body mass is often positively related to key life-history traits such as lifespan, survival or reproductive success in non-human species (Gaillard et al. 2000b, Milner et al. 2013, Ronget et al. 2018, Briga et al. 2019). Also, body mass is representative of the amount of stored fat (*i.e.* energy) available for an individual, which represents the main body reserve in birds and mammals, and informs on an individual nutritional status (Parker et al. 2009, Labocha and Hayes 2012, Monteith et al. 2014). Thus, body mass – usually corrected for an individual height or length for capital breeders –, provides a good indication of an animal body condition and integrates the balance between energy intakes and demands in response to varying physiological requirements according to environmental fluctuations (Parker et al. 2009). In contrast to capital breeders, which fuel reproductive energy demands by relying on their current body reserves, income breeders (such as the roe deer, *Capreolus capreolus*, the study system in this thesis) fuel reproduction by relying on environmental resources available at the time of breeding. In that regard, body mass alone reflects body condition as those animals do not store reserves

(Hewison et al. 1996, Andersen et al. 1998). In any case, longitudinal data on body mass are probably one of the easiest measures to collect on wild animals and provide a good estimation of body condition, health and fitness prospects of an individual.

ii. The implications of GCs on body mass

The relationship between GCs and body mass is complex. It seems that GC-driven body mass loss occurs only when GCs are elevated above seasonal baseline (*i.e.* physiological state C, see [section 1.a iii.](#)), which happens either during acute or chronic stress. However, whether elevated baseline GCs levels resulting from repeated or chronic exposure to stressors impact body mass in wild animals is not well understood. As mentioned above, at baseline levels, during normal life cycle, GCs promote body mass maintenance or body mass gain in preparation for predictable demanding life-history stage such as growth, hibernation, migration or breeding. However, at acute stress-induced levels, GCs bind to the low-affinity GR, mediating direct physiological effects on body mass. First, GCs affect feeding behaviours in different ways. GCs at baseline or seasonal levels promote normal feeding behaviours and can even increase food intake when GC levels are heightened in preparation migration or hibernation (Landys et al. 2006, Holberton et al. 2007, Hennin et al. 2016). However, when increased even more (*i.e.* at acute stress levels), GCs do not further augment food intakes but rather reduce it (Strack et al. 1995). Indeed, GCs at acute stress levels are associated with ‘fight or flight’ responses that favours cardiovascular and locomotor activities over feeding behaviours (Sapolsky et al. 2000). Additionally, from a metabolic perspective, GCs are considered as having catabolic effects. As a matter of fact, above baseline levels, GCs participate in making available amino acids to support hepatic gluconeogenesis so that plasma glucose increases and is available for working tissues (Dallman et al. 1993, Landys et al. 2006, Boudreau et al. 2019). This results in protein breakdown and utilisation, as well as muscle loss, to provide usable energy to support heightened physiological demands and allostatic (over)load

(Landys et al. 2006). Also, GCs at stress levels promote lipid availability through lipolysis in adipose tissue stores by decreasing re-esterification, increasing plasma free fatty acids in a dose-dependent fashion (Landys et al. 2006, Peckett et al. 2011). Moreover, GCs participate in fat deposition and lipogenesis in the liver where available amino acids, promoted by GCs, potentially contribute to the conversion of fats into usable energy resources to support heightened physiological states (Landys et al. 2006). Nevertheless, GCs also have a role in fat deposition, increased visceral fat and obesity (Landys et al. 2006, Peckett et al. 2011, Lee et al. 2014, Rabasa and Dickson 2016). This effect might be driven by insulin, with which GCs have either synergetic or antagonistic effects, and could depend on the level of GCs and on an individual's condition, with individual in a state of positive energy balance having heightened plasma insulin levels that promote the conversion of mobilised energy into fat depots (Baxter 1976, Dallman et al. 1993, Strack et al. 1995, Landys et al. 2006, Peckett et al. 2011, Lee et al. 2014, Rabasa and Dickson 2016).

Altogether, the actions of GCs on metabolism suggest that at high, acute stress levels, GCs contribute to body mass loss through energy mobilisation and structural tissue breakdown (*e.g.* Boonstra et al. 1998), but also through disruption of feeding behaviours. On the other hand, at (seasonal) baseline levels, GCs appear to be more related to the maintenance (or gain according to environmental or life-history demands) of body weight. Note, however, that GCs at stress levels might not always result in body mass loss, but rather in changes in body composition (Landys-Ciannelli et al. 2003).

Still, evidences for a relationship between GC levels and body mass are mixed in wild animals, showing either a negative relationship (*e.g.* George et al. 2014), a positive one (*e.g.* Hennin et al. 2016) or none (*e.g.* Boudreau et al. 2019). Indeed, this relationship can be confounded by the life-history stage of an animal (Crespi et al. 2013), its body condition or the availability and quality of resources (Henderson et al. 2017, Breuner and Berk 2019). In the

example of the positive relationship mentioned above, baseline GC levels actually increased in preparation for migration and promoted feeding behaviours and body mass gain (Hennin et al. 2016). In the example where no relationship was found, although predator-induced stress resulted in a continuous catabolic state in snowshoe hares (*Lepus americanus*), as evidenced by increased plasma glucose, compensatory physiological (*e.g.* decreased metabolic activity) and behavioural (*e.g.* decreased activity, feeding on higher quality resources) mechanisms are likely to occur in order to maintain body mass (Boudreau et al. 2019). While the effects of GCs at stress levels on body mass are well-documented in human and rodents in laboratory conditions, the effects of heightened baseline levels, as a result from repeated or chronic stress, on body mass is still not well understood in free-ranging animals (Crossin et al. 2016).

b) GCs and physiological parameters

i. Physiological parameters as proxies of body condition

Body condition describes both the physical and physiological status of an individual. It reflects the ability of the individual to acquire resources, since nutrition, body mass/size and physiology are intrinsically linked (Parker et al. 2009, Birnie-Gauvin et al. 2017). Appropriate nutritional intakes provide the organism with various nutrients (*i.e.* fatty acids, proteins, amino acids, minerals) that are metabolic fuels engaged in growth, maintenance, reproduction or immunity (Woodward 1998, Allen and Ullrey 2004), and which are therefore key for survival according to environmental conditions (Bright Ross et al. 2021).

Body mass represents the nutritional status of an individual across the past few days, weeks or months and can efficiently predict fitness outcomes. Nevertheless, it has been shown that physiological parameters can provide additional information to the body mass because they integrate shorter-term knowledge about an individual's nutritional status (Milner et al. 2003). Indeed, haematological and biochemical investigations may describe precisely the cellular and molecular content of blood, thus providing useful information on body condition and

physiological status (Milner et al. 2003, Ezenwa et al. 2012, Gilot-Fromont et al. 2012), and have also been shown to positively correlate with survival (Milner et al. 2003) and reproductive success (Ots et al. 1998, Nadolski et al. 2006).

Age-related variations in blood parameters have been mostly studied in humans and show consistent declines with advancing age, except for an increase in senescence- and inflammation-associated proteins (Eisenstaedt et al. 2006, Tanaka et al. 2020). However, assessments of age variations in blood parameters in wild animals are fewer, and most of them come from birds (*e.g.* Norte et al. 2009, Coughlan and Hollmén 2018). Studies of the senescence of blood physiological parameters in long-lived mammals are scarce and include the assessment of immunosenescence (*e.g.* Nussey et al. 2012, Cheynel et al. 2017) as well as the senescence of blood protein content (Jégo et al. 2014, Reichert et al. 2022). Still, a majority of these studies, on birds or free-ranging mammals, are cross-sectional and/or use age classes rather than continuous age, highlighting once more the need for longitudinal studies in such investigations (Nussey et al. 2008). The research of senescence in haematological parameters such as plasma protein content, being a valuable proxy of body condition, could provide significant insight into the underlying mechanisms explaining actuarial and reproductive senescence.

ii. Implications of GCs for haematological parameters

As described earlier, GCs play a key role in organising resource allocation in response to stressors (Sapolsky et al. 2000). This has several consequences on plasma composition. During acute stress, plasma glucose is increased because GCs promote gluconeogenesis (Landys et al. 2006). To support enhanced gluconeogenesis, GCs alter protein metabolism by favouring protein degradation and limit protein synthesis (Kuo et al. 2013). This mechanism provides the organism with amino acids that can readily be used for gluconeogenesis, so that plasma protein levels are decreased (Landys et al. 2006). Plasma free fatty acids also increase due to the effect of GCs on lipid mobilisation to fuel responses to stressors (Landys et al. 2006).

Catecholamines produced by the SNS during the early stage of the stress response decrease glomerular filtration rate (GFR, *i.e.* the rate at which the kidneys filter blood per unit of time; Gardoni 2019). However, chronic and acute GC levels increase renal activity, resulting in increased GFR, which is associated with a proteinuria and a paradoxical increase in plasma creatinine (Waters et al. 1997, Andreev et al. 1999). Creatinine is a by-product of the degradation of creatine in muscular cells and is directly related to muscle mass (Andreev et al. 1999). As GCs promote an increased GFR, a decrease in plasma creatinine would be expected during acute and chronic stress. However, acutely and chronically elevated GCs seem to be related to an increase in plasma creatinine, potentially resulting from the increased catabolism and a state of muscle loss (Andreev et al. 1999, Kuo et al. 2013). In addition, GCs can affect red blood cells (RBCs), the most numerous cells in plasma. RBCs carry haemoglobin, an iron-containing protein which role is to transport oxygen from the lungs to the rest of the organism. During acute stress, splenic contraction result in a significant release of blood rich in RBCs so that haematocrit (*i.e.* the volume percentage of RBC in blood) is increased and oxygen is transported more efficiently to the organism during the ‘fight or flight’ response (Gardoni 2019). However, on the longer-term GCs appear to disrupt RBC maturation by interfering with erythropoietin (EPO, *i.e.* a protein that stimulate the production of RBCs) through transcriptional mechanisms and through the binding of GC receptors and EPO receptors, inhibiting the signalling of EPO (Stellacci et al. 2009).

While the effects of acute stress on blood parameters are well described, the effects of chronically elevated levels of GCs are less understood and relate mostly to studies on human and laboratory rodents with administration of synthetic agonists of GCs (*e.g.* prednisone, dexamethasone).

c) GCs and immunity and immunosenescence

i. The immune system

Through their pathogenic effects, parasites act as a strong selective pressure on the host fitness in humans (Fumagalli et al. 2011, Adrian et al. 2019) and wild animals (Anderson and May 1982). Parasites are organisms that live in or on a larger different species, use part of the resources of their host for their own survival and reproduction, and may affect their host survival or reproductive success (Dimijian 1999, Penn 2001). Parasites include microparasites (*i.e.* viruses, bacteria, fungi and protozoan) and other macroparasites (*e.g.* helminths, ticks, mosquitoes). Since the pathogenicity (*i.e.* the capacity to cause disease to their host) is a common characteristic of parasites, the terms ‘pathogens’ and ‘parasites’ are frequently used interchangeably.

All living organisms are infected by parasites at one point in their lifetime. Therefore, organisms must have evolved defences to protect themselves against pathogens, ranging from behavioural spatial and/or temporal avoidance (*e.g.* Folstad et al. 1991), natural (physical and/or chemical and/or physiological) barriers (*e.g.* skin, cuticle; Moret and Moreau 2012) to complex physiological mechanisms such as the immune system of vertebrates (Boehm 2012, [Figure 7](#)). The vertebrate immune system is a complex two-armed physiological response to pathogens based on humoral and cellular effectors that regulate the host health and survival (Sorci et al. 2008, Shanley et al. 2009).

The two arms of the immune system include the innate immunity and the adaptive immunity. First, the innate immunity is a constitutive rapid and non-specific response to invading pathogens. Its activation, within hours following infection, is the first line of defence against pathogens (Klasing 2004). The innate immunity includes natural barriers and behavioural avoidance, but is more commonly regarded as the collection of various cells (*i.e.* phagocytic cells, such as neutrophils, monocytes and dendritic cells, and ‘Natural Killer’ (NK)

lymphocytes), and proteins that are recruited following infection. Phagocytic cells participate in eliminating pathogens through phagocytosis and in recruiting supplementary white blood cells. NK lymphocytes recognise and eliminate infected or deficient self-cells. Humoral innate immunity is constituted of a group of proteins, including the ‘complement’, that act in chain reactions to lyse exogeneous cells marked by antigen-antibody complexes (Matson et al. 2005, Turvey and Broide 2010), and the ‘natural antibodies’, which permit an early recognition of pathogens independently of previous immune challenge (Ochsenbein and Zinkernagel 2000). The innate immune response also promotes an inflammatory response, triggered through the complement proteins. The inflammatory response recruits inflammatory phagocytic cells (*i.e.* basophils, eosinophils), complement proteins, lysozymes and acute phase proteins to the site of injury or infection (Cray et al. 2009, Turvey and Broide 2010).

On the other hand, the adaptive immunity provides a slower (within days), but specific response to pathogens at first exposure with antigens. The response of this immune arm is mediated by T and B lymphocytes. T cells eliminate infected cells with intra-cellular pathogens through direct contact (cytotoxic T cells), coordinate the immune response by activating other cell types and producing cytokines (helper T cells) and participate in suppressing the immune response to maintain peripheral immune tolerance (regulatory T cells). B cells, when activated, differentiate into plasma cells that produce antigen-specific antibodies when exposed to an antigen. Specific antibodies (*i.e.* immunoglobulins) constitute the main humoral adaptive immunity and serve at eliminating pathogens and their products, enhancing phagocytic activity, inducing the activation of the complement and recruiting cells to the site of injury or infection. B cells also differentiate into memory cells that allow a rapid specific immune activation following a second exposure onward to a given pathogen (Bonilla and Oettgen 2010, [Figure 7](#)).

In general, innate immunity is quickly activated, but has non-specific action. In contrast, adaptive immunity is a slower response at first challenge, but is more specific and gets more rapid to build up and more efficient upon the second encounter with a specific antigen.

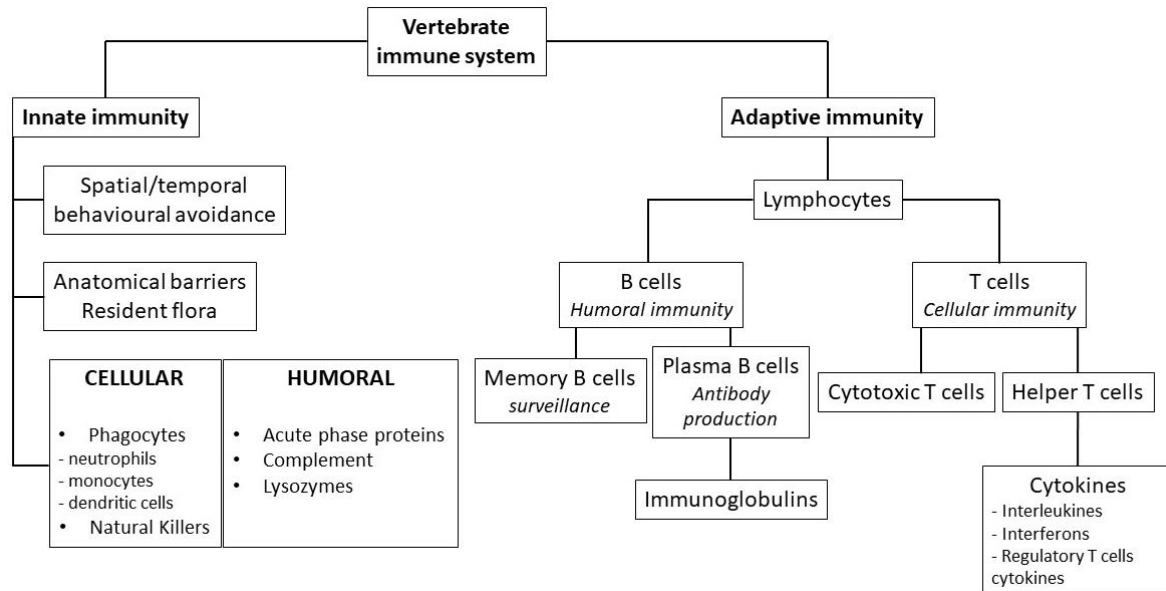


Figure 7. An overview of the vertebrate immune system. Adapted from Demas et al. 2011.

ii. The immune system as a life-history trait

Although essential, immune defences come at costs both for their development, maintenance and use, and for the organism. First, the development and maintenance of immunity has energetic costs, thus, as any other physiological function, immunity requires energetic and protein resources to be effective. Second, the use of immunity has energetic, but also metabolic costs (*i.e.* a shift of metabolic priorities towards immune response; Lochmiller and Deerenberg 2000) and immunopathological costs in case of dysregulation. Indeed, a dysregulated immune system can cause tissue damages and the disruption of other physiological processes. Each immune arm entails distinct costs for development, maintenance and use. Innate immunity is especially costly to activate and use, while its developmental costs are comparatively low. On the contrary, the activation costs of adaptive immunity are low, but it requires high energy to develop (Klasing 2004, Lee 2006, McDade et al. 2016). Therefore,

immunity is part of an individual life history and is subject to energy allocation trade-offs between immune arms and against other life-history traits such as somatic maintenance and repair, reproduction or growth (Sheldon and Verhulst 1996, Lochmiller and Deerenberg 2000, Lee 2006, Maizels and Nussey 2013, McDade et al. 2016).

Box 2. The development of eco-immunology

The perspectives that parasitism play a key role in the evolution of sexual selection (Hamilton and Zuk 1982) and that the immune system is a life-history trait that is subjected to energy allocation trade-offs between other physiological functions (Sheldon and Verhulst 1996), led to the development of the field of eco-immunology during the 1990s. The study of the immune system (*i.e.* immunology), initially restricted to human and laboratory investigations until the 1990s, eventually moved beyond controlled conditions and expanded to natural contexts, mixing insights from disease ecology, immunology, evolutionary biology and ecology. Eco-immunology is defined as the study of the causes and consequences of variations in immunity (Schoenle et al. 2018a) in environmentally relevant contexts, and account for the influence of genetic, individual, environmental and evolutionary factors, (Bowden et al. 2017). In a context where all animal species, including humans, are confronted to highly variable environmental conditions, show genetic diversity and are more and more exposed to infectious diseases (Jones et al. 2008), eco-immunology is highly relevant to better understand the drivers of immune variability.

However, the study of immunity in natural contexts requires to access to longitudinal data to account for the effects of age and infectious history on immunity. Eco-immunology also comes with technical and logistical constraints such as the transport and storage of biological samples or the need to consider the impact of capture and handling on animals and their immunity. It also requires assays designed for non-model species and the measurement of several markers, given the complexity of the immune system. Finally, ecologically relevant interpretations of such a broad set of measurements can be difficult (Martin et al. 2006, Schoenle et al. 2018a).

iii. Immunosenescence

Since the development of eco-immunology ([Box 2](#)), areas of investigations have extended to the study of the development of the immune system, immunoredistribution or immunosenescence, which were first also restricted to biomedicine (Martin et al. 2006). Immunosenescence (*i.e.* the progressive decline of immune functioning with advancing age) is

associated with increased infection diseases susceptibility in human (Pawelec et al. 2005, Hakim and Gress 2007) and the study of animal immunosenescence in natural contexts provides a promising opportunity to disentangle the proximate mechanisms underlying the ecology and evolution of senescence in the wild (Promislow et al. 2005, Peters et al. 2019, [Box 3](#)). A meta-analysis from 2019 collected 19 studies assessing immunosenescence in non-model mammal species and only 8 of them were conducted on wild populations. The remaining studies were conducted in captive contexts, and the majority of them were cross-sectional (Peters et al. 2019), whereas it is now clear that longitudinal studies are necessary to detect patterns of senescence (Nussey et al. 2008). Other studies of immunosenescence were mainly conducted on birds, or on humans and laboratory model species (Nussey et al. 2012, Peters et al. 2019).

In general, in human, variations of immune functions with age include a decrease in adaptive immunity (Hakim and Gress 2007) and an increase in the inflammatory response of the innate immune arm, leading to a low-grade inflammatory state named ‘inflammaging’ (Franceschi et al. 2000, 2007). Indeed, the production of inflammatory markers (*i.e.* pro-inflammatory cytokines) produced by senescent cells like macrophages increases (Franceschi et al. 2000). In parallel, the proliferation capacity of the hematopoietic stem cells of the bone marrow declines with age, leading to a decrease number of produced blood cells, impacting both innate and adaptive immunity. Also, thymic atrophy with advancing age results in a decreased production of naïve T cells, whereas the number and functioning of naïve B cells and antibodies also decline (Hakim and Gress 2007). Studies of immunosenescence in free-ranging and captive animals confirm these trends with an overall decline in immune function, coming mostly from a decline in adaptive immunity, and almost no changes in innate immunity, associated with an increased inflammatory response (Peters et al. 2019). In addition, immunosenescence is associated with an increase parasite burden with advancing age in mammals, including human (Hayward et al. 2009, 2015, Froy et al. 2019, Abdoli and Ardakani

2020). Changes in parasite burden with age are likely to transcribe the consequences of immunosenescence with direct effects on survival and reproductive success (*e.g.* Lynsdale et al. 2017), supporting that immunosenescence is expected to result in greater susceptibility to infectious diseases which can impact fitness in the wild (Sheldon and Verhulst 1996, Schmid-Hempel 2003).

iv. Implications of GCs for immunity and immunosenescence

A broad interest of eco-immunologists is devoted to unravel the link between stress and the immune system, which depends on previous and current environmental conditions (French et al. 2009). Since GCs play a major role in energy mobilization and allocation (Sapolsky et al. 2000, Reeder and Kramer 2005), they likely represent the proximate mechanism underlying trade-offs occurring between immune functions and other essential functions such as reproduction, for instance (French et al. 2009). Hence, the study of immunity in relation to stress is highly relevant in the field of eco-immunology (Schoenle et al. 2018a).

GCs at acute levels enhance immune functions (Martin 2009, Dhabhar 2014), an adaptive response proposed to be part of the ‘fight or flight’ response of an organism (Dhabhar 2002, 2009, [Figure 8](#)). GCs promote neutrophils, macrophages and T cells activity via an increased antigen presentation and phagocytic activity, mediated by cytokines (*i.e.* gamma interferon), a mediator of cellular immune response (Dhabhar et al. 2000, Dhabhar 2002). On the short-term, GCs trigger an increase in neutrophil counts, and a decrease in lymphocyte counts – a leukocyte profile typical of individuals exposed to stress (see [Chapter 1, section 3.a iii.](#)). If GC-mediated immuno-enhancement can be adaptive, it can also be maladaptive as an increased inflammatory response can increase pro-inflammatory and auto-immune diseases (Dhabhar 2014). On the other hand, chronic GC elevations result in immunosuppression (Dhabhar 2002, 2014, Martin 2009), throughout various mechanisms. GCs have the ability to inhibit the maturation, differentiation and proliferation of all immune cells, to induce apoptosis

in immature T and B cells, and mature T cells, to suppress the secretion of interleukin-1, which usually mediate inflammatory responses against pathogens, and to reduce lymphocytes, monocytes, and granulocytes chemotaxis and decrease leukocyte counts (Sapolsky et al. 2000, Martin 2009). Thus, chronic GCs, and associated allostatic overload (McEwen and Wingfield 2003), can have harmful consequences on the organism as it decreases resistance to infections (Martin 2009, Dhabhar 2014, [Figure 8](#)). As a consequence, GCs are overall positively related to parasite burden in humans and both wild and captive mammals (Defolie et al. 2020). Chronically elevated GCs seem to favour parasite infection through their immunosuppressive effects, whereas parasite infection act as a stressor eliciting the production of GCs (*i.e.* a 'vicious circle'; Beldomenico et al. 2008, Beldomenico and Begon 2010), and recent insights pinpoint the role of GCs in favouring parasite development (Herbert et al. 2022). However, GCs immunosuppression may also be beneficial as it restrains inflammation once infections have been brought under control (Dhabhar 2014, [Figure 8](#)).

Finally, both immunity and immunosenescence show between- and within-species variations, as well as inter-individual variations, all of which can largely be due to fluctuations in environmental conditions (McDade 2003, 2005). Thus, GCs provide a link between the environment and the immune system and can, at least partially, explain variations in observed immunosenescence patterns. Chronic GC elevations, and associated allostatic overload, can result in telomere attrition and accelerated cellular ageing (Casagrande et al. 2020, Lee et al. 2021, Bobba-Alves et al. 2023). Indeed, GCs promote oxidative stress (Costantini et al. 2011), leading to cellular damages, and contributing to telomere length shortening (Epel et al. 2004). Telomere shortening is supposed to be a major mechanism of senescence (López-Otín et al. 2013) in laboratory rodents, humans and wild vertebrates (Hausmann and Heidinger 2015, Angelier et al. 2018, Casagrande et al. 2020, Lee et al. 2021), including roe deer (Lemaître et al. 2021). Although the link between GCs and telomere shortening is not fully understood, it is

expected to involve oxidative stress. However, recent contributions also pinpoint the role of mitochondrial metabolic rate in telomere attrition and cellular ageing (Casagrande and Hau 2019, Casagrande et al. 2020, Bobba-Alves et al. 2023). Altogether, the role of GCs in regulating resource allocation trade-offs between immunity and other physiological functions (*cf.* Disposable Soma Theory, [Box 3](#)), and in impacting telomere attrition suggests that senescence is likely to be modulated according to the level of allostatic load, as measured by GC levels (Lee et al. 2021).

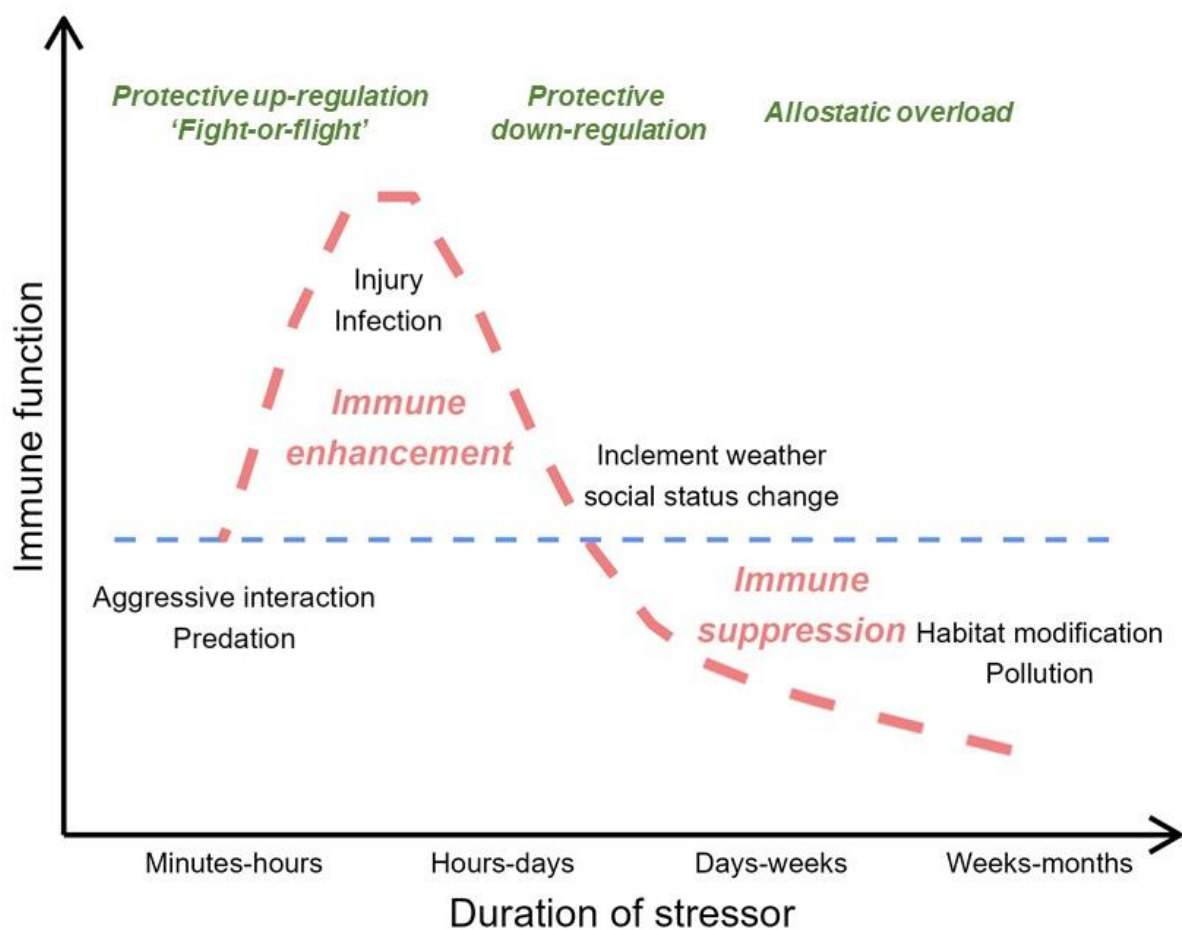


Figure 8. The immuno-enhancer and immunosuppressive effects of GCs depending on the duration of the stressor. Dashed blue line depicts the baseline immune functioning. The red dashed line depicts changes in immune function according to the duration of stressor exposure. Black text indicates examples of different stressors. Green italicised text at the top of the figure proposes evolutionary explanations for changes in immune functions according to the duration of stressors. Adapted from Martin 2009.

Box 3. The evolutionary theories of senescence

Senescence is defined as a decline in organismal functioning with age (Ricklefs 2008). It is a widespread phenomenon occurring in the quasi-entirety of the tree of life (Jones et al. 2014). The senescence of survival (*i.e.* actuarial senescence) and reproductive success (*i.e.* reproductive senescence) have been extensively studied in laboratory, captive and wild conditions, revealing that the onsets and rates of ageing differ between (Jones et al. 2014) and within species (Nussey et al. 2007, Lemaître et al. 2013), but also between traits within individuals in a so-called ‘mosaic ageing’ (Walker et al. 2005). A main challenge in current senescence research is to understand the sources of the observed diversity in the senescence onsets and rates, and to investigate the physiological mechanisms underpinning senescence.

Senescence has for long been considered an evolutionary paradox (Partridge and Barton 1993, Fabian and Flatt 2011). From the insight that the force of natural selection declines with advancing age (*i.e.* at older age, fewer individuals of a population are reproducing and contributing to the next generation; Fisher 1930), three main evolutionary theories of ageing have emerged:

- **The mutation accumulation theory** (MAT; Medawar 1952) posits that at the population level, few individuals would survive to old ages because of extrinsic mortality. Therefore, mutations with deleterious effects at old ages are not sorted out from the population gene pool and subsequently accumulate. In that respect, senescence is considered as a by-product of natural selection.
- **The antagonistic pleiotropy theory** (AP; Williams 1957) states that natural selection should favour the selection of beneficial early-acting genes, rather than the sorting of detrimental late-acting genes. This is because the magnitude of the effect of an allele on an individual’s fitness is compensated by the remaining probability of reproduction, which decreases with age. Thus, a small advantage of an allele at young ages outweighs a strong disadvantage of this allele at old ages. The AP considers that senescence is actively favoured by selection and implies that senescence is shaped by a trade-off between early benefits and late costs.
- **The disposable soma theory** (DST; Kirkwood 1977) posits that organisms accumulate resources from their environment and allocate them to different metabolic tasks (*i.e.* growth, reproduction, somatic repair). However, the pool of energy resources is limited, thus different resource allocation strategies can be adopted, and the question is therefore to determine the optimal strategy maximising fitness. Given the existence of extrinsic mortality over lifetime, resource allocation cannot be directed only towards somatic maintenance for optimal fitness outputs, so that a senescing life is inevitable (Kirkwood and Rose 1991). The DST is a special case and a physiological explanation of the AP where the focus is not population genetic processes but rather an optimisation theory approach based on physiological processes and associated molecular damages. The AP and DST are however not mutually exclusive and both have received support (Lemaître et al. 2015, Gaillard and Lemaître 2017, 2020).

Other theories exist, such as **the developmental theory of ageing** which supports the idea that senescence is shaped by the physiological processes optimised for early-life development, growth and reproduction, but not for late-life functions (Maklakov and Chapman 2019).

4. Synthesis and structure of the thesis

a) Synthesis and objectives

Humans and wild animals live in a fluctuating environment. Some of these fluctuations are predictable and part of the normal-life cycle of an individual, whereas some are demanding and unpredictable (Wingfield 2003, 2008). Glucocorticoids (GCs) are hormones produced through the hypothalamic-pituitary-adrenal (HPA) axis according to perceived demands associated to environmental conditions and life-history stages (Sapolsky et al. 2000). GCs play a key role in energy mobilisation and regulation of energy allocation among the different physiological functions of an organism (Sapolsky et al. 2000, Hau et al. 2016). At baseline levels, GCs regulate normal daily physiological processes (*i.e.* physiological state A; McEwen and Wingfield 2003, Landys et al. 2006). Baseline GC levels are heightened to support physiological processes associated with predictable life-history demands (*e.g.* breeding, moulting, migration) and predictable environmental demands (*e.g.* seasonal variations in resource availability and quality, climatic conditions) (*i.e.* physiological state B; McEwen and Wingfield 2003, Landys et al. 2006). As part of the stress response of an organism facing unpredictable and demanding conditions, (*i.e.* stressors such as predation, infection, injury, extreme climatic events, habitat destruction), the HPA axis elicits an enhanced production of GCs beyond baseline levels (*i.e.* physiological state C; McEwen and Wingfield 2003, Landys et al. 2006).

In the case where the stressor is temporary, the organism experiences an acute stress and GCs exert negative feedbacks on their own production and quickly (within minutes-hours) return to baseline levels (Sapolsky et al. 2000). This stress response is adaptive as it deviates energy resources away from non-immediately essential functions, such as growth or reproduction, and towards cardiovascular and locomotor functions that allow escaping the threat (*i.e.* ‘fight or flight’ response). However, if the stressor persists over longer periods of

time (days-months), the organism enters a state of chronic stress, a supposed maladaptive facet of the stress response during which GC levels are maintained elevated to support particular demands triggered by the stressor (Romero 2004, Koolhaas et al. 2011). In addition, baseline GC levels can be higher for an individual repeatedly or chronically stressed, compared to another individual with a lighter stress history. In the case of a repeatedly or chronically stressed individual, energy resources are deviated away from physiological functions such as growth, immunity, somatic maintenance or reproduction on the long-term, what is expected to have both detrimental immediate and long-term carry-over consequences on performances and fitness (Romero 2004, Monaghan and Haussmann 2015).

Physiological processes, such as the endocrine stress response, thus provide a relevant proximate mechanism to link the environment to individual performances and the shaping of life-histories, highlighting the relevance of integrating physiology, ecology and evolutionary biology (Ricklefs and Wikelski 2002). The immediate consequences of heightened baseline GC levels or chronically elevated GC levels on life-history traits have received attention, but results from observational studies in free-ranging animals are largely equivocal (Bonier et al. 2009a, Crespi et al. 2013). Also, the carry-over consequences of such GC levels have been far less studied. Especially, studies in natural settings and using longitudinal data obtained from long-lived animals (in particular mammals) are truly lacking. Longitudinal data are essentials to distinguish states of chronic stress from baseline GC measurements, and are essentials to account for individual and environmental information that could modulate both the stress response and its consequences on the organism (Crespi et al. 2013).

In the present thesis, I aim at filling this gap by assessing the immediate and carry-over consequences of baseline GC levels on different health parameters that are generally associated with viability and reproductive success. In particular, I have been using longitudinal data obtained from two roe deer (*Capreolus capreolus*) populations with contrasted environmental

conditions to assess the immediate and delayed relationships between baseline GC levels and body mass, physiological parameters, immune markers and their senescence.

b) Structure of the thesis

In a [first chapter](#), I will introduce the biology of the roe deer, present the two studied populations and describe the capture protocol of the capture-mark-recapture (CMR) program these populations are part of. I will also provide a description of all physiological and biological measurements performed on captured individuals, and detail the protocol of the laboratory manipulations. In particular, I will briefly describe the different types of measurements that can be obtained to estimate the activity of the HPA axis with a focus on faecal glucocorticoid metabolites (FGM) and the neutrophil:lymphocyte (N:L) ratio as an alternative proxy of the stress response. Finally, I will also describe the individual and environmental confounding variables that have been systematically accounted for in the analyses presented in this thesis.

The [second chapter](#) investigates whether baseline HPA activity is related to body mass in the two populations of roe deer. In such income breeders that do not store fat, body mass is a reliable proxy of body condition (Hewison et al. 1996, Andersen et al. 1998) that predicts survival and reproductive success (Gaillard et al. 2000a, b). The aim of this chapter is to test the prediction that a heightened baseline HPA activity negatively correlates to body mass, whether this negative immediate relationship persists on longer time-scales, and whether it is modulated by individual and environmental factors such as life-history stage, sex, population-specific environmental conditions or cohort-specific environmental conditions at birth. In a [first section](#) of this chapter, the focus is put on GCs to assess HPA activity, whereas in a [second section](#), the emphasize is put on the N:L ratio, where I discuss the relevance of this alternative/complementary measure.

In the [third chapter](#) I present the results of a study aiming at linking GCs and haematological parameters, arguably representative of the physiological and nutritional status

of an individual. The objective is to assess the immediate physiological consequences of baseline GCs on three blood parameters that cover different aspects of the physiological status of an individual, and to assess the carry-over effects of early-life GCs on the senescence of these parameters, that have already been shown to decline with advancing age in these two populations.

The [fourth chapter](#) explores the relationships between baseline GC levels, immunity and immunosenescence. Since the relationships between GCs and immunity are well described, the focus of this chapter was put on the delayed effects of GCs measured during the juvenile stage on adult immune performances and immunosenescence patterns. Here, immunity is evaluated using 12 immune markers that cover the humoral and cellular aspects of both adaptive and innate immunity, including the inflammatory response. It also includes parasite load variations with age, as parasite load is a direct translation of the consequences of declining immunity with advancing age. Such early-late effects of baseline GCs on immunity and immunosenescence has, as far as I know, never been studied, at least in a long-lived free-ranging mammal, and I provide here a first description of this relationship in two natural populations.

In the [discussion](#) of this thesis, I will first quickly summarise the results obtained in the previous chapters, emphasising the relevance of this work for ecophysiology. I will then emphasise the benefits for future research to use a large array of markers to fully capture a measure of allostatic load. In a subsequent paragraph, I will explore the links between GCs, body mass, immunity and physiology from a mechanistic perspective by integrating oxidative stress, mitochondrial metabolism and telomere attrition. I also briefly discuss preliminary results obtained on this topic. Finally, I will offer some recommendations if one hopes to investigate the consequences of the stress response on individual performances and fitness metrics.

For the bravest among the few that will read this thesis, you can still go through the [appendices](#) where I have hidden documents relating to the roe deer capture protocol (Appendix A), supplementary materials to the manuscript presented in the thesis (Appendix B-E), as well as side projects that I have been part of during this three roe-deer-centred years of my life (Appendix F-G).

CHAPTER 1 – Study system and Material & Methods



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1. Study System, the roe deer

a) General description and geographic distribution

The European roe deer (*Capreolus capreolus*) is a small ungulate from the Cervidae family, and belonging to the Artiodactyla order. Adults measure about 68-80 cm at shoulder, weigh between 18 and 32 kg (Lorenzini et al. 2022) and the species inhabits primarily forests but has also colonised a large variety of habitat, from all kind of forest types, to moorlands and marshes, in most of Europe (Andersen et al. 1998, [Figure 9](#)). Accordingly, roe deer can adapt to a wide range of climatic conditions, and has demonstrated behavioural plasticity, allowing them to tolerate habitat loss and fragmentation and human activities (Hewison et al. 2001). Although they favour habitats that provide them with covers from humans and predators, so that they avoid open habitats, roe deer also occupy agricultural areas which provide them with large amounts of rich food. However, such a varied habitat distribution and proximity with human activities exposes roe deer to a range of potential stressors, including anthropogenic disturbances (Hewison et al. 2001).

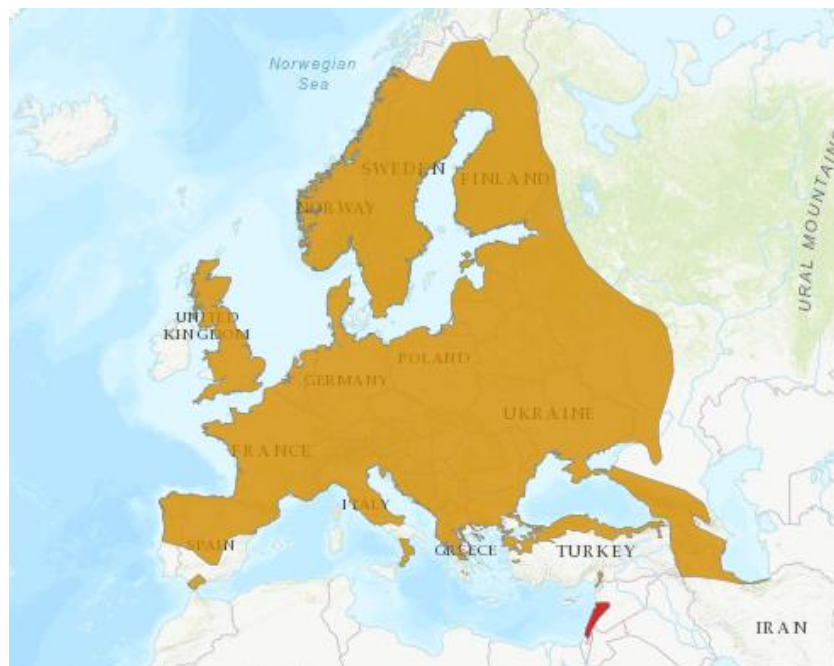


Figure 9. Geographic range of the European roe deer (*Capreolus capreolus*). Orange areas depict current distribution, and red area depicts areas where roe deer populations are extinct. Retrieved from the IUCN Red List of Threatened Species, version 2022-2 (Lovari et al. 2016).

b) Diet

Roe deer are generalist herbivores and considered income breeders (*sensu* Jönsson 1997). Individuals accumulate low body fat reserves and mostly rely on available food resources to complete their life cycle, so that body mass and physical condition do not vary widely between seasons (Hewison et al. 1996). As a consequence, roe deer, and especially fawns, are particularly sensitive to the quality and availability of food resources, which greatly impact their body condition, and environmental conditions can have strong influences on individual performances (Hewison et al. 1996, Douhard et al. 2013).

c) Survival

Roe deer is a long-lived species, with males living up to 14 years old, and females up to 18 years old in the wild. Prime-age survival (*i.e.* between 2 and 7 years old) is very high and constant (92-95 % and 83-86 % for females and males, respectively, Loison et al. 1999), but start decreasing from 8 years old onwards (Gaillard et al. 1993). Fawn survival is the most critical and variable during their first summer (20-80 %, Pettorelli et al. 2005), and is linked to the time of birth, early-growth rate and the mother quality (Plard et al. 2014). From their first winter, survival increases until stabilisation during prime-age.

d) Reproduction

This iteroparous species displays a weak sexual dimorphism, with males 5-10 % larger and on average 3 kg heavier than females, and only males display antlers (Andersen et al. 1998, [Figure 10](#)). Antlers in males are deciduous secondary sexual traits that grow each year, starting from the first year of life (called “buttons” then). Antlers grow from December until February-March when they are cleaned of velvet and have reached their maximum size (range 16-22 cm). Antlers are casted in October-November, a few months after the rut period, *i.e.* from mid-July to mid-August (Sempéré 1990).



Figure 10. Male (left) and female (right) roe deer, evidencing the weak sexual dimorphism characterising the species. Only males carry antlers. Photography: Andrea Dal Pian, retrieved from Lorenzini et al. (2022).

Males start reproducing at around 3 years old (Vanpé et al. 2009), male roe deer defend a territory from March (when antlers are cleaned of velvet) to August-September, and monopolise relatively few females during rut, *i.e.* from mid-July to mid-August (Andersen et al. 1998). During this 5-6 months period of territorial defence, males display aggressive, marking and patrolling behaviours, that coincide with variations in testosterone rates (Andersen et al. 1998). Females start reproducing from their second year of life and in most populations, the pregnancy rates is $> 95\%$ among females aged 3 to 10 years old, producing in general 1 to 3 fawns per litter. Afterwards, pregnancy rates decrease, and females aged 13 years old onwards usually stop reproducing 1 to 4 years before death (Lorenzini et al. 2022). Females display delayed embryonic implantations. A few days after mating, during the rut period, embryonic development stops and females enter a phase of diapause until December-January (Aitken 1974). Fawns are born in May and are fully weaned in October. They reach two third of their asymptotic mass at the beginning of their first winter, and reach their adult sizer between 3 and 4 years old (Lorenzini et al. 2022).

2. Two capture-mark-recapture programs

a) Study sites

Two French populations of roe deer (Chizé, CH; Trois-Fontaines, TF) have been intensively monitored by capture-mark-recapture (CMR) for nearly 50 years. The two CMR programs are conducted by the *Office Français de la Biodiversité* (OFB, French biodiversity office). The two sites display marked differences that impact the characteristics of both roe deer populations ([Figure 11](#)).

The site of Chizé (CH) is part of a CMR program since 1977. It is a 2,614 ha enclosed forest located in western France (46°05'N, 00°25'W, [Figure 11](#)). Since 2006 it has been classified as a *Réserve Biologique Intégrale* (RBI, integral biological reserve), so that no human interventions are authorised, with the exception of wild boar (*Sus scrofa*) hunting for management purposes, which can create disturbances for roe deer. Due to its location, the site displays a temperate oceanic climate with mild winter and frequent summer droughts. In addition to poor soils, the forest productivity is low (*i.e.* almost half of that of Trois-Fontaines), resulting in a habitat of overall poor quality in terms of food resources (Pettorelli et al. 2006). The habitat is heterogeneous with three distinct habitat types: a high-quality sector mainly composed of oaks (*Quercus* sp.), hornbeams (*Carpinus betulus*) and herbaceous species; an intermediate-quality sector composed mostly of oaks and maples (*Acer* sp.); and a poor-quality sector dominated by beeches (*Fagus sylvatica*). Such variability in habitat quality generates spatial variability in body mass and annual survival rates (Pettorelli et al. 2001, 2003).

The site of Trois-Fontaines (TF), followed by CMR since 1975, is a 1,360 ha enclosed forest in north-eastern France (48°43'N, 04°55'E, [Figure 11](#)). As a natural reserve and a *Territoire d'Etude et d'Expérimentation* (TEE, study and experimentation area), managing hunting is permitted during certain periods of the year (< 30 roe deer hunted per year). With a continental climate characterised by cold winters, warm rainy summers and rich soils, the forest

productivity is high (*i.e.* almost twice that of CH). Homogeneously composed of oaks and beeches, TF provides a habitat of high quality for roe deer (Pettorelli et al. 2006), as evidenced by high annual survival rates (Douhard et al. 2014).

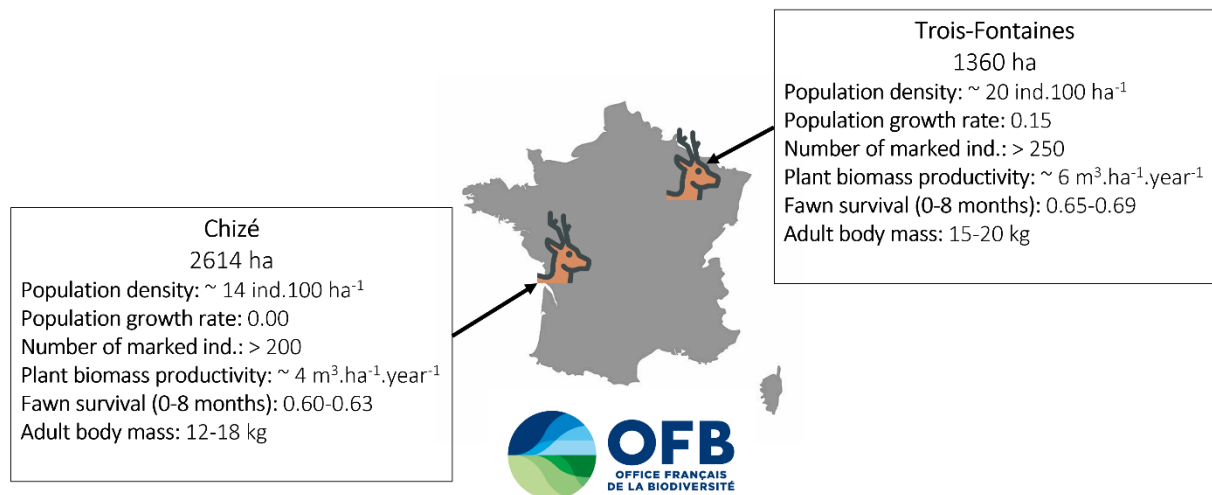


Figure 11. Location and characteristics of the two roe deer study sites of Chizé, in western France, and Trois-Fontaines, in north-eastern France, managed by the *Office Français de la Biodiversité* (OFB).

b) Capture protocol

Box 4. Authorisations and ethics

The research presented in the present thesis was done according to all institutional and/or national guidelines. For both populations (*i.e.* TF and CH), the protocol of capture and blood sampling of roe deer is under the authority of the *Office Français de la Biodiversité* (OFB) and was approved by the Director of Food, Agriculture and Forest (Prefectoral order 2009-14 from Paris). All procedures were approved by the Ethical Committee of Lyon 1 University (project DR2014-09, June 5th, 2014).

In both populations, captures take place every winter during 10-12 days spread from December (at TF) or January (at CH) to early March (Gaillard et al. 1993). Each year, the whole TF area is sampled, whereas about two third of the CH sites are sampled. Each capture year results in the capture of 200-300 individuals in each population, for a recapture probability of about 0.5 (Gaillard et al. 1993). Known-aged individuals represent more than 70 % of the individuals of both populations and are marked using visual collars, ear-tags and transponder

micro-chips, allowing to individually identify them. Age is known if individuals have been marked as fawns during a period of newborn research conducted in April-June, and during which maternal identity is determined from field observations. Otherwise, unmarked individuals caught during their first winter (*i.e.* 8-9 months old) can be aged from observation of their dentition eruption (Hewison et al. 1999).

A typical capture day requires the involvement of 150-350 volunteers to push roe deer towards 25 km of nets surrounding specific areas of each site. Net-caught roe deer are immediately handled by experienced technicians and transported in wood boxes to the laboratory for manipulations.

Manipulations consist of four experienced persons ([Figure 12](#)) performing contention for about 15 minutes during which the individual is weighed to the nearest 50 g, examined, identified and samples collected. Unmarked individuals identified as being within their first year of life are marked with ear-tags for future identification and allowing to collect cartilage for DNA analyses. On all individuals (*i.e.* known-aged and unknown-aged), morphologic measurements are collected (*i.e.* hind foot length (mm), antler length (mm)), as well as hair for DNA, heavy metals or endocrine analyses. Faeces are collected from direct rectal manipulations and separated in three different samples: ~ 20 pellets/individual for parasitic analyses, immediately stored at 4 °C; a similar quantity of pellets for diet analyses, immediately stored at 4 °C; and for known-aged individuals, a minimum of ~ 10 pellets/individuals for hormonal analyses (*i.e.* dosage of testosterone and glucocorticoid metabolites), immediately stored at -80 °C. The presence of external parasites is assessed in a standardised way based on the observation of the head and ears area. Behaviour during the manipulation is also recorded to evaluate acute stress response (see fieldwork form, [Appendix A](#)). In 2022, nasal swabs were collected for SARS-CoV-2 analyses (see [Appendix F](#)). Finally, on known-aged individuals blood is collected from the jugular vein (1 mL.kg⁻¹ with a maximum of 20 mL) using a 20 mL

syringe (TERUMO® SST20ES) and a 30 mm long needle with a diameter of 1.0 mm (TERUMO® NN-1030R). Blood samples are collected for different analyses: white blood cell counts are determined from whole blood samples preserved at 4 °C on EDTA and performed within 48 hours following sampling; measures of immune activity and estimation of humoral composition are performed on serum extracted by centrifugation immediately after collection and conserved at -20 °C; telomere length are measured from buffy coat containing leukocytes extracted from centrifuged tubes immediately after collection and stored at -20 °C. Following manipulations, individuals are released on their site of capture ([Figure 13](#)) and behaviour upon release is recorded according to detailed criteria ([Appendix A](#)). Note that all manipulations are validated and authorised by competent authorities ([Box 4](#)).



Figure 12. Manipulations of roe deer on the site of Trois-Fontaines. Photography on the left retrieved from the Journal de Haute-Marne (JHM, 2022), and photography on the right from François Débias.



Figure 13. Roe deer release following manipulations. A unique visual collar can be seen on the bottom picture. Photography: François Débias.

3. Physiological measurements

In the following sections I will detail the protocols of the different measurements collected on roe deer and used in the analyses presented in the current thesis. I will first give a brief description of the different matrices commonly used to estimate GC levels, with a particular focus on faecal glucocorticoid metabolites (FGMs), and a small presentation of an alternative measures of the adrenal activity, namely the Neutrophil:Lymphocyte (N:L) ratio. Then I will

describe the protocols and measurements that allowed to define the immune profile and the physiological condition of captured individuals.

a) Measuring stress in the wild

i. Blood measurements and other matrices

Measure of GCs is probably one of the most-used trait measured in wild animals to estimate the activity of the HPA axis and the stress response (Sapolsky et al. 2000, Busch and Hayward 2009, Sheriff et al. 2011). For long, blood GCs have been the favoured index measured in vertebrates, as it gives an immediate picture of the state of an organism at the time of collection. Blood GCs indicate a mixture of the daily and seasonal variations in GC levels, the acute response to a perceived stressor, and prior exposure to chronic stressors (Sheriff et al. 2011). Although invasive and requiring restraints and/or anaesthesia, blood GC measurements have the advantages of measuring directly the activity of the HPA axis (*i.e.* not a by-product of it), and it allows mutualise several blood analyses (*e.g.* immunity, protein content, other hormones, metabolism) to capture a full picture of an individual state. However, a main drawback of measuring GCs in blood samples is that it can be difficult to obtain ‘true’ baseline GC levels in wild settings. Indeed, following capture or restraint, blood GC levels will increase rapidly and sampling is advised to occur within 3 minutes in order to avoid measuring the stress response to acute stressors (Romero and Reed 2005). If the acute stress response might be an interesting trait to measure, depending on the focus, and if protocols and methods exist (*e.g.* hormonal challenge) to account or prevent the measurement of such acute responses (Sheriff et al. 2011, Lavergne et al. 2021), these methods cannot always be performed on wild animals, and the focus in these context is usually to measure baseline GC levels to evaluate the state of an individual (Busch and Hayward 2009, Sheriff et al. 2011).

Rapid and standardised protocols allowing the measurement of baseline blood GC levels in the two populations of roe deer presented above are not possible. Indeed, it is impossible to

predict when and where individuals will be caught and several trained persons are needed to sample them, making it impossible on the field to have a quick and standardised sampling protocol at the time of the capture of an individual. Hormonal challenge is also not an option as individuals cannot be kept for several hours after manipulation. Accordingly, field scientists and technicians might have to rely on alternative methods to measure GCs. GCs can be measured in other matrices than blood, such as saliva, excreta (*i.e.* urine, faeces), integumentary structures (*e.g.* hair, feathers) or milk. For the next section we will focus on GCs measured in faeces, the technic used in the present thesis but the technics used to measure GCs in these matrices and their advantages and drawbacks are reviewed elsewhere (Sheriff et al. 2011, Palme 2019).

ii. Faecal glucocorticoid metabolites (FGMs)

Faecal GC measurements consist in measuring the by-products of GCs resulting from their metabolization. Following exposure to stressors, the production of GCs by the HPA axis is increased, and plasma-circulating GCs (but other steroid hormones as well) are metabolised by the liver, primarily. Following this metabolization, GC conjugates are excreted *via* bile and urine. Conjugates excreted through bile are further metabolised by bacterial enzymes in the intestine and later excreted *via* faeces (Palme 2019). GC metabolites are detectable in faeces after a species-specific time delay which basically reflects the gut passage time of the species considered. In roe deer, the time delay between GCs production and GC metabolites excretion *via* faeces is roughly about 12 h, ranging from 6 to 23 h (Dehnhard et al. 2001). What is then measured are faecal GC metabolites (FGMs).

Steroid metabolites determination is generally achieved thanks to two methods: chromatography, combined with mass spectrometry, and immunoassays. The relative advantages and drawbacks of both technics have been reviewed (*e.g.* Wudy et al. 2018), but for faecal steroid metabolites, immunoassays are generally favoured because chromatography

requires knowledges about the chemical identity of the metabolites investigated, is expensive and not adapted to large scale studies (Palme 2019). Immunoassay methods used are competitive immunoassays on rely on the competition between the sample steroid metabolites and labelled steroids for binding to a limited number of antibodies. As sample, unlabelled, metabolites bind more readily to antibodies, the amount of metabolites in the sample tested is inversely proportional to the amount of labelled steroids bound. In the present studies, and almost exclusively now, immunoassays used to determine FGM concentrations are enzyme immunoassays (EIA, using enzyme as labels, as opposed to radioimmunoassays relying on radioactive labels). For a detailed protocol of FGMs extraction and quantification, see [Box 5](#). Note that FGMs determination requires species-specific analytical and physiological/biological validation. Analytical validations assess the accuracy, specificity, precision and sensitivity of the quantifying method, while physiological (or biological) validations aim at experimentally inducing changes in GCs production by stimulating the HPA axis and to determine the time delay between stimulation and peak increase in FGMs (Palme 2019), and both have been validated for roe deer (Dehnhard et al. 2001, Möstl et al. 2002, Zbyryt et al. 2018).

FGM levels are largely used in wild, captive or domestic animals (*e.g.* Palme and Möstl 1997, Möstl et al. 2002, Khan et al. 2002, Wong et al. 2016, Carbillet et al. 2020, Larm et al. 2021, Ukonaho et al. 2023) as it is a relevant integrative measure of GC concentrations arguably representative of baseline adrenocortical activity over a few hours (Palme 2019). It is also a non-invasive technic, that can be even obtained without seeing and disturbing individuals if identity is not required (but it requires fresh faeces). Moreover, FGMs are more reliable and more powerful predictors of chronic stress than baseline blood GC measurements, and this is of great interest for ecophysiologicals (Dickens and Romero 2013).

Box 5. FGMs extraction and quantification protocol

FGMs extraction

For each sample, approximately one third of five pellets are delicately cut into small particles to limit compaction. We place $0.5 \text{ g} \pm 0.005 \text{ g}$ of this mixture in a numbered Pyrex tube. To avoid any bacterial contamination, it is preferable to keep the tubes on ice until adding 5 ml of 80% methanol in the hood. The samples are vortexed for 1 minute for homogenisation. Samples are then placed 15 minutes in the centrifuge at 2500 g, and after centrifugation, 1 ml of the supernatant is collected and placed in an Eppendorf tube, which will bear the numbering of the tube and the plate. Finally, the samples are stored at -20°C and transported under cold conditions to Vienna where quantification occurs (a 24-hour journey).

FGMs extractions were performed at the *Laboratoire de Biométrie et Biologie Evolutive* (LBBE)/UMR 5558, Lyon, France. I personally performed FGMs extraction for samples obtained from the 2020-2023 capture sessions of TF and CH, with help from Amandine Herrada (Ph.D. student) and Benjamin Rey (research engineer).

FGMs quantification

FGMs-methanol extracts are diluted 10 times in assay buffer. Analyses were performed using the group-specific 11-oxoetiocholanone enzyme immunoassay (EIA). The EIA was performed on microtiter plates with a double antibody technic, and was conceived as to detect specific groups of metabolites characterised by the presence of 11,17-dioxoandrostane structures. Measurements are carried out in duplicate and results are expressed as nanograms of metabolites per grams of wet faeces (ng.g^{-1}). Intra- and inter-assay coefficients were lower than 10 and 15 %, respectively.

FGMs quantifications were carried out at the University of Veterinary Medicine, in Vienna, Austria, by Rupert Palme (associate professor). Many thanks to him and all technicians involved.

iii. N:L ratio

In the present thesis, we propose a short analysis aiming at testing the relevance of another index increasingly used to estimate the activity of the HPA axis ([Chapter 2B](#)). Accordingly, the utilisation of leukocyte profiles as a complementary or alternative measure for assessing GC levels has gained increasing popularity among ecophysiologists, particularly in

aquatic vertebrates and birds (Davis et al. 2008, Davis and Maney 2018). Indeed, exposure to stressors leads to an elevated production of GCs, which, in turn, triggers changes in the composition of circulating leukocytes. Neutrophils (or heterophils in birds and reptiles) increase, while lymphocytes decrease in response to this stressor-induced rearrangement. It is believed that this shift serves an adaptive purpose, as elevated circulating neutrophil levels prepare the organism for potential infections (Dhabhar 2002, 2009, Goessling et al. 2015) while avoiding wasting unnecessary resources of the adaptive arm of the immune system. Consequently, the Neutrophil:Lymphocyte (N:L) ratio has garnered increasing attention over the past decade, as an elevated N:L ratio is indicative of a stress response (Dhabhar 2006, Davis et al. 2008).

This metric is touted to offer several advantages over traditional GCs indices. First, it appears that the GCs response to environmental stressors diminishes with the duration and repetition of the stressor, whereas the N:L ratio response remains consistent over time (Goessling et al. 2015, Davis and Maney 2018). Second, the N:L ratio response to stressors occurs later than that of GCs, obviating the need for rapid sampling as is the case for plasma GCs (Montané et al. 2007). Consequently, the N:L ratio is arguably a reliable indicator of chronic stress, as it is easier to measure at baseline and maintains its sensitivity in the face of repeated or chronic stressors. Finally, the analysis of N:L ratios is faster, simpler, and cheaper compared to GC metabolites in alternative matrices like hair, feathers, or faeces (Davis et al. 2008). Remarkably, N:L ratios have seen limited application in studies involving wild mammals (*e.g.* Seltmann et al. 2017, Cohas et al. 2018, Carbillet et al. 2019, Ukonaho et al. 2023), and they have been even less frequently employed to elucidate inter-individual variations in life-history traits, in contrast to the extensive research conducted on GCs (Davis and Maney 2018).

The protocol for the determination of N:L ratios used in the current thesis is detailed in the following parts dealing with the measurements of immune traits, as well as in the manuscript presented in [Chapter 2B](#).

b) Physiological measurements

Physiological condition of roe deer was assessed using three haematological parameters that reflect the nutritional state and physiological functioning of individuals. Namely, haematocrit (HCT), albumin (ALB) and creatinine (CREA) were investigated. HCT (%) represents the proportion of red blood cells (RBCs) within the bloodstream. It provides insights into an individual nutritional state and the blood ability to carry oxygen to the muscles (DelGiudice et al. 1992). ALB, on the other hand, is the most abundant protein in the blood and regulates osmotic pressure and fluid transfers. Variations in ALB levels indicate the availability of serum protein resources and an individual nutritional condition. ALB is considered a reliable indicator of the availability of environmental resources (Sams et al. 1998). Finally, CREA is a by-product produced during the breakdown of creatine in muscle cells. It is eliminated through glomerular filtration in the kidneys, and its concentration in the blood provides information about both muscle mass and renal filtration efficiency (Andreev et al. 1999).

Blood samples collected on the field were stored between 4 and 6 °C and analysed within 48 hours at the Biochemical and Endocrinological laboratory, Biovelys, VetAgro Sup, France. Haematological analyses were performed as described in Gilot-Fromont et al. 2012 and Jégo et al. 2014. Briefly, RBCs were enumerated by impedance technology and the percentage of red blood cells within blood sample (*i.e.* HCT, %) were determined considering bovine sample parameters. Refractometry and automatic agarose gel electrophoresis permitted to determine ALB concentrations (g/L). Creatinine concentrations ($\mu\text{mol/L}$) were measured using a Konelab 30i automaton (Fisher Thermo Scientific, Cergy-Pontoise, France) with Thermo Scientific reagents.

c) Immune measurements

During the present thesis, I characterised immune phenotypes of individuals captured between 2010 and 2022, by describing the composition and activity of their immune system using 12 immune traits that reflect both the adaptive and innate arms (including markers of the inflammatory response), and by measuring both their humoral and cell-mediated components.

i. Innate immunity

Cellular innate immunity is assessed by describing the composition of the white blood cell (WBC) population. The first hundred WBCs are counted in Wright-Giemsa-stained blood smears to determine the proportion of the different types of WBCs and expressed as concentrations (10^3 cells/mL). Five different cell types compose WBCs. 1) Neutrophils, the most numerous WBCs and 2) monocytes, are key components of the innate arm of the immune system through their antimicrobial/biotoxic and phagocytic activity (Auffray et al. 2009, Burn et al. 2021). 3) Basophils, the least abundant WBCs, have a specific role in the inflammatory response and are particularly active at sites of ectoparasite infections (*e.g.* ticks) and communicate with the adaptive arm of the immune system (Siracusa et al. 2010, Karasuyama et al. 2011). 4) Eosinophils are involved in the inflammatory response and reaction to allergies (with basophils and mast cells). In particular, they play a role in the defence against internal parasites (*e.g.* helminths), and modulate the innate and adaptive immunity (Rothenberg and Hogan 2006). Finally, 5) lymphocytes, involved in the cellular adaptive immunity, are described below.

Box 6. HA-HL assay protocol, adapted from Matson et al. (2005)

Hemagglutination (HA) measures the ability of an individual to agglutinate exogenous cells, as representative of the concentration of circulating natural antibodies (NABs). Similarly, hemolysis (HL) measures the activity of the complement, a group of proteins acting in chain reactions to provoke the lysis of exogenous cells in the presence of antigen-antibody complexes (Matson et al. 2005). Plasma samples coloration is determined before the test to estimate its HL level (**Figure a**).

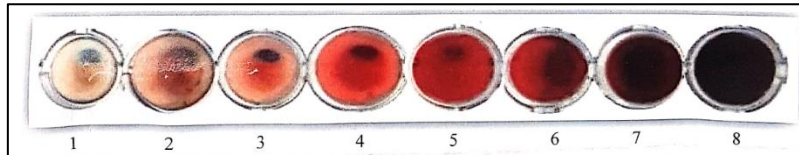


Figure a. Standard colorations to compare to plasma coloration to determine hemolysis level of samples before the test.

The HA-HL test is performed using serum samples stored at -20 °C in 96-well plates (12 columns, 8 rows). Columns 1 and 12 serve as positive controls, row H serves as negative control. 25 µL of ten plasma samples are pipetted into the first two wells of columns 2-11, respectively. Columns 1 and 12 are pipetted similarly but with rabbit serum. 25 µL of 0.01 M PBS are added to all wells of rows B-H. With a multi-channel pipetter, the content of row 1 is serially-diluted (1:2) down to row G, so that dilution is multiplied by 2 from row A (1:1) to row G (1:64) and each well contains 25 µL (row H contains only PBS). 25 µL of a 1 % chicken blood cell suspension is added to all wells and plates are sealed with Parafilm and covered. Plates are gently vortexed during 10 seconds, and incubated for 90 minutes at 37 °C in a water bath. After incubation, plates are tilted to 45° along their length for 20 min at room temperature. Plates are then scanned to determine HA scores. After scanning, plates are left at room temperature for another 70 minutes, and scanned again to read HL scores (**Figure b**). HA and HL scores are therefore expressed as $10^{-2} \log(\text{dilution})$.

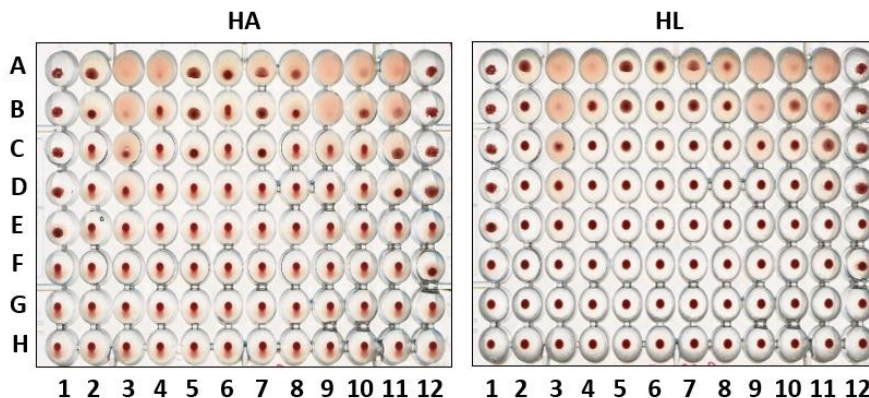


Figure b. Determination of HA and HL scores from a scanned test plate performed on 10 individuals (columns 2-11), with two positive controls (columns 1 and 12) and a negative control (row H).

During my Ph.D I performed HA-HL assays for samples from CH and TF obtained in 2021 and 2022, with help from Emmanuelle Gilot-Fromont (Ph.D. supervisor), at the LBBE/UMR 5558, Lyon, France.

The humoral part of the innate immune system was evaluated by determining the concentrations (mg/mL) of alpha1-globulins, alpha2-globulins and beta-globulins. These globulins are inflammatory proteins involved in the acute phase response, a part of the early innate immune system (Cray et al. 2009). Protein concentrations were assessed by refractometry and automatic agarose gel electrophoresis (HYDRASYS, Sebia, Evry, France) to separate albumin and alpha1-, alpha2-, beta- and gamma-globulins. Haptoglobin concentrations (mg/mL), were also determined through a Konelab 30i automaton (Fisher Thermo Scientific, Cergy-Pontainse, France) using phase haptoglobin assay chromogenic kit (Tridelta Development LTD, County Kildare, Ireland). Haptoglobins are part of the alpha2-globulin fraction and produced in case of chronic inflammation and infection. Hemagglutination-hemolysis (HA-HL) assays were also performed, as described in Matson et al. (2005) and previously used on roe deer blood samples (Gilot-Fromont et al. 2012, Cheynel et al. 2017, Carbillet et al. 2022, 2023a, [Box 6](#)).

ii. Adaptive immunity

Cellular adaptive immunity was determined by measuring the concentration of lymphocytes (10^3 cells/mL). Lymphocytes include both B and T cells. T lymphocytes have many functions, including the recognition of exogeneous or infected cells and the induction of apoptosis. B lymphocytes play a crucial role in the humoral adaptive immunity, by being the primary producers of antibodies.

Humoral adaptive immunity was assessed by determining the concentration (mg/mL) of gamma-globulins (or immunoglobulins). Gamma-globulins constitute the majority of circulating antibodies and were measured by refractometry and automatic agarose gel electrophoresis.

Haematological parameters were obtained at Biovelys, VetAgro Sup, Marcy-l'Etoile, France under the supervision of Gaël Baral, Cyrille Debard and Eve Ramery. Leukocyte formula and HA-HL assays have been performed at the LBBE/UMR 5558, Lyon, France, by Corinne Régis.

d) Parasitic loads

We enumerated the faecal propagules from four parasites that are commonly found in roe deer populations, as described in Cheynel et al. (2017). This enumeration serves as a reliable indicator of parasite abundance in roe deer during the capture period in these populations. The investigated parasites included pulmonary nematodes (protostrongylids), gastrointestinal (GI) nematodes (GI strongyles and *Trichuris* sp.), and GI protozoa (coccidia, *Eimeria* sp.). For GI nematodes and coccidia, we determined the counts of eggs per gram (EPG) and oocysts per gram (OPG) of faeces, respectively, employing the McMaster protocol (Raynaud 1970). In the case of pulmonary nematodes, we calculated the count of stage 1 larvae per gram of faeces (LPG) using the Baermann faecal technique (Baermann 1917). Briefly, the McMaster protocol aims at determining faecal propagule counts using a specific plate with a known volume of faecal suspension, examined microscopically. The Baermann faecal technique relies on the migration of larvae: faeces are suspended in water, so that larvae migrate into the water and sink at the bottom of the device where it can be collected and identified.

Parasitic counts are conducted at the laboratory of parasitology of VetAgro Sup, Marcy-l'Etoile, France, by Slimania Benabed and Marie-Thérèse Poirel under the supervision of Gilles Bourgoïn. Before 2014, faeces analyses were performed by Hubert Ferté at the EA4688 VECPAR from the University of Reims, France.

4. Individual characteristics and environmental conditions

As mentioned in Introduction of the current thesis, individual characteristics and environmental conditions are likely to modulate relationships between GCs and life-history or fitness traits. Such factors can explain part of the variability in the directions and strengths of these relationship, and accounting for those factors can prove useful for future research. In the following analyses, conducted during my Ph.D., I took advantage of individual and environmental information available on the two populations of roe deer investigated to account as much as possible for those sources of variations.

All analyses presented below include at least the information of the sex and the age of the individual. When it was relevant, I also performed analyses separately for juveniles and adults, or separated individuals according to their growth period (early growth *v.* late growth *v.* prime age). Analyses also included information about overall environmental conditions (*i.e.* population) and current and early-life environmental conditions (*i.e.* year of capture, and cohort environmental quality, respectively).

In those two populations of roe deer, cohort environmental quality, referred as ‘cohort quality’ hereafter, is reliably estimated as the cohort-specific average juvenile body mass corrected for the median date of capture (Gaillard et al. 1996). From the birth of an individual (typically in May) up to its capture during its first winter, environmental conditions can greatly influence roe deer condition. This metric of environmental quality integrates information about variations in environmental conditions (*e.g.* climate, resource availability and quality, roe deer density) as well as information about individual quality (*e.g.* mother condition, maternal care, itself partly dependent on environmental quality). Such an integrative metric of natal environmental conditions allows to avoid the problem of defining the exact metric to consider to describe environmental conditions (Toïgo et al. 2006).

***CHAPTER 2 –
Glucocorticoids and N:L
ratios in relation to body mass***



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1. Glucocorticoids negatively relate to body mass on the short-term in a free-ranging ungulate



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Research article

Glucocorticoids negatively relate to body mass on the short-term in a free-ranging ungulate

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Environmental fluctuations force animals to adjust glucocorticoids (GCs) secretion and release to current conditions. GCs are a widely used proxy of an individual stress level. While short-term elevation in GCs is arguably beneficial for fitness components, previous studies have documented that the relationship between long-term baseline GCs elevation and fitness components can vary according to ecological and individual factors and according to the life-history of the species studied. Using longitudinal data on roe deer *Capreolus capreolus* from two populations facing markedly different environmental contexts, we tested whether baseline GC levels negatively correlate with body mass – a trait positively associated with demographic individual performance – on the short- to long-term. In support, higher baseline GC concentrations were associated to lighter body mass, both measured during the same capture event, in adults of both populations. Overall, we showed that despite the marked environmental and demographic differences between populations and despite the between-sex differences in life history (i.e. reproductive tactics), the relationship between body mass and GCs is consistent across environmental contexts, but might differ according to the life history stage of an individual. This work opens promising perspectives to further explore the relationship between GC and fitness-related traits according to life history stages in free-ranging mammals across seasonal and environmental contexts. The timing and context-dependence of GC levels highlight the complexity of studying stress responses in the wild.

Keywords: body condition, faecal cortisol metabolites, individual performance, stress response, ungulates



Introduction

Throughout their life, individuals have to adjust their morphology, physiology and/or behaviour to cope with environmental variation. Part of this variation is predictable as individuals can rely on environmental cues (e.g. photoperiod or temperature at the daily or seasonal scales) to anticipate future conditions (Wingfield 2003, 2008). Whereas predictable variation is not considered as stressful per se (i.e. eliciting a stress response), an unpredictable perturbation can disrupt life cycles and trigger a stress response through the activation of the hypothalamic-pituitary-adrenal (HPA) axis and the release of glucocorticoids (GCs), departing them from the baseline level (Reeder and Kramer 2005). The primary role of these hormones (namely cortisol and corticosterone) is to maintain an organism's energy balance according to its requirements in its current environment (Wingfield 2013, Hau et al. 2016), meaning that GCs promote allostasis (i.e. achieving stability through change) by ensuring energy homeostasis despite predictable and unpredictable environmental and life history variation (McEwen and Wingfield 2003, 2010, Romero et al. 2009). On the short-term, an elevation of GC concentration in an individual facing a challenging situation is beneficial because it promotes processes enhancing survival through reallocation of stored energy from non-immediately essential functions (e.g. growth, reproduction) towards cardiovascular functions or locomotion and foraging activities (Sapolsky et al. 2000). After a temporary perturbation, the GC concentration often quickly returns to the baseline level (Reeder and Kramer 2005). However, baseline GC concentration may remain elevated on the long-term (i.e. days to weeks) as a result from exposure to repeated or long-lasting perturbations such as prolonged inclement weather or food shortage (McEwen and Wingfield 2010, Hau et al. 2016). Such elevated GC concentration in the long-term can have adverse consequences on individual performance through disruption of the immune function (Dhabhar 2014), inhibition of growth, or decreased body condition (Reeder and Kramer 2005), with an intensity depending on the species (Boonstra 2013). Indeed, long-term GC elevation can cause 'allostatic overload' (i.e. when the energy intake is lower than the energy required to support daily and seasonal activities and to cope with unpredictable perturbations, McEwen and Wingfield 2010). Although variations in GC concentration does not reflect only stress (MacDougall-Shackleton et al. 2019), GC levels are a widely used proxy for evaluating an individual physiological stress response (Palme 2019).

While many studies analysed the effect of an increase of GC beyond the baseline level (i.e. acute stress-induced GC concentration) on individual performance, fewer have considered how GCs at baseline concentration (i.e. homeostatic levels of hormones) affect the performance of animals in the wild. Results from these rare studies remain equivocal (Rogovin et al. 2003, 2008, Altmann et al. 2004, Ethan Pride 2005, Cabezas et al. 2007, Pauli and Buskirk 2007, Pedersen and Greives 2008, Pinho et al. 2019), which could be explained by the complex influence of environmental

conditions (Henderson et al. 2017). When resources are limited, increased energy demands can be detrimental by resulting in high levels of GC and allostatic overload. Nevertheless, this detrimental effect may not be observed when resources are abundant, as high energy input can contribute reducing allostatic load (McEwen and Wingfield 2003, 2010, Romero et al. 2009). Food availability (i.e. energy availability) or behavioural flexibility stimulating foraging activity can thus compensate for elevated GC levels and associated higher energy requirements (Henderson et al. 2017). Animals inhabiting suboptimal habitats (e.g. exposed to human disturbance, predation, intra- or interspecific competition or pathogens) experience continuous stressful conditions and generally show a higher GC concentration compared to conspecifics inhabiting optimal areas (Dantzer et al. 2013, 2014, Formenti et al. 2018, Boudreau et al. 2019, Carbillet et al. 2020, Dulude-de Broin et al. 2020).

Additionally, there is evidence that the direction of the relationship between GCs and performance can also depend on individual attributes, such as body condition, sex or reproductive status and reproductive tactics (Tilbrook et al. 2000, Ricklefs and Wikelski 2002, Wey et al. 2015, Blumstein et al. 2016, Vuarin et al. 2019). Under high energy demands triggered by elevated GC concentration, high quality individuals are more likely to prevent themselves from reaching allostatic overload and may perform better than poor quality ones. In African striped mice *Rhabdomys pumilio*, elevated baseline GC concentration was related to the selective disappearance of light individuals (Vuarin et al. 2019). Since the relationship between GCs and performance is complex and involves individual attributes and environmental context, both sources of variation have to be investigated to disentangle their respective roles (Crespi et al. 2013, Dantzer et al. 2014).

Focusing on body mass provides a relevant approach to investigate the detrimental effects of prolonged exposure to a high GC concentration. Indeed, body mass is often positively linked to fitness components (Gaillard et al. 2000; Ronget et al. 2018) and is a trait easily measured in the field that constitutes a reliable proxy of body condition in many species, especially income breeders (Hewison et al. 1996, Andersen et al. 1998). High levels of GCs are known to have a crucial role on body mass by increasing protein and amino-acid catabolism and mobilising lipids (promoting gluconeogenesis), consequently decreasing body mass (Boonstra et al. 1998, Dallman et al. 1999, Hodges et al. 2006, Rabasa and Dickson 2016).

However, the relationship between baseline GC concentration and body mass is complex. Indeed, within seasonal baseline levels, GCs actually result in increased feeding behaviours potentially promoting fat storing and body mass gain, as opposed to the previously-mentioned GC actions on protein and amino-acid catabolism and lipid mobilisation (Landys et al. 2006). Case studies have shown that the relationship between baseline GCs and body mass or condition can be positive, negative or null (George et al. 2014, Wey et al. 2015, Hennin et al. 2016, Boudreau et al. 2019). For instance, low body mass and poor body condition were

associated with higher baseline cortisol concentrations in Eurasian badgers *Meles meles* (George et al. 2014). On the other hand, experimental increase in GC concentration through predation risk manipulation resulted in no changes in body condition in snowshoe hares *Lepus americanus* (Boudreau et al. 2019). Thus, more exploratory research are needed in natural settings to better understand which factors can modulate this relationship.

In this exploratory study, we aimed to contribute to a better understanding of how high baseline GC concentration relates to body mass in roe deer *Capreolus capreolus*, an income breeder for which body mass is a good proxy for body condition (Hewison et al. 1996, Andersen et al. 2000, Pettorelli et al. 2006). We took advantage of the detailed and intensive monitoring data collected through capture–mark–recapture for more than a decade at the individual level in two populations subject to markedly different ecological contexts (i.e. one inhabiting a good quality habitat and one population being strongly food-limited; Pettorelli et al. 2006). To avoid measuring the capture-induced GC response (Romero and Reed 2005), longer-term, indirect measures of GC concentration can be obtained from biological matrices other than blood (Sheriff et al. 2011). Indeed, blood GC concentration is a point in time measure and circulating GCs concentration typically increases drastically within 3 min following an individual's capture (Sapolsky et al. 2000, Romero and Reed 2005), which makes it difficult to obtain reliable measures of the HPA baseline activity in free-ranging animals (but see Sheriff et al. 2011, Lavergne et al. 2021). In this context, levels of faecal glucocorticoid metabolites (FGMs) are particularly relevant as they represent an integrative measure of GC concentration several hours before the capture event (i.e. baseline stress; Palme 2019). Thus, we measured FGMs, as arguably representative of baseline adrenocortical activity over a few hours, with a time delay of 12 h on average in roe deer (ranging from 6 to 23 h, Dehnhard et al. 2001).

As chronic stress can have both immediate and long-term consequences on fitness components (Monaghan and Haussmann 2015), we tested the immediate relationship between concomitant body mass and FGMs (i.e. short-term relationship), the relationship between FGMs in a given year and the change of body mass between two consecutive years (i.e. medium-term relationship) and the relationship between FGM measured on juveniles (i.e. individuals in their first year of life) and body mass during the prime-age and senescent life stages (i.e. long-term relationship). We first analysed the relationship between FGMs and body mass measured at the same capture event. We expected individuals with higher FGM concentration to be lighter than individuals with low FGM concentration. As roe deer are income breeders that do not store fat (Hewison et al. 1996, Andersen et al. 1998), we expected the catabolic actions of GCs to directly impact body mass and condition and to overcome the effects of increased feeding behaviours promoted by GCs. Then, since chronically elevated FGMs are expected to be deleterious on the medium to long-term, we tested whether FGMs measured at a given capture influenced body mass changes across lifespan.

Thus, we first assessed the relationship between FGMs measured in a given year and a given body mass change between two consecutive years, separately for growing individuals and prime-aged adults having reached their full mass (i.e. between 4 and 10 years old; Douhard et al. 2017). We expected that higher FGM concentration should result in a smaller mass gain for growing individuals, and in a decrease in body mass for prime-aged adults, between two consecutive years, as chronic stress can be linked to inhibited growth or decreased body condition (Reeder and Kramer 2005). Finally, we tested for the effect of the stress experienced during the first year of life on the individual body mass relative to the population and sex at a given age, from the second year of life onwards. We predicted that individuals with higher FGMs during the first year of life should be lighter later in life, as late-life performance in roe deer is affected by early-life environmental conditions (Gaillard et al. 2003). Lastly, in all analyses we expected the negative relationship between FGMs and body mass to be steeper in the food-limited population due to higher allostatic load (McEwen and Wingfield 2003, Henderson et al. 2017), and we expected sexes to respond differently to higher FGMs levels due to the different reproductive tactics of males and females (Ricklefs and Wikelski 2002).

Material and methods

Study population and sample collection

We studied roe deer in two populations inhabiting closed forests at Trois-Fontaines (TF – 1360 ha) in north-eastern France (48°43'N, 04°55'E) and at Chizé (CH – 2614 ha) in western France (46°05'N, 00°25'W). Roe deer are medium-sized ungulates, weighing around 25 kg and common in lowland woodlands throughout most of Europe. Roe deer display weak sexual selection, with adult males only 10% heavier than females and party size less than three females per buck. They are income breeders (Andersen et al. 2000), and to meet the markedly increased energy needs during the late gestation and early lactation periods, adult females rely on food resources rather than body reserves. Both sexes allocate high energy expenditure to reproduction. Females produce twins every year from 2 to 12 years old (Andersen et al. 1998), and males allocate heavily in territory defence for 5–6 months during the rut period (Johansson 1996). The TF population is characterised by a homogeneous habitat of good quality on a broad spatial scale, with high forest productivity due to rich soils. On the other hand, the CH population inhabits a less suitable heterogeneous habitat comprising three types of varying quality (Pettorelli et al. 2001), with low forest productivity due to poor soils and frequent summer droughts (Pettorelli et al. 2006). In both study sites, large carnivores are absent, but hunting occurs occasionally to control population growth. Since 1975 in TF and 1977 in CH, a capture–mark–recapture (CMR) program has taken place every winter, and CMR analyses show that

both populations were quite below carrying capacity during the present study period (2010–2021 in TF, 2013–2021 in CH, unpubl.). Captures take place during 10–12 days each year, which are spread across December (at TF) or January (at CH) and March (Gaillard et al. 1993) and consists of drive-netting captures with 30–100 beaters pushing individuals towards nets surrounding specific areas of the forests. Given that captures took place each year over three months, we might expect endocrine activity to vary throughout the capture season according to environmental conditions (i.e. populations) and sex (Dantzer et al. 2010, Sheriff et al. 2012). Therefore, we tested whether FGM levels varied as a function of the Julian date, population and sex and found that FGMs increased throughout the season in a population-specific manner (Supporting information). Thus, all FGM measurements were standardised for the median Julian date of capture (9 February) specifically for each population. Successive capture events within a capture season may also have consequences on FGM measurements during the following captures. However, most capture days were more than 48 h apart while in roe deer, FGMs peak from 6 to 23 h after an ATCH challenge and return to baseline levels between 28 and 31 h after treatment (Dehnhardt et al. 2001). In some cases, captures took place during two consecutive days, but were then conducted on opposite areas of the forest to minimise disturbances. Likewise, morning and afternoon captures of a given day took place in different areas within both forests. We thus considered that FGM levels were not influenced by previous captures. Concerning the potential impact of the capture event itself, since roe deer were captured by drive-netting and most animal manipulations occurred between 1 and 4 h after capture, we tested for a possible impact of this delay on FGM levels. We did not detect any increase or decrease in FGMs (log-transformed) along with the time between capture and sampling (linear regression accounting for repeated measurements of a given individual: $0.006 \text{ h}^{-1} \pm 0.01 \text{ SE}$, $t=0.44$, $p=0.7$), even when the delay was $> 6 \text{ h}$ (mean FGMs 6 h onwards after capture compared to mean FGMs measured within 6 h following capture, accounting for repeated measurement of a given individual, $-0.06 \pm 0.06 \text{ SE}$, $t=-1.05$, $p=0.3$) (Dehnhardt et al. 2001). Individuals of known age (i.e. captured within their first year of life) were weighed, and faecal matter has been collected since 2010. During the capture period, diet is similar and mostly composed of brambles *Rubus* sp. and ivy *Hedera helix* in the two deciduous forests (Tixier and Duncan 1996), so FGM measurements between populations should not be biased according to diet composition. During this period, females can be gestating, and differences in female reproductive status could result in FGM variability (Brunton et al. 2008, Dantzer et al. 2010). Nevertheless, we did not account for females' reproductive status in our models, since 1) we have information about reproductive status (i.e. pregnant or not and number of foetuses) only at CH where ultrasounds are performed on captured females, 2) most of them (i.e. 92%) were gestating at that time and 3) due to the delayed embryonic implantation (Aitken 1974), gestation is at a very early stage during

the capture season. Body mass measured on juveniles (i.e. at about eight months of age) depends on the date of capture. Juveniles gained on average 12 and 24 g day^{-1} in CH and TF, respectively, during the capture period (Douhard et al. 2017). Therefore, we standardised juvenile body mass with a linear regression, using the above-mentioned Julian date–body mass relationship. Thus, we computed the individual body mass expected on the 9 February, the median date of captures at CH and TF. In these two populations, cohort quality is reliably measured by the cohort-specific average juvenile mass corrected for the Julian date of capture (Gaillard et al. 1996). This proxy of environmental quality from the birth of an individual (typically in May) to its capture as juvenile in winter was obtained for all years, and will be referred to as 'cohort quality' hereafter.

Faecal glucocorticoid metabolites (FGMs)

Baseline adrenocortical activity was estimated through FGMs (Palme 2019). We collected faecal matter rectally at capture from 2010 to 2021. Faeces were frozen at -20°C within 24 h prior to 2013 in CH and 2017 in TF, and immediately frozen at -80°C after collection since then. The time between faecal sampling and freezing, as well as freezing temperature, may impact FGM values, with longer delays and higher temperature likely to result in lower FGM levels due to bacterial activity (Lexen et al. 2008, Hadinger et al. 2015, Carbillat et al. 2023b). In CH, the first FGM values available in our dataset are post-2013, so all samples were immediately frozen at -80°C . However, in TF, FGM values are effectively higher when samples were immediately frozen ($0.43 \pm 0.06 \text{ SE}$, $t=7.62$, $p < 0.0001$). This is accounted for in our models by adding the year of capture as a random effect. Extraction of FGM followed a methanol-based procedure and the analysis was performed using a group-specific 11-oxoetiocholanolone enzyme immunoassay (EIA), a method previously described in details (Möstl et al. 2002) and validated for roe deer (Zbyryt et al. 2018). In brief, 0.5 g (± 0.005) of faeces were vortexed in 5 ml of 80% methanol before being centrifuged for 15 min at 2500 g (Palme et al. 2013). The amount of FGM was determined in an aliquot of the supernatant diluted 10 times in assay buffer. Measurements were done in duplicate with intra- and inter-assay coefficients lower than 10 and 15%, respectively. FGMs are expressed as nanograms per grams of wet faeces (ng g^{-1}). The data were then log-transformed for the statistical analyses (henceforth called FGMs).

Statistical analyses

All analyses were performed using R ver. 4.2.2 (www.r-project.org). Two raw FGM measures were unusually high: 5192 and 6194 ng g^{-1} , for a female in CH aged 10, and a female in TF aged 1, respectively (Supporting information). Results exclude these extreme values (analyses including them are reported in the Supporting information), but we specify whether adding them yielded different conclusions or not.

FGM repeatability

Individual FGM repeatability was calculated for all individuals, and separately for each population, using the 'rptR' package (Stoffel et al. 2017). Repeatability analysis included individuals sampled only once to improve estimates of the within-individual variance (Martin et al. 2011). Within-individual FGM repeatability was detectable but weak in both CH ($r=0.14$, 95% CI=[0.03, 0.26]) and TF ($r=0.15$ [0.01, 0.29]).

Model structure

We tested the hypothesis that body mass is related to FGM levels, and that this relationship may depend on individual (i.e. sex) and environmental (i.e. population and condition at birth) characteristics, using linear mixed effect models (LMMs) with a normal error distribution. All continuous covariates were mean-centred so that the intercept is the relative individual body mass for the mean cohort quality and/or mean FGMs. Visual inspection of model residuals confirmed that they fulfilled the assumptions of Gaussian distribution and homoscedasticity.

Short-term relationships between FGMs and body mass measured at the same capture event

We first assessed the relationship between FGMs and body mass measured the same year (i.e. at the same capture) to test the hypothesis that higher baseline FGMs result in short-term adverse consequences on body mass. Individuals in their first year of life (i.e. juveniles) were analysed separately from individuals in their second year of life onwards, because juveniles have to allocate to growth and are much more susceptible to any environmental harshness than adults, making the first year of life is the critical period of roe deer population dynamics (Hamel et al. 2009, Gaillard et al. 2013). In addition, metabolic rate is generally higher in juveniles than in adults due to the costs of growth (Glazier 2005) and metabolic rates can alter hormones metabolism and excretion, making it difficult to compare juvenile and adult FGM concentrations (Goymann 2012). Since juveniles are not yet reproductively active, they also likely display different endocrine profiles than adults (Dantzer et al. 2010). Finally, adult roe deer habituate faster than juveniles to stress, which can in turn affect the relationship between FGM and fitness-related trait (Bonnot et al. 2018). Body mass varies with age, and at a given age, varies between sexes and according to environmental conditions at birth (i.e. cohort effects, Hamel et al. 2016). We thus tested the relationship between FGMs measured in a given year and individual body mass relative to the average body mass of all individuals of the same age, population and sex, considering only ages for which we had data from at least three individuals. Therefore, the average body mass was calculated up to 10 and 15 years old in males and females at CH, respectively, and up to 12 and 13 years old in males and females at TF, respectively. For juveniles, we analysed the relationship between FGMs and body mass measured the same year on 368 juveniles (78 females and 96 males in CH, 91 females and 103 males in TF). The response variable

was the mass corrected for the date of capture (above) and fixed explanatory variables included FGMs (corrected for the date of capture and the population), cohort quality, population, sex and the two-way interactions between FGMs and the other covariables. Cohort quality was expressed as a relative cohort quality (i.e. the difference between mean cohort quality in each population and an individual cohort quality), so there is no redundancy with the population variable. The year of birth of the individuals (i.e. cohort) was included as a random effect. For individuals aged two years or older, the dataset included 655 observations on 377 individuals: 104 females (218 observations) and 83 males (136 obs.) in CH, and 106 females (164 obs.) and 84 males (137 obs.) in TF. The response variable used to test for the FGMs–mass relationship was the individual body mass minus the average mass of roe deer of the same population, same sex and same age, regardless of their year of capture (hereafter 'relative body mass'). Fixed effects included continuous variation in FGMs, relative cohort quality, population, sex and the two-way interactions between FGMs and other covariables. Random effects of the cohort and of the year of capture were also included. Random effect of the individual identity (ID) was also included to account for repeated measurements on the same individuals.

Medium-term relationships between FGMs and body mass change between two consecutive years

Baseline FGMs can reflect medium-term consequences of GCs on body mass. The relationship between FGMs and the change of body mass between two consecutive years was analysed separately for early-growing individuals (i.e. between age one and two), late-growing individuals (i.e. between the second year of life and adulthood at four years old, Hewison et al. 2011) and for prime-aged adults which had reached their full body mass (i.e. from 4 to 10 years old, Hewison et al. 2011). The response variable was the change in relative body mass. Briefly, the change in relative mass expresses the change in body mass of a given individual between two ages in relation to the change in body mass of all individuals of the same sex and population between the same ages. The relative mass was calculated as the difference between the mass of an individual at age x , and the mean mass of all individuals of age t of the same sex and the same population. The change in relative body mass was calculated as the difference between the relative mass at age $t+1$ and the relative mass at age t . Fixed effects included relative cohort quality, population, sex, FGMs (either measured at age t or considered as the average of the FGM values measured at age t and age $t+1$), body mass at age t (i.e. initial body mass) and all two-way interactions between FGMs and the other covariables. Random effect of the cohort was included, and for late-growing and prime-aged individuals, random effects of the year of capture and individual ID were also included. For growing individuals, the dataset included 99 individuals with FGMs measured as juveniles, and 67 individuals when including FGMs measured during the first and second years of life to calculate the mean value. For both late-growing individuals and adults,

we kept a single observation per individual since the inclusion of individual ID as a random effect created singularities. For individuals with several observations, we kept the one for which the individual's age was the closest to the mean age of all individuals with a unique observation according to sex and population, among observations with complete data. The final dataset comprised 85 individuals in their late-growth period and 90 prime-aged adults with FGMs measured at age t , and 63 late-growing individuals and 71 prime-aged adults for which we had measurements at age t and $t + 1$.

Long-term relationships between FGMs during early life and body mass later in life

As elevated baseline FGMs are expected to have negative effects on body mass on the long-term, and as environmental conditions during the first year of life have carry-over effects on performance later in life in roe deer (Gaillard et al. 2003), we tested whether FGMs during development are associated with adult body mass. Only individuals for which we measured FGMs as juvenile and body mass beyond the second year of life were analysed. We used 345 observations on 159 individuals for which we measured FGMs as juvenile and body mass as adult: 34 females (78 obs.) and 44 males (87 obs.) in CH, and 42 females (99 obs.) and 39 males (81 obs.) in TF. The response variable was the relative individual body mass as defined above. Fixed effects included FGMs measured when juvenile, relative cohort quality, population, sex and the two-way interactions between FGMs and the other covariables. Random effects of the cohort and the year of capture were included, as well as individual ID to account for repeated measurements on the same individuals.

Model selection

Final models were selected based on the second order Akaike information criterion (AICc, i.e. Akaike information criterion corrected for small sample sizes). We compared all sub-models included in the full model described above and for each model AICc scores were computed with the 'MuMIn' package ver. 1.47.5 (Baroń 2023). We retained the best-fitting model as the one with the lowest AICc score (Burnham and Anderson 2002), or the simplest model (i.e. with the lowest number of parameters) within the set of models within $7 \Delta\text{AICc}$. Indeed, models within $7 \Delta\text{AICc}$ are plausible models, become increasingly equivocal up to $14 \Delta\text{AICc}$, and implausible afterwards (Burnham et al. 2011). For each variable we estimated its effect size (β) with 95% confidence intervals (95% CI) and calculated marginal and conditional R^2 . We provide full model selection tables in the Supporting information.

Results

FGM concentrations ranged from 8 to 3428 ng g⁻¹ with a median value of 689 ng g⁻¹. The median FGMs was 674 ng g⁻¹ (range: 34–3275 ng g⁻¹) in CH, and 715 ng g⁻¹ (range: 8–3428 ng g⁻¹) in TF.

Short-term relationships between FGMs and body mass measured at the same capture event

Juveniles

The retained model highlighted the expected positive relationship between cohort quality and body mass ($\beta_{\text{Cohort quality}} = 1.08 [0.84, 1.32]$, Table 1, Supporting information), but no relationship between FGMs and body mass could be evidenced (Fig. 1a, Table 1, Supporting information). Results were similar when including female of TF with a high FGM value (SI). Note that since the random effect of the cohort created singularities, it was removed and models therefore consisted of linear models.

Adults

We found support for a short-term negative relationship between individual relative body mass and FGMs in adults ($\beta_{\text{FGM}} = -0.32 [-0.46, -0.18]$, Fig. 1b, Table 1, Supporting information). Results were similar when including the high FGM value measured on a female of CH (SI). Since the random effect of the cohort created singularities, the models only included individual ID and year of capture as random effects.

Medium-term relationships between FGMs and change in body mass between two consecutive years

Early-growing individuals

We did not find any evidence for a relationship between FGMs, either measured in juveniles or considered as the mean FGM value between the first and second years of life, and the change in relative body mass for growing individuals (Fig. 2a, Table 1, Supporting information). Results were similar when including the female of CH with the highest FGM value (Supporting information). All models included cohort as random effect.

Late-growing individuals

We found a negative relationship between body mass gain and initial mass ($\beta_{\text{mass}} = -0.15 [-0.25, -0.05]$, Table 1, Supporting information) for the dataset accounting for FGMs measured in juveniles. With the dataset including the mean FGM value between FGMs measured at age t and age $t + 1$, we also found a negative relationship between late growth and body mass measured at age t ($\beta_{\text{mass}} = -0.17 [-0.27, -0.07]$, Table 1, Supporting information). However, in both cases, we found no support for a relationship between FGMs and change in relative body mass (Fig. 2b, Table 1, Supporting information). Models resulted in singularities when the random effect of the cohort was included so that only the year of capture was included as a random effect in the models.

Prime-aged adults

In no cases (i.e. neither when accounting for FGM measured at age t or for the mean FGM value between age t and age $t + 1$) was a relationship found between FGMs and change in relative body mass (Fig. 2c, Table 1, Supporting information). Similarly, we found no evidence that mass measured at age

Table 1. Linear and linear mixed effect models selected for the short-, medium- and long-term relationships between relative body mass and faecal glucocorticoid metabolites (FGMs). Models accounted for sex, population and for relative cohort quality (Qcohort, the difference between the mean cohort quality in each population and an individual cohort quality). Models were selected through model selection based on AICc. 95% CI: 95% confidence intervals, V: variance, SD: standard deviation.

SHORT-TERM									
Juveniles			Adults						
Random effects	V	SD	Random effects	V	SD				
			Individual ID	3.45	1.86				
			Year of capture	0.29	0.54				
Fixed effects	Estimate	95% CI	Fixed effects	Estimate	95% CI				
Intercept	-0.06	[-0.28, 0.16]	Intercept	-0.02	[-0.39, 0.35]				
Qcohort	1.08	[0.84, 1.32]	FGM	-0.32	[-0.46, -0.18]				
Marginal R ²	0.18		Marginal R ²	0.02					
Conditional R ²			Conditional R ²	0.81					
MEDIUM-TERM									
FGM measured at age <i>t</i>									
Early-growth			Late-growth			Prime-aged			
Random effects	V	SD	Random effects	V	SD	Random effects	V	SD	
Cohort	0.65	0.81	Year of capture	0.41	0.64	Year of capture	0.24	0.49	
Fixed effects	Estimate	95% CI	Fixed effects	Estimate	95% CI	Fixed effects	Estimate	95% CI	
Intercept	-0.35	[-0.92, 0.22]	Intercept	-0.55	[-1.02, -0.08]	Intercept	-0.19	[-0.60, 0.22]	
			Initial mass	-0.15	[-0.25, -0.05]				
Marginal R ²	-		Marginal R ²	0.09		Marginal R ²	-		
Conditional R ²	0.21		Conditional R ²	0.32		Conditional R ²	0.15		
Mean FGM (FGMt and FGMt + 1)									
Early-growth			Late-growth			Prime-aged			
Random effects	V	SD	Random effects	V	SD	Random effects	V	SD	
Cohort	0.90	0.95	Year of capture	0.32	0.56	Year of capture	0.18	0.42	
Fixed effects	Estimate	95% CI	Fixed effects	Estimate	95% CI	Fixed effects	Estimate	95% CI	
Intercept	-0.37	[-1.11, 0.37]	Intercept	-0.47	[-0.94, -0.00]	Intercept	-0.28	[-0.71, 0.15]	
			Initial mass	-0.17	[-0.27, -0.07]				
Marginal R ²	-		Marginal R ²	0.13		Marginal R ²	-		
Conditional R ²	0.26		Conditional R ²	0.32		Conditional R ²	0.10		
LONG-TERM									
Random effects	V			SD					
Individual ID	3.65			1.91					
Year of capture	0.63			0.79					
Fixed effects	Estimate			95% CI					
Intercept	-0.06			[-0.63, 0.51]					
Marginal R ²	-								
Conditional R ²	0.83								

t, cohort quality, population or sex were related to changes in relative body mass (Table 1, Supporting information). Models resulted in singularities when the random effect of the cohort was included, thus, only the random effect of the year of capture was included in the models.

Long-term relationships between FGMs during early-life and body mass later in life

We found no evidence for a relationship between FGM measured in juveniles, cohort quality, population or sex, and adult relative body mass (Fig. 3, Table 1, Supporting information). Results were similar when including the three observations of the adult female of CH with the highest FGM value measured as juvenile (Supporting information). We only included

individual ID and year of capture as random effects, because including the cohort created singularities.

Discussion

The relationship between GCs and fitness-related traits such as body mass is yet to be fully deciphered. Recent reviews indicate that studies in the wild have led to contrasting results by showing either negative, positive or no relationships between measures of stress and studied traits (Bonier et al. 2009, Crespi et al. 2013, Dantzer et al. 2014). A salient conclusion from these studies is that the relationship depends on an individual's ecological context and internal state. In the present study, the longitudinal monitoring of individual roe deer in

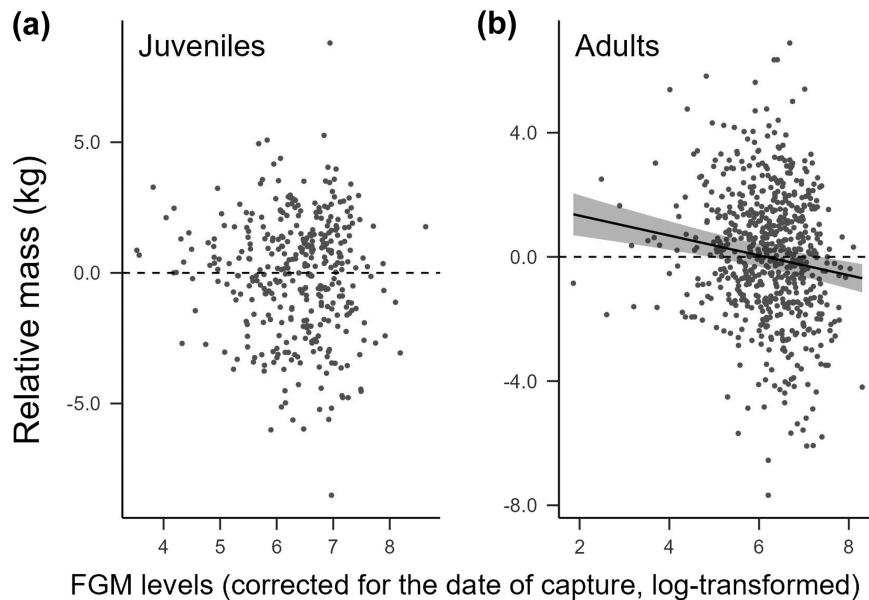


Figure 1. Relationship between faecal glucocorticoid metabolites (log-transformed FGMs, corrected for the date of capture and population) and individual relative body mass (individual body mass (corrected for the date of capture for juveniles) minus the average body mass of all individuals of the same age, population and sex) for (a) juveniles ($n = 368$ individuals) and for (b) adults aged two years old onwards ($n = 655$ observations on 377 individuals). Points are raw data and line is the prediction from the retained model with 95% confidence intervals.

two populations exposed to markedly different environmental contexts – one facing limiting resources and the other benefiting from a higher availability and quality of resources – allowed us to analyse the relationship between FGMs and body mass, while accounting for ecological factors and individual attributes such as sex and age. On the short-term, we found clear evidence for a negative relationship between baseline adult FGMs and adult body mass, an important driver of fitness in this species (Gaillard et al. 2000).

We could not detect any relationship between FGMs and the change in relative body mass between two consecutive years, neither in growing or prime-aged individuals. In late-growing individuals only, a negative relationship between initial body mass and relative body mass gain appeared, so that lighter individuals of age t tended to gain more weight from one year to the other, suggesting the existence of compensatory growth mechanisms in these populations (Metcalf and Monaghan 2001). Likewise, we found no support for a relationship between early-life FGM levels and adult body mass. Indeed, we found that FGMs and body mass were negatively associated only on the short-term and in adult individuals only. This suggests that FGMs relate to body mass, but without any carry-over effects, and that FGMs are related to body mass differently according to life-history stages. High viability selection at the juvenile stage in the two populations (Toïgo et al. 2006, Douhard et al. 2014) could explain that we could not observe any carry-over effects of FGMs on body mass, and an absence of evidence for a short-term relationship between FGMs and body mass in juveniles.

Roe deer body mass correlated negatively with FGM levels on the short-term, in adults, with no detectable population or sex differences. This result suggests that the relationship

between FGM and body mass is consistent across individuals and between environmental contexts in this species. Roe deer are income breeders, relying mostly on acquired resources rather than on stored energy (Andersen et al. 2000). As GCs stimulate activity through the mobilisation of energy stored (Sapolsky et al. 2000, Reeder and Kramer 2005), the physiological costs of elevated GC levels are expected to directly impair body condition as individuals do not have body reserves to buffer elevated energy requirements (Henderson et al. 2017). However, given marked between-population differences in environmental context we would have expected individuals at TF to be able to mitigate the physiological costs of high GC levels by reducing their allostatic load (McEwen and Wingfield 2010, Henderson et al. 2017), compared to individuals at CH which were expected to enter allostatic overload, thus expecting different allostatic load in the two populations (McEwen and Wingfield 2010). Both populations differ in terms of overall habitat quality, with CH being characterised by a low forest productivity due to poor soils, mild winters, but frequent droughts during summer, and TF by a high forest productivity due to rich soils and relatively wet summers (Pettorelli et al. 2006). Accordingly, life history parameters differ between populations. This is especially the case for juvenile survival (Gaillard et al. 1993), body condition (Gilot-Fromont et al. 2012), senescence patterns (haematological parameters: Jégo et al. 2014, body mass: Douhard et al. 2017, immune system: Cheynel et al. 2017), and the relation between FGMs and immune traits (Carbillet et al. 2023a). Thus, differences in the relationship between FGMs and body mass was expected to occur between populations, with the strongest negative association in CH, the poorer habitat in which individuals would be

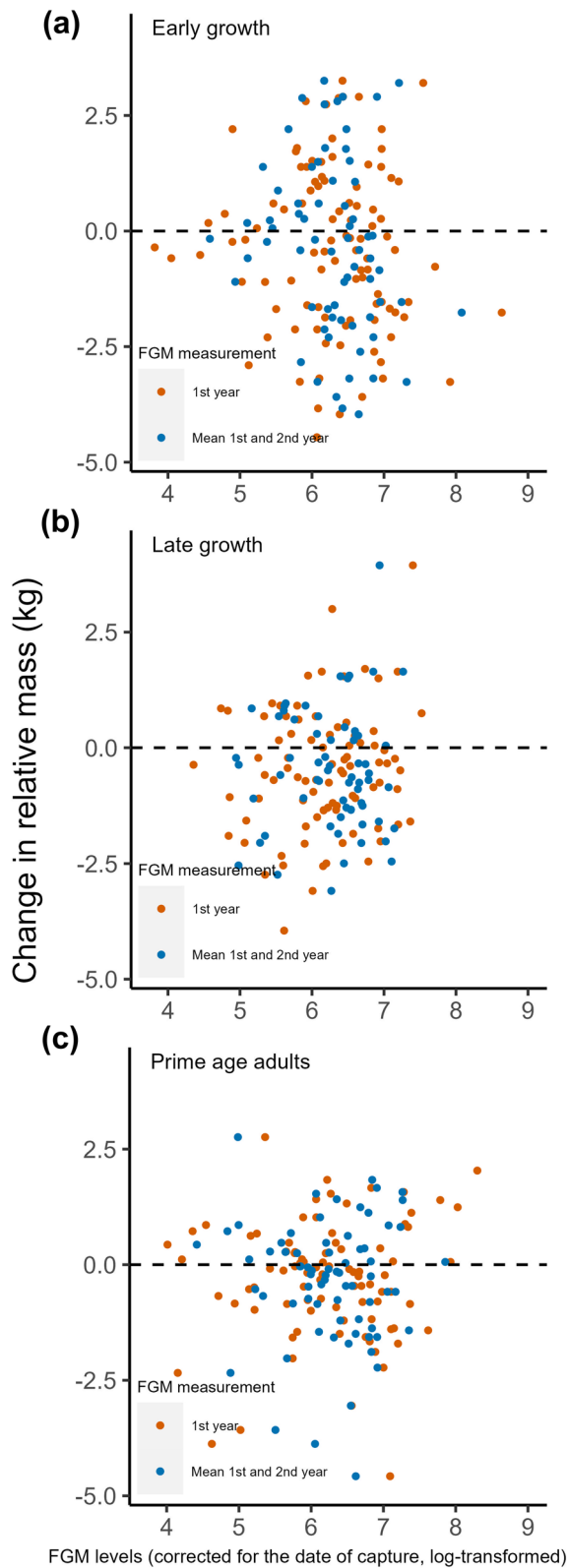


Figure 2. Relationship between faecal glucocorticoid metabolites (log-transformed FGMs, corrected for the date of capture and population) and change in relative body mass between two consecutive years for (a) early-growing individuals (between the first and second years of life), with FGMs either measured the first year of life

more at risk of allostatic overload (McEwen and Wingfield 2010, Henderson et al. 2017). Red deer *Cervus elaphus* showed different allostatic loads across populations with different densities and environmental conditions, with individuals facing high density or poor-quality habitat having higher FGM levels and lower body mass (Caslini et al. 2016). Our results rather suggest that both roe deer populations are in a state of allostatic overload. During the winter season, roe deer face more severe winters at TF than at CH, which can in turn mitigate, during this period, the overall better resource quality in TF. This could partly explain why no population differences were detected. It also suggests that allostatic load can change across seasons, as shown in wild grey mouse lemurs *Microcebus murinus* (Hämäläinen et al. 2015), and that this variation could operate in a population-specific way. It is therefore of great interest to assess the GC-fitness relationship across different environmental contexts, including different years or climatic conditions, and through various life history stages. Finally, the absence of large predators and the weak hunting pressure in both population might at least partly account for the absence of support for a steeper relationship at CH. Studies of roe deer populations facing varying conditions in terms of presence/absence of large predators and hunting pressure, in Poland, showed that hunted populations without large predators had highest FGM levels with largest variation (Zbyryt et al. 2018). In support, observed variation in FGM levels was high in both populations (CV = 59.7 and 55.3% in CH and TF, respectively), which could mitigate the relationship between body mass and FGMs and differences between populations.

Similarly, we could not detect any sex difference, despite the marked differences in the stress response in males and females reported in mammals, due in part to the interaction between the HPA and reproductive axes (Viau 2002, Wilson et al. 2005, Toufexis et al. 2014, Novais et al. 2016). This absence of sex-differences also suggests that during this period of the year, female reproductive status does not influence the relationship between FGMs and body mass. In reindeer *Rangifer tarandus*, cortisol levels did not differ between males, non-pregnant or pregnant females, but showed seasonal variation (Bubenik et al. 1998). Accordingly, red deer showed no sex-differences in their levels of FGMs but also showed seasonal variation (Huber et al. 2003). Thus, it would be interesting to collect FGM data of roe deer during different seasons as the stress response can vary accordingly (Bubenik et al. 1998, Vera et al. 2011). It would also provide an opportunity to investigate whether the different reproductive tactics of males and females can affect the relationship

($n=99$, orange points) or considered as the mean FGMs between the first and second years ($n=67$, blue points); for (b) late-growing individuals (between two and four years old) with FGMs either measured at age t ($n=85$, orange points) or considered as the mean FGMs between years t and $t+1$ ($n=63$, blue points) and for (c) prime-aged adults which had reached their full body mass (from 4 years old to 10 years old), with FGMs either measured at a given age t ($n=90$, orange points) or considered as the mean FGM between age t and age $t+1$ ($n=71$, blue points). Points are raw data.

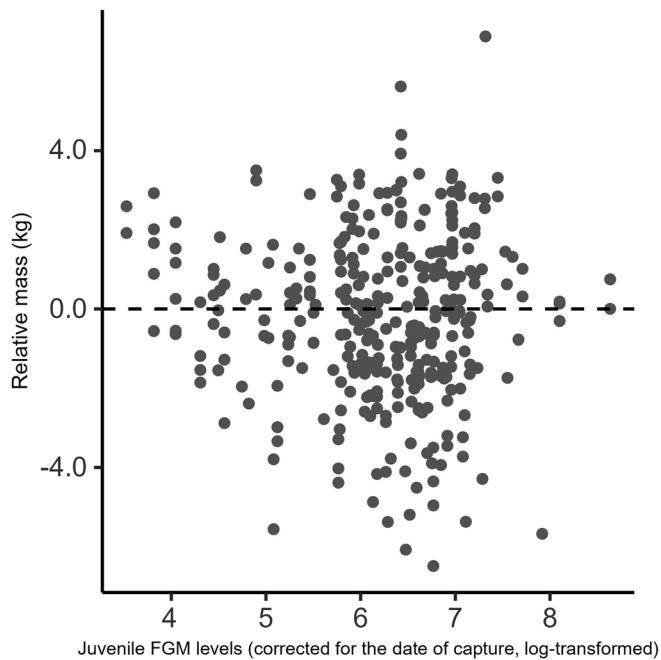


Figure 3. Relationship between faecal glucocorticoid metabolites (log-transformed FGMs, corrected for the date of capture and population) measured in juveniles and individual relative body mass (i.e. individual body mass minus the average body mass of all individuals of the same age, sex and population) ($n = 345$ observations on 159 individuals). Points are raw data.

between GC and fitness-related traits. Females of an iteroparous, long-lived species such as roe deer are expected to trade reproduction for survival when environmental conditions are poor (Hirshfield and Tinkle 1975). Thus, females are expected to be stress-responsive during their reproductive period to be able to switch between alternative physiological states according to whether they reproduce or not, due to their future opportunities of reproducing (Ricklefs and Wikelski 2002). On the other hand, males allocate heavily to reproduction by defending territories for half of the year (Johansson 1996). Territory defence results in elevated physiological damages, whatever their reproductive success. Therefore, females could show a larger variability in the way they respond to GC than males which are more constrained. This provides promising perspectives to better understand the relationship between GC levels and fitness-related traits according to sex-specific life history stages. It would also be interesting to investigate whether offspring have different life-history trajectories according to their mothers' baseline FGMs during gestation, as shown in North American red squirrels *Tamiasciurus hudsonicus* (Dantzer et al. 2013), or according to whether mother were captured or not during gestation to further investigate the impact of capture on individuals.

FGMs have been previously used as proxies to evaluate the homeostatic level or allostatic load in wild populations, but the results should be interpreted with caution. FGMs do not fully reflect an individual's stress response because GCs only represent one part of the complex endocrine pathway involved in this response (MacDougall-Shackleton et al.

2019). Moreover, although GCs are often referred to as 'stress hormones', at baseline levels, their primary role is to acquire, deposit and mobilise energy (Busch and Hayward 2009). Thus, unlike acute GC concentrations, baseline GCs do not only provide information on how an individual responds to stressors, but also on its physiology and activity (Reeder and Kramer 2005). It has been reported that the acute GC response tends to be attenuated in individuals repeatedly exposed to stressors (Ader et al. 1968, Kant et al. 1983, Vera et al. 2011). However, baseline GC levels can also, but not always, be lower in individuals chronically exposed to environmental stressors, making it difficult to interpret FGM levels in the wild. Indeed, whether low baseline GCs actually represent a non-stressed individual rather than an individual chronically exposed to stressors is difficult to tell (Davis and Maney 2018).

In conclusion, we found that high baseline FGMs may have immediate adverse consequences on body mass and that the relationship between body mass and FGM levels seems to depend on an individual life-stage, rather than on the environmental context or sex. Through this work, we therefore emphasise the need to account for both environmental and individual factors, including life history traits, to better capture the relationship between the stress response and fitness-related traits. We also put emphasis on the idea to collect FGM across different temporal and environmental contexts to evaluate how the GC–fitness relationship can be modulated according to the seasonal and environmental conditions. To do so, it appears critical to have access to longitudinal data. Measuring proxies of stress together with fitness-related traits throughout the life of individuals is likely to be the only way to properly refine our understanding of the implications of stress for fitness.

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Author contributions

Lucas D. Lalande: Conceptualization (equal); Data curation (equal); Formal analysis (lead); Funding acquisition (equal); Investigation (equal); Methodology (equal); Visualization

(lead); Writing – original draft (lead). **Emmanuelle Gilot-Fromont**: Conceptualization (equal); Data curation (equal); Funding acquisition (equal); Investigation (equal); Project administration (equal); Resources (equal); Supervision (equal); Writing – review and editing (equal). **Jeffrey Carbillat**: Data curation (equal); Investigation (equal); Writing – review and editing (equal). **François Debias**: Investigation (equal). **Jeanne Duhayer**: Investigation (equal). **Jean-Michel Gaillard**: Data curation (equal); Funding acquisition (equal); Investigation (equal); Project administration (equal); Writing – review and editing (equal). **Jean-François Lemaître**: Data curation (equal); Funding acquisition (equal); Investigation (equal); Project administration (equal); Writing – review and editing (equal). **Rupert Palme**: Investigation (equal); Resources (equal); Writing – review and editing (equal). **Sylvia Pardonnet**: Investigation (equal). **Maryline Pellerin**: Funding acquisition (equal); Investigation (equal); Project administration (equal). **Benjamin Rey**: Investigation (equal); Writing – review and editing (equal). **Pauline Vuarin**: Conceptualization (equal); Funding acquisition (equal); Investigation (equal); Supervision (equal); Writing – review and editing (equal).

Data availability statement

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.pnvx0k6sk> (Lalande et al. 2023).

Supporting information

The Supporting information associated with this article is available with the online version.

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2. N:L ratio and body mass in a long-lived ungulate: A relevant alternative?



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Neutrophil:Lymphocyte ratio may reflect body condition and growth rates rather than stress response in a wild ungulate

In preparation

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Abstract

Ecophysiologicalists and conservation physiologists need reliable ways of measuring stress in the wild to assess individual and population health and welfare. Glucocorticoids (GCs) have largely predominated this field of research, but relationships between GCs and individual performances are equivocal. Given the complexity of the stress response, stress should be estimated by using a large array of indices in order to fully capture an individual physiological status. Leukocyte profiles (Neutrophil:Lymphocyte (N:L) ratios) have gained popularity during the last decades as a reliable proxy of the stress response. However, their use is mainly restricted to birds and aquatic vertebrate species. They have rarely been used to assess stress in wild mammal populations, and even more rarely to test for relationships with individual performances. A previous study evidenced a short-term negative relationship between baseline GCs levels and body mass in adults of two populations of roe deer (*Capreolus capreolus*). Here, we aim at using N:L ratios as an alternative to GCs for measuring stress response and test whether similar relationships with body mass can be found in the same populations. We found that results of the two studies differed. N:L ratios did not relate to body mass on the short-term, as GCs did. We evidenced that mean N:L ratios between two consecutive years negatively related to body mass gain during late growth only (*i.e.* between 2 and 4 years old). The higher the average N:L ratios between two consecutive years during late growth, the less mass gain between those two years. We suggest that this discrepancy stems from differences in the temporality of GCs and N:L ratio responses to stressors, and from the role of N:L ratio in predicting body condition and potentially reflecting costs of high growth rates.

Key-words: leukocyte profile, stress response, heterophils, ungulates, growth

Introduction

Since the emergence of the fields of conservation physiology and ecophysiology (Walker et al. 2005, Stevenson et al. 2005, Wikelski and Cooke 2006, Wingfield et al. 2008), reliable ways of measuring stress in wild populations of vertebrates have become increasingly needed. Both immunological and endocrine signals have been used in order to identify stress in free-ranging animals. However, to date, most the stress research focused on the measurement of glucocorticoids (GCs; Busch and Hayward 2009). Indeed, the use of GCs to estimate the activity of the hypothalamus-pituitary-adrenal (HPA) axis has been largely documented and used by ecophysiologicalists as a main mediator of allostasis (*i.e.* the support of homeostasis through changes in steady physiological states according to environmental conditions and life-history-associated demands; Sterling and Eyer 1988, McEwen and Wingfield 2003, 2010) and to estimate chronic stress in wild animal populations (Walker et al. 2005, Wikelski and Cooke 2006).

However, the use of GCs as a proxy of the stress response also has its drawbacks. First, ecophysiologicalists and conservation physiologists are generally more interested in assessing baseline or chronic stress to evaluate animal and population welfare (Wikelski and Cooke 2006, Bonier et al. 2009). Since plasma GCs rise sharply within minutes following exposure to a stressor (Romero and Reed 2005), blood samples taken after capture are generally not appropriate to assess baseline adrenal activity. Consequently, measurements of faecal GC metabolites (FGMs), that integrate GC levels over extended periods of time, depending on the species, has gained popularity over the past decades (Sheriff et al. 2011, Palme 2019). Second, reviews evidence that the relationship between baseline GC levels and individual or population performances is equivocal being either positive, neutral or negative, because these relationships are likely to depend on individual (*e.g.* life-history stage, sex, body condition) and environmental contexts (*e.g.* past and current environmental conditions, resource availability)

that need to be taken into account (Breuner et al. 2008, Bonier et al. 2009, Schoenle et al. 2018, Breuner and Berk 2019). This highlights the need for longitudinal data, through which such individual and environmental information can be obtained.

The stress response is complex and focusing on one proxy of it might represent a limitation into the comprehension of the mechanisms linking stress, performances and welfare. It is now clear that ecophysiologicalists need to integrate a variety of mediators to fully capture the allostatic load (*i.e.* the cumulative costs of allostatic processes) of an individual and draw conclusions about potential consequences on fitness and performances (Edes et al. 2018, Seeley et al. 2022). Among this range of primary mediators, we can cite neuroendocrine, behavioural, metabolic, cardiovascular, and immune markers. However, the measurement of such mediators still raises protocol issues for free-ranging animals. For example, cardiovascular and behavioural markers, as well as catecholamines which are produced in response to a stressor and disappear within seconds-minutes; might not be possible to collect in wild animals that require to be captured as part of longitudinal programs.

Immune markers might prove useful to estimate stress in free-ranging animals. Namely, the use of leukocytes as a complementary/alternate measure of GC levels has become more and more popular among ecophysiologicalists, especially in aquatic vertebrates and birds (Davis et al. 2008, Davis and Maney 2018). In fact, the increased production of GCs following stressor exposures causes changes in circulating leukocytes, with neutrophils (or heterophils in birds and reptiles) increasing and lymphocyte decreasing. This re-arrangement is thought to be adaptive as increased levels of circulating neutrophils prepare the organism for imminent infections (Dhabhar 2009, Goessling et al. 2015). Therefore, the Neutrophil:Lymphocyte (N:L) ratio has been increasingly investigated during the last decade because an increase in the N:L ratio reflects a stress response (Dhabhar 2006, Davis et al. 2008). This index has been advocated to have several advantages over GC indices. First, it appears that the GC response to

environmental stressors attenuates with the duration and repetition of the stressor, whereas N:L ratio response does not decrease over time (Goessling et al. 2015, Davis and Maney 2018). Then, the response of N:L ratio to stressors occurs later than that of GCs, and thus does not require such rapid sampling (Montané et al. 2007). Consequently, N:L ratio is arguably a reliable estimate of chronic stress as it is easier to measure at baseline, and since the response does not wane with repeated or chronic stress. Finally, N:L ratios are quicker, easier and cheaper to analyse than GC metabolites in alternative matrices such as hair, feathers or faeces (Davis et al. 2008).

N:L ratios have rarely been used in wild mammals (Seltmann et al. 2017, Cohas et al. 2018, Carbillet et al. 2019), and even more rarely used to explain inter-individual variations in life-history traits (Davis and Maney 2018), by contrast to GCs. Therefore, the aim of the present study is to investigate whether N:L ratio provides a consistent alternative to GCs to estimate baseline stress or whether both indexes provide different results. To do so, we based the present study on a previous study that evaluated the short- to long-term relationships between baseline GCs and body mass in two populations of roe deer (*Capreolus capreolus*; Lalande et al. 2023). We wish to evaluate whether N:L ratios are a relevant proxy of the stress response and whether it yields similar or different results than this previous study. Hence, we used the exact same data than described in Lalande et al. (2023) to assess whether N:L ratio and body mass were related on the short-to-long-term, in two populations of roe deer (*Capreolus capreolus*) with contrasted environmental conditions.

Material and Methods

The study populations and the biology of the roe deer are described in details in Lalande et al. (2023).

Neutrophil-to-lymphocyte ratio

From 2010 to 2021, blood samples were collected from the jugular vein up to 1 mL/kg and preserved at 4°C with EDTA to prevent coagulation. The concentration of total leukocytes was assessed at the Clinical Pathology laboratory (Biovelys) of VetAgro-Sup and the percentage of each leukocyte type was estimated by trained technicians counting each type of cell among the first 100 leukocytes on Wright-Giemsa-stained blood smears (see Cheynel et al. 2017). Since we intended to measure the N:L ratio that results from baseline stress levels, we corrected the observed values to obtain more comparable measures. First, the capture is a stressful event and associated short-term production of GCs can affect the N:L ratio (Martin 2009, Dhabhar 2014). In free-ranging roe deer, neutrophils increased significantly, and lymphocytes decreased significantly within 2 hours following capture, so that N:L ratio is overall increased (Montané et al. 2007). As a consequence, blood samples collected several hours after capture are expected to show higher N:L ratios than samples collected immediately after capture. Hence, for the analyses, we used the residuals of the linear relationship between the delay from capture to sampling and the log-transformed N:L ratio (hereafter called NLR). Also, captures took place each year over 3 months. Thus, endocrine activity can vary throughout the capture season according to environmental conditions (*i.e.* population effects) and sex (Dantzer et al. 2010, Sheriff et al. 2012). We tested whether NLR varied as a function of the Julian date, population and sex. We found that NLR increased in TF throughout the season, and that NLR first decreased up to mid-season and then increased until the end of the season in CH (Figure S1). Thus, all FGM measurements were standardised for the median Julian date of capture (*i.e.* 9th of February) specifically for each population. Finally, immunosenescence has been evidenced in those two populations (Cheynel et al. 2017), showing an overall increase in neutrophil counts and decrease in lymphocyte counts with advancing age. Accordingly, NLR increased with age in a linear manner (Figure S2). For adults of the short-term analysis, and for

the medium-term analysis (see the different analyses below), NLR were standardised for the median age of all individuals (3 years old).

Statistical analyses

All analyses were performed using R version 4.3.0 (R Core Team 2023). One NLR value was unusually high (*i.e.* 40.6, for a female in CH aged 3; see Figure S3), thus, results exclude this extreme value. Nevertheless, analyses including it are reported in Supplementary Information (SI, Table S1), and we specified whether adding it yielded different conclusions or not.

NLR repeatability

Individual NLR repeatability was calculated for all individuals, but separately in the two populations, using the ‘rptR’ package (v. 0.9.22, Stoffel et al. 2017). Individuals sampled only once were included to improve estimates of within-individual variance (Martin et al. 2011). Within-individual NLR repeatability was detectable but weak both in CH ($r = 0.16$, 95% CI = [0.07, 0.26]) and TF ($r = 0.08$ [0.00, 0.16]). The coefficient of variations for both populations were high (1184% in CH, 928% in TF).

Model structure

The relationship between NLR and body mass was analysed on the short-, medium- and long-terms. The model structures for all three analyses were exactly the same as for FGM, presented in Lalande et al. (2023). Briefly, we hypothesised that body mass was negatively related to NLR and that this relationship may be dependent on sex and life-history stage (*i.e.* juvenile *v.* adults, early-growth *v.* late-growth *v.* prime age), as well as on environmental conditions (*i.e.* population, environmental quality at birth). Therefore, all models considered body mass as the response variable, while fixed explanatory variables included modulating variables (sex, population and cohort quality, as well as initial body mass for the medium-term analysis) and their two-way interactions with NLR. Models also included year of capture and

individual identity (ID) as random effects. Details of the models can be found in Lalande et al. (2023).

Short-term relationships between NLR and body mass measured at the same capture event

Individuals in their first year of life (*i.e.* juveniles) were analysed separately from individuals in their second year of life onwards, because juveniles have to allocate to growth and are much more susceptible to any environmental harshness than adults, making the first year of life the critical period of roe deer population dynamics (Hamel et al. 2009, Gaillard et al. 2013). Also, the vertebrate immune system is not fully developed and efficient at birth, and progressively develops with age, in particular the adaptive immunity (McDade et al. 2016). As a consequence, in roe deer, individuals in their first year of life carry a higher parasite load than older individuals (Body et al. 2011). This immature immune system is also expected to affect the NLR, making it not comparable to the adult one. Finally, adult roe deer habituate faster than juveniles to stress (Bonnot et al. 2018), which can in turn affects the HPA axis activity and NLR, and thus the relationship between NLR and fitness-related traits.

We analysed the relationship between NLR and body mass measured the same year on 470 juveniles (79 females and 112 males in CH, 131 females and 148 males in TF). For individuals aged 2 years or older, the dataset included 1033 observations on 488 individuals: 129 females (310 observations) and 116 males (204 obs.) in CH, and 132 females (288 obs.) and 111 males (231 obs.) in TF.

Medium-term relationships between NLR and body mass change between two consecutive years

The relationship between NLR and the change of body mass between two consecutive years was analysed separately for early-growing individuals (*i.e.* between age 1 and 2), late-

growing individuals (*i.e.* between the second year of life and adulthood at 4 years old; Hewison et al. 2011) and for prime-aged adults which had reached their full body mass (*i.e.* from 4 to 10 years old; Hewison et al. 2011). The response variable was the change in relative body mass as it is described in Lalande et al. (2023). Briefly, it is the change in body mass of a given individual between two ages, relative to the change in body mass of all individuals of the same sex, population and age.

For early-growing individuals, the dataset included 105 individuals with NLR measured as juveniles, and 93 individuals when including NLR measured during the first and second years of life to calculate the mean value. For both late-growing individuals and adults, we kept a single observation per individual since the inclusion of individual ID as a random effect created singularities. For individuals with several observations, we kept the one for which the individual's age was the closest to the mean age of all individuals with a unique observation according to sex and population, among observations with complete data. The final dataset comprised 112 individuals in their late-growth period and 127 prime-aged adults with NLR measured at age t , and 92 late-growing individuals and 120 prime-aged adults for which we had measurements at age t and $t+1$.

Long-term relationships between NLR during early life and body mass later in life

Only individuals for which we measured NLR as juvenile and body mass beyond the second year of life were analysed. We used 331 observations on 159 individuals: 33 females (76 obs.) and 45 males (87 obs.) in CH, and 43 females (94 obs.) and 38 males (74 obs.) in TF.

Model selection

Final models were selected based on the second order Akaike Information Criterion (AICc, *i.e.* Akaike Information Criterion corrected for small sample sizes) and was exactly the same as previously described in Lalande et al. (2023). Briefly, we retained the simplest model

(i.e. with the lowest number of parameters) among the set of models within 7 Δ AICc (Burnham and Anderson 2002, Burnham et al. 2011), using the “*MuMIn*” package (v. 1.47.5, Bartoń 2023). For each retained variable we estimated its effect size (β) with 95% confidence intervals (95% CI) and calculated the marginal and conditional R^2 of the selected models. We provided full model selection tables in SI (Tables S2-4).

Results

Overall, NLR ranged from -2.89 to 15.97 with a median value of -0.41. The median NLR was -0.27 (range: -2.89 – 13.83) in CH, and -0.58 (range: -2.72 – 15.97) in TF.

Short-term relationships between NLR and body mass measured at the same capture event

Juveniles

The retained model highlighted a positive relationship between cohort quality and body mass ($\beta_{\text{Cohort quality}} = 0.97$ [0.74, 1.19]; Table 1, Table S2), but no relationship between NLR and body mass. Note that since the random effect of the year of capture created singularities, it was removed and models therefore consisted of linear models.

Adults

We did not detect any relationship between individual relative body mass and NLR in adults (Table 1, Table S2). Results were similar when including the high FGM value measured on a female of CH (Table S1). Since the random effect of the cohort created singularities, the models only included individual ID and year of capture as random effects.

Medium-term relationships between NLR and change in body mass between two consecutive years

Early-growing individuals

We did not evidence any relationship between NLR and the change in relative body mass for growing individuals, neither when NLR was measured in juveniles nor when considering the mean NLR between the first and second years of life (Table 1, Table S3). All models included cohort as a random effect.

Late-growing individuals

With the dataset including the mean NLR between age t and age $t+1$, we found support for negative relationships between initial mass with the gain of body mass between two consecutive years during late growth ($\beta_{\text{mass}} = -0.19$ [-0.29, -0.09]; Table 1, Table S3), and between NLR and the gain of body mass ($\beta_{\text{NLR}} = -0.27$ [-0.46, -0.08]; Figure 1, Table 1, Table S3). Models resulted in singularities when including cohort as random effect, so only the year of capture was included as a random effect.

Prime-aged adults

When we accounted for NLR measured at age t , we found no relationship between change in body mass and any explanatory variable. When accounting for mean NLR between age t and $t+1$, we only found a negative relationship between initial mass and the gain of body mass between two consecutive years ($\beta_{\text{mass}} = -0.13$ [-0.21, -0.04]; Table 1, Table S3). Models resulted in singularities when the random effect of the cohort was included, thus, only the random effect of the year of capture was included in the models.

Long-term relationships between NLR during early-life and body mass later in life

We found no evidence for a relationship between NLR measured in juveniles, cohort quality, population or sex, with adult relative body mass (Table 1, Table S4). We only included individual ID and year of capture as random effects, because including the cohort created singularities.

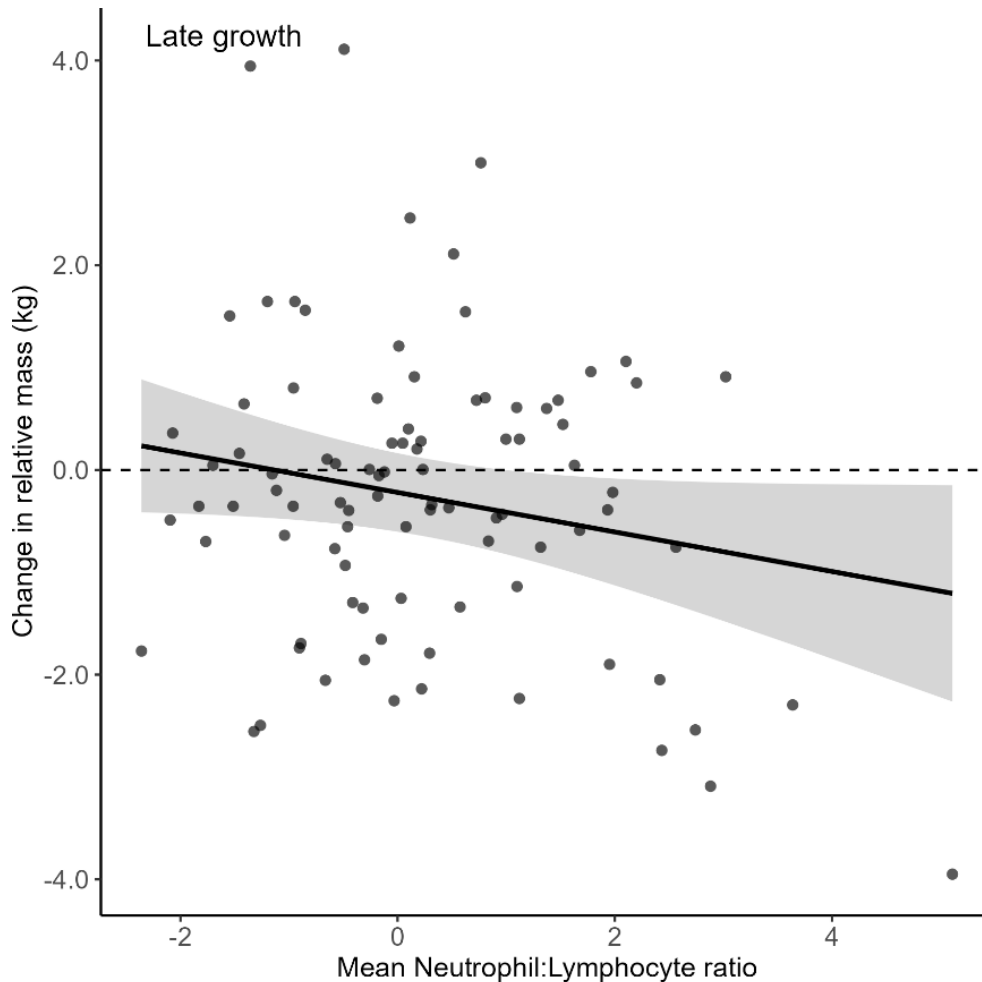


Figure 1. Relationship between Neutrophil:Lymphocyte ratio (NLR, corrected for the time between capture and sampling, the date of capture and age) and change in relative body mass between two consecutive years for late-growing individuals (between 2 and 4 years old). Here, NLR is the average NLR value obtained from the mean NLR value measured year t and measured year $t+1$. $N = 92$ mean N:L ratios on 92 individuals. Points are mean N:L ratios and line is the selected model prediction with 95% confidence intervals (shaded areas).

Table 1. Linear and linear mixed effect models selected for the short-, medium- and long-term relationships between relative body mass and Neutrophil:Lymphocyte (N:L) ratio. Models accounted for sex, population and relative cohort quality (Qcohort, the difference between the mean cohort quality in each population and individual cohort quality). Models were selected through model selection based on AICc. 95%CI: 95% confidence intervals, V: variance, SD: standard-deviation.

SHORT-TERM					
Juveniles			Adults		
Random effects	V	SD	Random effects	V	SD
			Individual ID	3.44	1.85
			Year of capture	0.31	0.55
Fixed effects	Estimate	95%CI	Fixed effects	Estimate	95%CI
Intercept	0.03	[-0.16, 0.22]	Intercept	-0.06	[-0.42, 0.30]
Qcohort	0.97	[0.74, 1.19]			
Marginal R ²	0.13		Marginal R ²	-	
Conditional R ²			Conditional R ²	0.80	

MEDIUM-TERM								
FGM measured at age t								
Early-growth			Late-growth			Prime-aged		
Random effects	V	SD	Random effects	V	SD	Random effects	V	SD
Cohort	0.41	0.64	Year of capture	0.07	0.26	Year of capture	0.34	0.58
Fixed effects	Estimate	95%CI	Fixed effects	Estimate	95%CI	Fixed effects	Estimate	95%CI
Intercept	-0.32	[-0.81, 0.18]	Intercept	-0.34	[-0.62, -0.06]	Intercept	-0.10	[-0.50, 0.30]
			Initial mass	-0.18	[-0.27, -0.09]			
Marginal R ²	-		Marginal R ²	0.12		Marginal R ²	-	
Conditional R ²	0.13		Conditional R ²	0.15		Conditional R ²	0.19	

Mean FGM (FGMt and FGMt+1)								
Early-growth			Late-growth			Prime-aged		
Random effects	V	SD	Random effects	V	SD	Random effects	V	SD
Cohort	0.23	0.48	Year of capture	0.08	0.27	Year of capture	0.26	0.51
Fixed effects	Estimate	95%CI	Fixed effects	Estimate	95%CI	Fixed effects	Estimate	95%CI
Intercept	-0.17	[-0.61, 0.28]	Intercept	-0.26	[-0.57, 0.05]	Intercept	-0.28	[-0.71, 0.15]
			N:L ratio	-0.27	[-0.46, -0.08]	Initial mass	-0.13	[-0.21, -0.04]
			Initial mass	-0.19	[-0.29, -0.09]			
Marginal R ²	-		Marginal R ²	0.17		Marginal R ²	0.07	
Conditional R ²	0.08		Conditional R ²	0.21		Conditional R ²	0.22	

LONG-TERM		
Random effects	V	SD
Individual ID	3.28	1.81
Year of capture	0.31	0.56
Fixed effects	Estimate	95%CI
Intercept	-0.07	[-0.42, 0.29]
Marginal R ²	-	
Conditional R ²	0.74	

Discussion

Ecophysiologicals and conservation physiologists need reliable ways of measuring stress in the wild to investigate animal health and welfare. If GCs have been extensively used in that purpose (Busch and Hayward 2009), and more particularly to evaluate stress consequences on individual fitness and performances (Bonier et al. 2009), it has become clear that this proxy of stress has its drawbacks. Alternative or complementary indices of the stress response should be developed to estimate allostatic load in wild animals (Edes et al. 2018, Seeley et al. 2022). Over the past decades, the use of leukocyte profiles to estimate the stress response has increasingly gained popularity but has been mainly restricted to birds and aquatic vertebrates (Davis and Maney 2018). Based on previous study conducted on two contrasted populations of roe deer aiming at assessing the relationship between baseline GC levels and body mass on the short-to-long term (Lalande et al. 2023), we tested the relevance of N:L ratios as a proxy of the stress response. We showed that GCs and N:L ratios provided different results and that these two indices were not interchangeable.

In the previous study by Lalande et al. (2023), baseline GCs were shown to negatively relate to body mass on the short-term (*i.e.* body mass and FGMs measured at the same time) in adults, but not juveniles, in both populations and both sexes. Here, mean N:L ratio between two consecutive annual measurements was associated to lower body mass gain between those two years, for individuals in their late-growth stage (*i.e.* between 2 and 4 years old; Hewison et al. 2011). These results suggest that these two proxies of the stress response have consequences on body mass on different time scales: the former affects body mass on the short-term, and the latter relates to body mass over longer time scales. Also, it suggests that N:L ratios need to be elevated over longer periods of time, compared to GCs, to be able to detect consequences on body mass. Indeed, higher mean N:L ratios over two consecutive years related to lower body

mass gain, but elevated N:L ratios during one year did not relate to concomitant body mass or body mass gain.

High N:L ratios are associated with higher body condition in those two populations of roe deer (Gilot-Fromont et al. 2012). Good-quality individuals tend to orientate their immunity towards the innate component, the costliest one to maintain and use, while individuals of poorer quality are expected to invest more in adaptive immunity, costly to develop but cheapest to maintain and use (Klasing 2004, Lee 2006). Thus, those individuals with higher body condition and N:L ratios would not need to invest as much in growth to reach their prime-age body mass as individuals with lower initial body mass. In an income breeder species such as the roe deer, in which body mass is a key parameter predicting future fitness (Gaillard et al. 2000), compensatory growth (Hector and Nakagawa 2012) is likely to occur. Therefore, we could expect lighter individuals to gain more body mass during growth, compared to already heavier individuals, with associated fitness benefits of being heavier at prime age, but with later physiological costs that can ultimately affect body mass senescence (Douhard et al. 2017) or longevity (Metcalf and Monaghan 2001). Finally, in line with this, elevated N:L ratios during late growth could be associated to fast early-growth rates and would worth being investigated in these populations. Fast growth rates can have physiological repercussions (Rollo 2002, Metcalfe and Monaghan 2003), such as decreased investment towards the development of adaptive immunity, which is energetically costly (Klasing 2004), hence resulting in low lymphocyte counts. Parallely, rapid growth results in greater levels of oxidative stress (Rollo 2002, Metcalfe and Monaghan 2003) and favours low-grade auto-immune and inflammatory processes (Rollo 2002) that can translate into higher neutrophil counts.

Overall, in the context of the present study, N:L ratio and GC responses are not interchangeable indices of the stress response. It is likely that our results actually highlight the link between immune phenotype, growth rate and body condition, rather than the link between

N:L ratios and the stress response. Theoretically, N:L ratio offers an alternative proxy of stress levels in free-living animals, as being a reliable indicator of chronic stress (Davis et al. 2008). Yet, although we had similar predictions for the relationship between N:L ratios or GCs and body mass, we did not find such consistency and the reasons are at least twofold. First, both proxies have different temporal dynamics. GC response to stressors tends to wane down in individuals repeatedly exposed to stressors, while there are evidences that the acute NLR response tends to persist over longer periods (Goessling et al. 2015). Secondly, NLR not only reflect the stress response but also the immune status of an individual. Thus, the relationship between NLR and fitness may involve physiological processes involved in the stress response as well as immune components, but could also potentially result from high growth rates. Accordingly, we encourage the use of N:L ratios in natural populations in order to estimate body conditions and immune phenotype, but we do not advise to use it as a reliable estimate of the stress response. N:L ratios and GCs should not be used interchangeably, but rather complementarily if one hope to draw inferences about the physiological status of an individual.

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***CHAPTER 3 –
Glucocorticoids and the
senescence of physiological
condition***



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Glucocorticoids covary positively with haematological indices of body condition, but do not modulate patterns of haematological senescence in a wild ungulate

In preparation

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Abstract

Body condition reflects both an individual's energy requirements, and its ability to acquire resources, hence describing both its physical and physiological status. Although body mass is often used to estimate body condition, specific haematological reflect body condition and are considered good predictor of survival and reproductive success in wild animals. As such, they are interesting candidates as proximate mechanisms of actuarial (*i.e.* survival) and reproductive senescence, but evidences for senescence of haematological parameters are rare in long-lived, free-ranging mammals. In addition, the stress response to unpredictable environmental fluctuations, estimated through glucocorticoid (GC) levels, provides a relevant mechanism through which environmental conditions can modulate patterns of senescence. Here, we aim at determining the immediate and carry-over effects of baseline GC levels on three haematological parameters (*i.e.* haematocrit, albumin and creatinine) in two populations of roe deer (*Capreolus capreolus*) longitudinally monitored. We found that GC levels were positively related to albumin and creatinine on the short-term, but that early-life GCs did not have carry-over effects on haematological parameters, nor on their senescence patterns. These results support previous works showing that GC effects on health parameters are mainly restricted to the short-term in those populations. Furthermore, we show that the increase in creatinine concentrations together with GCs can be explained by an increased catabolic state in individuals displaying higher levels of baseline GCs. Finally, we also suggest that increases in ALB concentrations with GC levels can result from stressor exposure prior to capture.

Keywords: faecal cortisol metabolites, ungulates, aging, serum proteins, body condition indices

Introduction

Body condition describes both the physical and physiological status and reflects an individual's energy requirements, and its ability to acquire resources (DelGiudice et al. 1992, Parker et al. 2009). Body mass, often corrected for body size, is a widely-used proxy of body condition and has been shown to predict survival and reproductive success in several species (Gaillard et al. 2000a, b, Milner et al. 2013, Ronget et al. 2018). However, body mass integrates the nutritional status of an individual over the past days-months, while other indicators of body condition provide shorter-term and complementary information about the physiological status of an individual (Milner et al. 2003), hence reflecting shorter-term responses to environmental fluctuations. Moreover, no single index can accurately assess body condition, so that multiplying indices to comprehensively describe body condition is necessary (Delgiudice et al. 1990). Accordingly, haematological parameters are valuable indices of body condition (DelGiudice et al. 1992, Milner et al. 2003, Gilot-Fromont et al. 2012), and are good predictors of survival and reproductive success in wild animals (Milner et al. 2003, Nadolski et al. 2006).

The predictive role of haematological parameters for fitness renders them interesting candidates as proximate mechanisms of actuarial (*i.e.* survival) and reproductive senescence – a progressive decline in survival and reproductive success with advancing age, respectively (Ricklefs 2008). However, evidences for senescence of haematological parameters are rare from long-lived wild mammals, as they come mostly from birds, humans and laboratory model species (Norte et al. 2009, Coughlan and Hollmén 2018, Tanaka et al. 2020). Also, most of these studies were cross-sectional with age classes studies (*e.g.* Reichert et al. 2022), rather than longitudinal studies. Still, the few haematological senescence studies conducted in the wild show consistent declines of immune functions with advancing age (Peters et al. 2019), and a decrease in plasma proteins and red blood cells content (Jégo et al. 2014).

Evolutionary theories of senescence are based on the idea of trade-offs, including population genetic trade-offs between early- and late-life (the Antagonistic Pleiotropy theory, Williams 1957), and physiological trade-offs in resource allocations between growth, reproduction and somatic maintenance (the Disposable Soma theory, Kirkwood 1977, Kirkwood and Rose 1991). The stress response provides a relevant mechanism through which environmental conditions can modulate patterns of senescence. Indeed, glucocorticoids (GCs) are largely involved in the response of the organism to stressors, since they orchestrate resource allocation to competing functions in order to cope with environmental challenges (Sapolsky et al. 2000, Wingfield 2013). When exposed to an acute stressor, baseline GC levels increase sharply within minutes through the activation of the hypothalamus-pituitary-adrenal (HPA) axis. This increase favours locomotor and cardiovascular functions over competing ones (*e.g.* growth, immunity, reproduction) to escape the stressor, after what, GCs return to baseline (Sapolsky et al. 2000). By contrast, long-term perturbations (*i.e.* days-weeks) of an individual normal life-cycle requires to reach new homeostatic setpoints, *i.e.* steady physiological states adjusted to cope with perturbations. Homeostatic setpoints are reached through allostatic processes partly mediated by lasting increase of GCs, resulting in elevated baseline GC levels (McEwen and Wingfield 2003, 2010). Such long-term GC elevations deviate resources away from life-history functions on the long-run, resulting in allostatic overload (*i.e.* when resources intake is lower than energy requirements to support unpredictable environmental demands, McEwen and Wingfield 2010) with potential detrimental consequences on individual performances (*e.g.* immunosuppression, growth inhibition, altered reproduction; Romero 2004). Because of their role in resource allocation, and their link to oxidative stress (Costantini et al. 2011), increased telomere attrition rates and accelerated cellular ageing (Casagrande et al. 2020, Lee et al. 2021, Bobba-Alves et al. 2023), GCs are likely to have both immediate and

carry-over consequences on haematological parameters. They are also expected to integrate the response to environmental perturbations into the modulation of senescence patterns.

Here, we aim at determining the immediate and carry-over effects of baseline GC levels on haematological parameters in two populations of roe deer (*Capreolus capreolus*) intensively monitored. This long-term monitoring allowed to describe patterns of actuarial (Gaillard et al. 1993, Loison et al. 1999), reproductive (Vanpé et al. 2009), body mass (Douhard et al. 2017), immune (Cheynel et al. 2017) and haematological (Jégo et al. 2014) senescence. Yet, whether physiological factors modulate these patterns remain to be investigated. We first described senescence trajectories for haematocrit (HCT), albumin (ALB) concentrations and creatinine (CREA) concentrations accounting for sex, population (Jégo et al. 2014) and environmental quality at birth (Cooper and Kruuk 2018). HCT (%) is the percentage of red blood cells (RBCs) composing blood. It informs about the nutritional status and oxygen-carrying capacity to muscles (DelGiudice et al. 1992). ALB is the blood most abundant protein and changes in its concentrations reflect the level of available serum protein resources, as well as individual nutritional status as ALB is likely to be a reliable indicator of environmental resources availability (Sams et al. 1998). Their role involves the maintenance of osmotic pressure and fluid transmission from blood to tissues. Lastly, CREA is a by-product of the catabolism of creatine in muscular cells, eliminated through glomerular filtration in kidneys, and its blood concentrations informs both about muscle mass and renal filtration efficiency (Andreev et al. 1999). We then assessed *i*) whether early-life GCs modulated patterns of senescence of these three haematological traits, and *ii*) whether concomitant GC levels covaried with these traits. In the two studied roe deer populations, RBC counts, ALB and CREA have all been shown to positively covary and to be reliable proxies of body condition in this species (Gilot-Fromont et al. 2012). We expected high GC levels during early-life to result in accelerated rates and/or earlier onset of senescence of haematological parameters for the following reasons: *i*) GCs play

a critical role in resource allocation, *ii*) favour protein degradation over protein synthesis, and *iii*) high GC levels during early-life can have long-term consequences on an individual physiological status (Braun et al. 2013, Monaghan and Hausmann 2015). Finally, we expected ALB to be negatively related to concomitant GC levels because GC promote an increased glomerular filtration rate (Waters et al. 1997, Andreev et al. 1999), so that ALB plasma concentrations should decrease (whereas urine concentrations should increase). Consequently, CREA concentrations may also decrease although they could actually increase due to the role of GCs in favouring catabolism and muscle loss (Andreev et al. 1999, Kuo et al. 2013), resulting potentially in a null or even positive relationship between CREA and concomitant GCs. We also expected HCT to decrease with GC levels because lasting GC elevations interfere with the maturation of RBCs (Stellacci et al. 2009).

Material and Methods

Study Populations

Roe deer are ungulates commonly found in European lowland woodland, and weighing 25 kg on average. In this study, we used data obtained on two free-ranging populations, *i.e.* Trois-Fontaines (TF - 1360 ha) in north-eastern France (48°43'N, 4°55'E) and Chizé (CH - 2614 ha) in western France (46°05'N, 0°25'W), as part of a Capture-Mark-Recapture (CMR) program taking place every winter since 1975 and 1977, respectively. The forest of TF is highly productive due to rich soils and provides a homogeneous and high-quality habitat for roe deer. In contrast, soils are poor and summer drought are frequent in the CH forest. Accordingly, forest productivity is low and CH provides a less suitable, heterogeneous habitat for roe deer (Pettorelli et al. 2006). Natal environmental conditions are assessed as the cohort-specific average juvenile mass adjusted for the Julian date of capture in both populations (Gaillard et al. 1996). Termed 'cohort quality' hereafter, this metric accounts for the impact of variations in

environmental conditions (e.g., climate, resource availability, roe deer density) and maternal quality (e.g., mother's condition, maternal care) on roe deer condition from birth (typically in May) up to an individual first winter, thus avoiding the need to define specific metrics for describing natal environmental conditions (Toïgo et al. 2006). Cohort quality is categorized either as poor or good based on the median cohort quality value in each population. Captures are conducted annually over 10-12 days, spanning from December (TF) or January (CH) to early March (Gaillard et al. 1993). To minimize disturbance and stress for the animals, capture days are typically scheduled at least 48 hours apart and, for captures occurring on two consecutive days, or for morning and afternoon captures of a given day, they are conducted in different areas of the forests. CMR analyses indicate that both populations remained below their carrying capacity during the study period from 2010 to 2022. Since 2010, faecal matter and blood samples are collected from known-sex and known-age individuals.

Faecal Glucocorticoid Metabolites (FGMs)

The baseline activity of the HPA axis was estimated using faecal glucocorticoid metabolites (FGMs), as collecting blood immediately after capture to measure baseline circulating GC concentrations was not feasible in our setting (Romero and Reed 2005). FGMs provide an integrated measure of baseline GC concentrations, reflecting adrenocortical activity several hours before sampling (Palme 2019). In roe deer, there is an average delay of 12 hours (ranging from 6 to 23 hours) between GC secretion following adrenocortical activation and FGM detection (Dehnhard et al. 2001). However, FGM collection in free-ranging animals is subject to various factors that can affect their levels. These include the time between faecal sampling and freezing and freezing temperature (Hadinger et al. 2015, Carbillet et al. 2023), seasonal variation in FGM levels across the capture period (Bubenik et al. 1998, Huber et al. 2003), and the time delay between capture and sampling. We tested whether these factors influenced FGM levels for *i*) the 162 individuals for which we measured FGMs during their

first winter (*i.e.* early-late effects of FGMs on haematological senescence analysis), and for *ii*) the 775 FGM measurements obtained on 437 individuals of all ages for which we had both FGMs and haematological parameters measured at the same capture events (*i.e.* analysis of the covariation between FGMs and haematological parameters). For both analyses separately, we computed a linear mixed effect model (LMM) accounting for *i*) the Julian date of capture (linear and quadratic), *ii*) the time delay (minutes) between capture and sampling, and *iii*) whether faeces were immediately frozen at -80 °C or frozen at -20 °C within 24 hours. LMMs also included individual identity as a random effect to account for repeated measurement on the same individuals. In the first analysis, we found that none of the above-mentioned factors affected FGM levels (Table S1). For the second analysis, only the Julian date of capture affected FGM levels, with FGM levels linearly increasing throughout the capture season (Table S2, Figure S1).

FGMs were extracted by vortexing 0.5 g (± 0.005) of faeces in 5 mL of 80% methanol and then centrifuging the mixture for 15 minutes at 2500 g (Palme et al. 2013). The amount of FGM was determined according to a method previously described and validated for roe deer (Möstl et al. 2002, Zbyryt et al. 2018). Briefly, an aliquot of the supernatant was diluted 10 times in assay buffer and the dosage was performed using a group-specific 11-oxo-aetiocholanolone enzyme immunoassay (EIA). Measurements were duplicated, and intra- and inter-assay coefficients of variation were lower than 10 and 15%, respectively. Results are expressed as nanograms per gram of wet feces (ng/g). For the analyses, FGMs were log-transformed.

Haematological parameters

Blood was collected on known-aged individuals at the jugular vein, up to 1 mL/kg. Blood samples were stored between 4 and 6 °C and analysed within 48 hours at the Biochemical and Endocrinological laboratory, VetAgro Sup, France. Haematological analyses were

performed as described in Gilot-Fromont et al. 2012 and Jégo et al. 2014. Briefly, impedance technology was used to enumerate red blood cells and determine the percentage of red blood cells within blood sample (*i.e.* haematocrit, %), considering bovine sample parameters. Albumin concentrations (g/L) were determined by separating total protein content through electrophoresis, using an automatic agarose gel electrophoresis processor HYDRASYS (Sebia, Evry, France). Creatinine concentrations ($\mu\text{mol/L}$) were measured using a Konelab 30i automaton (Fisher Thermo Scientific, Cergy-Pontoise, France) with Thermo Scientific reagents.

Statistical Analysis

All statistical analyses were conducted using R version 4.3.0 (R Core Team 2023). To account for small sample sizes, individuals aged 10 and older were grouped together. In any case, haematological senescence has been shown not to start later than 7 years old in these populations (Jégo et al. 2014).

Haematological senescence trajectories

Age trajectories

Each haematological parameter was set as the response variable in a specific model to define its senescence trajectory. Various trajectories were evaluated using linear mixed models (LMMs), including a linear effect of age, a quadratic effect, and a full-age structure that encompassed 9 age categories ranging from 2 to 10 years old and beyond. Additionally, three threshold models were also tested, varying in whether pre- and post-threshold trajectories were held constant or not. The first model featured a constant pre-threshold trajectory and a free post-threshold slope, the second model had a free pre-threshold slope and a constant post-threshold trajectory, and the third model included both free pre- and post-threshold slopes. The definition of threshold models followed methodologies previously outlined in Briga et al. (2019) and Douhard et al. (2017). The thresholds ranged from 3 to 9 years old with one-year increments,

and the best-fitting threshold was determined as described in the 'Model Selection' section below.

We anticipated that age-specific changes in haematological parameters might vary according to population and sex, as already demonstrated in those populations (Jégo et al. 2014). Also, early-life environmental conditions might affect senescence patterns (Nussey et al. 2007, Cooper and Kruuk 2018). Therefore, models included the fixed effects of age (either linear, quadratic, full-age, or threshold), population, sex, and cohort quality, along with two-way interactions between age term(s) and the three other variables. Four potential confounding variables were also included: the fixed effect of body mass (standardized for the Julian date of capture for juveniles, see Douhard et al. 2017) because body mass, body condition and haematological traits are intrinsically related in roe deer (Gilot-Fromont et al. 2012); the fixed effect of the age at last observation/capture to account for selective disappearance (van de Pol and Verhulst 2006, van de Pol and Wright 2009); the Julian date of capture to take into account potential changes in haematological parameters throughout the capture season (DeGiudice et al. 1992); and the time delay between capture and blood sampling to consider changes in haematological parameters due to the heightened activity and stress induced by captures. These four additional fixed effects are referred to as 'confounding effects' hereafter. Finally, all models encompassed the random effects of individual identity ('ID') to address repeated measurements on the same individuals, and the year of capture nested within the population to account for year-to-year variations in demographic and environmental conditions in both populations. Random slopes for age terms were not included, as they led to perfect correlations between the random intercepts and slopes. Random slopes allow to control for type I error and provide more power to detect among individual variation (Harrison et al. 2018).

Model Selection

For each haematological parameter, model selection was carried out based on the second-order Akaike Information Criterion corrected for small sample size (AICc), using the 'MuMIn' package (Bartoń 2023). Model selection was performed on the linear, quadratic, full-age, and threshold models, separately. In essence, among the models within $2 \Delta\text{AICc}$ for each senescence trajectory and for each haematological parameter, the simplest model (*i.e.* with the fewest parameters) was selected as the supported model, adhering to principles of parsimony (Burnham and Anderson 2002). To determine the most appropriate threshold for threshold models, we retained the threshold that resulted in the lowest AICc. Note that for these models, one parameter was added to account for the threshold, which effectively represents an extra parameter that is not explicitly included in the models. Following identification of senescence trajectory for each trait, this trajectory is used as the base models for the following analyses.

Early-life FGMs effect on haematological senescence

We tested whether early-life FGMs levels (log-transformed here and after) influenced the retained senescence trajectories for each of the three haematological parameters. Thus, we added the interaction between log-transformed early-life FGMs and the various age terms in the models.

Immediate relationship between FGMs and haematological parameters

We tested whether FGMs, measured at the same capture event than haematological parameters, had an effect on parameter values, once they were corrected for age, sex, population, cohort quality and confounding variables. For each trait, the model structure (*i.e.* fixed and random effects) was kept as previously found when describing senescence trajectories. In addition, models included the two-way interactions between FGMs and age terms, population, sex, cohort quality and mass, as all could modulate the relationship between FGMs and haematological parameters (Crespi et al. 2013, Dantzer et al. 2014, Henderson et al.

2017). One FGM value measured on a 10 years old female of CH was surprisingly high (>5000 ng/g). We performed analyses with and without this value but chose to present the results that excluded it. However, we systematically mention whether adding this value yielded different results or not.

For both analyses (*i.e.* effect of early-life FGMs and immediate effect of FGM) the same model selection procedure than previously described was used to decide whether to retain the additive and interacting effects of early-life FGMs with the age terms and to test for the immediate additive effect of FGMs on haematological parameters.

Table 1. Selected linear mixed effects models for the 3 haematological traits according to age (either linear or quadratic), population (Chizé: CH, Trois-Fontaines: TF), sex (Females: F, Males: M), and cohort quality (Qcoh; good quality: G, poor quality: P). Models also accounted for potential confounding effects (Mass: body mass, Age-last: age at last observation, Delay: time delay between capture and sampling, Julian: Julian date of capture). Individuals aged 10 years old and older were pooled. All models included the random effects of individual identity and the year of capture nested within the population. SE: standard error, R²m – R²c: marginal - conditional model variance, respectively.

Physiological trait	Model	Fixed effects	Estimate	SE	t-value	R ² m	R ² c
Haematocrit (N = 1046 observations, n = 488 individuals)	Linear	Intercept	47.08	1.79	26.30	0.12	0.42
		Age	-0.66	0.08	-8.60		
		Sex(M)	-3.59	0.45	-7.92		
		Mass	0.52	0.09	6.04		
Albumin (N = 1068 obs., n = 492 ind.)	Quadratic	Intercept	27.55	1.75	15.72	0.10	0.57
		Age ²	-0.06	0.01	-7.94		
		Sex(M)	-3.13	0.35	-8.89		
		Mass	0.42	0.07	6.00		
		Delay	0.04.10 ⁻¹	0.02.10 ⁻¹	3.22		
		Age-last	0.15	0.07	1.84		
Creatinine (N = 1069 obs., n = 490 ind.)	Quadratic	Intercept	81.22	8.60	9.44	0.10	0.56
		Age ²	-0.28	0.03	-8.32		
		Qcoh(P)	-2.95	1.82	-1.62		
		Pop(CH)	-3.99	5.21	-0.77		
		Sex(M)	-9.46	1.83	-5.18		
		Age ² :pop(CH)	0.17	0.05	3.63		
		Mass	2.18	0.34	6.45		
		Delay	0.02	0.01	2.26		

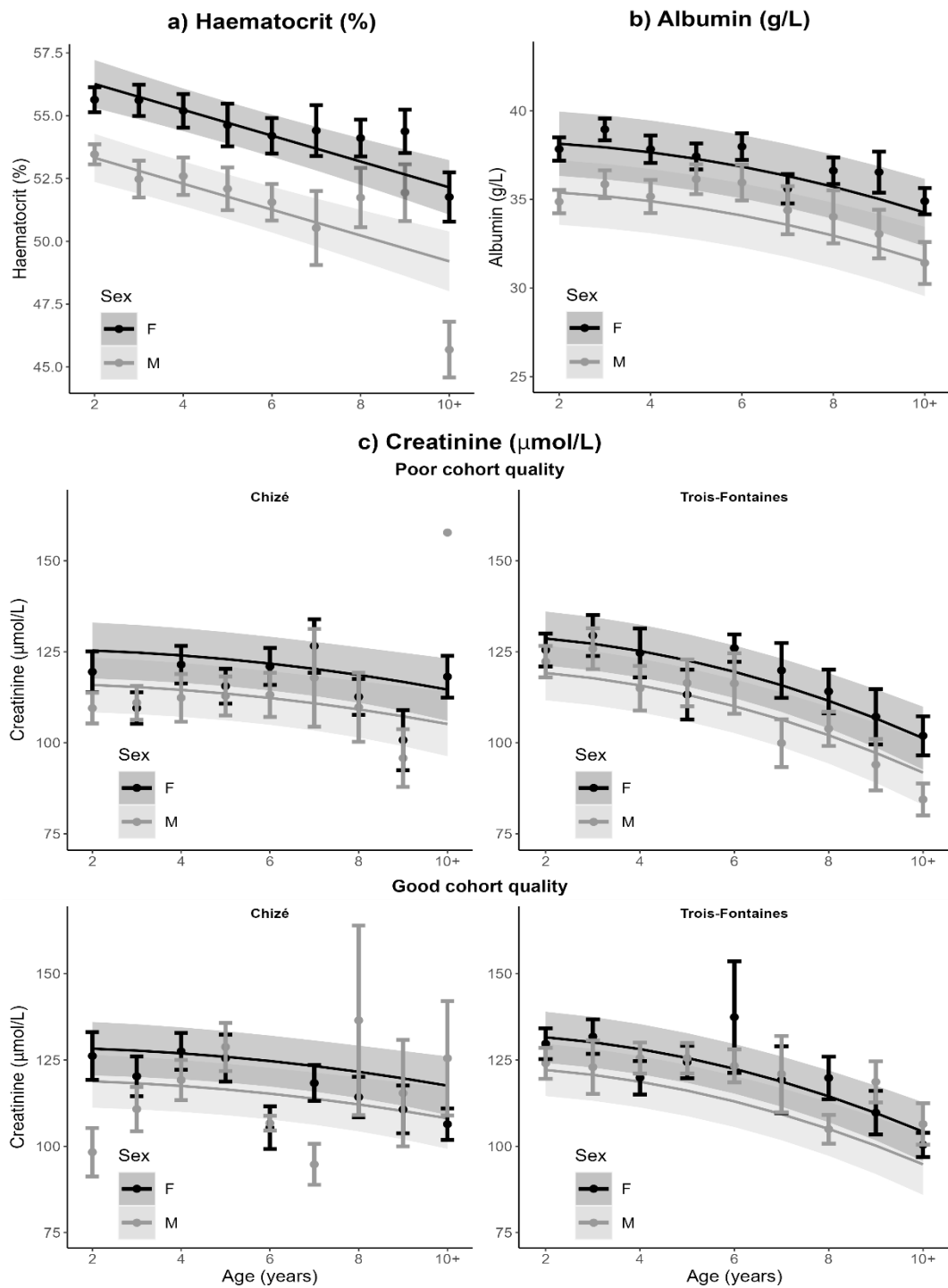


Figure 1. Predicted roe deer haematological senescence trajectories in Chizé (CH) and Trois-Fontaines (TF), accounting for sex and cohort quality. Lines are retained model predictions for each haematological parameter (excluding retained confounding variables for graphical representation) and shaded areas are 95% CIs. Points are age-specific average trait value \pm standard errors. All individuals aged 10 years old and older are pooled within the “10+” age class.

Table 2. Linear mixed effects models (LMM) selection table for the early-late relationship between early-life faecal glucocorticoid metabolites (FGMs, log-transformed) and haematological senescence trajectories. Models included the additive effect of FGMs and the interaction between FGMs and age term(s) retained in the senescence trajectories. M: Males, Qcoh(P): poor cohort quality (as opposed to good cohort quality), CH: population of Chizé, Age-last: age at last observation, Delay: time between capture and sampling (minutes). Values correspond to the parameter coefficient and values between brackets to the standard-errors. ‘I’: Intercept, ‘df’: number of parameters, ‘Log-lik’: log-likelihood, ‘Delta’: difference of AICc between the candidate model and the model having the lowest AICc, and ‘w’: AIC weight of each model. Retained models are in bold. Dashed lines separate models below and above 2 Δ AICc.

Haematocrit (N = 270 observations, n = 142 individuals)																
I	Age	Sex(M)	Mass	FGM	Age:FGM	df	Log-lik	AICc	Delta	w						
51.23 (2.71)	-0.88 (0.19)	-2.76 (0.76)	0.33 (0.13)			7	-837.38	1689.19	0.00	0.64						
51.37 (4.68)	-0.88 (0.19)	-2.75 (0.76)	0.33 (0.13)	-0.02 (0.60)		8	-837.38	1691.32	2.12	0.22						
59.78 (8.81)	-3.24 (2.11)	-2.71 (0.76)	0.31 (0.14)	-1.30 (1.28)	0.39 (0.34)	9	-836.75	1692.20	3.01	0.14						
Albumin (N = 268 obs., n = 140 ind.)																
I	Age ²	Sex(M)	Age-last	Delay	Mass	FGM	Age ² :FGM	df	Log-lik	AICc	Delta	w				
24.67 (3.06)	-0.06 (0.02)	-2.89 (0.66)	0.07 (0.18)	0.01 (0.00)	0.49 (0.14)			9	-815.36	1649.41	0.00	0.61				
21.65 (4.74)	-0.06 (0.02)	-2.92 (0.66)	0.08 (0.18)	0.01 (0.00)	0.50 (0.14)	0.47 (0.56)		10	-815.01	1650.87	1.46	0.29				
22.20 (5.82)	-0.09 (0.23)	-2.91 (0.66)	0.08 (0.18)	0.01 (0.00)	0.49 (0.14)	0.39 (0.74)	0.01 (0.04)	11	-815.00	1653.02	3.61	0.10				
Creatinine (N = 269 obs., n = 141 ind.)																
I	Age ²	Qcoh(P)	Delay	Mass	Pop(CH)	Sex(M)	Age ² :pop(CH)	FGM	Pop(CH):FGM	Age ² :FGM	Age ² :pop(CH):FGM	df	Log-lik	AICc	Delta	w
92.40 (17.20)	-0.10 (0.10)	-9.57 (3.83)	0.01 (0.02)	1.96 (0.73)	-2.64 (6.58)	-14.07 (3.67)	0.12 (0.20)					11	-1252.47	2527.96	0.00	0.37
137.25 (32.07)	-0.14 (0.10)	-9.28 (3.81)	0.01 (0.02)	2.01 (0.73)	-73.55 (38.84)	-13.62 (3.64)	0.19 (0.20)	-7.20 (4.24)	11.16 (6.03)			13	-1250.67	2528.76	0.80	0.25
102.90 (26.52)	-0.11 (0.10)	-9.54 (3.83)	0.01 (0.02)	1.94 (0.73)	-2.90 (6.64)	-13.92 (3.67)	0.11 (0.20)	-1.59 (3.04)				12	-1252.33	2529.89	1.92	0.14
123.41 (35.33)	1.00 (1.19)	-9.25 (3.82)	0.01 (0.02)	2.10 (0.74)	-77.71 (39.12)	-13.85 (3.66)	0.18 (0.20)	-5.28 (4.71)	11.89 (6.08)	-0.19 (0.19)		14	-1250.21	2530.07	2.10	0.13
90.48 (31.43)	0.78 (1.19)	-9.53 (3.84)	0.01 (0.02)	2.01 (0.74)	-2.58 (6.66)	-14.13 (3.69)	0.09 (0.20)	0.17 (3.86)		-0.15 (0.19)		13	-1252.06	2531.54	3.58	0.06
126.86 (38.25)	0.71 (1.70)	-9.41 (3.87)	0.01 (0.02)	2.08 (0.74)	-84.39 (48.43)	-13.80 (3.65)	0.74 (2.39)	-5.77 (5.13)	12.96 (7.63)	-0.14 (0.28)	-0.09 (0.39)	15	-1250.18	2532.26	4.29	0.04

Table 3. Linear mixed effects models (LMM) selection table for the short-term relationship between faecal glucocorticoid metabolites (FGMs, log-transformed) and haematological parameters measured at the same capture event. Models included the additive effect of FGMs and the two-way interactions between FGMs and age term(s) retained in the senescence trajectories, population, sex, cohort quality and mass. M: Males, Qcoh(P): poor cohort quality (as opposed to good cohort quality), CH: population of Chizé, Age-last: age at last observation, Delay: time between capture and sampling (minutes). Values correspond to the parameter coefficient and values between brackets to the standard-errors. ‘I’: Intercept, ‘df’: number of parameters, ‘Log-lik’: log-likelihood, ‘Delta’: difference of AICc between the candidate model and the model having the lowest AICc, and ‘w’: AIC weight of each model. Retained models are in bold. Only models up to delta AICc = 2 are displayed.

Haematocrit (N = 603 observations, n = 362 individuals)																			
I	Age	Sex(M)	Mass	FGM	Pop(CH)	Qcoh(P)	Age: FGM	Sex(M): FGM	Pop(CH): FGM	Qcoh(P): FGM	Mass: FGM	df	Log-lik	AICc	Delta	w			
48.00 (2.58)	-0.63 (0.10)	-3.56 (0.59)	0.42 (0.12)		1.49 (0.87)							8	-1927.71	3871.67	0.00	0.11			
49.91 (2.31)	-0.61 (0.10)	-3.49 (0.59)	0.36 (0.11)									7	-1929.07	3872.33	0.66	0.08			
45.36 (3.76)	-0.64 (0.10)	-3.58 (0.59)	0.44 (0.12)	0.37 (0.38)	1.60 (0.89)							9	-1927.25	3872.81	1.13	0.06			
39.91 (5.63)	0.30 (0.74)	-3.53 (0.59)	0.44 (0.12)	1.20 (0.75)	1.63 (0.89)		-0.15 (0.11)					10	-1926.43	3873.23	1.56	0.05			
48.11 (2.61)	-0.64 (0.10)	-3.55 (0.59)	0.42 (0.12)		1.50 (0.88)	-0.15 (0.59)						9	-1927.68	3873.66	1.99	0.04			

Albumin (N = 610 obs., n = 360 ind.)																				
I	Age	Age ²	Sex(M)	Age-last	Delay	Mass	FGM	Pop(CH)	Qcoh(P)	Age: FGM	Age ² : FGM	Sex(M): FGM	Mass: FGM	Pop(CH): FGM	Qcoh(P): FGM	df	Log-lik	AICc	Delta	w
51.92 (16.90)	-0.74 (0.34)	-0.00 (0.03)	-10.77 (3.94)	0.31 (0.12)	0.01 (0.00)	-0.71 (0.78)	-3.93 (2.56)						1.20 (0.61)	0.18 (0.12)		13	-1827.38	3681.38	0.00	0.03
49.97 (16.95)	-0.77 (0.34)	0.00 (0.03)	-10.93 (3.95)	0.31 (0.12)	0.01 (0.00)	-0.68 (0.78)	-3.87 (2.56)	2.81 (1.92)					1.22 (0.61)	0.18 (0.12)		14	-1826.39	3681.48	0.10	0.03
26.68 (3.36)	-0.75 (0.34)	0.00 (0.03)	-11.79 (3.89)	0.30 (0.12)	0.01 (0.00)	0.47 (0.10)	-0.07 (0.38)						1.35 (0.60)			12	-1828.54	3681.61	0.23	0.02
25.00 (3.55)	-0.77 (0.34)	0.00 (0.03)	-11.94 (3.89)	0.30 (0.12)	0.01 (0.00)	0.48 (0.10)	-0.06 (0.38)	2.85 (1.92)					1.37 (0.60)			13	-1827.52	3681.65	0.27	0.02
40.24 (7.66)	-6.07 (2.72)	0.38 (0.21)	-10.36 (4.02)	0.31 (0.12)	0.01 (0.00)	0.47 (0.10)	-2.18 (1.13)			0.82 (0.42)	-0.06 (0.03)		1.13 (0.62)			14	-1826.55	3681.82	0.44	0.02
38.48 (7.75)	-6.09 (2.72)	0.38 (0.21)	-10.49 (4.03)	0.31 (0.12)	0.01 (0.00)	0.48 (0.10)	-2.15 (1.13)	2.85 (1.94)		0.82 (0.42)	-0.06 (0.03)		1.14 (0.62)			15	-1825.55	3681.90	0.52	0.02
57.78 (17.36)	-0.72 (0.34)	-0.00 (0.03)	-9.92 (3.96)	0.32 (0.12)	0.01 (0.00)	-0.82 (0.78)	-4.83 (2.64)		-6.25 (3.93)				1.07 (0.61)	0.20 (0.12)	0.91 (0.60)	15	-1825.92	3682.64	1.26	0.01
55.86 (17.41)	-0.74 (0.34)	-0.00 (0.03)	-10.07 (3.96)	0.31 (0.12)	0.01 (0.00)	-0.79 (0.78)	-4.78 (2.64)	2.88 (1.93)	-6.32 (3.93)				1.09 (0.61)	0.19 (0.12)	0.92 (0.60)	16	-1824.89	3682.69	1.31	0.01

51.58 (16.89)	-0.71 (0.34)	-0.00 (0.03)	-10.64 (3.95)	0.32 (0.12)	0.01 (0.00)	-0.69 (0.78)	-3.84 (2.56)		-0.38 (0.46)		1.18 (0.61)	0.18 (0.12)	14	-1827.04	3682.79	1.41	0.01	
49.63 (16.95)	-0.73 (0.34)	-0.00 (0.03)	-10.79 (3.95)	0.31 (0.12)	0.01 (0.00)	-0.66 (0.78)	-3.78 (2.56)	2.82 (1.92)	-0.38 (0.46)		1.20 (0.61)	0.17 (0.12)	15	-1826.04	3682.88	1.50	0.01	
55.79 (17.10)	-5.33 (2.81)	0.33 (0.21)	-9.70 (4.07)	0.32 (0.12)	0.01 (0.00)	-0.37 (0.83)	-4.55 (2.59)			0.71 (0.43)	-0.05 (0.03)	1.03 (0.63)	0.13 (0.13)	15	-1826.04	3682.89	1.51	0.01
26.98 (3.38)	-0.71 (0.34)	-0.00 (0.03)	-11.61 (3.90)	0.30 (0.12)	0.01 (0.00)	0.46 (0.10)	-0.08 (0.38)		-0.41 (0.46)		1.33 (0.60)		13	-1828.14	3682.90	1.52	0.01	
25.30 (3.56)	-0.74 (0.34)	-0.00 (0.03)	-11.76 (3.90)	0.30 (0.12)	0.01 (0.00)	0.48 (0.10)	-0.06 (0.38)	2.86 (1.92)	-0.41 (0.46)		1.35 (0.60)		14	-1827.11	3682.93	1.55	0.01	
53.90 (17.15)	-5.36 (2.81)	0.34 (0.21)	-9.83 (4.08)	0.32 (0.12)	0.01 (0.00)	-0.35 (0.83)	-4.50 (2.59)	2.83 (1.93)		0.71 (0.43)	-0.05 (0.03)	1.04 (0.63)	0.13 (0.13)	16	-1825.04	3683.00	1.62	0.01
39.62 (7.67)	-7.06 (2.67)	0.46 (0.20)	-3.08 (0.46)	0.32 (0.12)	0.01 (0.00)	0.46 (0.10)	-2.09 (1.14)			0.98 (0.41)	-0.07 (0.03)			13	-1828.20	3683.01	1.63	0.01
40.68 (7.67)	-6.10 (2.72)	0.38 (0.21)	-10.14 (4.03)	0.31 (0.12)	0.01 (0.00)	0.46 (0.10)	-2.20 (1.13)		-0.43 (0.45)	0.83 (0.42)	-0.06 (0.03)	1.10 (0.62)		15	-1826.11	3683.03	1.65	0.01
38.91 (7.75)	-6.12 (2.72)	0.38 (0.21)	-10.26 (4.03)	0.31 (0.12)	0.01 (0.00)	0.47 (0.10)	-2.17 (1.13)	2.86 (1.93)	-0.43 (0.45)	0.83 (0.42)	-0.06 (0.03)	1.12 (0.62)		16	-1825.09	3683.10	1.72	0.01
54.64 (16.89)	-0.71 (0.34)	-0.00 (0.03)	-3.05 (0.46)	0.33 (0.12)	0.01 (0.00)	-0.97 (0.77)	-4.35 (2.56)					0.22 (0.12)		12	-1829.31	3683.13	1.76	0.01
29.33 (4.93)	-1.17 (0.67)	-0.00 (0.03)	-12.13 (3.92)	0.31 (0.12)	0.01 (0.00)	0.46 (0.10)	-0.48 (0.67)			0.07 (0.09)		1.40 (0.60)		13	-1828.27	3683.15	1.77	0.01
37.88 (7.77)	-7.10 (2.67)	0.47 (0.20)	-3.10 (0.46)	0.32 (0.12)	0.01 (0.00)	0.48 (0.10)	-2.07 (1.14)	2.80 (1.95)		0.98 (0.41)	-0.07 (0.03)			14	-1827.24	3683.18	1.80	0.01
37.71 (7.79)	-6.56 (2.77)	0.42 (0.21)	-10.72 (4.03)	0.32 (0.12)	0.01 (0.00)	0.48 (0.10)	-2.02 (1.14)	6.53 (4.65)		0.89 (0.42)	-0.06 (0.03)	1.18 (0.62)	-0.57 (0.65)	16	-1825.17	3683.25	1.87	0.01
27.54 (5.08)	-1.17 (0.67)	0.00 (0.03)	-12.26 (3.92)	0.30 (0.12)	0.01 (0.00)	0.48 (0.10)	-0.44 (0.67)	2.82 (1.93)		0.06 (0.09)		1.42 (0.60)		14	-1827.28	3683.26	1.88	0.01
28.08 (4.13)	-0.74 (0.34)	0.00 (0.03)	-11.23 (3.91)	0.30 (0.12)	0.01 (0.00)	0.49 (0.10)	-0.53 (0.52)	2.91 (1.93)	-5.58 (3.91)			1.27 (0.60)	0.80 (0.60)	15	-1826.23	3683.27	1.89	0.01
29.75 (3.99)	-0.72 (0.34)	-0.00 (0.03)	-11.09 (3.91)	0.30 (0.12)	0.01 (0.00)	0.47 (0.10)	-0.54 (0.52)		-5.50 (3.91)			1.25 (0.60)	0.79 (0.60)	14	-1827.29	3683.28	1.91	0.01
52.80 (16.94)	-0.74 (0.34)	-0.00 (0.03)	-3.07 (0.46)	0.32 (0.12)	0.01 (0.00)	-0.94 (0.77)	-4.30 (2.56)	2.73 (1.94)				0.22 (0.12)		13	-1828.38	3683.37	1.99	0.01
51.55 (16.93)	-0.93 (0.69)	-0.00 (0.03)	-10.99 (4.00)	0.32 (0.12)	0.01 (0.00)	-0.64 (0.81)	-3.87 (2.57)			0.03 (0.10)		1.23 (0.61)	0.17 (0.12)	14	-1827.33	3683.37	1.99	0.01

Creatinine (N = 615 obs., n = 362 ind.)

I	Age ²	Sex(M)	Pop(CH)	Qcoh(P)	Mass	Delay	Age ² : pop(CH)	FGM	Age ² : FGM	Sex(M): FGM	Qcoh(P): FGM	Mass: FGM	df	Log-lik	AICc	Delta	w
81.15 (16.00)	-0.25 (0.04)	-37.36 (17.48)	-3.61 (5.87)	-2.23 (2.50)	1.69 (0.44)	0.02 (0.01)	0.15 (0.06)	1.60 (1.71)		4.48 (2.69)			13	-2785.39	5597.38	0.00	0.08
68.73 (18.62)	-0.26 (0.04)	-38.26 (17.47)	-4.26 (5.84)	20.45 (17.70)	1.64 (0.44)	0.02 (0.01)	0.16 (0.06)	3.77 (2.38)		4.61 (2.68)	-3.52 (2.72)		14	-2784.55	5597.80	0.42	0.06

70.66 (14.75)	-0.25 (0.04)	-8.54 (2.52)	-3.81 (5.92)	-2.37 (2.51)	1.70 (0.44)	0.02 (0.01)	0.15 (0.06)	3.20 (1.41)				12	-2786.77	5598.05	0.67	0.06
58.60 (17.70)	-0.26 (0.04)	-8.55 (2.52)	-4.43 (5.90)	19.10 (17.70)	1.66 (0.44)	0.02 (0.01)	0.16 (0.06)	5.29 (2.21)		-3.33 (2.72)		13	-2786.02	5598.64	1.26	0.04
32.50 (77.56)	-0.25 (0.04)	-38.83 (17.62)	-3.40 (5.87)	-2.32 (2.50)	3.96 (3.57)	0.02 (0.01)	0.14 (0.06)	9.02 (11.70)	4.70 (2.71)		-0.35 (0.54)	14	-2785.18	5599.06	1.68	0.03
6.87 (79.63)	-0.25 (0.04)	-40.15 (17.62)	-4.06 (5.84)	22.01 (17.80)	4.48 (3.59)	0.02 (0.01)	0.15 (0.06)	13.22 (12.07)	4.90 (2.71)	-3.77 (2.73)	-0.44 (0.54)	15	-2784.23	5599.27	1.89	0.03
77.24 (18.87)	-0.17 (0.22)	-35.84 (17.89)	-3.56 (5.87)	-2.27 (2.50)	1.68 (0.44)	0.02 (0.01)	0.15 (0.06)	2.20 (2.30)	-0.01 (0.03)	4.25 (2.74)		14	-2785.31	5599.32	1.94	0.03

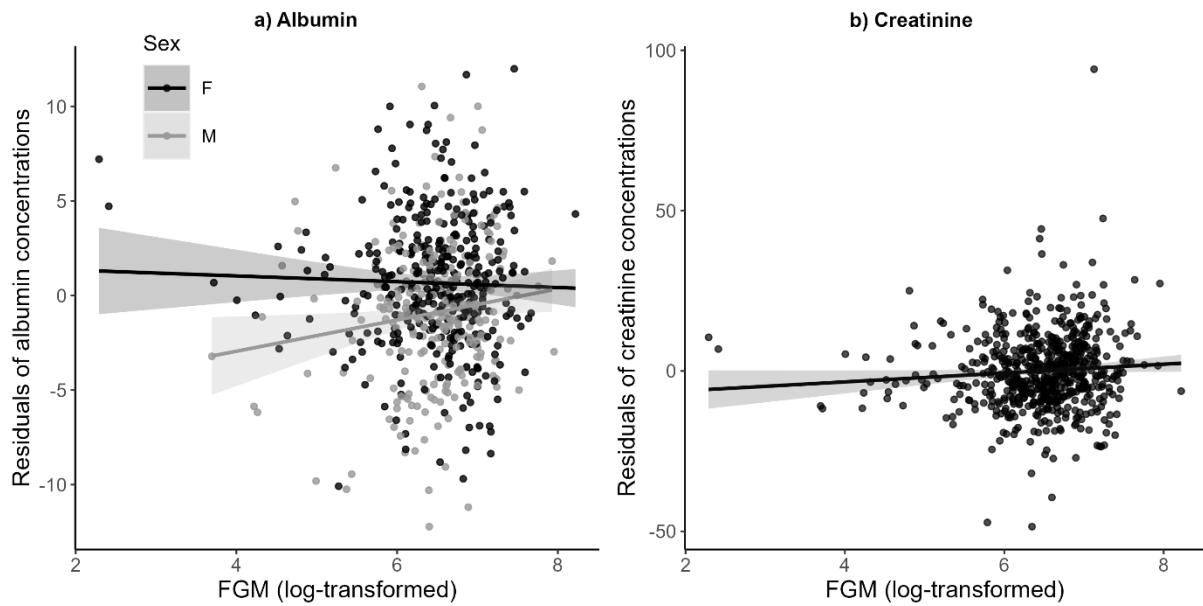


Figure 2. Predicted roe deer haematological concentrations (residuals of the retained senescence trajectories). a) Relationship between albumin and faecal glucocorticoid metabolites (FGMs, log-transformed) measured at the same capture events according to sex (females: F, gray points and line, males: M, black points and line), and accounting for age, mass, age at last observation and delay between capture and sampling. b) Relationship between creatinine and FGMs, accounting for age, sex, population, cohort quality, mass and delay between capture and sampling. Lines are model predictions and shaded areas are 95% CIs.

Results

Haematological senescence

Haematological senescence trajectories were computed from 1046 HCT values measured on 488 individuals, 1068 ALB concentrations from 492 individuals and 1069 CREA concentrations from 490 individuals. As expected, we found evidence of senescence for the three parameters. HCT decreased linearly with advancing age similarly in both sexes and both populations ($\beta_{\text{age}} = -0.66 \pm 0.08$, Table 1, Figure 1a). Albumin decreased quadratically with age in a similar fashion in both sexes and both populations ($\beta_{\text{age}^2} = -0.06 \pm 0.01$, Table 1, Figure 1b). We found support for a slight increase in ALB concentrations with increasing delay between capture and blood sampling ($\beta_{\text{delay}} = 0.04 \cdot 10^{-1} \pm 0.02 \cdot 10^{-1}$, Table 1), and found that the age at last observation was positively related to ALB concentrations, suggesting the existence of selective disappearance for this trait ($\beta_{\text{age-last}} = 0.15 \pm 0.07$, Table 1). Lastly, CREA decreased

with advancing age in a quadratic and population-specific manner ($\beta_{\text{age}^2} = -0.28 \pm 0.03$, $\beta_{\text{pop(CH)}} = -2.95 \pm 1.82$, $\beta_{\text{age}^2:\text{pop(CH)}} = 0.17 \pm 0.05$, Table 1, Figure 1c), with individuals of TF displaying a higher rate of senescence than those of CH. More specifically, individuals of TF displayed higher CREA concentrations than individuals of CH up to 6 years old, after what CREA concentrations was lower in TF than CH until the end of life (Figure 1c). CREA also increased with the delay between capture and blood sampling ($\beta_{\text{delay}} = 0.02 \pm 0.01$, Table 1). All three parameters increased with body mass (HCT: $\beta_{\text{mass}} = 0.52 \pm 0.09$, ALB: $\beta_{\text{mass}} = 0.42 \pm 0.07$, CREA: $\beta_{\text{mass}} = 2.18 \pm 0.34$, Table 1), and were higher in females than in males (HCT: $\beta_{\text{sex(M)}} = -3.59 \pm 0.45$, ALB: $\beta_{\text{sex(M)}} = -3.13 \pm 0.35$, CREA: $\beta_{\text{sex(M)}} = -9.46 \pm 1.83$, Table 1, Figure 1).

Early-life FGM and haematological senescence

For HCT, we obtained 270 observations from 142 individuals for which we had FGMs measured during the first year of life and adult HCT measurements. Similarly, we obtained 268 ALB measurements from 140 individuals, and 269 CREA measurements from 141 individuals. For none of the three parameters we found support for an additive or an interactive effect of early-life FGMs on haematological senescence trajectories.

Immediate relationship between FGMs and haematological parameters

We collected 603 observations from 362 individuals for which we had both FGMs and HCT measurements obtained during the same capture event. For ALB, we obtained 610 observations from 360 individuals, and 615 observations from 362 individuals for CREA. No relationship between FGMs and HCT was found (Table 3). However, we found support for an interacting effect of FGMs and sex on ALB concentrations ($\beta_{\text{FGM}} = -0.07 \pm 0.38$, $\beta_{\text{sex(M)}} = -11.79 \pm 3.89$, $\beta_{\text{sex(M):FGM}} = 1.35 \pm 0.60$, Table 3, Figure 2a), once accounting for ALB senescence trajectory. The relationship between FGMs and ALB was positive for males, and slightly negative for females (but note the large standard-error: $\beta_{\text{FGM}} = -0.07 \pm 0.38$, Table 1, Figure

2a). We also found a positive relationship between FGMs and CREA ($\beta_{\text{FGM}} = 3.20 \pm 1.41$, Table 3, Figure 2b). Results were similar for ALB and CREA when including the particularly high FGM value mentioned earlier. Also note that the observed relationships could be driven by two low female FGM values (visible on the left of both panels in Figure 2). We tested whether removing these two FGM values changed results and found that it was not the case for CREA. For ALB, however, the interaction between sex and FGM was no longer retained. Instead, a positive interaction between body mass and FGM was detected ($\beta_{\text{FGM}} = -5.00 \pm 2.56$, $\beta_{\text{mass}} = -1.33 \pm 0.77$, $\beta_{\text{mass:FGM}} = 0.26 \pm 0.12$; Table S3, Figure S2).

Discussion

Our study aimed at describing the immediate and carry-over effects of baseline GCs on the values and senescence of haematological parameters reflecting individual body condition. We took advantage of a longitudinal dataset collected on two contrasted populations of a long-lived mammal, the roe deer. We first confirmed that senescence of three haematological parameters (*i.e.* HCT, ALB, CREA) occurs in those populations (Jégo et al. 2014), and demonstrated that environmental quality at birth impacts CREA concentrations. Individuals from good cohorts displayed higher CREA concentrations than those from cohorts of poorer quality. Most importantly, our results demonstrated that GC levels, measured as FGMs, were related to two indices of physiological condition (*i.e.* ALB and CREA) on the short-term but that early-life GCs did not have carry-over effects on haematological parameters nor on their senescence patterns.

The absence of carry-over or delayed effects of GCs on physiological status have already been acknowledged in these roe deer populations. Indeed, early-life GCs do not modulate patterns of immunosenescence (Lalande et al. *submitted*) and do not relate to adult

body mass either (Lalande et al. 2023). The fact that GCs and haematological parameters relate on the short-term only is thus consistent with previous works on these populations. The reasons for an absence of such carry-over effects can be manifold and include the limitations of using only GCs to assess the stress response. GCs have pleiotropic effects and are not the sole mediators involved in the stress response, so that allostatic states should ideally be measured using multiple indices (*e.g.* metabolic, immune, cardiovascular markers) to fully capture the physiological state of an individual coping with environmental challenges (Edes et al. 2018, Seeley et al. 2022). Also, although Monaghan and Hausmann (2015) reviewed the carry-over negative consequences of early-life GCs, and evidenced negative consequences that were mainly restricted to longevity, telomere attrition and behaviours. It is possible that the carry-over effects of GCs are trait-dependent.

We evidenced that GCs were positively related to CREA concentrations, on the short-term. Such a positive relationship was expected for CREA because, although GCs promote increased glomerular filtration rates, and thus higher excretion rates of CREA, GCs also favour protein catabolism and muscle loss (Andreev et al. 1999, Kuo et al. 2013). Indeed, GCs are usually positively related to plasma CREA concentrations (van Acker et al. 1993, Andreev et al. 1999) because they are likely to enhance the degradation rate of the muscular creatine. Increased plasma CREA concentrations could actually reflect both a renal dysfunction (Perrone et al. 1992) or a state of increased catabolic state (Andreev et al. 1999). Also, high population density – generally associated with stress and increased GC levels (*e.g.* Dantzer et al. 2013) – has been shown to translate into increased plasma CREA concentrations and lower body mass in a white-tailed deer (*Odocoileus virginianus*) population (Sams et al. 1998). This suggests that reduced body mass in response to increased population density could increase plasma CREA concentrations due to an enhanced catabolic state.

The sex-specific association between FGMs and ALB concentrations can relate to differences in reproductive status between males and females at the time of capture. ALB concentrations increased with FGMs in males, but slightly decreased in females. Progesterone is an hormone involved in maintaining pregnancy, and which binds to plasma ALB (Abboud et al. 2017). In winter, roe deer females are (almost) all gestating (92% in CH, unpublished data), and plasma progesterone concentrations increase across the capture season (Sempéré et al. 1989). The fact that progesterone binds to ALB could explain the sex-specific relationship between FGMs and circulating ALB, and specifically why females show a decrease in ALB concentrations as GCs increase, whereas it is not the case in males. Note however, that when removing two low female FGM values, we found support for a positive relationship between FGMs and ALB independently of sex, and modulated by body mass, with heavier individual displaying greater increases in ALB concentrations with increasing FGMs. This could suggest that heavier individuals, *i.e.* of better body condition, are able to compensate adverse consequences to maintain their condition as shown in wild European badgers (*Meles meles*; Bright Ross et al. 2021). In both cases, however, we found that ALB increased with FGMs in some contexts.

An increase in plasma ALB concentration with GC levels was unexpected. High white-tailed deer population density was associated to reduced plasma ALB concentrations due to an impoverishment of the nutritional status of individuals competing for acquiring resources (Sams et al. 1998). However, as mentioned, GCs are expected to increase protein degradation rates and to decrease protein synthesis. This should result in decreased plasma proteins concentrations to fuel demanding activities, by providing the organism with amino acids that can readily be used for gluconeogenesis (Landys et al. 2006, Kuo et al. 2013). However, the role of glucocorticoids in affecting the synthesis of albumin proteins is actually not as clear as expected, with GCs maintaining ALB concentrations *in vitro*, but without regulating ALB

synthesis *in vivo* (Moshage et al. 1985). Finally, haemoconcentration can occur in response to stress, resulting in increased blood ALB concentrations (Marco and Lavín 1999, Navarro et al. 2019). High FGM levels reflect either individuals chronically stressed and for which baseline GC levels are heightened, or individuals that experienced stress few hours (*ca.* 12 h; Dehnhard et al. 2001) prior to capture. Consequently, the positive relationship between FGMs and ALB could reflect individuals that have been recently exposed to stressors prior to capture.

The conclusion relating to an increase ALB concentration with FGMs emphasises the difficulty of studying stress in wild populations and the challenges of detecting states of chronic stress when stressor exposure history is generally unknown. A solution would be to rely on several measures of the GC response, such as integrated measures (*e.g.* FGMs), circulating blood GCs and efficacy of the negative feedback system of the stress response, which together give a better predictive power to estimate chronic stress (Dickens and Romero 2013). This would allow to disentangle cases where FGM levels could reflect either chronically stressed individuals or individuals recently exposed to stressors.

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CHAPTER 4 – Early-life glucocorticoids and immunosenescence



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Glucocorticoids and immunosenescence patterns in a wild ungulate

In preparation

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Abstract

Immunosenescence describes the progressive decline of immune functions with age. Although it is critical to understand what modulates such decline *in nature*, immunosenescence remains poorly documented in free-ranging populations. Glucocorticoids (GCs) are good candidates to modulate patterns of immunosenescence, especially in early-life since they can then have long-term consequences on individual physiology. Indeed, these hormones are part of the stress response triggered by unpredictable events, act as regulators of energy allocation in order to ensure allostasis, and have immunosuppressive effects when chronically elevated. Here, we used longitudinal data from two roe deer (*Capreolus capreolus*) populations to investigate whether early-life baseline GC levels can modulate patterns of immunosenescence. We first updated immunosenescence trajectories previously described in these populations, exhibiting contrasted environmental conditions. We did not find any evidence that early-life GC levels modulate these trajectories. However, GC levels were related to the abundance of lung parasites during adulthood. Our results emphasise the temporality of GCs consequences on life-history traits, suggesting that GCs have short-, but not long-term effects on immunity.

Keywords: Faecal glucocorticoid metabolites, parasite, aging, immunity, stress, allostatic load

Introduction

The immune system is a key component of health and viability (Schmid-Hempel 2003). It is constituted of two interacting arms: the innate immunity, providing a rapid, generalist response to pathogens and including the inflammatory response, and the adaptive immunity, characterised by a slower but more specific, long-term protection against pathogens (Klasing 2004). The development, maintenance and use of the immune system is costly both in terms of energy allocation and tissue damages (Klasing 2004). Consequently, allocation to each arm of the immune system must be traded-off against other essential functions such as growth, somatic maintenance or reproduction (Sheldon and Verhulst 1996, Lee 2006, Martin 2009, Maizels and Nussey 2013, McDade et al. 2016). Inevitably, these costs and the subsequent trade-offs can explain that immune functioning cannot be maximised throughout life and declines with increasing age, a process defined as ‘immunosenescence’ (Shanley et al. 2009). Coming mostly from human and laboratory mammal studies (Bauer and Fuente (2016), but see Peters et al. (2019) for a review in free-ranging and captive animals), the literature shows consistent patterns of decline of the adaptive arm and no clear patterns for the innate arm, although its inflammatory component shows consistent increase (leading to a continuous low-grade inflammatory state, named ‘*inflammaging*’, source of tissue damages, Franceschi et al. 2000). These age-associated declines in immune functioning are generally the underlying causes of an increased susceptibility to pathogens (such as parasites) with age in mammals, including humans (Hayward et al. 2009, 2015, Froy et al. 2019, Abdoli and Ardakani 2020; but see also Hämäläinen et al. 2015), associated with higher morbidity and mortality risks with potential consequences on survival and reproductive success (Sheldon and Verhulst 1996, Bauer and Fuente 2016, Froy et al. 2019). Accordingly, because of these consequences on wild populations, it is important to investigate what could modulate immune responses and their senescence in natural context.

The allocation trade-off between immune and other essential functions (Sheldon and Verhulst 1996, McDade et al. 2016) is expected to be mediated, at least partially, by glucocorticoids (GCs). GCs are steroid hormones with pleiotropic effect. Although they are mainly known for their involvement in the stress response (Wingfield 2013, Hau et al. 2016), their primary role is to regulate an organism energy balance, and as a result, modulate energy allocation between growth, reproduction, and somatic maintenance and repair (Hau et al. 2016). Therefore, GCs contribute to allostasis (*i.e.* achieving stability through change) by ensuring energy homeostasis (McEwen and Wingfield 2003, 2010, Romero et al. 2009), according to predictable and unpredictable events. Unpredictable environmental perturbations elicit a stress response which manifests by the activation of the hypothalamic-pituitary-adrenal (HPA) axis and the release of GCs. Short-term, acute GC elevation (*i.e.* within minutes to hours) promotes reallocation of stored energy to processes enhancing survival through the activation of ‘*fight-or-flight*’ responses (Sapolsky et al. 2000), after what GCs quickly return to baseline levels to support normal daily activities. Following exposure to repeated or long-lasting perturbations, however, GC concentrations can remain elevated for longer periods of times (*i.e.* days to weeks). Long-term GC elevation can cause ‘allostatic overload’, meaning that the energy required to ensure daily and seasonal activities, and to cope with the perturbations, is higher than the energy intake (McEwen and Wingfield 2010). Allostatic overload can have various negative effects [*e.g.* increased metabolic rate, telomere attrition, accelerated cellular ageing (Seeman et al. 2001, Casagrande et al. 2020, Lee et al. 2021, Bobba-Alves et al. 2023), body growth inhibition, weakening of body condition (Reeder and Kramer 2005)], including the disruption of immune functions due to the immunosuppressive effects of GCs (Lee 2006, Martin 2009, Dhabhar 2014, Hau et al. 2016). Indeed, chronic GC elevations decreases the production, activity and functioning of immunoprotective cells and lead to inflammaging and immune dysregulation (Dhabhar 2014).

While GCs have been evidenced to suppress immunity, the carry-over consequences of GCs on immunosenescence remain to be studied in wild populations. Baseline GC levels and immune parameters have effectively been shown to covary in a wild ungulate (*Capreolus capreolus*, Carbillet et al. 2022). Similarly, GCs and parasite burden show a general positive correlation on the short-term (Defolie et al. 2020). The underlying mechanisms are not fully elucidated, but could involve the suppressive effects of GCs on host immunity and their supporting role in parasite development (Herbert et al. 2022). Generally, the consequences of acute stress-induced GC concentrations on individual performances have been largely studied (*i.e.* the ‘*emergency life-history stage*’, Wingfield et al. 1998), and the immunosuppressive effects of GCs during chronic stress are well documented (Lee 2006, Martin 2009, Dhabhar 2014). By contrast, the consequences of elevated baseline GC levels (resulting from repeated or chronic stress exposure) on life-history traits are more equivocal, showing either positive, null or negative relationships (*e.g.* Ethan Pride 2005, Pauli and Buskirk 2007, Cabezas et al. 2007, Wey et al. 2015). Similarly, the carry-over effects of GCs (O’Connor et al. 2014, Sopinka et al. 2015) during early-life on adult performances have been poorly studied in wild mammals, although chronic GC exposure during early-life can have profound long-term detrimental effects on longevity and telomere length in birds (*e.g.* Spencer et al. 2009, Monaghan et al. 2011, Herborn et al. 2014). The literature on stress exposure/chronically elevated GC levels and immunosenescence tended to evidence negative relationships (Bauer 2005, 2008, Garrido et al. 2022), but most studies focused on humans, and less frequently on laboratory rodents, while none on wild animals. Importantly, these studies are more related to covariations (or relationships over short period of times relative to lifespan) between chronic stress exposure/GC levels and changes in immune parameters in elderly, rather than to the study of early-life stress and carry-over changes in immune functioning.

Here, we took advantage of a longitudinal dataset obtained from an intensive capture-mark-recapture (CMR) program in two populations of roe deer (*Capreolus capreolus*) living in habitats with contrasted environmental conditions (Pettorelli et al. 2006). We aimed at exploring the long-term effects of GC levels on immunosenescence. Immunosenescence trajectories had been previously described in those two populations, showing that immunosenescence was mainly driven by population-level characteristics (Cheynel et al. 2017). However, how physiological markers, and more particularly GCs, modulate those patterns of immunosenescence has never been investigated. We first described and updated trajectories of immunosenescence for 12 immune traits representative of both adaptive and innate immune components, and for susceptibility to 4 parasites. We accounted for confounding factors that could modulate trajectories such as population (Cheynel et al. 2017), sex (Brooks and Garratt 2017, Lemaître et al. 2020) or natal environmental conditions (Nussey et al. 2007, Reed et al. 2008, Lemaître et al. 2015, Cooper and Kruuk 2018). Then, we used baseline GC levels (estimated through faecal glucocorticoid metabolites, FGMs) measured during the first year of life to investigate how early-life GCs affected immunosenescence patterns. Early-life is a major determinant of future performance in this income breeder (Gaillard et al. 2003) and early-life stress can have detrimental consequences on adult performances (Monaghan and Hausmann 2015). We expected individuals exposed to higher levels of stress during early-life to display earlier and/or accelerated immunosenescence due to the role of GCs in resource allocation. High levels of stress during early-life were also expected to result in immunosuppression and enhanced parasite development during late-life (Herbert et al. 2022).

Material and methods

Study populations

Roe deer is a medium-sized ungulate (*ca.* 25 kg) commonly found in European lowland woodlands. Two populations, Trois-Fontaines (TF - 1360 ha) in north-eastern France (48°43'N, 4°55'E) and Chizé (CH - 2614 ha) in western France (46°05'N, 0°25'W), have been monitored through a CMR program every winter since 1975 and 1977, respectively. At TF, forest productivity is high due to rich soils and the habitat is homogeneous and of good-quality. By contrast, at CH, poor soils and frequent summer droughts result in overall low forest productivity and heterogeneous habitat quality (Pettorelli et al. 2006), which is less suitable for roe deer. In both populations, natal environmental conditions are measured as the cohort-specific average juvenile mass corrected for the Julian date of capture (Gaillard et al. 1996). This metric of environmental quality allows us to account for the influence of environmental fluctuations on roe deer condition, from the birth of an individual (typically in May) up to its capture during its first winter, and is referred to as 'cohort quality' hereafter. Such an integrative metric of natal environmental conditions encompasses variations in environmental conditions (*e.g.* climate, resource availability and quality, roe deer density) as well as information about individual quality (*e.g.* mother condition, maternal care, itself partly dependent on environmental quality) while avoiding the problem of defining the exact metric to consider to describe environmental conditions (Toïgo et al. 2006). Cohort quality was included as a categorical variable with two levels (poor and good cohort quality) split based on the median cohort quality in each population. Captures take place each year during 10-12 days, spread across December (at TF) or January (at CH) to early March (Gaillard et al. 1993). In order to minimise disturbance and stress caused by human intervention, most capture days are organised more than 48 hours apart. In some cases, captures can take place during two consecutive days, but are then conducted on opposite areas of the forest. Likewise, morning and afternoon

captures of a given day take place in different areas within both forests. According to CMR analyses, both populations were below carrying capacity during the present study period (2010-2022, unpublished data). Since 2010, faecal matter and blood have been collected on weighed individuals of known sex and age (*i.e.* captured within their first year of life).

Faecal Glucocorticoid Metabolites

Baseline HPA activity was estimated through faecal glucocorticoid metabolites (FGMs, Palme 2019). The measurement of circulating GC concentrations would require sampling blood shortly after capture (*i.e.* generally within 3 minutes) to be a reliable measure of the HPA baseline activity in free-ranging animals (Romero and Reed 2005, Sheriff et al. 2011, Lavergne et al. 2021), which is impossible in our capture setting. Alternatively, FGMs represent an integrative measure of baseline GC concentrations and thus baseline adrenocortical activity several hours before sampling (*i.e.* baseline stress; Palme, 2019), with a delay of 12 hours on average in roe deer (ranging from 6 to 23 hours, Dehnhard et al. 2001). Thus FGMs are widely used as a proxy for evaluating the baseline activity of the HPA axis of an individual (Palme 2019).

Extraction of FGM consisted in 0.5 g (\pm 0.005) of faeces vortexed in 5 mL of 80 % methanol and centrifuged for 15 minutes at 2500 g (Palme et al. 2013). The amount of FGM was determined in an aliquot of the supernatant diluted 10 times in assay buffer and was performed using a group-specific 11-oxoetiocholanolone enzyme immunoassay (EIA), according to a method previously described in Möstl et al. (2002) and validated for roe deer (Zbyryt et al. 2018). Measurements were done in duplicate and intra- and inter-assay coefficients of variation were lower than 10% and 15%, respectively. Results of FGMs dosage are expressed as nanograms per gram of wet faeces (ng/g).

FGMs collection in free-ranging animals is subject to several issues that might impact FGM levels. First, we collected faecal matter rectally from 2010 to 2022. In both populations, faeces were immediately frozen at -80 °C after collection, except prior to 2017 in TF where faeces were stored at 4 °C and frozen at -20 °C within 24 hours. The time between faecal sampling and freezing, as well as freezing temperature, can impact FGM levels due to different bacterial activity (Lexen et al. 2008, Hadinger et al. 2015, Carbillet et al. 2023b). Second, FGMs are likely to show variation throughout the capture period (Bubenik et al. 1998, Huber et al. 2003). Finally, as animals are manipulated and sampled from 1 to 4 hours following capture on average, the variation in the time delay between capture and sampling can also impact FGM levels. We first tested whether these three protocol issues affected the FGM levels measured during the first winter of 162 individuals. A linear mixed effect model (LMM) accounting for *i*) the Julian date of capture (linear and quadratic), *ii*) the time delay (min) between capture and sampling, and *iii*) whether faeces were immediately frozen at -80 °C or frozen at -20 °C within 24 hours, showed no effect of these confounds on FGM levels (Table S1). We did not account for differences in reproductive status between and among males and females, as FGMs used in the present study are exclusively measured on juveniles (*i.e.* measured at 8-9 months old), when individuals are thus not reproducing yet. During the capture period, roe deer's diet is mostly composed of brambles (*Rubus* sp.) and ivy (*Hedera helix*) and similar in both study sites (Tixier and Duncan 1996), so FGM differences between populations are not expected to be biased according to diet composition. Note that for the analyses, FGM values were log-transformed (henceforth called FGMs).

Immune traits

In order to assess immunosenescence of both the adaptive and innate components of the immune response, we considered 12 immune traits of both the humoral and cellular immune

activity, (as described in Cheynel et al. 2017), that were measured between 2010 and 2021 (as well as 2022 for hemagglutination (HA) and hemolysis (HL) scores).

Cellular innate immunity

Cellular innate immunity was assessed by describing the composition of the white blood cell (WBC) population. We counted the first hundred WBC in Wright-Giemsa-stained blood smears to evaluate the proportion of the different types of WBC and brought back proportions to concentrations (10^3 cells/mL). WBC is composed of five different cell types: neutrophils are the most numerous WBC and, together with monocytes, are key components of the innate arm of the immune system through their antimicrobial/biotoxic and phagocytic activity. Moreover, neutrophils and monocytes have recently been identified as having a role in auto-immune and inflammatory diseases when their activation is dysregulated (Auffray et al. 2009, Burn et al. 2021). Basophils are the least abundant WBC, but have a specific role in the inflammatory response, being particularly active at sites of ectoparasite infections such as ticks and communicating with the adaptive arm of the immune system (Siracusa et al. 2010, Karasuyama et al. 2011). Eosinophils are involved in the inflammatory response and reaction to allergies with basophils and mast cells. They also play a role in the defence against internal parasites such as helminths, and in the modulation of the innate and adaptive immunity. If dysregulated, their cytotoxic actions can have detrimental consequences on the organism (Rothenberg and Hogan 2006). Finally, lymphocytes are involved in the cellular adaptive immunity and are described below.

Humoral innate immunity

To evaluate the humoral part of the innate immune system, we performed hemagglutination-hemolysis (HA-HL) assays, as described in Matson et al. (2005) and previously used on roe deer blood samples (Gilot-Fromont et al. 2012, Cheynel et al. 2017,

Carbillet et al. 2022, 2023a). Briefly, hemagglutination [HA, 10^{-2} log(dilution)] measures the ability of an individual to agglutinate exogeneous cells, as representative of the concentration of circulating natural antibodies (NAbs). Similarly, hemolysis [HL, 10^{-2} log(dilution)] measures the activity of the complement, a group of proteins acting in chain reactions to provoke the lysis of exogeneous cells in the presence of antigen-antibody complexes (Matson et al. 2005). We also assessed the concentrations (mg/mL) of alpha1-globulins, alpha2-globulins and beta-globulins to follow the inflammatory proteins involved in the acute phase response, a part of the early innate immune system (Cray et al. 2009). Inflammation was assessed by determining alpha1-, alpha2-, beta- and gamma-globulin protein concentrations using refractometry and automatic agarose gel electrophoresis (HYDRASYS, Sebia, Evry, France) that separates blood proteins in 5 fractions: albumin, alpha1, alpha2, beta and gamma fractions. Haptoglobin is a protein produced in case of chronic inflammation and infection and is part of the alpha2 fraction. Haptoglobin concentrations (mg/mL) were thus measured with a Konelab 30i automaton (Fisher Thermo Scientific, Cergy-Pontoise, France) using phase haptoglobin assay chromogenic kit (Tridelta Development LTD, County Kildare, Ireland).

Cellular adaptive immunity

Cellular adaptive immunity was assessed by measuring the concentration of lymphocytes (10^3 cells/mL). Lymphocytes include both B and T cells. T lymphocytes have many functions, among which the recognition of exogeneous or infected cells and provoke cell death. B lymphocytes are an essential part of the humoral adaptive immunity, being the main antibody-producing cells. Lymphocytes also include natural killer (NK) cells, as part of the innate immunity. However, NK cells are a minority among lymphocytes (*e.g.* 0.5-10 % in cattle, (Kulberg et al. 2004) and could be neglected here.

Humoral adaptive immunity

Humoral adaptive immunity was measured through the concentration (mg/mL) of gamma-globulins (or immunoglobulins), which represent the majority of circulating antibodies. Gamma-globulins were measured according to the protocol described above.

Parasitic traits

We counted the number of faecal propagules of 4 parasites commonly occurring in roe deer (as described in Cheynel et al. 2017), since it is a reliable estimator of the abundance of parasite in roe deer in those populations during the capture period. Investigated parasites encompassed lung nematodes (protostrongylids), gastro-intestinal (GI) nematodes (GI strongyles and *Trichuris* sp.) and GI protozoan (coccidia, *Eimeria* sp.). For GI nematodes and coccidia, we obtained the counts of eggs per gram (EPG) and oocysts per gram (OPG) of faeces, respectively, using a modified McMaster protocol (Raynaud 1970) with a solution of zinc sulfate ($ZnSO_4$, s.g. = 1.36) and by counting in the whole chambers of the McMaster slide (*i.e.* quantitative examination with a theoretical sensitivity of 15 EPG/OPG). We also centrifuged a 14 mL tube with the remaining solution, covered with a coverslip, before to seek for the presence of parasite propagules not detected on the McMaster slide (*i.e.* control slide, qualitative examination). For pulmonary nematodes, we obtained the count of stage 1 larvae per gram of faeces (LPG) using the Baermann faecal technique (Baermann 1917).

Statistical analyses

All analyses were performed using R version 4.3.0 (R Core Team 2023). For the 12 immune and 4 parasitic traits described above, we pooled individuals aged 10 and older owing to small sample sizes (Table S1). Actually, immunosenescence has been shown to begin earlier than 10 years old for all these traits (Cheynel et al. 2017).

Immunosenescence trajectories

Immunity and parasite count trajectories

For all 12 immune and 4 parasitic traits, we tested different ageing trajectories using linear mixed models (LMMs): a linear effect of age, a quadratic effect and a factor model (including 9 age classes from 2 years old to 10 years old onwards). We also tested three threshold trajectories differing by whether pre- and post-threshold trajectories were set as constant or not: the first model described a constant pre-threshold trajectory and a free post-threshold slope, the second described a free pre-threshold slope and a constant post-threshold trajectory, and the third described free pre- and post-threshold slopes. Threshold models were implemented following the approaches previously described in Briga et al. (2019) and Douhard et al. (2017). Briefly, thresholds varied between 3 and 9 years old with one-year increments and we identified the best-fitting threshold as described in the *Model selection* subsection below.

Immunosenescence and parasite count trajectory models included each immune and parasitic trait as the response variable. Parasite counts were log-transformed as $\ln(n+1)$ to reach a normal distribution. For HL scores, we used the residuals of the regression of the score on the serum coloration as a response variable, to correct for the ability of the complement to cause hemolysis according to the level of hemolysis of the serum.

We expected immunosenescence to differ according to population (Cheynel et al. 2017) and sex (Brooks and Garratt 2017). Early-life environmental conditions may also influence sex-specific immunosenescence, since cohort-specific environmental quality leads to the selection of different adult phenotypes between males and females (Douhard et al. 2013, Garratt et al. 2015) and to different adult female survival responses (Douhard et al. 2014) in the two populations. Models included the additive fixed effects of age (linear, quadratic, full-age or threshold), population, sex and cohort quality, and the two-way interactions between age terms

and the three other variables. Models also included four possible confounding variables as fixed effects: 1) the body mass (standardised for the Julian date of capture for juveniles, Douhard et al. 2017) as body condition affects immune phenotype (Gilot-Fromont et al. 2012) and since parasitism is negatively linked to body mass in roe deer (Body et al. 2011); 2) the age at last observation/capture to account for selective disappearance (van de Pol and Verhulst 2006, van de Pol and Wright 2009); 3) the Julian date of capture to account for potential seasonal variations in immune parameters and parasite exposure; and 4) the time delay between capture and blood sampling, for immune, but not parasite traits, to account for changes in immune parameters in response to hormonal changes triggered by the stress of capture. We refer to these four additional fixed effects as ‘confounding effects’ hereafter. Finally, all models included random effects of the individual identity (‘ID’) to account for repeated measurements on the same individuals, as well as the year of capture nested within the population, to account for year-to-year variations in demographic and environmental conditions in both populations. We did not include random slopes of age terms as they resulted in perfect correlations between the random intercepts and slopes (Harrison et al. 2018).

Model selection for senescence trajectories

To select the most relevant threshold for threshold models, we performed model selection based on the second-order Akaike Information Criterion corrected for small sample size (AICc) as implemented in the package ‘MuMIn’ (Bartoń 2021). For each threshold model, we retained the threshold resulting in the lowest AICc. Note that for all threshold trajectories, we added one parameter (and thus 2 to the AICc of the models) to account for the extra-parameter that is the threshold but which is not explicitly included in the models. After having identified the best threshold for each threshold model, we performed model selection on the linear, quadratic, full-age and retained threshold models. Briefly, among the set of models within $2 \Delta AICc$ for each age trajectory, we considered the simplest model (*i.e.* with the lowest

number of parameters) as the supported model to satisfy parcimony rules (Burnham and Anderson 2002). We then compared the retained models for each age trajectory and retained the trajectory for which the AICc score was the lowest and/or the simplest model within 2 Δ AICc.

Early-life FGMs and immunosenescence

We then tested whether early-life FGMs levels modulated the retained immunosenescence patterns. Because having individuals with both FGMs measured during their first year of life and adult immune/parasite traits measured as adults substantially decreased sample sizes (Table S2), we kept the structure of the previously found immunosenescence trajectories and added the additive effect of FGMs and the interaction between FGMs and the different age terms of the models. If constant ageing trajectories were found, we only added the additive effect of early-life FGMs on the immune/parasite traits, in addition to the other covariables that had already been selected. The random effect structure was also kept as described above, except for alpha1- and alpha2-globulin for which the inclusion of ID created singularities. Therefore, when testing the effect of early-life FGMs on the trajectories of these two traits, we included only one observation per individual, keeping the observation for which the age at measurement was the closest to the mean age of all individuals that were sampled only once.

Once the trajectory identified for all 12 immune and 4 parasitic traits, we used the same model selection approach to retain or not the additive and interacting effects of early-life FGMs with the age terms.

Results

Immunity

For innate traits, neutrophil levels were higher in the population of CH than in TF but similarly increased with age in both populations, without sex or cohort differences (Table 1, Figure 1a). Monocytes decreased until 5 years old and stabilised afterwards until the end of life (Table 1, Figure 1b). Basophils, eosinophils, and HA and HL scores remained constant throughout life, although eosinophil levels were higher in females than males, without population or cohort differences (Table 1, Figure 1c-f). For the inflammatory response (*i.e.* alpha1-, alpha2-, beta-globulins and haptoglobins), all traits showed an overall increase in their concentrations until the end of life (Table 1, Figure 1g-j), except in males for alpha1-globulins that remained constant throughout life, and for haptoglobins that increased markedly from 2 years old to 4 years old before slightly declining until the end of life (Table 1, Figure 1g and j). Note that for both alpha1-globulins and haptoglobins, female inflammatory marker concentrations were lower than those of males throughout life and reached male levels in late life. Alpha2-globulins decreased from 2 years old up to around 7 years old, after what they slightly increased until late life in a similar fashion for both populations and sexes, and with a higher level in females than males (Table 1, Figure 1h). Beta-globulin concentrations increased linearly with age and similarly in both sexes, with higher levels in males than females, and without population or cohort differences (Table 1, Figure 1i). Models for adaptive immunity showed that lymphocyte concentrations decreased in the first part of life (until *ca.* 6 years old) in both populations, but that lymphocyte counts were constantly higher in the good quality population of TF compared to CH (Table 1, Figure 1.k). Gamma-globulin concentrations increased throughout life similarly in both populations, with higher concentrations in CH than in TF (Table 1, Figure 1l). Our results did not suggest any sign of selective disappearance (Table 1). Most of immune trait values (*i.e.* neutrophils, basophils, eosinophils, HL, alpha1-globulins,

lymphocytes) were impacted by the delay between capture and sampling, showing an overall increase in trait levels with time, whereas body mass was negatively associated to inflammatory innate markers mostly, suggesting that individuals of poorest condition had the strongest inflammatory response (Table 1). Only alpha1-globulins showed seasonal variation throughout the capture season, with a trend to decrease across the season (Table 1).

For none of the 12 immune traits we could evidence an effect of early-life FGMs on immunosenescence trajectories (Table S3). Models including either the additive or interacting effect of FGMs with age displayed $\Delta\text{AICc} > 2$ compared to the retained immunosenescence trajectories (*i.e.* neutrophils, basophils, eosinophils, alpha1-, alpha2-, beta-, gamma-globulins and haptoglobins, Table S3) or, when they were within 2 ΔAICc , the simplest model, without FGM effects, was systematically within 2 ΔAICc (*i.e.* monocytes, HA, HL, lymphocytes, Table S3).

Parasitism

Our results evidenced that the abundance of GI strongyles increased with age in both sexes and both populations. However, abundance was always higher in males than females (Table 2, Figure 2.a). Abundance of *Trichuris* sp. increased more strongly with age in males than in females and was always higher in males compared to females, and in CH compared to TF (Table 2, Figure 2.c). Protostrongylids abundance increased linearly from 2 to 9 years old at a similar rate for both sexes and populations, with higher abundances in males than in females, and higher in CH than in TF. From 9 years old, protostrongylids abundance increased more strongly in males of both populations (Table 2, Figure 2.d). We found no change in abundance of coccidia in either population or sex (Table 2, Figure 2.b). We detected signs of selective disappearance only for GI strongyles (Table 2). All parasite trait values were impacted by body mass, showing that heavier individuals tended to be less parasitised (Table 2).

As for immune traits, we found no effect of early-life FGMs on GI nematodes and protozoan abundance trajectories (Table S3). Models including either the additive and interacting effect of FGMs with age showed $\Delta\text{AICc} > 2$ compared to the retained parasitism trajectories (GI strongyles, *Trichuris* sp., Table S3) or, when they were within 2 ΔAICc , the previously retained model was simpler and within 2 ΔAICc (Coccidia, Table S3). For protostrongylids, the dataset included only two individuals older than 9 years old (a male of TF aged 10 years old and a female of TF aged 11 years old). Individuals of CH were all aged 8 years old or younger. As the trajectory retained included a threshold at 9 years old, we tested only for the effect of early-life FGM on the slope of the linear relationship before the threshold. We retained the additive effect of early-life FGMs on adult protostrongylid abundance, suggesting that increased FGMs in early-life resulted in greater abundances of this lung parasite during adulthood ($\beta = 0.25$, standard-error = 0.10, R^2 marginal = 0.09, R^2 conditional = 0.41, Table S3, Figure 3).

Table 1. Selected linear mixed effect models for the 12 immune traits according to age (linear, quadratic, full-age, threshold), population (Chizé: CH, Trois-Fontaines: TF), sex (Females: F, Males: M), and cohort quality. Models accounted for the following potential confounding effects: body mass (Mass), age at last observation (Age-last), time delay between capture and sampling (Delay) and julian day of capture (Julian). All models included the random effects of the individual identity and the year of capture nested within the population. SE: standard error, $R^2m - R^2c$: marginal – conditional model variance, respectively.

Immune trait	Model	Fixed effects	Estimate	SE	t-value	R^2m	R^2c
INNATE IMMUNE SYSTEM							
Neutrophil (N = 1021 obs., n = 484 ind.)	Quadratic	Intercept	4.80	0.26	18.36	0.08	0.52
		Age ²	0.01	0.02.10 ⁻¹	3.59		
		Pop(CH)	-0.79	0.26	-3.06		
		Delay	0.04.10 ⁻¹	0.06.10 ⁻²	6.85		
Monocyte (N = 1021 obs., n = 484 ind.)	Threshold (5 yo)	Intercept	0.25	0.04	6.09	0.01	0.41
		Age.1	-0.02	0.07.10 ⁻²	-3.35		
Basophil (N = 1021 obs., n = 484 ind.)	Constant	Intercept	0.08	0.01	6.92	0.01	0.30
		Delay	0.08.10 ⁻³	0.03.10 ⁻³	-2.79		
Eosinophil (N = 1021 obs., n = 484 ind.)	Sex	Intercept	0.26	0.02	15.90	0.09	0.19
		Sex(M)	-0.04	0.01	-8.77		
		Delay	-0.04.10 ⁻²	0.05.10 ⁻³	-4.14		
Hemagglutination (N = 1201 obs., n = 556 ind.)	Constant	Intercept	3.90	0.15	26.81	-	0.38
Hemolysis (N = 1191 obs., n = 555 ind.)	Constant	Intercept	-0.16	0.18	-0.89	0.04.10 ⁻¹	0.49
		Delay	0.08.10 ⁻²	0.03.10 ⁻²	2.65		
INFLAMMATORY SYSTEM							
Alpha1-globulin (N = 1029 obs., n = 485 ind.)	Quadratic	Intercept	3.97	0.20	20.03	0.06	0.46
		Age ²	0.04.10 ⁻¹	0.07.10 ⁻²	5.07		
		Sex(M)	0.34	0.05	6.47		
		Age ² :sex(M)	-0.03.10 ⁻¹	0.01.10 ⁻¹	-2.52		
		Mass	-0.04	0.01	-5.08		
		Julian	-0.03.10 ⁻¹	0.01.10 ⁻¹	-3.17		
		Delay	0.03.10 ⁻²	0.02.10 ⁻²	1.78		
Alpha2-globulin (N = 1029 obs., n = 485 ind.)	Quadratic	Intercept	6.37	0.26	24.60	0.01	0.24
		Age	-0.22	0.09	-2.55		
		Age ²	0.02	0.01	2.44		
		Sex(M)	-0.28	0.10	-2.84		
Beta-globulin (N = 1029 obs., n = 485 ind.)	Linear	Intercept	7.82	0.55	14.21	0.11	0.40
		Age	0.26	0.02	11.34		
		Sex(M)	0.69	0.13	5.44		
		Mass	-0.11	0.03	-4.13		
Haptoglobin (N = 1034 obs., n = 483 ind.)	Threshold (4 yo)	Intercept	1.19	0.37	3.23	0.04	0.19
		Age.1	-0.01	0.07	-0.08		
		Age.2	0.09	0.03	3.51		
		Sex(M)	-0.48	0.33	-1.47		
		Age.1:sex(M)	0.30	0.10	2.92		
		Age.2:sex(M)	-0.13	0.04	-2.91		
		Mass	-0.04	0.02	-2.64		
ADAPTIVE IMMUNE SYSTEM							
Lymphocyte (N = 1021 obs., n = 484 ind.)	Quadratic	Intercept	3.34	0.20	16.66	0.14	0.48
		Age	-0.21	0.05	-4.08		
		Age ²	0.02	0.05.10 ⁻¹	4.13		
		Pop(CH)	-0.49	0.19	-2.57		
		Sex(M)	-0.18	0.07	-2.72		
		Age ² :pop(TF)	-0.06.10 ⁻¹	0.02.10 ⁻¹	-3.23		
		Delay	-0.01.10 ⁻¹	0.03.10 ⁻²	-3.90		
Gamma-globulin (N = 1029 obs., n = 485 ind.)	Quadratic	Intercept	18.01	1.49	12.08	0.23	0.64
		Age ²	0.04	0.04.10 ⁻¹	9.11		
		Pop(CH)	3.78	1.04	3.63		
		Mass	-0.16	0.06	-2.85		

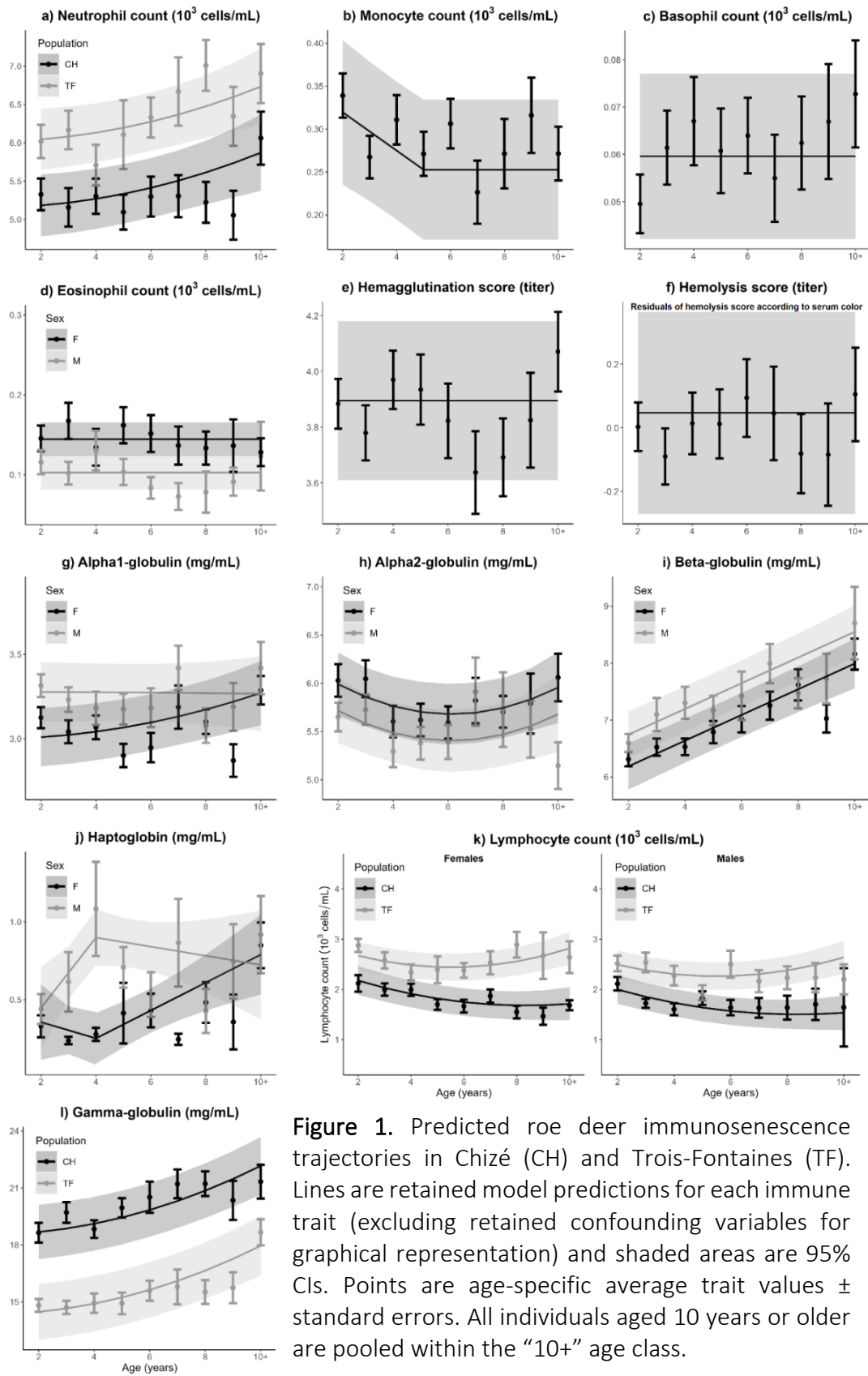


Figure 1. Predicted roe deer immunosenescence trajectories in Chizé (CH) and Trois-Fontaines (TF). Lines are retained model predictions for each immune trait (excluding retained confounding variables for graphical representation) and shaded areas are 95% CIs. Points are age-specific average trait values \pm standard errors. All individuals aged 10 years or older are pooled within the “10+” age class.

Table 2. Selected linear mixed effect models for the 4 parasitic traits according to age (linear, quadratic, full-age, threshold), population (Chizé: CH, Trois-Fontaines: TF), sex (Females: F, Males: M), and cohort quality. Models accounted for the following potential confounding effects: body mass (Mass), age at last observation (Age-last) and julian day of capture (Julian). All models included the random effects of the individual identity and the year of capture nested within the population. SE: standard error, R²m – R²c: marginal – conditional model variance, respectively.

Immune trait	Model	Fixed effects	Estimate	SE	t-value	R ² m	R ² c
Gastro-intestinal strongyles (N = 976 obs., n = 469 ind.)	Quadratic	Intercept	3.67	0.50	5.42	0.07	0.35
		Age ²	0.02	0.02.10 ⁻¹	6.23		
		Sex(M)	0.41	0.12	2.70		
		Mass	-0.07	0.02	3.49		
		Age-last	-0.13	0.03	-6.20		
Coccidia (N = 975 obs., n = 469 ind.)	Constant	Intercept	1.17	0.07	16.31	-	0.11
Trichuris sp. (N = 938 obs., n = 450 ind.)	Quadratic	Intercept	3.70	0.56	6.59	0.31	0.51
		Age	-0.28	0.11	-2.53		
		Age ²	0.03	0.01	3.84		
		Pop(CH)	1.01	0.26	3.86		
		Sex(M)	-0.01	0.24	-0.04		
		Age:sex(M)	0.16	0.04	3.84		
		Age ² :pop(CH)	-0.01	0.03.10 ⁻¹	2.07		
		Mass	-0.14	0.03	-5.36		
Protostrongylids (N = 918 obs., n = 450 ind.)	Threshold (9 yo)	Intercept	1.45	0.25	5.82	0.14	0.39
		Age.1	0.06	0.01	5.41		
		Age.2	0.16	0.10	1.55		
		Pop(CH)	-0.21	0.08	-2.69		
		Sex(M)	0.23	0.06	3.85		
		Age.2:sex(M)	0.83	0.18	4.87		
		Mass	-0.07	0.01	-6.24		

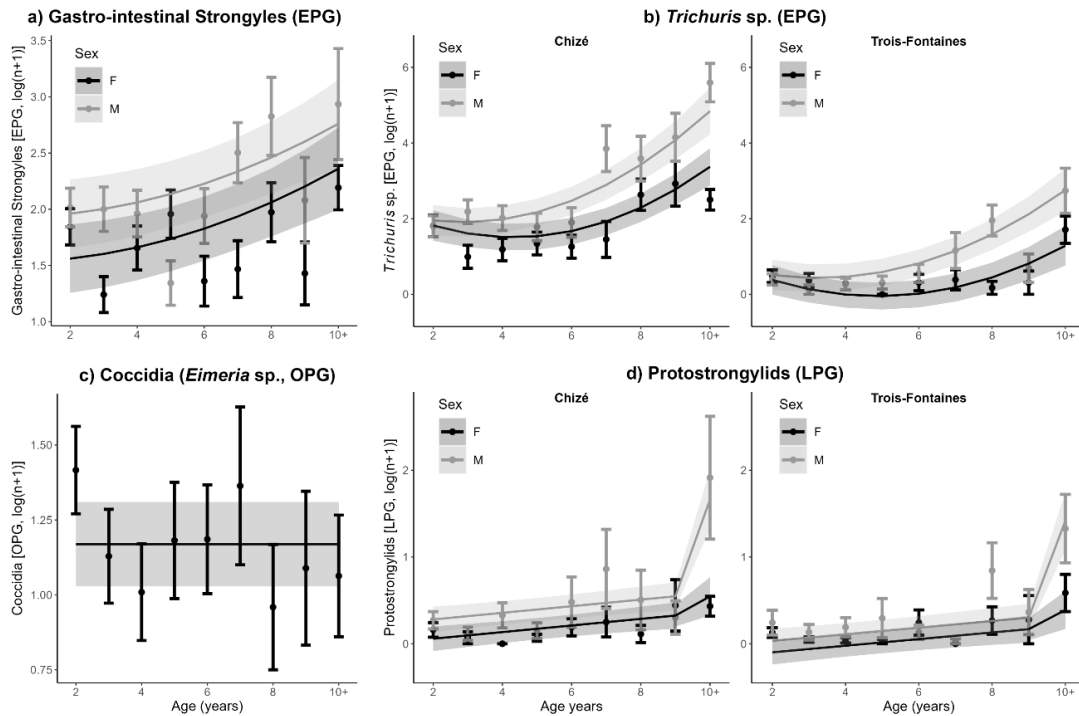


Figure 2. Predicted roe deer parasite abundance trajectories in Chizé (CH) and Trois-Fontaines (TF). Lines are retained model predictions for each parasitic trait (excluding retained confounding variables for graphical representation) and shaded areas are 95% CIs. EPG: eggs per gram, LPG: larvae per gram, OPG: oocysts per gram. Points are age-specific average trait values \pm standard errors. All individuals aged 10 years or older are pooled within the “10+” age class.

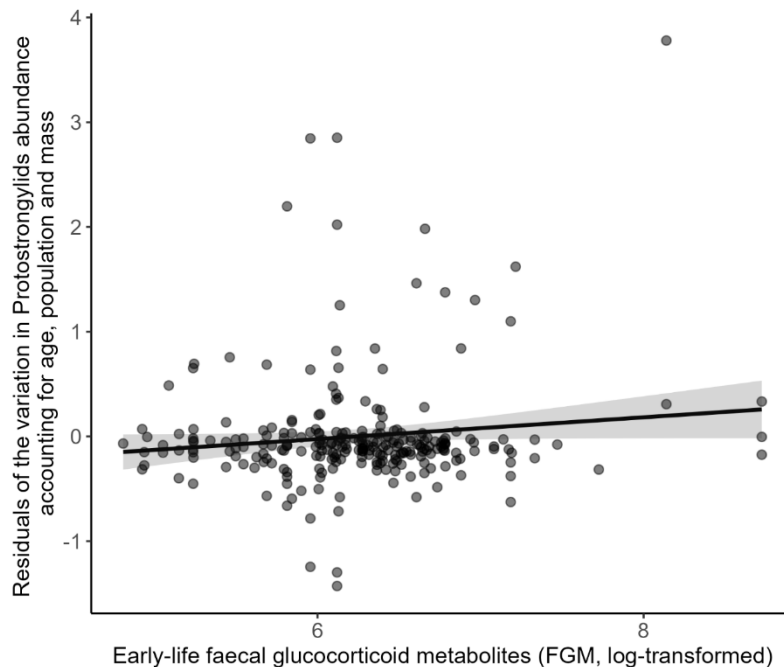


Figure 3. Predicted roe deer protostrongylids abundance accounting for age population and body mass (residuals of the retained parasite trajectory) according to faecal glucocorticoid metabolites (FGM, log-transformed) measured during the first year of life. Line is the model prediction and shaded area is 95% CIs. Due to limited sample size, only observations collected on individuals 9 years old or younger have been considered (see Results section).

Discussion

There is a gap in the literature on early-life stress exposure/chronically elevated GC levels and immunosenescence in wild mammals. Studies that tended to evidence negative relationships between GCs and immunosenescence focused mostly on humans (Bauer 2005, 2008, Garrido et al. 2022), and these studies did not explore the carry-over effects of early-life chronic stress in early-life on immunosenescence. Only few studies have assessed the effect of early-life stress exposure or GC levels on late-life fitness, evidencing negative relationships, but they were all conducted on birds, whose physiology likely differs from that of mammals (Spencer et al. 2009, Monaghan et al. 2011, Herborn et al. 2014, Monaghan and Hausmann 2015, Casagrande et al. 2020, but see also Spencer and Verhulst 2008). Here, we explored whether early-life GCs can modulate patterns of immunosenescence in free-ranging roe deer. Using a larger dataset including confounding factors, we found that, overall, our patterns of immunosenescence followed the same direction than those previously described by Cheynel et al. (2017) in roe deer, although we note some differences and novelties (*e.g.* sex effects, different trajectories). Most importantly, our results are opposed to our prediction that early-life FGMs modulate immunosenescence trajectories. We found no evidence for a long-term relationship between FGMs measured during the first year of life and immune trait variations with age. Nevertheless, early-life FGMs affected the abundance of pulmonary parasites (*i.e.* protostrongylids), measured at adulthood, but without modulating the trajectory of these parasites load over age.

This overall lack of effect of early-life FGMs on immunosenescence can be due to a certain number of limitations when dealing with GCs. First, although often referred to as ‘stress hormones’, variations in GC concentration do not reflect only stress (MacDougall-Shackleton et al. 2019). GCs have pleiotropic effects, and the stress response does not only involve the HPA axis and GCs, but also other hormones, cytokines and neurotransmitters (Sapolsky et al.

2000). Similarly, GCs are not the sole contributors to allostasis, and estimating allostatic load should ideally be done using several markers to summarise the level of physiological activity (including markers of neuroendocrine, immune, metabolic, and cardiovascular functions, Seeman et al. 1997, 2001, Romero et al. 2009, Juster et al. 2010). Second, it is admitted that acute/short-term GCs elevations can promote immune functions, whereas high baseline GC levels or chronic GCs elevation often result in immunosuppression (Martin 2009, Dhabhar 2014). However, in some instances, chronic/long-term GC elevation has also been described to decrease the responsiveness of macrophages to GCs, resulting in an up-regulation of immune activity (Martin et al. 2005, Martin 2009). Such a pattern may be expected for individuals living at risks of disease or infection threats to ensure competent immune system at all times (Martin et al. 2005). Finally, the consequences of GCs on certain traits, such as immune traits, could be restricted to immediate or relatively short-coming consequences (*i.e.* days-weeks), but could be lifelong on others, such as longevity or telomere dynamics (*e.g.* Monaghan and Hausmann 2015). Recently, a study on the same two populations of roe deer has shown that baseline GC levels negatively covaried with individual body mass, but GCs measured during the first year of life showed no delayed relationship with adult body mass (Lalande et al. 2023). Similarly, baseline GC levels have been shown to covary with immunity over time in this species (Carbillet et al. 2022), but we were not able to show carry-over effects of GCs on immunity and immunosenescence.

Despite the lack of impact of early-life FGMs on the immune traits investigated, early-life FGMs were positively related to abundance of lung parasites during adulthood. Indeed, once we accounted for age, sex, population and body mass, we found evidence for a positive relationship between early-life FGMs and abundance of lung parasites. More stressed individuals during their first year of life exhibited higher counts of protostrongylids during adulthood than comparable individuals. Protostrongylids are weakly pathogenic parasites,

except when individuals are heavily infected, and they have a long period of patency (> 2 years) and do not enable the final host to develop acquired immunity (Adem 2016). Thus, innate immunity might be the only line of defence against such lung parasites. As individuals during early-life are expected to rely more heavily on innate immunity, rather than adaptive immunity (McDade et al. 2016), and because adaptive immunity does not seem to defend the host against protostrongylids, high FGM levels during early-life could disrupt early innate immunity and favour infection by protostrongylids, resulting in individuals more infected during adulthood.

Our results showed that selective disappearance occurred for GI strongyles, but not for other parasite traits. Thus, individuals heavily parasitised by GI strongyles are not represented in our dataset and this can mask a potential effect of early-life GCs on adults for this parasite trait. Potentially, the selective pressure of parasites for individuals to maintain an efficient immunity, regardless of GC levels, is higher for GI parasites than for lung parasites, *Trichuris* sp. or coccidia. Indeed, GI strongyle species are diverse in both populations, and certain of these species are highly pathogenic (*e.g.* *Bunostomum trigonocephalum*, *Haemonchus contortus*, (Beaumelle et al. 2021). Interestingly these two pathogenic GI nematodes were found only in TF. Although out of the scope of the present study, the link between parasitism and survival would deserve more attention in future research on those populations.

By considering a larger dataset and more confounding factors, we expected to refine the immunosenescence trajectories previously found in roe deer by Cheynel et al. (2017). Overall, our patterns of immunosenescence follow the same direction than those previously described, although some traits exhibit slightly different shapes (*i.e.* betaglobulins, gammaglobulins, GI strongyles, *Trichuris* sp. and protostrongylids). However, some differences and novelties deserve to be noted. First, some traits that previously showed no changes with age now showed evidence of senescence (*i.e.* monocytes, alpha2-globulins). Second, contrary to what was previously found, population did not appear to be the main modulator of immunosenescence

patterns. Cheynel et al. (2017) showed population-specific trajectories for 6 traits. Here, only lymphocyte counts and *Trichuris* sp. abundances showed limited differences between-population trajectories. Methodological differences may explain that simpler models were selected in our study. In particular, individuals aged 10 years old onward were pooled here to avoid the common issue of having few old individuals and as immunosenescence has been shown to start no later than 10 years old for all traits studied (Cheynel et al. 2017). Third, we found no effect of natal environmental conditions on the value of immune traits during adulthood, or on immunosenescence trajectories. Environmental conditions at the time of birth likely affect resource availability and quality, which can interfere with life-history trajectories (Nussey et al. 2007, Cooper and Kruuk 2018), and notably the immune system (Lochmiller and Deerenberg 2000, McDade 2005, Rauw 2012, McDade et al. 2016).

Our results evidence that sex also appeared to modulate immunosenescence trajectories, a result that was not documented in those populations previously. In females, alpha1-globulins and haptoglobins displayed lower concentrations at young age but increased later in life until reaching male values, although this trend is subtle due to large between-age variations. The previously documented sex-specific trajectory for neutrophils disappeared in the current study, while it remained similar for *Trichuris* sp. and protostrongylids. Our results thus extend the work of Cheynel et al. (2017) and suggest that population and sex could actually drive patterns of immunosenescence, or at least immune trait values, in this long-lived mammal. The existence of sex differences in immunocompetence have for long been accepted. According to the immunocompetence handicap hypothesis, males should display a weaker immunity due to allocation trade-offs between immunity and reproduction (Zuk 1990) and the negative effects of male sex hormones on immunity (Foo et al. 2017). However, empirical evidences for a stronger female immunocompetence are mixed, and a recent meta-analysis actually showed no significant bias towards females (Kelly et al. 2018). Similarly, although sex-specific patterns

of senescence are expected from sex-specific life-histories (Brooks and Garratt 2017), a meta-analysis showed no bias towards an earlier or accelerated immunosenescence in males (Peters et al. 2019) compared to females. In line with this, we showed that, throughout life, immune trait values were higher (*i.e.* alpha1-globulins, beta-globulins, haptoglobins) or lower (*i.e.* eosinophils, alpha2-globulins, lymphocytes) in males compared to females, or similar between sexes (*i.e.* neutrophils, monocytes, basophils, HA and HL scores, gamma-globulins and coccidia), in this weakly sexually dimorphic species (Andersen et al. 1998). However, except for coccidia, parasite load was constantly greater in males than in females, and even increased more rapidly with age for males than for females (*i.e.* *Trichuris* sp. and protostrongylids). This is unlikely due to sex-specific behaviours that could result in different parasite exposure between males and females, as both sexes do not segregate spatially (Bonenfant et al. 2007). This rather suggests that although immune trait values do not strongly differ between sexes, resistance to parasite could actually be stronger in females than in males, as it has previously been suggested (Poulin 1996, Zuk and McKean 1996). Here, parasite load arguably reflects the consequences of immunosenescence, suggesting that immune functioning declines with age in the two roe deer populations, and more rapidly in males than females.

Concerning adaptive immunity, our results are partly in accordance with the view that adaptive immunity declines with age (Peters et al. 2019). Lymphocyte counts decreased with advancing age in CH, a decline that could be expected due to the diminished capacity to generate naïve B and T lymphocytes (Hakim and Gress 2007). In the good-quality population of TF, lymphocyte counts remained overall constant, suggesting that the expected decline in lymphocyte counts can actually be dampened by favourable environmental conditions (McDade et al. 2016). In sharp contrast, gamma-globulin concentrations increased with age in both populations. Immunosenescence has been found to be accompanied by a decrease in the production of high-affinity antibodies (Frasca et al. 2011), but an increase in the production of

autoantibodies which can be in some cases associated to autoimmune diseases (Hakim and Gress 2007, Kovaïou et al. 2007, Lleo et al. 2010, Bachi et al. 2013). This suggests a dysregulation of the adaptive immunity, rather than a mere decline in adaptive immunity cell counts with age. In addition, lymphocyte counts are lower in the poor-quality population of CH than TF, whereas it is the opposite for gamma-globulins. Thus, B lymphocytes from individuals of CH produce more antibodies relative to their number compared to TF, potentially resulting in a stronger auto-immunity in CH than in TF. Here, however, we do not have sufficient information about the lymphocyte pool composition and we do not know about relative proportions of NK cells and B and T lymphocytes or whether produced antibodies are efficient, directed against certain parasites or produce an autoimmune response. Still, altogether, it suggests that the adaptive immunity functioning could actually be altered in both populations, and this could be in line with a decline in global adaptive immunity (Peters et al. 2019).

Finally, immunosenescence trajectories for innate and inflammatory traits are consistent with the trajectories described in the literature on wild-animals, with innate traits showing no particular ageing directions, and inflammatory markers increasing with advancing age (Peters et al. 2019). Also, neutrophil counts were higher in the good-quality population of TF than in CH, suggesting a poorer phagocytic capacity in the latter population, and potentially a poorer immunocompetence, and supporting the fact that resources quality and availability can impact some immune traits (McDade et al. 2016). Similarly, parasite load was greater in CH than TF at all ages for *Trichuris* sp. and protostrongylids, but not for coccidia and GI strongyles (showing no population-specific differences), a pattern recently described in those two populations (Bariod et al. 2023). Whether it is due to different pathogen exposure between populations or it is a consequence of a poorer immunocompetence in CH compared to TF remains to be assessed.

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DISCUSSION



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1. Summary of the results and outline

The stress response allows organisms to cope with unpredictable environmental challenges. It includes a suite of behavioural and physiological processes, mediated in part by GCs, that permit an individual to escape threatening situations or to support biological processes associated with unexpected energy requirements (*i.e.* stressors). If stressors are short-lasting and temporary, the stress response provides an evolutionary advantage promoting survival. However, long-lasting and/or recurrent stressors can put the organism in a state of chronic stress and allostatic overload, during which resources are deviated away from non-essential functions in favour of those aiming at coping with the stressor, through the actions of GCs. Thus, states of chronic stress are likely to result in adverse consequences for an individual performance. However, evidences of this so-called Cort-Fitness hypothesis are lacking, and require longitudinal data to account for environmental and individual factors that are likely to modulate this expected negative relationship. In addition, information concerning the detrimental consequences of elevated baseline GCs on a variety of traits, and the timing of such effects are still missing.

The aim of this thesis was to fill in this gap by evaluating the relationships between baseline GC levels and various traits measured through a longitudinal survey in two populations of roe deer with contrasted environmental conditions. I investigated both the immediate and carry-over consequences of varying baseline GCs on different physiological parameters that reflect an individual health status, and on their senescence patterns, while accounting for individual characteristics (*i.e.* age, sex, life-history stage, body mass) and environmental conditions (*i.e.* current or early-life). By using longitudinal data on two populations of a free-ranging mammal, allowing to account for the influence of individual and environmental conditions on the relationships between baseline GC levels and fitness-related physiological traits and their senescence, I aimed at contributing in bridging ecology, demography and

physiology, by proposing potential mechanisms linking the environment to individual performances.

The results of the different studies conducted as part of my thesis are summed up in [Table 1](#), and evidenced that elevated baseline GC levels mainly impacted health parameters on short time scales (*i.e.* body mass, [Chapter 2A](#); albumin and creatinine, [Chapter 3](#)). This relationship can depend on life-history stage (*i.e.* juveniles *v.* adults, [Chapter 2A](#)) or sex and/or condition (*i.e.* albumin, [Chapter 3](#)). I found no evidences for carry-over effects of GCs on either age-, sex-, population- or cohort-specific body mass ([Chapter 2A](#)), physiological parameters ([Chapter 3](#)) or immune markers, except for a lung parasite with low pathogenicity ([Chapter 4](#)). On the other hand, N:L ratios might be negatively associated to body mass on longer time scales than GCs are ([Chapter 2B](#)). In the following sections, I propose to incorporate these results within the development of perspectives that our laboratory and others could benefit from.

In the following section, I will put emphasis on the relevance of the longitudinal data used here into contributing importantly to the field of ecophysiology and I will advocate once again towards the use of such longitudinal data. Then I will discuss two perspectives that, accompanied with longitudinal data, can greatly contribute to ecophysiology.

First, I develop the idea that future research should estimate states of chronic stress based on a set of biomarkers rather than being restricted to GCs. I discuss some drawbacks of using (only) GCs, and in particular FGMs, to estimate the stress response and advise to fully recognise and account for the potential biases associated with these proxies. Then, I explore the benefits of developing species-specific allostatic load indices using several biomarkers (Edes et al. 2018).

Table 1. Summary of the results obtained during this Ph.D.

Investigated traits		Short-term (same capture event)	Medium-term (between two consecutive years)	Long-term (early-late)
Chapter 2 – Glucocorticoids and N:L ratios in relation to body mass				
Body mass	GCs	<ul style="list-style-type: none"> ➤ Negative relationship in adults ➤ No relationship in juvenile 	<ul style="list-style-type: none"> ➤ No relationship whatever the life-history stage, sex, population or cohort quality 	<ul style="list-style-type: none"> ➤ No relationship
	N:L ratios	<ul style="list-style-type: none"> ➤ No relationship 	<ul style="list-style-type: none"> ➤ Negative relationship between the mean N:L ratios between two consecutive years and body mass gain during these two years during late growth (<i>i.e.</i> between 2 and 4 years old) 	<ul style="list-style-type: none"> ➤ No relationship
Chapter 3 – Glucocorticoids and the senescence of physiological condition				
Physiological parameters		<ul style="list-style-type: none"> ➤ Positive relationship between GCs and creatinine ➤ Sex-specific positive relationship between GCs and albumin <p><i>This relationship could also involve an effect of body condition</i></p>	-	<ul style="list-style-type: none"> ➤ No relationship
Chapter 4 – Early-life glucocorticoids and immunosenescence				
Immunological parameters		-	-	<ul style="list-style-type: none"> ➤ Positive relationship between early-life GCs and the abundance of weakly pathogenic lung parasites (<i>i.e.</i> protostrongylids) during adulthood

Second, I argue that a better understanding of the mechanisms linking GCs and physiological traits can provide valuable insights to better predict associations between GCs and performances. To do that, I focus here on physiological mechanisms usually associated to senescence and decreased survival (*i.e.* telomere, oxidative stress, metabolic rates, immunity).

Finally, I will briefly discuss some points that deserve to be addressed if one hopes to evaluate and identify relationships between GCs and life-history traits or even fitness in natural contexts. Namely, I emphasise the need to put GC-fitness relationships within a resource framework, to rely on longitudinal studies and experimental manipulations, to be careful about the direct translocation of laboratory insights to natural contexts and to rely on metrics that directly measure fitness.

2. A consolidation of the physiology/life-history nexus

a) The evolutionary ecology relevance of the stress response

The present thesis explored the relationship between the stress response (estimated by GCs) and different fitness-related traits that reflect the overall health and performances of an individual. This work also evaluated whether this physiological response can have long-term consequences on senescence. In a context where ageing human populations are a societal problematic worldwide (Flatt and Partridge 2018), the study of senescence has gained growing interest over the last decades and we need to understand the mechanisms underlying the large diversity of patterns observed in humans and wild animals (Promislow et al. 2005, Lemaître et al. 2013, Jones et al. 2014, Rando and Wyss-Coray 2021). As such, this work integrates proximate physiological mechanisms and ultimate eco-evolutionary processes.

The physiology/life-history nexus (Ricklefs and Wikelski 2002) proposes a bridge between physiologists and ecologists who have for long separated their fields of research. Integrating physiology and ecology provides an opportunity to better understand the evolution of life-history traits by incorporating physiological constraints, and to better understand

physiological systems by adopting an evolutionary perspective. Indeed, selection shapes physiological mechanisms that will in turn allow the adjustment of individual performances relatively to their environmental constraints. Eventually, such adjustments may impact populations composition and natural selection acting on subsequent generations, which could in turn feedback on environmental conditions (Ricklefs and Wikelski 2002, Wingfield et al. 2008, [Figure 6](#)). Stress ecology is the field of research in which environmental stress is considered as an ‘ecological driving force and key player in evolution’ (Steinberg 2012). As such, the stress response, and other hormonal control mechanisms, are interesting candidates for evolutionary endocrinologists, aiming at identifying how hormones shape life-history traits and fitness, as they act as a first step toward the evolution of novel traits and adaptation to novel environments (Moczek et al. 2011, Perry et al. 2018, Lema 2020). The role of GCs in energy regulation is mediated by the regulation of thousands of genes, producing alterations of the epigenetic landscape (Mourtzi et al. 2021). Evaluating the potential of the stress response in participating to evolutionary processes in natural context could further consolidate the bridge between physiologists and evolutionary ecologists, and the present work provides some answers by showing that GCs relate to fitness-related traits on short time scales.

b) On the importance of longitudinal studies

Previous works and reviews highlighted the importance of longitudinal studies in order to correctly investigate relationships between GCs and life-history traits or fitness, as those relationships are likely to be life-history- and context-dependent, *i.e.* modulated by individual and environmental factors (Crespi et al. 2013, Dantzer et al. 2016). Indeed, age, life-history stages, sex, body condition, current and past environmental conditions can mask expected patterns linking GCs and investigated traits, and are thus essential to account for for a better predictive power (Crespi et al. 2013, Henderson et al. 2017). Similarly, we need information about a population stressor exposure history. Production of GCs can be reduced following

repeated or chronic exposure to stressors and can result in either null or positive relationships (Rich and Romero 2005, Newediuk and Bath 2023). Such downregulations of the GC response can either reflect an adaptive strategy to mitigate the pathogenic effects of heightened GC expression (Rich and Romero 2005) or reflect habituation to a stressor an individual has already been exposed to (Tennessen et al. 2018). Thus, having information about past stress exposure can help in identifying whether a negative or null/positive GC-fitness relationship should be expected. Finally, baseline GC levels repeatability is overall moderate in captive and wild vertebrates, although it is better for integrated GC measures (*i.e.* in feathers and faeces) or stress-induced GC levels (Taff et al. 2018). Consequently, according to when a given individual is sampled, GC-fitness relationships are likely to vary, but increased sampling frequency through intensive longitudinal monitoring improves repeatability of GC samples (Taff et al. 2018). Longitudinal studies thus allow to consider much of the above-mentioned sources of confounds that could explain a large part of the variation observed in GC-fitness relationships, and are a useful tool to rely on for ecophysiologicalists (Dantzer et al. 2016).

During my Ph.D., I had the opportunity to investigate the relationships between GCs and physiological fitness-related traits, using longitudinal data, in two populations of a long-lived mammal. This allowed to *i)* assess relationships between GCs and a large set of physiological fitness correlates, and *ii)* evaluate the influence of individual characteristics and environmental conditions on these relationships. Such an approach allows to assess the ecological and evolutionary relevance of proximate mechanisms that modulate individual performances relative to their environment, and provides an important contribution to the field of stress ecology. On the other hand, it is important to acknowledge that such longitudinal data, although virtuous, also have limitations resulting from logistical constraints. Namely, captures take place only during winter, so that what happens between two captures is unknown and seasonal variation in the production of GCs cannot be accounted for; captures and

manipulations impact the stress response and physiological systems and can bias observations; even though it is rare to have access to longitudinal data on one species inhabiting two habitat with different environmental conditions, the range of environmental variations is still limited to two populations; and observational studies such as the ones presented here can bring confusion about correlated environmental factors that cannot be disentangled.

3. Towards a more comprehensive estimation of chronic stress

GCs provide a relevant way of estimating responses to stressors because of their prevalence in triggering the stress response and their role in mediating energy regulation (Sapolsky et al. 2000). GCs are also relevant because they are an easily measurable trait (compared to catecholamines for example), hence allowing comparisons with most studies in this field, as they are, by far, the most largely used estimate of the stress response and allostatic load (McEwen and Wingfield 2003, Seeley et al. 2022). However, it is important to acknowledge the limitations raised by the use of GCs and integrated measures of GCs.

a) GCs do not equate to stress

As pedagogically put by MacDougall-Shackleton et al. (2019), ‘glucocorticoids and “stress” are not synonymous’. Emphasis should be put on the fact that the primary role of GCs is energy mobilisation and regulation (Sapolsky et al. 2000) rather than mediating the stress response or being the cause of stress. GCs have pleiotropic effects and these effects are mediated through receptors – mostly MR and GR, but also membrane receptors – that form homo- and hetero-dimers with other steroid hormone receptors to effectively regulate the transcription of thousands of genes (MacDougall-Shackleton et al. 2019). In addition, if GCs are indeed involved during the stress response, there are only one part of it. The stress response encompasses a large suite of physiological processes including the activation of the HPA axis (leading to the increased production of GCs) but also the SNS (leading to the production of catecholamines), the hypothalamic-pituitary-thyroid axis (producing thyroid hormones) or the

release of endorphins, together with behavioural adjustments (Reeder and Kramer 2005, Peter 2011). It is important to remember that measuring only GCs allows to draw conclusion about the effects of GCs, and more largely of the HPA axis, on an organism, but not to conclude about the effects of the overall stress response (MacDougall-Shackleton et al. 2019).

Moreover, all GC elevations do not relate to stress. Indeed, McEwen and Wingfield (2003) defined stress as the state of an individual facing events (*i.e.* stressors) that elicit behavioural and physiological responses (*i.e.* stress response) in addition of those imposed by its normal life cycle. Landys et al. (2006) described three physiological states defined as follow: A) a resting, undisturbed state during which physiological processes simply support the fundamental requirements of life; B) a heightened physiological state that supports increased demands in the face of predictable environmental and life-history events, as part of the normal life cycle of an individual; and C) a facultative physiological state that allows to cope with unpredictable, threatening events. Only the last one would reflect a stress response (McEwen and Wingfield 2003, Landys et al. 2006, Koolhaas et al. 2011). Predictability (*i.e.* the possibility to produce an anticipatory response to a stimulus) and controllability (*i.e.* the ability, or perceived ability, to have control over a stimulus) are key concepts that should be evaluated before considering an event as a stressor and before drawing conclusions about changes in a given biomarker as part as the stress response (Koolhaas et al. 2011). In the roe deer populations followed, it is impossible to know whether an event is (perceived as) stressful, and whether GC levels are increased in a predictable, ‘unstressed’ manner (*i.e.* physiological state B), or within a stress response context (*i.e.* physiological state C). Experimental manipulations (see [section 5.b](#) below) could help in identifying factors that are considered stressful from an animal perspective and reduce variability in the detection of GC-fitness relationships.

b) Estimating chronic stress in the wild

In eco-physiology, measuring chronic states in wild animals is often estimated through measures of baseline GC levels (Walker et al. 2005, Wikelski and Cooke 2006, Bonier et al. 2009a). Indeed, GCs are largely relevant in doing so, due to their central role in mediating energy mobilisation during and out of the normal life cycle (Sapolsky et al. 2000). They are also by far the most used proxy of the stress response (Seeley et al. 2022), making studies more easily comparable. However, although it is assumed that baseline GC levels should increase as a result from exposure to chronic stressors, evidence for such a relationship are controverted, showing a large variability in the way they respond to different chronic stress protocols, making baseline blood GC levels a limited predictor of chronic stress (Dickens and Romero 2013). Indeed, repeatedly or chronically stressed individuals can also display a reduced response to acute stress and lower baseline GC levels, potentially to avoid the detrimental consequences of chronically elevated GC levels (Rich and Romero 2005, Davis and Maney 2018), as it was shown to be the case in about 20% of the studies included in a recent meta-analysis (Newediuk and Bath 2023). Still, both long-term GC values below and above the normal GC baseline levels can be seen as detrimental, as it could either reflect an individual with insufficient baseline levels to support basic requirements and to cope with additional stressors in the former case, or an individual chronically exposed to stressors in the latter (Brown et al. 2005, Dickens and Romero 2013).

On the other hand, Dickens and Romero (2013) showed that integrated measures of GC levels, such as those measured in faeces (*i.e.* FGM), gave far more predictive value to estimate chronic stress than blood GC levels. Integrated measures of GCs capture the activity of the adrenocortical activity over extended periods of times, which duration depends on the species (Palme 2019). In roe deer, FGMs provide an integrated measure of circulating GC levels over an average of 12h (ranging from 8-23h, Dehnhard et al. 2001). Variations in these integrated

GC measures can confidently reflect variations in GCs production, but limits the capacity to distinguish which part of the HPA axis is altered during chronic stress. Indeed, such integrated measures include circulating GC level variations due to the normal life cycle, exposure to acute and chronic stressors and negative feedback mechanisms (Dickens and Romero 2013). An alternative option to estimate chronic stress would be through measuring decreases in negative feedback efficacy during acute stress (*i.e.* increased time between peak GC production and return to baseline levels), because negative feedback mechanisms are effectively dysregulated during states of chronic GC stress (Sapolsky et al. 2000, Romero 2004, Dickens and Romero 2013). Therefore, I highly recommend to use multiple aspects of the GC response to identify more confidently states of chronic stress in wild animal populations, including the two populations of roe deer investigated here, in the near future. These aspects could encompass measure of integrated GC levels, of negative feedback efficacy or the magnitude of the GC response to acute stress (see [section 3.e](#)) and [Table 2](#)).

c) Early-life GCs and later fitness

One of the main highlights of the studies conducted as part of my Ph.D. is that we found overall no support for relationships between early-life GCs levels and adult fitness-related, except for lung parasites. In a study on the European white stork (*Ciconia ciconia*), baseline GC levels in early-life did not relate to later survival or reproductive success. However, stress-induced GC levels measured during early-life did relate negatively to later survival and reproductive success (Blas et al. 2007). In other words, the magnitude of the stress response to a standardised stressor exposure during early-life (but not baseline GCs) was negatively related to survival and recruitment probability. This suggests that it is the way individuals respond to a stressor, rather than stressor exposure *per se*, which predicts future individual performances. On the other hand, chronic stress in wild rabbits was negatively related to short-term body condition and positively to short-term survival (Cabezas et al. 2007). Although some long-term

associations can be found between baseline or chronic GC levels, and performances (*e.g.* Brown et al. 2005) these links can be blurred by interindividual differences in exposure to stressors which ultimately affect baseline GC levels and stress response (Rich and Romero 2005). This suggests that baseline GC levels, as a measure of chronic stress in unmanipulated wild animal populations, are more likely to predict individual performances on short time scales, whereas the magnitude of the GC response, even more during early-life, can predict more accurately individual performances over longer time scales.

d) Methodological drawbacks of using FGMs

As we noted, FGMs, as an integrated measure of GC levels, provide a better predictive power to detect states of chronic stress, compared to mere baseline blood GC levels (Dickens and Romero 2013). However, the sources of confounds are numerous when measuring FGMs because of methodological limitations and because of individual and environmental characteristics that can make delicate FGMs comparisons between individuals or populations.

First, the storage and preservation of faecal matter after collection is an important step that sets the stage for all subsequent laboratory and statistical analyses (Palme 2019). Indeed, bacterial activity have been shown to metabolise steroids rapidly following excretion (within hours), and field studies face logistic difficulties that may prevent scientists and technicians to immediately freeze faecal samples after collection (Möstl et al. 1999, Hadinger et al. 2015, Carbillet et al. 2023b). Also, the impact of bacterial activity on FGM levels might depend on the species, so that it is important to have good understanding of the biology and ecology of the species under investigation (Hadinger et al. 2015). For example in baboons (*Papio cynocephalus*), FGMs remained stables for about 30 days after collection when kept in ethanol at room temperature, and increased significantly afterwards to peak at about 120 days following collection (Khan et al. 2002). In Arctic foxes (*Vulpes lagopus*), FGMs remained stables for 48 h at 20-25 °C (Larm et al. 2021), whereas in Asian elephants (*Elephas maximus*), FGMs

increased starting 8 h following faeces excretion, peaking 1 day after collection, and decreased from the second day onwards (Wong et al. 2016). In roe deer, FGM levels decreased by 25% every 24h following collection when stored at +4 °C compared to samples immediately frozen at -20 °C (Carbillet et al. 2023b). In all analyses presented in the current thesis, we accounted inter-annual changes in the FGM storage method by adding the year of capture in the random effect model structures, as samples were frozen at -20 °C within 24 h prior to 2013 and 2017 in CH and TF, respectively, and immediately stored at -80 °C in both populations since then.

Second, as previously shown in some species, metabolites might not be distributed homogeneously with faeces samples (*e.g.* Millspaugh and Washburn 2003). In addition, within-sample coefficients of variations appear to be species-dependent (Hadinger et al. 2015). As described in [Box 5](#), FGM extraction for a given individual was performed using one third of 5 different pellets of that individual in order to account for such within-sample variation.

Lastly, several individual and environmental factors can affect FGM levels. The annual period of sample collection can affect FGM as GCs levels are expected to vary seasonally according to environmental conditions to support heightened energy demands associated with lower resource availability and temperature (McEwen and Wingfield 2003, Landys et al. 2006). Accordingly, FGM levels have been shown to vary throughout the year in red deer (*Cervus elaphus*), with a peak during winter in December and January, and to increase with snow depth and as minimum ambient temperature lowered (Huber et al. 2003). In the present thesis, we systematically tested whether FGMs levels varied across the capture season, as captures take place from December to March, and corrected FGM levels accordingly. Individual characteristics also can impact an individual FGM level. FGMs can vary according to sex or reproductive status (Goymann 2012) – although no effect of sex or reproductive status could be found in red deer (Huber et al. 2003) or in reindeer, *Rangifer tarandus* (Bubenik et al. 1998). Diet and gut microbial community can also metabolise steroids differently during gut path

between populations or individuals (Goymann 2012, Hadinger et al. 2015). Here, in the two studied populations, females are (almost) all pregnant (~ 92% in CH, not estimated in TF), and due to delayed embryonic implantation, gestation is at a very early stage during the capture season (Aitken 1974) so the reproductive status of females was not accounted in the studies conducted in this thesis, while accounting for sex. Similarly, diet is largely similar during the capture season (Tixier and Duncan 1996) and unlikely to result in FGM differences between the two studied populations.

e) Measuring allostasis

If the precedent section argues in favour of measuring of several components of the GC production and regulation to detect more accurately chronic stress in wild animal populations, I also encourage measuring different aspects of the stress response, other than GC-related markers, to fully assess allostasis (Edes et al. 2018, Seeley et al. 2022). As previously mentioned, the stress response is complex and engages several behavioural and physiological systems (Reeder and Kramer 2005). In biomedical research, the construction of an allostatic load index from several markers is the norm (Seeman et al. 1997, 2001, Juster et al. 2010) whereas the study of stress in animal research mainly relies on one allostatic marker to estimate allostatic load, usually GCs (Seeley et al. 2022). Allostatic load describes the cumulative costs caused by physiological systems that are activated during states of chronic stress (McEwen and Wingfield 2003), and allostatic overload reflects the ‘wear and tear’ of physiological systems, with pathological consequences, because energy requirements to fuel these systems exceed energy acquirement (McEwen and Wingfield 2010). Thus, what ecophysiologicalists aim at measuring in the wild is allostatic load, as occurring during chronic stress. However, allostatic load cannot be directly measured but can be estimated through diverse biomarkers that deviate from baseline levels (Seeman et al. 1997, Seeley et al. 2022). In a recent meta-analysis, Seeley et al. (2022) noted 63 publications over 572 that explicitly estimated allostatic load, the

remaining ones only mentioning the concept of allostasis in introduction/discussion. About half of these 63 studies estimated allostatic load using only GCs, while 6 estimated it by computing an allostatic load index, as recommended in biomedical research.

The authors of the meta-analysis gave two examples illustrating the benefits of both using several biomarkers to evaluate allostatic load, and to use them in a comprehensive index. First, the allostatic load index developed in western lowland gorillas (*Gorilla gorilla gorilla*) reflected chronic stress while accounting for age, sex or rearing conditions, and became more powerful to predict morbidity and mortality risks as authors incorporated additional biomarkers in their index (Edes et al. 2016a, b, 2020). Second, whereas individually-evaluated biomarkers could not predict neuronal deficit in rats, gathering them into a comprehensive index gave a powerful predictor of neurological deficit (McCreary et al. 2019). In contrast, in the case of roe deer, I evidenced in [Chapter 2](#) that GCs and N:L ratios used separately in relation to body mass showed contrasted results. If both measures are arguably estimates of the stress response, they do not respond similarly to stressors and relate to body mass in different ways and timing (Davis et al. 2008, Davis and Maney 2018). Future work on these roe deer populations could involve evaluating the relevance of combining both indices as a starting point for the construction of an allostatic load index.

Despite the recommendations from human research that allostatic load indices should include biomarkers that reflect neuroendocrine, immune, cardiovascular, metabolic and behavioural processes (Seeman et al. 1997, Romero et al. 2009), achieving to collect such indices in free-ranging animals can be challenging. In particular, the aim of developing allostatic load indices is to evaluate states of chronic stress. However, in wild animals, it can be difficult to disentangle states of acute and chronic stress for all biomarkers investigated through blood samples. In addition, it is likely that the set of biomarkers recommended for humans (*e.g.* Seeman et al. 1997, see [Table 2](#)) cannot be generalised to all taxa and that allostatic load indices

should be taxa- or species-specific (Seeley et al. 2022). Future research would gain in trying to evaluate a set of relevant biomarkers that could accurately describe allostatic load. In the two roe deer populations investigated here, we already possess measurement of potential complementary biomarkers (*e.g.* albumin, leukocyte counts, creatinine, testosterone; [Table 2](#)) and evaluating their relevance to estimate chronic stress in those populations would provide valuable tools for future research in our laboratory.

4. Mechanisms linking GCs and physiological determinants of senescence

As part of the current thesis, I assessed the impact of GCs on the senescence of different physiological systems. A main result of this work was that early-life GCs were not predictive of accelerated or earlier physiological senescence. I discussed above some of the reasons that could in part explain such absence of relationship ([section 3.c](#) above), despite the mechanistic link between GCs and the different traits investigated (*i.e.* body mass, immunity, physiological parameters). I also briefly proposed future research directions to better estimate chronic stress, and that can have higher predictive power for linking individual performances to the environment. Aiming at pursuing investigating the link between chronic stress and senescence, I argue here for a mechanistic approach. This approach focuses on GCs as they are the most relevant biomarker of chronic stress available in the followed populations until now (but should ideally be performed using an allostatic load index). The aim here is to investigate the link between baseline GC levels and mechanisms proposed to be hallmarks of senescence processes (López-Otín et al. 2013), while incorporating more recent insights about the way GCs can influence senescence (Casagrande and Hau 2019). This work is essentially conceptual, and despite some early, preliminary results are presented, they would deserve more attention before to draw firm conclusions.

Table 2. Non-exhaustive list of biomarkers that can be used to establish an allostatic load index, and associated physiological systems. The ten biomarkers annotated with * are the ones initially recommended in biomedical research (Seeman et al. 1997, 2001). Bold-italic: biomarkers for which we already have data in Chizé and Trois-Fontaines roe deer populations. ACTH: adrenocorticotrophic hormone, AMP/ATP: adenosine mono/triphosphate, CRH: corticotropin-releasing hormone, DBP/SBP: diastolic/systolic blood pressure, DHEA-S: dehydroepiandrosterone-sulfate, GCs: glucocorticoids, HDL: high density lipoprotein, IL: interleukines, MDA: malodialdehyde, NLR: neutrophil:lymphocyte ratio, RBCs: red blood cells, ROMs: reactive oxygen metabolites, SOD: superoxide dismutase, TNF: tumor necrosis factors, TOR: target of rapamycin, WHR: waist:hip ratio.

Physiological system	Physiological biomarkers	References
Neuroendocrine	<i>GCs (plasma baseline/stress levels, integrated levels)*</i>	Seeman et al. 1997, Dickens and Romero 2013
	GCs negative feedback efficacy	Dickens and Romero 2013
	Magnitude of the GCs response to acute stressors	Dickens and Romero 2013
	Epinephrine*	Seeman et al. 1997
	Norepinephrine*	Seeman et al. 1997
	ACTH	Romero et al. 2009, Seeley et al. 2022
	CRH	Seeley et al. 2022
	Catecholamines	
	DHEA-S*	Seeman et al. 1997, Seeley et al. 2022
	<i>Testosterone</i>	Seeley et al. 2022
	Oestradiol	Seeley et al. 2022
	Growth hormones	Seeley et al. 2022
	<i>Thyroid hormones</i>	
Cardiovascular	SBP*	Seeman et al. 1997
	DBP*	Seeman et al. 1997
	Heart rate	Romero et al. 2009, Seeley et al. 2022
	Heart rate variability	Romero et al. 2009, Seeley et al. 2022
Metabolic	<i>Body mass</i>	Seeley et al. 2022
	WHR*	Seeman et al. 1997
	Serum HDL*	Seeman et al. 1997
	Total cholesterol*	Seeman et al. 1997
	Plasma glycosylated haemoglobin*	Seeman et al. 1997
	Glucose	Seeley et al. 2022
	<i>Total protein</i>	Seeley et al. 2022
	<i>Creatinine</i>	Seeley et al. 2022
	<i>Albumin</i>	Seeley et al. 2022
	Triglycerides	Seeley et al. 2022
	Free amino acids	Seeley et al. 2022
	<i>Oxidative stress – damages (MDA, protein carbonyl, ROMs)</i>	
	<i>Oxidative stress – defenses (glutathione, SOD activity)</i>	
	AMP/ATP	Casagrande and Hau 2019
	TOR	Casagrande and Hau 2019
	Mitochondrial activity	Casagrande and Hau 2019
<i>RBC</i>	Seeley et al. 2022	
<i>Haemoglobin</i>		
Immunity	Prostaglandin	Romero et al. 2009
	T-cell activation	Romero et al. 2009
	<i>Leukocyte counts</i>	Seeley et al. 2022
	<i>Antibody titers</i>	Romero et al. 2009
	<i>NLR</i>	Seeley et al. 2022
	Cytokines (IL, TNF)	Romero et al. 2009, Seeley et al. 2022
Behavioural	Foraging/feeding	Romero et al. 2009
	<i>Locomotion</i>	Romero et al. 2009, Seeley et al. 2022
	Migration	Romero et al. 2009
	Conspecific aggression	Romero et al. 2009

a) Chronic stress and senescence

Chronic stress is a source of large interest in human research, as it is considered to favour accelerated senescence and the development of diseases. Therefore, ‘successful’ ageing would depend on the ability to provide an efficient response to ‘the dynamic challenges of being alive’ (Juster et al. 2010). With this in mind, ecophysiologicalists now invest in the development of allostatic load indices to monitor animal health for conservation purposes (Edes et al. 2018, Seeley et al. 2022), as recommended for biomedical research (Seeman et al. 1997, 2001, Juster et al. 2010, Guidi et al. 2020). Providing a better understanding of the mechanisms linking markers of stress to senescence processes could help in identifying relevant biomarkers to achieve the construction of such indices.

The mechanisms linking GCs to accelerated senescence and decreased survival have been identified and include, among others, oxidative stress, increased telomere attrition (Epel et al. 2004, Costantini et al. 2011, Reichert and Stier 2017, Lee et al. 2021) and decreased immune functions (Promislow et al. 2005, Bauer 2008). Long-term GC elevation can cause allostatic overload which can result in the disruption of immune functions (Dhabhar 2014), as well as in increased metabolic rates and telomere attrition, leading eventually to accelerated cellular ageing (Casagrande and Hau 2019, Casagrande et al. 2020, Lee et al. 2021, Bobba-Alves et al. 2023). Indeed, GCs promote oxidative stress (Costantini et al. 2011, Casagrande and Hau 2019), leading to cellular damages, and thought to be linked to telomere length shortening (Epel et al. 2004). Telomeres are short DNA sequences (TTAGGG) at the end of chromosomes. Telomeres maintain the chromosomes integrity through the protection of the coding sequences of the DNA. Indeed, chromosomes progressively shorten due to the incomplete replication throughout cell divisions. Beyond a critical length threshold, telomeres are no longer functional and trigger cell senescence. Telomere shortening is a supposed mechanism of senescence (López-Otín et al. 2013), and the link between telomere shortening

and GCs has been studied in laboratory rodents, humans, or wild vertebrates (Hausmann and Heidinger 2015, Angelier et al. 2018, Casagrande et al. 2020, Lee et al. 2021) and most recently in roe deer (Lemaître et al. 2021). However, the exact mechanisms involved in the relationship between GCs and telomere shortening are not fully elucidated, and although they are thought to involve oxidative stress, correlative studies provided equivocal support for this hypothesis (Reichert and Stier 2017). Recent papers point to a possible link between high chronic GC levels and telomere attrition through reallocation of energy away from telomere maintenance, towards increased mitochondrial metabolic rate (Casagrande and Hau 2019). This would result in accelerated cellular aging (Casagrande et al. 2020, Bobba-Alves et al. 2023), thus providing a potential explanation for immune cells' senescence. Thus, senescence is likely to be modulated according to the level of allostatic load, as measured here by levels of GCs. GCs and associated allostatic overload may be the underlying physiological mechanisms explaining part of the observed diversity of senescence patterns.

b) A conceptual framework linking GCs and cellular senescence

Here, I propose a conceptual framework linking baseline GCs, oxidative stress, (mitochondrial) metabolism, telomere length and immunity ([Figure 14](#)), that would allow to disentangle *i*) the relative roles of oxidative stress and metabolism in mediating the effects of GCs on telomere dynamics, and *ii*) the relative roles of GCs, telomere shortening and oxidative stress in modulating immunity. GCs, and more largely chronic stress, are considered as pro-oxidants and should alter the balance between oxidative damages and defences (Costantini and Verhulst 2009, Costantini et al. 2011). In turn, the resulting state of oxidative stress is hypothesised to accelerate telomere length shortening. Oxidative stress is proposed to accelerate telomere loss because reactive oxygen species (ROS, *i.e.* highly reactive molecules generated in the mitochondria) produce DNA damages that are not repaired in this area of chromosomes (Hewitt et al. 2012, Monaghan and Ozanne 2018). Thus, telomere shortening eventually leads

to cell senescence, among which immune cells (Blackburn et al. 2015). The inflammatory response of the immune system also promotes an increased oxidative stress as inflammatory mediators trigger immune cells to produce and release ROS (Isaksson 2015). GCs have also been evidenced to directly accelerate telomere loss by inhibiting the ‘target of rapamycin’ (TOR) kinase (Casagrande and Hau 2019). TOR is a kinase involved in physiological trade-offs at the cellular level. It regulates cell differentiation, growth and proliferation, and favours energy allocation towards cellular maintenance over growth during the stress response (Wei et al. 2013). The inhibition of TOR downregulates proteins and enzymes that normally maintain telomere length, including telomerase (Zhou et al. 2003). Telomerase is an RNA-dependent DNA polymerase involved in the maintenance of telomere length, which shows a reduced activity when exposed to GCs (Choi et al. 2008). Increased telomere length is hypothesised to result in dysfunctional mitochondria that produces more ROS and fewer antioxidants (*i.e.* increased oxidative stress), which in turn accelerates telomere loss and cell senescence (Casagrande and Hau 2019, Casagrande et al. 2020). The role of dysregulated mitochondria could be to reduce allocations toward telomere length maintenance through mitochondria hypermetabolism, as part of a transition toward a state that prioritises immediate survival over longer-term benefits (Casagrande and Hau 2019). Indeed, GCs have been shown to accelerate metabolic rates, in particular mitochondrial metabolic rates both *in vitro* and *in vivo* (Du et al. 2009, Casagrande et al. 2020, Bobba-Alves et al. 2023). Finally, chronic GC levels directly impact immunity through their immunosuppressive effects, and can also promote an increase in pro-inflammatory cytokines, that will in turn favour the production of GCs and oxidative stress (Dhabhar 2014).

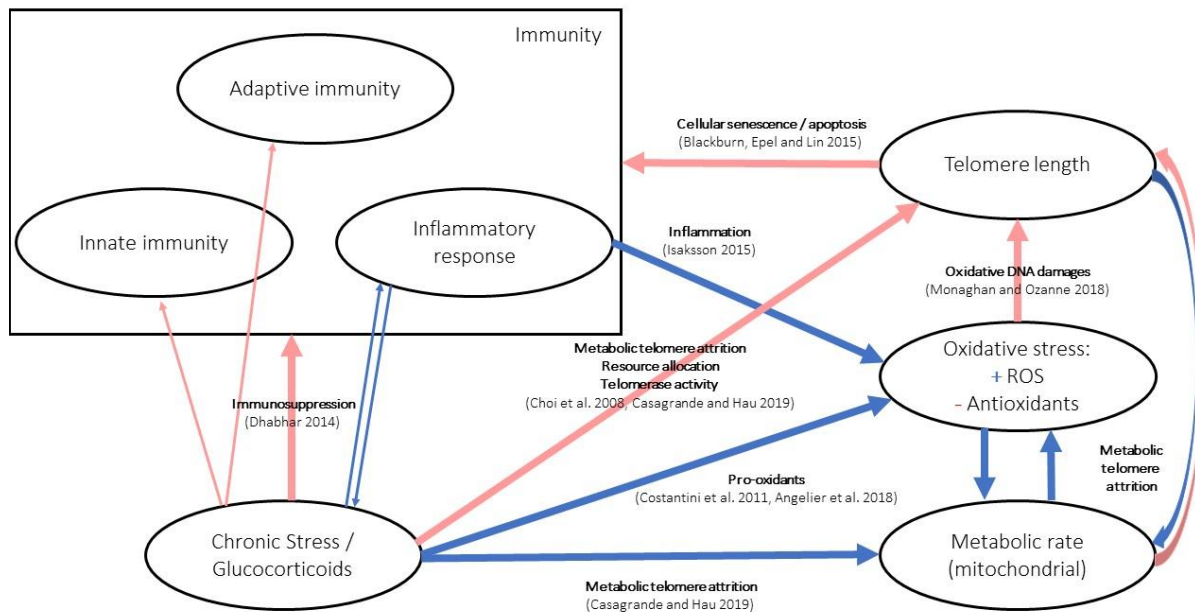


Figure 14. Conceptual mechanistic schema of the relationships between glucocorticoids (GCs) and senescence, summarising possible mechanistic links between GCs, oxidative stress, immunity, metabolism and telomere attrition. Pink arrows depict a theoretical negative relationship between two traits. Blue arrows depict a theoretical positive relationship between two traits. Oxidative stress should ideally be measured as the balance between pro- and anti-oxidants. ROS: reactive oxygen species. Thin arrows between GCs and immune components simply illustrate the detail of the large arrow between GCs and immunity. Texts in bold describe the main mechanism or concept that explains the expected relationship. Texts in brackets are references for the concepts mentioned and can be found in the main text.

c) Exploration of the link between GCs, oxidative stress and telomere length

i. Methods

To assess relationships between GCs, oxidative stress and telomere length I ran partial least square path modelling (PLS-PM) analyses (Sanchez 2013). PLS-PM is a multivariate analysis method that allows to assess relationships between multiple sets of variables. This analysis is particularly recommended when dealing with highly correlated variables, such as expected here. I built four blocks of variables (*i.e.* four latent variables): 1) GCs (one observed variable: FGMs as described in [Chapter 1](#)), 2) oxidative stress damages (3 observed variables: reactive oxygen metabolites content, protein carbonylation, malondialdehyde concentrations), 3) oxidative stress defences (2 observed variables: superoxide dismutase activity, antioxidant

capacity), and 4) telomere length (one observed variable: relative leukocyte telomere length obtained by real-time quantitative PCR; (Lemaître et al. 2021)). I expected observed variables (*i.e.* variables within latent variables) to reflect the corresponding latent variable (*i.e.* reflective model). The structural model was defined as follows: telomere length on GCs and oxidative stress damages and defences. Oxidative stress damages and defences depend on GCs. We then ran two PLS-PM analyses: one for juveniles (8-9 months old, 50 observations) and one for adults (second year of life onwards, 110 observations) to test for the effect of growth on expected relationships (Costantini et al. 2011, Monaghan and Ozanne 2018).

Preliminary analyses of this mechanistic scheme presented below are restricted to investigations of the link between GCs, oxidative stress and telomere length for several reasons. Firstly, data on metabolism are limited in those two roe deer populations, since we do not have direct biomarkers of mitochondrial activity but only data about thyroid hormones (*i.e.* T3 and T4), that are tightly linked to mitochondria metabolism (Harper and Seifert 2008). Secondly, such path modelling analyses require specific statistical skills that I could not have the time to fully develop yet, especially to account for repeated measurements of a given individual, stratified data or to implement recursive models (*i.e.* loop relationship between two blocks). Lastly, due to these limitations, simplifying path models allowed to test for differences between juveniles and adults, limiting the dataset to one observation per individuals, because there is evidence that GCs, oxidative stress and telomere length are linked differently in growing *versus* grown individuals (Costantini et al. 2011, Casagrande et al. 2020).

ii. Results

Preliminary analyses on 50 juvenile roe deer (8-9 months old) and 110 adults (2 years old and older) are summarised in [Figure 15](#). Analyses show that GCs are significantly and positively related to oxidative stress damages in both subsets (juveniles: 0.31 [0.04, 0.58], adults: 0.19 [0.01, 0.38]). Although it is not significant, there is a trend for a negative

relationship between GCs and oxidative defences (juveniles: -0.07 [-0.36, 0.21], adults: -0.06 [-0.25, 0.13]). Also, these results show that neither oxidative damages nor oxidative defences significantly relate to telomere shortening. Nevertheless, a trend for oxidative damages to negatively relate to telomere length (juveniles: -0.03 [-0.32, 0.26], adults: -0.07 [-0.26, 0.13]), and for oxidative defences to positively relate to telomere length (juveniles: 0.07 [-0.21, 0.35], adults: 0.06 [-0.14, 0.25]) can be observed. Finally, GCs negatively related to telomere length in juveniles (although not significantly: -0.25 [-0.55, 0.04]), but not in adults (0.02 [-0.17, 0.22]).

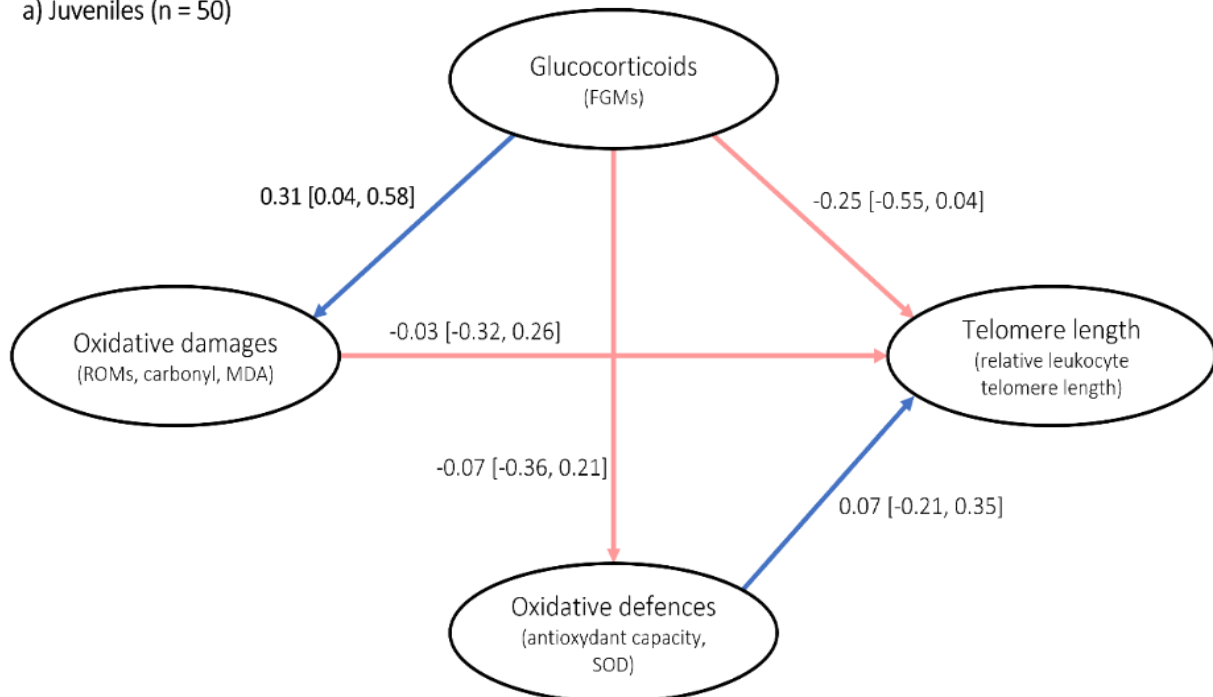
iii. Interpretation, conclusion and perspectives

These preliminary results support the idea that GCs favour oxidative stress by disrupting the balance between anti- and pro-oxidants (Costantini and Verhulst 2009, Costantini et al. 2011). These results also go in the direction of what previously found in the literature, namely that the pro-oxidant effects of GCs are stronger in growing individuals than in adults (Costantini et al. 2011), as seen with the trend for a stronger relationship in juveniles than adults. It is difficult to really conclude about this trend but it would deserve to be more profoundly investigated using a larger dataset. Analyses show that GC levels participate in the reduction of telomere length more in juveniles than in adults. In both cases, however, the relationship between oxidative stress damages and telomere attrition is extremely weak and not significant ([Figure 15](#)). This is consistent with previous findings showing equivocal support for the effects of oxidative stress on telomere shortening (Reichert and Stier 2017). These results rather suggests that GCs are related to telomere length independently of oxidative stress. This could support the ‘metabolic telomere attrition’ hypothesis (Casagrande and Hau 2019), which suggests that telomere attrition resulting from GC levels is part of a transition toward an emergency state promoting immediate survival over longer-term benefits. Telomere attrition would therefore be associated with an increased mitochondrial metabolic rate, rather than

oxidative stress, as it has been thought for a long time. Results are also consistent with the findings of Casagrande et al. (2020) that showed decreased telomere length in relation to mitochondrial metabolic activity mediated by early-life GC levels. Next steps will involve to include mitochondrial metabolic information to explicitly test for the metabolic telomere attrition hypothesis. Finally, the results presented suggest that accelerated telomere shortening is more likely to occur during the juvenile stage than during adulthood. This could support the idea that growth is costly for telomere length maintenance (Monaghan and Ozanne 2018, Casagrande et al. 2020) and it would deserve explicit tests of the relationships between telomere shortening and growth rates in the studied populations.

Correlational studies, such as the ones presented in this thesis, are required to investigate the relationships between GCs and animal health in natural contexts that are likely to differ from laboratory ones (Boonstra 2013), and to provide useful insights for conservation biologists. An approach focusing on the proximate mechanisms underlying observed correlations can have important predictive power. The conceptual mechanistic processes presented above ([Figure 14](#)) mainly come from laboratory model species, humans or birds. Here, the CMR program on these two free-ranging populations of roe deer provide a unique opportunity to further explore the mechanisms linking GCs and fitness prospects. I therefore highly recommend to further explore such links using relevant measures of mitochondrial and metabolic activity (*e.g.* production of adenosine-mono/triphosphate (AMP/ATP), TOR kinase, telomerase, number of mitochondria; Casagrande and Hau 2019). This approach will also permit to identify relevant biomarkers varying with GC levels, with the aim of developing an allostatic load index for roe deer (Edes et al. 2018, Seeley et al. 2022).

a) Juveniles (n = 50)



b) Adults (n = 110)

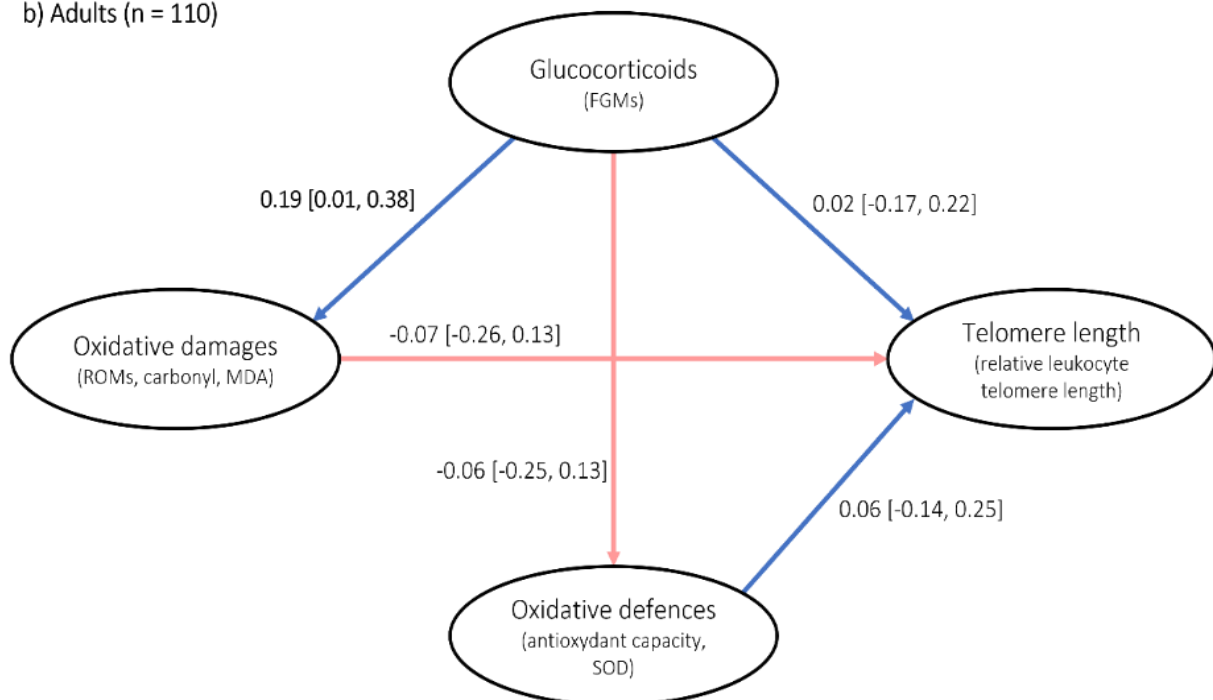


Figure 15. Path diagram showing the relationships between baseline glucocorticoid levels (expressed as FGMs: faecal glucocorticoid metabolites), oxidative damages (expressed as the content of ROMs: reactive oxygen metabolites, carbonyl: carbonyl groups and MDA: malondialdehyde), oxidative defences (expressed as the antioxidant capacity and SOD: superoxide dismutase activity), and telomere length (measured by real-time quantitative PCR as the relative leukocyte telomere length) in a) juveniles (8-9 months old, n = 50), and b) adults (2 years old and older, n = 110). Values are estimated direct effects with 95% confidence intervals given between brackets. Values in bold indicate a significant relationship ($p < 0.05$).

5. Recommendations to detect associations between chronic stress and fitness

A major conclusion of the literature in stress ecology is that there is no consensus about the strength and the direction of the relationship between chronic GCs and life-history traits or in natural contexts. Biomedical research has largely focused on chronic stress, because of its implications in stress-related diseases in humans. However, whether chronic stress, resulting in allostatic overloads, does occur in wild animals, and whether the biomedical view of chronic stress can be directly translated to natural contexts with ecological and evolutionary implications remains unclear (Sapolsky 1994, Romero 2004, Boonstra 2013, Beehner and Bergman 2017). While expectations for a relationship between GCs and fitness to be negative are dominating, empirical results show that it is often not the case, and numerous reviews and conceptual studies were written during the past 15 years in order to understand the sources of these inconsistencies (Breuner et al. 2008, Bonier et al. 2009a, Crespi et al. 2013, Dantzer et al. 2016, Beehner and Bergman 2017, Vitousek et al. 2018, Schoenle et al. 2018b, 2021, Breuner and Berk 2019). Below, I will try to briefly summarise key messages from previous works, aiming for a better understanding of the mechanisms and factors that might impact expected relationships.

a) Life-history contexts and resource acquisition

A first step towards a better evaluation of GC-fitness relationships requires accounting for individual characteristics, such as age, life-history stage or sex, and environmental conditions that likely modulate these relationships. The best way of doing so is definitely to rely on longitudinal individual-based monitoring (Bonier et al. 2009a, Crespi et al. 2013, Dantzer et al. 2016). But to get even farther, a good knowledge of the biology and the life-history strategy of the species, or population(s), under consideration might help predict the direction of GC-fitness relationships (Crespi et al. 2013, Dantzer et al. 2016, Schoenle et al.

2018b, 2021). For instance, two populations of black-legged kittiwakes (*Rissa tridactyla*), one with a slow-living life-history strategy, and one with a fast-living one, responded differently to experimentally increased GC levels. The response to experimental manipulations resulted in more nest desertion, more pronounced reduction in chick growth and lower reproductive success for the slow-living population, than for the fast-living one (Schultner et al. 2013). In addition, environmental conditions need to be accounted for when evaluating GC-fitness relationships (*i.e.* food abundance, predation pressure, population density; Dantzer et al. 2016), and a particular attention should be paid to resource availability and resource acquisition. Indeed, during periods of low resource availability, allostatic overload are more likely to occur (McEwen and Wingfield 2003). Breuner and Berk (2019) proposed to use the van Noordwijk and de Jong resource framework (1986) to test predictions about GC-fitness relationships according to the three main GCs hypotheses (*i.e.* Cort-Fitness hypothesis, Cort-Adaptation hypothesis and Cort-Tradeoff hypothesis, [Box 1](#)). Briefly, the idea is that GC levels determine individual allocation strategies between survival and reproduction, and depending on resource availability and acquisition, positive, null or negative GC-fitness relationships might be expected and relationships can be predicted to fall under one of the three hypotheses mentioned (Breuner and Berk 2019).

b) Experimental manipulations

A main issue in stress ecology is to identify whether fitness is reduced due to the effects of stressors, or due to the effects of the stress response (Beehner and Bergman 2017). This is problematic as it can create false interpretation of observed relationships across contexts (Dantzer et al. 2016, [Figure 16](#)). Let us consider three years of monitoring of a population, during which density increases each year. GCs increase as density increases, and if density negatively impact fitness, then we would observe a negative relationship between GCs and fitness across the three years of monitoring, as illustrated in [Figure 16](#). However, we might

observe that, within years, GC levels are not related to fitness for years of weak or moderate density. By contrast, during years of high density, individuals with elevated GC levels appear to have better fitness prospects. Such pattern is actually what is occurring in free-ranging red squirrels (*Tamiasciurus hudsonicus*) for which elevated GC levels during years of high density result in females producing pups with greater post-natal growth rates, providing them with greater survival and recruitment probability (Dantzer et al. 2013, 2016).

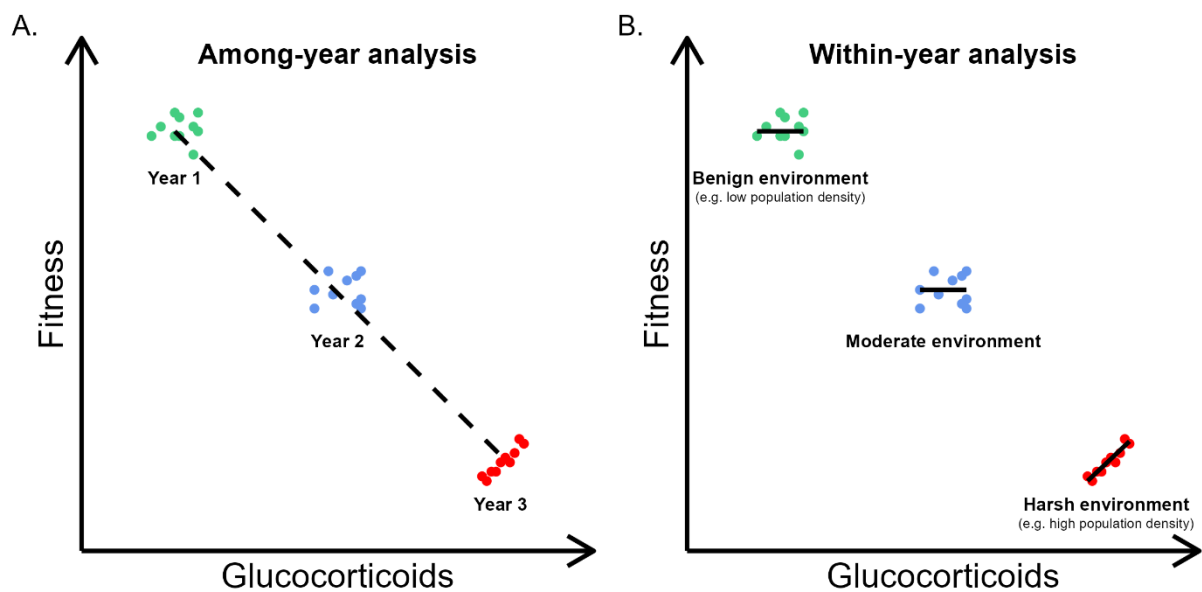


Figure 16. Covariance between environment and endocrine measures (*i.e.* glucocorticoids, GCs, here) can yields false interpretations concerning GC-fitness relationships, depending on how data are analysed. A comparison among years (A) shows a negative relationship between GCs and fitness, however, this results from the effect of the stressor (*i.e.* increase in population density over years), rather than from the effect of the stress response (*i.e.* GC levels). Within-years analyses (B) reveal that the GC-fitness relationship is actually positive during years of high population density. Experimental environmental or endocrine manipulations are a way to avoid such issue by identifying whether fitness is altered because of the stressor or because of the stress response. Adapted from Dantzer et al. (2016).

One way of avoiding such false interpretations could be to rely on the establishment of experimental endocrine or environmental manipulations in natural contexts (Dantzer et al. 2016, Schoenle et al. 2021). Such experimental manipulations can involve artificial manipulations of population density (real or perceived), increases of maternal GCs during gestation and/or lactation or use of exogenous GCs pellets. Experimental manipulations allow

disentangling the direct effects of GCs on life-history traits or fitness-related traits, from the effects of the environments, the latter possibly covarying with GCs (or any other biomarker of the stress response). Drawbacks of such approaches are that environmental manipulations might be logistically challenging to perform, and manipulating one aspect of the stress response is likely to impact other aspects of it, making it hard to disentangle the causes and mechanisms of relationships observed. Moreover, different aspects of the stress response can potentially have conflicting consequences on fitness. Thus, an excellent knowledge of the study system biology is necessary to avoid such undesired effects of experimental manipulations. With such manipulations also comes the benefit of identifying more causal relationships linking GCs and fitness outputs. Focusing on exploring mechanisms linking GCs and investigated traits can greatly improve our predictive powers if we are able to understand by which processes, or intermediate traits, the stress response can impact fitness. This can provide insights about targets of selection and be of great interests for evolutionary ecologists (Dantzer et al. 2016), and it can allow identifying potential relevant biomarkers to estimate chronic stress in wild settings.

c) Is chronic stress a reality in the wild?

In his book '*Why zebras don't get ulcers*', Sapolsky (1994) posits that wild animals do not experience chronic stress, as it is a more recent innovation restricted to primates and humans. The statement is not that wild individuals are not exposed to long-term stressors, but rather to know whether chronic stress can result in allostatic overload with expected detrimental consequences on fitness. Whether wild vertebrates suffer from states of chronic stress is actually a complicated question, as measuring states of allostatic overload is not straightforward (Boonstra 2013, Beehner and Bergman 2017). In addition, a main difference between studies in the field and studies in laboratory conditions is that in the former, the stress response has evolved in response to ecological challenges, while in the latter, the stress response has evolved

in a direction imposed by researchers. Genetic and phenotypic variations, with associated fitness outputs, are likely to be largely greater in the former case than in the latter (Boonstra 2013). Given that biomedical research here aims at studying the consequences of chronic stress on stress-related human diseases, model species are selected to specifically to investigate this. The consequences of chronic stress on obesity, the risk of strokes or the development of ulcers are unlikely to be possible to study in wild settings. Biomedical research brought many benefits for ecophysiologicalists, notably by describing the physiological processes associated to the stress response, but also heavily influenced ecophysiologicalists in thinking that chronic stress is pathological. Actually, it is difficult to argue that such chronic stress would have evolved if it was indeed pathological, and discussion emerged about whether chronic stress is adaptive or not (Sapolsky 1994, Boonstra 2013).

One way to answer such question is to not rely solely on the measurement of GCs but, as largely advocated previously, to rather rely on a set of biomarkers that encompasses much of (if not all) the aspects of the stress response from neuroendocrine, immune, metabolic, behavioural or cardiovascular systems (Seeman et al. 1997, Edes et al. 2018, Seeley et al. 2022, [Table 2](#)). These aspects of the stress response also include different aspects of the GC response (*e.g.* negative feedback efficacy, magnitude of the GC response, baseline and acute GC levels; Dickens and Romero 2013, [Table 2](#)). For example, a study on tree swallows (*Tachycineta bicolor*) evidenced that fitness was related to trade-offs in GCs regulation: females that displayed low baseline GCs coupled with high acute stress GCs, and females displaying high baseline GCs coupled with low acute stress GCs, had higher reproductive success than females displaying both baseline and acute GCs at low or high levels (Vitousek et al. 2018). Focusing only on one GC aspect (*e.g.* baseline or response to acute stress) would probably have resulted in null relationships. Similarly, measurements of the stress response would be improved by including measures of the availability and numbers of GC receptors and binding proteins that

can modulate the effects of GCs and which can render measurements of circulating GC levels irrelevant (Crespi et al. 2013). One other point that would deserve to be considered more carefully concerns the reference levels of GCs measured. Whether slightly elevated baseline levels, or long-term GC levels above baseline ranges are expected to have the same effects on reproduction and survival is not obvious. Indeed, GCs are increased during reproduction, but up to what point this can disrupt reproductive functions should be assessed in a species-specific manner (Wingfield and Sapolsky 2003, Moore and Jessop 2003, Breuner and Berk 2019).

d) Fitness metrics

Finally, a particular focus should be paid to the fitness metrics under investigation during studies aiming at investigating GC-fitness relationships. Indeed, in several instances, relationships between GC and fitness-related traits differed according to the traits under investigation, although traits were supposed to relate to fitness in similar ways (*e.g.* Sorenson et al. 2017). This observation suggests two recommendations to avoid contradictory and unexpected results. First, if the aim is to evaluate relationships between GCs and fitness, we need to rely on direct measures of survival and reproduction and not solely on proxies of it to limit intermediates that can modulate expected relationships (Breuner et al. 2008, Bonier et al. 2009a, Crespi et al. 2013). Second, and in complement of the previous point, I advocate for the investigation of several traits in relation to GC levels. These traits include life-history traits that can vary between individuals and populations and that can determine an individual life-history strategy (*e.g.* growth rate, age at first reproduction, longevity, fecundity) and thus the relationship between GCs and fitness (*e.g.* Schultner et al. 2013). Investigated traits could also include behavioural (*e.g.* territoriality) and physiological (*e.g.* secondary sexual traits such as antlers in roe deer) fitness correlates (Crespi et al. 2013). This should allow drawing a more comprehensive picture of what is occurring in relation to GCs, and could help to identify potential mechanisms involved in GC-fitness relationships.

CONCLUSION

The current thesis proposes an evaluation of the immediate and carry-over consequences of GCs on a variety of fitness-related traits that reflects health. This was permitted by a longitudinal monitoring of two free-ranging populations of a long-lived mammal, the roe deer, and evidenced that GCs are more likely to explain short-term variations in individual fitness-related traits compared to later performances and senescence. I showed that individual characteristics (*e.g.* life-history stage, sex) can modulate these relationships. Based on my results, I then discussed future research directions for future studies in ecophysiology by *i*) emphasising the need for measuring several biomarkers of the stress response to estimate allostatic load/chronic stress, and *ii*) by exploring physiological mechanisms linking GCs and senescence. I finally detailed some recommendations to improve our understanding of the GC-fitness relationships in wild animals. Namely, I argued that considering the life history of a given species or population and integrating resource acquisition information can improve our ability to predict the direction and strength of these relationships. Also, I encouraged future research to rely on longitudinal monitoring and on experimental manipulations to disentangle the various contributions of individual characteristics and environmental conditions in driving GC-fitness associations.

Still, whether elevated baseline GC levels reflect individuals that are challenged by their environments and that suffer from chronic stress, with expected low fitness, or whether they reflect individuals properly responding to challenges, thus with good fitness, is not fully elucidated (Monaghan and Spencer 2014). Moving away from the biomedical conception that chronic stress is maladaptive, including in natural contexts, and following the above-mentioned recommendations can help researchers to better predict the direction of GC-fitness relationships.

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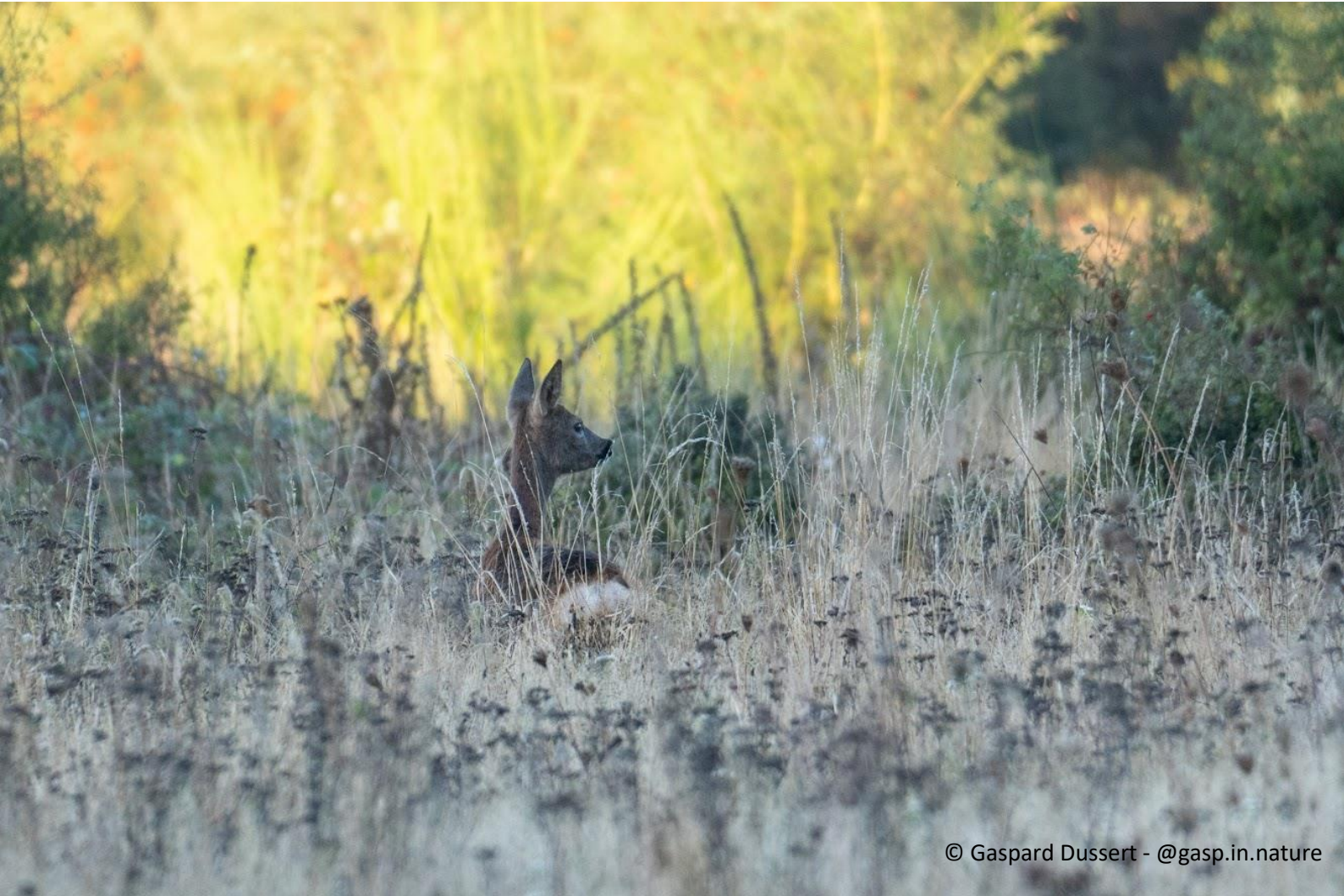
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APPENDICES



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Appendix A – Capture form

Example of a filled in capture form from Trois-Fontaines



OFB
OFFICE FRANÇAIS
DE LA BIODIVERSITÉ



978101081527804

VetAgro Sup

Fiche de renseignement capture chevreuil

OFB - CNRS - VETAGRO SUP
ANIMAUX d'AGE CONNU

2022-51 026

N°ordre ONCFS : ..22.53.....

DATE: ..13../01../2022... matin / après-midi

LIEU: Chizé Trois-Fontaines SECTEUR:

Boucle (n°/couleur): 1. 948. bleu 2. 966. bleu..

N° collier (plaque, GPS...): 27

Age: 1 Poids: 16.4 kg Sexe: ♂

LPA (gauche): 357 LPA (droite): 357

Chevrillard non capturé faon (pas d'hémato-bioch):

Veil animal d'âge non connu (pas d'hémato-bioch):

Tiques: nombre de tiques fixées sur la tête : 10
(Nombre exact jusqu'à 20 tiques puis > 20)

Prélèvement de tiques: NON

Autres ectoparasites (ex : Lipoptena (non fixés),...): oui non

Heure de la prise de sang: ...14H40.....

Heure de la centrifugation du plasma: ...19H48.....

Nombre de personnes pendant la manip: 8

TEST COVID

N° kit: N° ordre ONCFS : ...22:53.....

Mesure du stress

Dans le sabot:

N° sabot:

55

(entourer les scores donnés ci-dessous)

Comportement sur la table

Sur la table:

Score Mesure subjective des réactions de l'animal sur la table de manipulation

- 0 Calme - Sans résistance - Pas de cri - Pas de coup de pattes
- 1 Calme - Pas plus de deux cris - Presque aucun coups de pattes
- 2 Animal qui crie et donne des coups de pattes mais qui reste relativement calme entre ces événements
- 3 Animal stressé, beaucoup de cris et de coups de pattes mais la manipulation reste possible
- 4 Animal extrêmement stressé. Très difficile à manipuler / impossibilité de prendre des mesures précises
- 5 Animal exténué/ Aucune résistance, facile à manipuler. Ce score diffère de 0 car ici l'animal est stressé

Lâché:

Comportement au lâché

Heure : ...17h55.....

De nuit - de jour

Score Mesure subjective du comportement de l'animal lors du lâcher

Nombre de personnes présentes : ...15.....

- 0 Quitte le site lentement. S'arrête plusieurs fois
- 1 Quitte le site en courant mais s'arrête après une courte distance
- 2 Quitte le site en courant et ne s'arrête que lorsqu'il ne peut plus être vu
- 3 Animal qui tombe ou saute en quittant le site / qui essaye d'arracher son collier / ou de s'échapper
- 4 Animal qui reste allongé et qui ne peut plus tenir debout seul

Animal sorti spontanément de la boîte :
oui / non

Nom de la personne ayant mesuré le stress :

Table : ...J.F.L..... Lâché : ...LALANDE.....

***Appendix B – Supplementary information: Glucocorticoids
and body mass***

Supporting Information to:

*Glucocorticoids negatively relate to body mass on the short-term in a
free-ranging ungulate (Lalande et al. 2023. Oikos)*

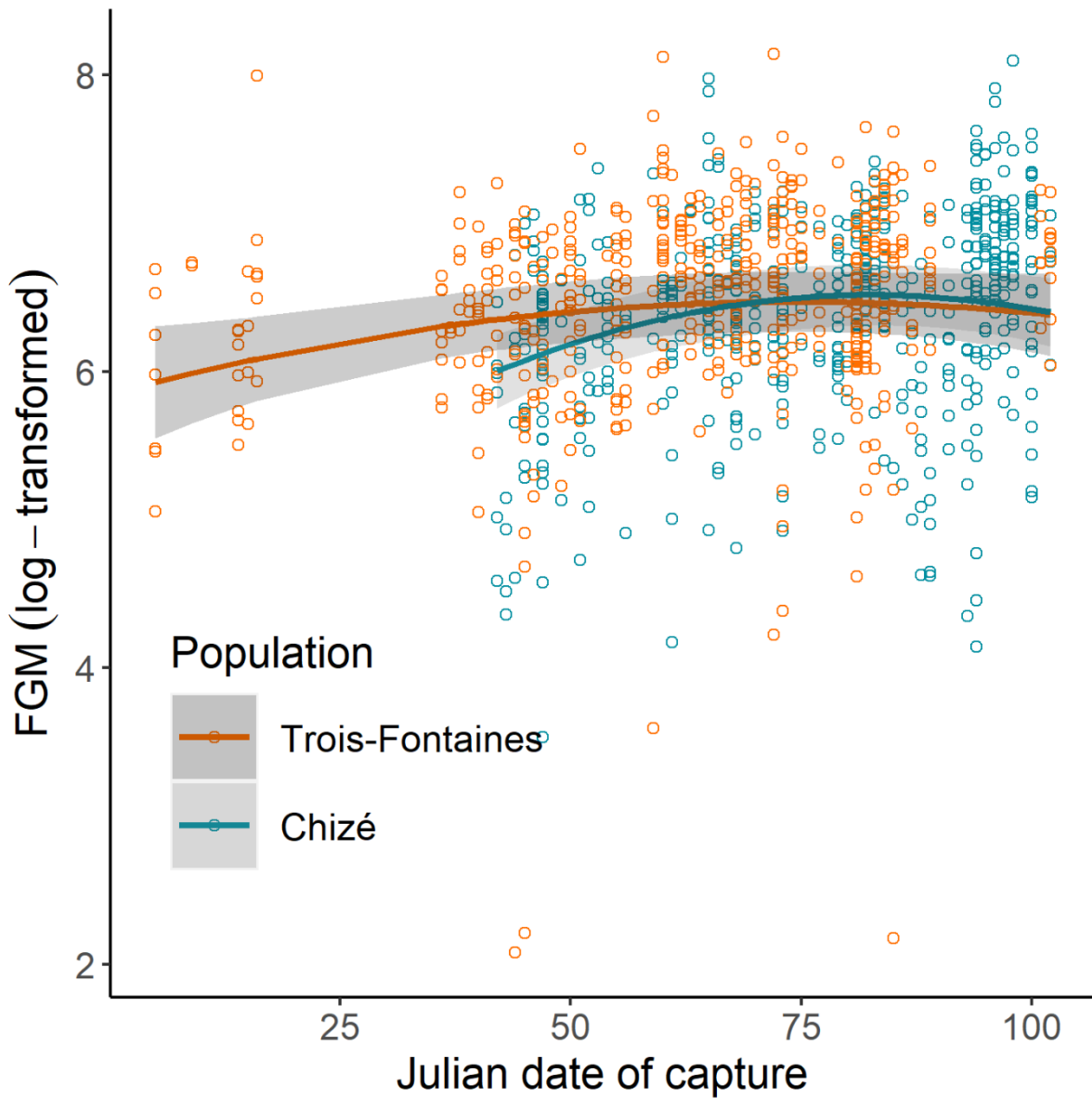


Figure S1. Seasonal variation of faecal glucocorticoid metabolites (FGMs, log-transformed) according to Julian date of capture (starting early December, ending early March) and the population (Trois-Fontaines: orange, Chizé: blue). FGMs increase across the capture season and slightly decrease at the end of the season, in a population-specific manner. Points are raw data and lines are predictions from the selected model with 95% confidence intervals.

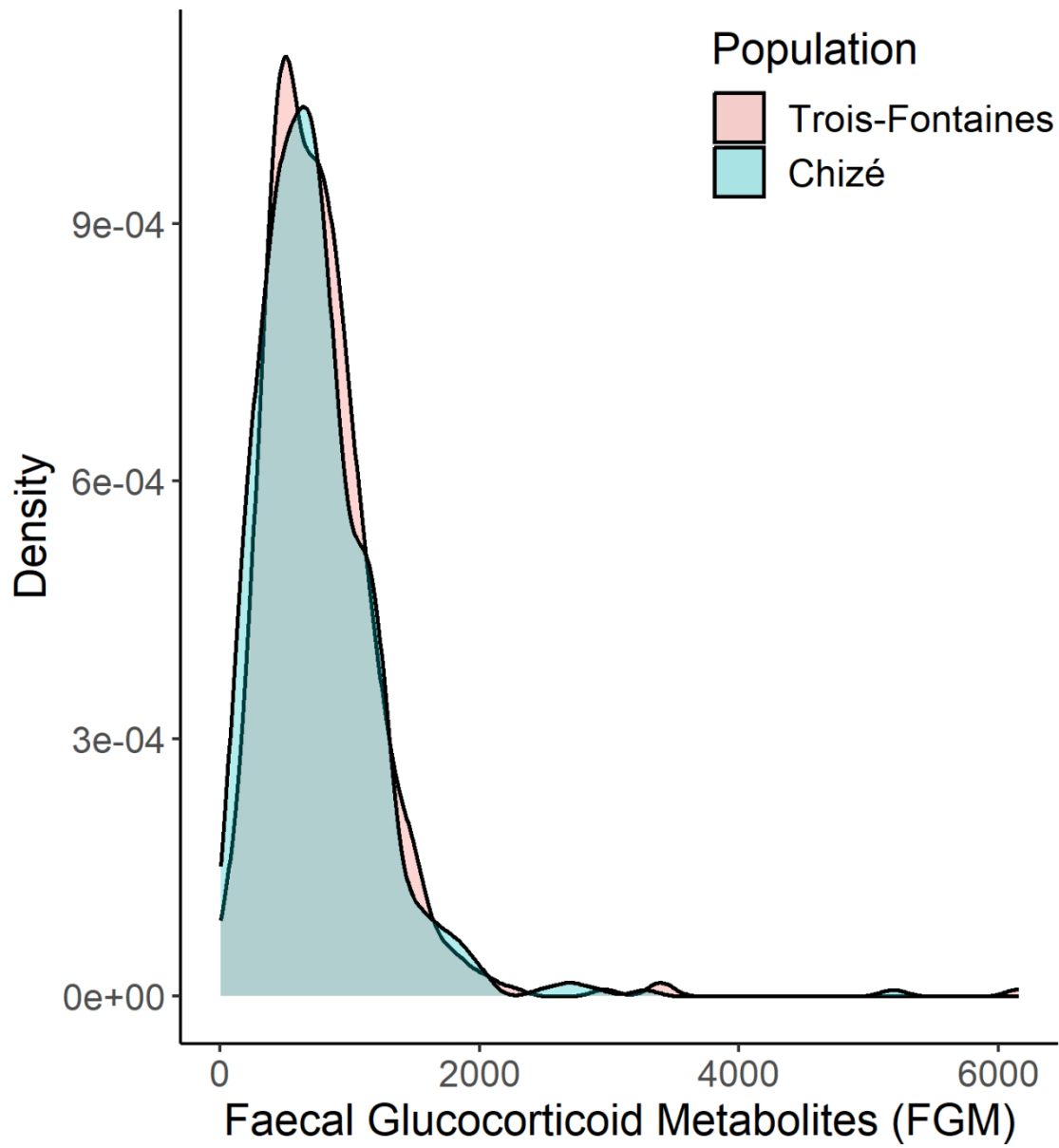


Figure S2. Density plot of faecal glucocorticoid metabolites (FGMs) according to population, evidencing particularly high FGM values.

Table S1. Linear and linear mixed effect models selected for the short-, medium- and long-term relationships between relative body mass and faecal glucocorticoid metabolites (FGM), including two high FGM values. Models accounted for sex, population (CH: Chizé) and for cohort quality (Qcoh). Models were selected through model selection based on AICc and parameters estimated through model averaging. 95%CI: 95% confidence interval, V: variance, SD: standard-deviation.

SHORT-TERM					
Juveniles			Adults		
Random effects	V	SD	Random effects	V	SD
			Individual ID	3.45	1.86
			Year of capture	0.29	0.53
Fixed effects	Estimate	95% CI	Fixed effects	Estimate	95% CI
Intercept	-0.06	[-0.28, 0.16]	Intercept	-0.02	[-0.39, 0.35]
Qcoh	1.08	[0.84, 1.32]	FGM	-0.33	[-0.47, -0.19]
Marginal R ²	0.18		Marginal R ²	0.02	
Conditional R ²			Conditional R ²	0.81	

MEDIUM-TERM (Early-growth)					
FGM measured year <i>t</i>			Mean FGM (FGM _{<i>t</i>} and FGM _{<i>t</i>+1})		
Random effects	V	SD	Random effects	V	SD
Cohort	0.72	0.85	Cohort	0.99	0.99
Fixed effects	Estimate	95%CI	Fixed effects	Estimate	95% CI
Intercept	-0.32	[-0.91, 0.27]	Intercept	-0.33	[-1.09, 0.43]
Marginal R ²	-		Marginal R ²	-	
Conditional R ²	0.22		Conditional R ²	0.27	

LONG-TERM		
Random effects	V	Random effects
Individual ID	3.69	1.92
Year of capture	0.65	0.80
Fixed effects	Estimate	95% CI
Intercept	-0.04	[-0.61, 0.53]
Marginal R ²	-	
Conditional R ²	0.83	

Table S2. Linear mixed effects model (LMM) selection table for the short-term relationship between relative body mass for juveniles and adults (*i.e.* aged 2 years old and older) and concomitant faecal glucocorticoid metabolites ('FGM'). We accounted for cohort quality ('Qcoh'), population (reference level is Chizé: CH, and is compared to Trois-Fontaines: TF), and sex (reference level is male: M, and is compared to females: F). Values give the parameter coefficient and values between brackets in grey rows are the standard-errors. 'I' is the Intercept, 'df' is the number of parameters, 'Log-lik' is the log-likelihood, 'delta' is the difference of AICc between the candidate model and the model having the lowest AICc, and 'weight' the AIC weight of each model. Retained model is in bold. Only models up to delta AICc = 7 are displayed.

SHORT-TERM (JUVENILES, N = 368)												
I	FGM	Qcoh	Pop(CH)	Sex(M)	FGM:Qcoh	FGM:Pop(CH)	FGM:Sex(M)	df	Log-lik	AICc	Delta	Weight
-0.06	-0.23	1.09						4	-785.24	1578.59	0.00	0.23
(0.11)	(0.13)	(0.12)										
-0.06		1.08						3	-786.72	1579.52	0.93	0.15
(0.11)		(0.12)										
-0.06	-0.22	1.08			-0.07			5	-785.16	1580.49	1.90	0.09
(0.11)	(0.14)	(0.12)			(0.18)							
-0.08	-0.23	1.09	0.03					5	-785.23	1580.63	2.04	0.08
(0.15)	(0.14)	(0.12)	(0.22)									
-0.06	-0.23	1.09		-0.01				5	-785.24	1580.64	2.05	0.08
(0.16)	(0.13)	(0.12)		(0.22)								
-0.12		1.08	0.12					4	-786.58	1581.27	2.68	0.06
(0.15)		(0.12)	(0.22)									
-0.05		1.08		-0.04				4	-786.71	1581.53	2.94	0.05
(0.16)		(0.12)		(0.22)								
-0.10	-0.12	1.09	0.04			-0.15		6	-785.09	1582.41	3.82	0.03
(0.15)	(0.24)	(0.12)	(0.22)			(0.29)						
-0.08	-0.22	1.08	0.03		-0.07			6	-785.15	1582.54	3.95	0.03
(0.15)	(0.14)	(0.12)	(0.22)		(0.18)							
-0.06	-0.22	1.08		-0.00	-0.07			6	-785.16	1582.56	3.97	0.03
(0.16)	(0.14)	(0.12)		(0.22)	(0.18)							
-0.06	-0.19	1.09		-0.01			-0.09	6	-785.19	1582.60	4.01	0.03
(0.16)	(0.19)	(0.12)		(0.22)			(0.27)					
-0.07	-0.23	1.09	0.03	-0.01				6	-785.23	1582.69	4.10	0.03
(0.19)	(0.14)	(0.12)	(0.22)	(0.22)								
-0.10		1.08	0.12	-0.04				5	-786.57	1583.30	4.71	0.02
(0.19)		(0.12)	(0.22)	(0.22)								
-0.10	-0.12	1.08	0.04		-0.06	-0.15		7	-785.02	1584.35	5.77	0.01
(0.15)	(0.24)	(0.12)	(0.22)		(0.18)	(0.30)						
-0.09	-0.12	1.09	0.04	-0.01		-0.16		7	-785.09	1584.49	5.90	0.01
(0.19)	(0.24)	(0.12)	(0.22)	(0.22)		(0.30)						
-0.06	-0.17	1.09		-0.00	-0.08		-0.10	7	-785.09	1584.49	5.90	0.01
(0.16)	(0.20)	(0.12)		(0.22)	(0.18)		(0.27)					
-0.08	-0.22	1.08	0.03	-0.01	-0.07			7	-785.15	1584.61	6.03	0.01
(0.19)	(0.14)	(0.12)	(0.22)	(0.22)	(0.18)							
-0.07	-0.18	1.09	0.03	-0.01			-0.09	7	-785.18	1584.66	6.07	0.01
(0.19)	(0.19)	(0.12)	(0.22)	(0.22)			(0.27)					
-0.09	-0.12	1.08	0.04	-0.01	-0.06	-0.15		8	-785.02	1586.44	7.85	0.00

(0.19)	(0.24)	(0.12)	(0.22)	(0.22)	(0.18)	(0.30)						
-0.09	-0.09	1.09	0.04	-0.01		-0.15	-0.08	8	-785.05	1586.50	7.91	0.00
(0.19)	(0.27)	(0.12)	(0.22)	(0.22)		(0.30)	(0.27)					
-0.07	-0.17	1.09	0.03	-0.00	-0.08		-0.10	8	-785.08	1586.56	7.97	0.00
(0.19)	(0.20)	(0.12)	(0.22)	(0.22)	(0.18)		(0.27)					
SHORT-TERM (ADULTS, N = 655 obs., n = 377 ind.)												
I	FGM	Qcoh	Pop(CH)	Sex(M)	FGM:Qcoh	FGM:Pop(CH)	FGM:Sex(M)	df	Log-lik	AICc	Delta	Weight
-0.01	-0.32	0.26						6	-1268.53	2549.18	0.00	0.17
(0.18)	(0.07)	(0.14)										
-0.00	-0.32	0.25			0.10			7	-1267.99	2550.16	0.98	0.10
(0.18)	(0.07)	(0.14)			(0.10)							
-0.02	-0.32							5	-1270.38	2550.85	1.67	0.07
(0.18)	(0.07)											
-0.07	-0.31	0.26	0.14					7	-1268.35	2550.87	1.69	0.07
(0.21)	(0.07)	(0.14)	(0.23)									
-0.01	-0.40	0.25		0.01			0.20	8	-1267.39	2551.01	1.83	0.07
(0.20)	(0.09)	(0.14)		(0.21)			(0.14)					
-0.01	-0.32	0.26		0.01				7	-1268.52	2551.22	2.04	0.06
(0.20)	(0.07)	(0.14)		(0.21)								
-0.06	-0.31	0.24	0.13		0.10			8	-1267.84	2551.91	2.73	0.04
(0.21)	(0.07)	(0.14)	(0.23)		(0.10)							
-0.00	-0.39	0.24		-0.00	0.10		0.20	9	-1266.89	2552.05	2.87	0.04
(0.20)	(0.09)	(0.14)		(0.21)	(0.10)		(0.14)					
-0.00	-0.32	0.25		0.00	0.10			8	-1267.99	2552.21	3.03	0.04
(0.20)	(0.07)	(0.14)		(0.21)	(0.10)							
-0.10	-0.23	0.26	0.15			-0.12		8	-1268.00	2552.23	3.05	0.04
(0.22)	(0.12)	(0.14)	(0.23)			(0.15)						
-0.01	-0.41			-0.01			0.22	7	-1269.07	2552.32	3.14	0.04
(0.21)	(0.08)			(0.21)			(0.14)					
-0.09	-0.31		0.16					6	-1270.13	2552.40	3.22	0.03
(0.22)	(0.07)		(0.23)									
-0.08	-0.39	0.25	0.14	0.01			0.21	9	-1267.20	2552.67	3.49	0.03
(0.23)	(0.09)	(0.14)	(0.23)	(0.21)			(0.14)					
-0.01	-0.32			-0.01				6	-1270.38	2552.89	3.71	0.03
(0.21)	(0.07)			(0.21)								
-0.08	-0.31	0.26	0.14	0.02				8	-1268.35	2552.91	3.73	0.03
(0.23)	(0.07)	(0.14)	(0.23)	(0.21)								
-0.09	-0.24	0.25	0.14		0.08	-0.09		9	-1267.64	2553.56	4.38	0.02
(0.21)	(0.12)	(0.14)	(0.23)		(0.10)	(0.15)						
-0.07	-0.39	0.23	0.13	0.00	0.09		0.20	10	-1266.72	2553.78	4.60	0.02
(0.23)	(0.09)	(0.14)	(0.23)	(0.21)	(0.10)		(0.14)					
-0.12	-0.23		0.18			-0.11		7	-1269.82	2553.82	4.64	0.02
(0.22)	(0.12)		(0.23)			(0.15)						
-0.09	-0.40		0.17	-0.01			0.22	8	-1268.81	2553.84	4.66	0.02
(0.24)	(0.09)		(0.23)	(0.21)			(0.14)					
-0.07	-0.31	0.25	0.13	0.00	0.10			9	-1267.84	2553.96	4.78	0.02

(0.23)	(0.07)	(0.14)	(0.23)	(0.21)	(0.10)								
-0.11	-0.23	0.26	0.15	0.02		-0.12		9	-1268.00	2554.28	5.10	0.01	
(0.23)	(0.12)	(0.14)	(0.23)	(0.21)		(0.15)							
-0.10	-0.32	0.25	0.16	0.01		-0.09	0.19	10	-1267.01	2554.37	5.19	0.01	
(0.24)	(0.14)	(0.14)	(0.23)	(0.21)		(0.15)	(0.14)						
-0.09	-0.31		0.16	-0.01				7	-1270.13	2554.44	5.26	0.01	
(0.24)	(0.07)		(0.23)	(0.21)									
-0.11	-0.34		0.18	-0.01		-0.08	0.21	9	-1268.66	2555.60	6.42	0.01	
(0.24)	(0.14)		(0.23)	(0.21)		(0.15)	(0.14)						
-0.09	-0.24	0.25	0.14	0.01	0.08	-0.09		10	-1267.64	2555.62	6.44	0.01	
(0.23)	(0.12)	(0.14)	(0.23)	(0.21)	(0.10)	(0.15)							
-0.08	-0.34	0.24	0.14	0.00	0.09	-0.06	0.19	11	-1266.63	2555.68	6.50	0.01	
(0.24)	(0.14)	(0.14)	(0.23)	(0.21)	(0.10)	(0.15)	(0.14)						
-0.12	-0.23		0.18	-0.01		-0.11		8	-1269.82	2555.87	6.69	0.01	
(0.24)	(0.12)		(0.23)	(0.21)		(0.15)							

Table S3. Linear mixed effects model (LMM) selection table for the medium-term relationship between the change of body mass between two consecutive years for early-growing (*i.e.* between the first and second years of life), late-growing (*i.e.* between 2 and 4 years old) and prime age (*i.e.* between 4 and 10 years old) individuals, and faecal glucocorticoid metabolites ('FGM') measured age t ('FGM t ') or FGMs considered as the mean FGM value of the FGMs measured at age t and age $t+1$ ('mean FGM'). We accounted for cohort quality ('Qcoh'), population (reference level is Chizé: CH, and is compared to Trois-Fontaines: TF), and sex (reference level is male: M, and is compared to females: F) and the initial body mass (*i.e.* measured year t , 'Mass'). Values give the parameter coefficient and values between brackets in grey rows are the standard-errors. 'I' is the Intercept, 'df' is the number of parameters, 'Log-lik' is the log-likelihood, 'delta' is the difference of AICc between the candidate model and the model having the lowest AICc, and 'weight' the AIC weight of each model. Retained model is in bold. Only models up to delta AICc = 7 are displayed.

MEDIUM-TERM (EARLY GROWTH, FGMt, N = 99)														
I	FGM	Qcoh	Pop(CH)	Sex(M)	Mass	FGM:Qcoh	FGM:Pop(CH)	FGM:Sex(M)	FGM:Mass	df	Log-lik	AICc	Delta	Weight
0.08			-1.03		-0.19					5	-187.73	386.10	0.00	0.11
(0.31)			(0.42)		(0.08)									
0.14	-0.24		-1.16		-0.20					6	-187.09	387.09	0.99	0.07
(0.30)	(0.21)		(0.43)		(0.08)									
0.41		-0.30	-1.75		-0.16					6	-187.22	387.35	1.24	0.06
(0.45)		(0.29)	(0.83)		(0.09)									
0.05			-1.03	0.07	-0.19					6	-187.70	388.32	2.22	0.04
(0.34)			(0.42)	(0.32)	(0.08)									
0.48		-0.50	-1.90							5	-188.87	388.39	2.29	0.04
(0.46)		(0.28)	(0.86)											
0.20	-0.50		-1.20		-0.21		0.41			7	-186.64	388.52	2.41	0.03
(0.30)	(0.34)		(0.43)		(0.08)		(0.43)							
0.42	-0.22	-0.26	-1.78		-0.17					7	-186.69	388.61	2.50	0.03
(0.43)	(0.21)	(0.29)	(0.81)		(0.09)									
0.55	-0.41	-0.33	-1.98		-0.15	-0.38			0.25	9	-184.40	388.82	2.72	0.03
(0.44)	(0.22)	(0.29)	(0.82)		(0.09)	(0.18)			(0.14)					
-0.35										3	-191.34	388.94	2.83	0.03
(0.29)														

0.12	-0.24		-1.16		-0.20			0.06	7	-186.95	389.13	3.02	0.02
(0.31)	(0.21)		(0.43)		(0.08)			(0.11)					
-0.33					-0.10				4	-190.37	389.17	3.06	0.02
(0.29)					(0.07)								
-0.14			-0.54						4	-190.42	389.26	3.16	0.02
(0.31)			(0.39)										
-0.36		0.23			-0.17				5	-189.32	389.28	3.18	0.02
(0.28)		(0.15)			(0.09)								
0.09	-0.24		-1.18	0.10	-0.21				7	-187.03	389.30	3.19	0.02
(0.34)	(0.21)		(0.43)	(0.32)	(0.08)								
0.20	-0.72		-1.22		-0.20		0.74	0.16	8	-185.90	389.40	3.30	0.02
(0.31)	(0.39)		(0.43)		(0.08)		(0.51)	(0.13)					
0.47	-0.30	-0.25	-1.79		-0.17	-0.17			8	-185.99	389.58	3.47	0.02
(0.42)	(0.21)	(0.27)	(0.79)		(0.09)	(0.14)							
0.39		-0.29	-1.74	0.03	-0.16				7	-187.21	389.66	3.55	0.02
(0.49)		(0.29)	(0.84)	(0.32)	(0.09)								
0.46	-0.48	-0.24	-1.79		-0.18		0.41		8	-186.26	390.12	4.01	0.01
(0.42)	(0.34)	(0.28)	(0.80)		(0.09)		(0.43)						
0.48	-0.15	-0.48	-1.92						6	-188.62	390.15	4.04	0.01
(0.45)	(0.21)	(0.28)	(0.84)										
0.10	-0.47		-1.22	0.10	-0.20		0.50		8	-186.28	390.16	4.05	0.01
(0.33)	(0.27)		(0.43)	(0.31)	(0.08)		(0.41)						
0.42	-0.22	-0.28	-1.83		-0.17			0.07	8	-186.50	390.60	4.50	0.01
(0.44)	(0.21)	(0.29)	(0.83)		(0.09)			(0.11)					
0.49		-0.50	-1.91	-0.02					6	-188.87	390.66	4.55	0.01
(0.50)		(0.28)	(0.86)	(0.32)									
-0.36	-0.19	0.27			-0.19				6	-188.91	390.73	4.63	0.01
(0.27)	(0.21)	(0.16)			(0.09)								
-0.11	-0.17		-0.62						5	-190.09	390.82	4.71	0.01
(0.31)	(0.21)		(0.39)										
0.16	-0.50		-1.21	0.07	-0.21		0.40		8	-186.62	390.84	4.73	0.01
(0.34)	(0.34)		(0.43)	(0.32)	(0.08)		(0.44)						
0.51	-0.71	-0.28	-1.91		-0.17		0.75	0.17	9	-185.42	390.86	4.75	0.01
(0.44)	(0.39)	(0.29)	(0.82)		(0.09)		(0.51)	(0.13)					
-0.35	-0.10								4	-191.23	390.89	4.79	0.01
(0.29)	(0.21)												
-0.36		0.06							4	-191.25	390.92	4.81	0.01
(0.29)		(0.13)											
0.37	-0.22	-0.25	-1.77	0.07	-0.18				8	-186.67	390.93	4.83	0.01
(0.47)	(0.21)	(0.29)	(0.82)	(0.32)	(0.09)								
-0.36				0.03					4	-191.34	391.10	5.00	0.01
(0.33)				(0.32)									
0.53	-0.24	-0.47	-1.92			-0.17			7	-187.94	391.12	5.02	0.01
(0.44)	(0.22)	(0.27)	(0.82)			(0.14)							
-0.33	-0.10				-0.10				5	-190.26	391.17	5.07	0.01
(0.29)	(0.21)				(0.07)								

0.50	-0.41	-0.32	-1.96	0.08	-0.16	-0.38			0.26	10	-184.37	391.24	5.13	0.01
(0.48)	(0.22)	(0.29)	(0.82)	(0.31)	(0.09)	(0.18)			(0.14)					
0.55	-0.36	-0.33	-1.98		-0.15	-0.40	-0.09		0.25	10	-184.39	391.29	5.18	0.01
(0.44)	(0.45)	(0.29)	(0.82)		(0.09)	(0.28)	(0.77)		(0.14)					
0.05	-0.25		-1.17	0.13	-0.20				0.07	8	-186.86	391.32	5.22	0.01
(0.34)	(0.21)		(0.43)	(0.32)	(0.08)				(0.11)					
-0.35				0.04	-0.10					5	-190.36	391.37	5.27	0.01
(0.34)				(0.32)	(0.07)									
-0.15			-0.54	0.04						5	-190.41	391.47	5.37	0.01
(0.35)			(0.39)	(0.32)										
-0.40		0.23		0.08	-0.18					6	-189.29	391.49	5.38	0.01
(0.32)		(0.15)		(0.32)	(0.09)									
0.14	-0.73		-1.23	0.12	-0.21		0.74		0.16	9	-185.83	391.69	5.59	0.01
(0.34)	(0.39)		(0.43)	(0.32)	(0.08)		(0.51)		(0.13)					
0.17	-0.71		-1.24	0.07	-0.20		0.38	0.49		9	-185.89	391.81	5.71	0.01
(0.33)	(0.38)		(0.43)	(0.32)	(0.08)		(0.43)	(0.41)						
-0.32	-0.37	0.26			-0.17	-0.34			0.22	8	-187.14	391.89	5.78	0.01
(0.28)	(0.23)	(0.16)			(0.09)	(0.19)			(0.14)					
-0.31	-0.26	0.26			-0.19	-0.16				7	-188.33	391.89	5.78	0.01
(0.27)	(0.22)	(0.16)			(0.09)	(0.15)								
0.52	-0.37	-0.47	-1.92				0.34			7	-188.33	391.89	5.78	0.01
(0.44)	(0.34)	(0.27)	(0.83)				(0.44)							
0.06	-0.50		-1.22	0.14	-0.19			0.56	0.09	9	-185.94	391.91	5.81	0.01
(0.34)	(0.28)		(0.43)	(0.32)	(0.08)			(0.41)	(0.11)					
0.37	-0.45	-0.23	-1.77	0.06	-0.17			0.49		9	-185.95	391.92	5.81	0.01
(0.46)	(0.27)	(0.28)	(0.80)	(0.32)	(0.09)			(0.41)						
0.15	-1.03		-1.29	0.12	-0.19		0.79	0.60	0.20	10	-184.74	391.98	5.88	0.01
(0.34)	(0.44)		(0.43)	(0.31)	(0.08)		(0.51)	(0.41)	(0.13)					
0.47	-0.26	-0.25	-1.79		-0.17	-0.20	-0.09			9	-185.98	391.99	5.89	0.01
(0.42)	(0.45)	(0.28)	(0.79)		(0.09)	(0.26)	(0.79)							
0.46	-0.30	-0.25	-1.79	0.02	-0.18	-0.17				9	-185.99	392.00	5.89	0.01
(0.46)	(0.21)	(0.28)	(0.79)	(0.32)	(0.09)	(0.14)								
0.48	-0.65	-0.30	-1.97	0.09	-0.15	-0.37		0.53	0.28	11	-183.52	392.07	5.96	0.01
(0.47)	(0.29)	(0.28)	(0.81)	(0.31)	(0.09)	(0.18)		(0.40)	(0.14)					
0.48	-0.15	-0.48	-1.92	0.00						7	-188.62	392.46	6.36	0.00
(0.49)	(0.21)	(0.28)	(0.85)	(0.32)										
0.44	-0.48	-0.24	-1.78	0.03	-0.18		0.40			9	-186.25	392.53	6.43	0.00
(0.47)	(0.34)	(0.28)	(0.80)	(0.32)	(0.09)		(0.43)							
-0.06	-0.38		-0.63				0.33			6	-189.82	392.55	6.44	0.00
(0.31)	(0.35)		(0.39)				(0.45)							
-0.36	-0.13	0.08								5	-191.07	392.79	6.68	0.00
(0.28)	(0.21)	(0.13)												
-0.38	-0.20	0.26			-0.18				0.06	7	-188.78	392.80	6.69	0.00
(0.28)	(0.21)	(0.16)			(0.09)				(0.11)					
-0.42	-0.20	0.27		0.11	-0.19					7	-188.85	392.92	6.82	0.00
(0.32)	(0.21)	(0.16)		(0.32)	(0.09)									

0.36	-0.23	-0.27	-1.81	0.10	-0.17				0.07	9	-186.45	392.93	6.83	0.00
(0.48)	(0.21)	(0.29)	(0.83)	(0.32)	(0.09)				(0.11)					
-0.14	-0.18		-0.62	0.06						6	-190.07	393.05	6.95	0.00
(0.35)	(0.21)		(0.39)	(0.32)										
-0.37	-0.10			0.04						5	-191.23	393.10	6.99	0.00
(0.33)	(0.21)			(0.32)										
MEDIUM-TERM (EARLY GROWTH, mean FGM, N = 67)														
I	FGM	Qcoh	Pop(CH)	Sex(M)	Mass	FGM:Qcoh	FGM:Pop(CH)	FGM:Sex(M)	FGM:Mass	df	Log-lik	AICc	Delta	Weight
0.32	-0.85		-1.57		-0.27					6	-127.12	267.65	0.00	0.10
(0.34)	(0.34)		(0.54)		(0.10)									
0.16	-0.92		-1.75	0.46	-0.28					7	-126.48	268.87	1.22	0.06
(0.37)	(0.34)		(0.52)	(0.39)	(0.09)									
0.75	-0.98	-0.37	-2.43		-0.21	-0.72			0.47	9	-123.91	268.97	1.32	0.05
(0.47)	(0.34)	(0.30)	(0.91)		(0.10)	(0.31)			(0.20)					
0.54	-0.85	-0.24	-2.11		-0.23					7	-126.77	269.45	1.80	0.04
(0.43)	(0.34)	(0.28)	(0.85)		(0.11)									
0.40	-1.07		-1.68		-0.29		0.44			7	-126.95	269.79	2.14	0.04
(0.33)	(0.48)		(0.53)		(0.10)		(0.70)							
0.30	-0.84		-1.53		-0.26				0.07	7	-127.01	269.92	2.27	0.03
(0.35)	(0.34)		(0.55)		(0.10)				(0.14)					
-0.37										3	-131.93	270.23	2.58	0.03
(0.38)														
0.03			-0.94		-0.21					5	-129.73	270.45	2.80	0.03
(0.39)			(0.55)		(0.10)									
-0.37					-0.12					4	-130.93	270.51	2.87	0.02
(0.37)					(0.09)									
-0.34	-0.47									4	-130.99	270.63	2.98	0.02
(0.36)	(0.34)													
0.54	-1.03	-0.33	-2.43	0.40	-0.22	-0.72			0.48	10	-123.35	270.63	2.98	0.02
(0.49)	(0.33)	(0.29)	(0.88)	(0.38)	(0.10)	(0.31)			(0.20)					
-0.35	-0.49				-0.13					5	-129.89	270.77	3.12	0.02
(0.36)	(0.33)				(0.09)									
0.57	-0.90	-0.21	-2.09		-0.25	-0.23				8	-126.23	270.95	3.30	0.02
(0.41)	(0.34)	(0.27)	(0.82)		(0.11)	(0.21)								
0.24	-1.17		-1.88	0.48	-0.30		0.49			8	-126.26	271.01	3.36	0.02
(0.36)	(0.47)		(0.51)	(0.39)	(0.10)		(0.70)							
0.34	-0.90	-0.18	-2.10	0.40	-0.24					8	-126.28	271.05	3.40	0.02
(0.47)	(0.34)	(0.27)	(0.82)	(0.40)	(0.11)									
0.14	-0.92		-1.72	0.48	-0.27				0.09	8	-126.32	271.12	3.47	0.02
(0.38)	(0.34)		(0.54)	(0.40)	(0.09)				(0.14)					
0.53	-0.67	-0.52	-2.10							6	-128.89	271.19	3.54	0.02
(0.49)	(0.35)	(0.28)	(0.94)											
0.45	-1.36		-1.73		-0.29		1.00		0.20	8	-126.40	271.29	3.64	0.02
(0.36)	(0.58)		(0.56)		(0.10)		(0.90)		(0.18)					
0.63	-0.62	-0.35	-2.23		-0.19	-0.91	-0.75		0.47	10	-123.75	271.42	3.77	0.02
(0.51)	(0.69)	(0.31)	(0.98)		(0.10)	(0.45)	(1.31)		(0.20)					

0.16	-0.94		-1.76	0.46	-0.28			0.05		8	-126.48	271.44	3.80	0.02
(0.37)	(0.44)		(0.52)	(0.39)	(0.10)			(0.67)						
-0.34	-0.62	0.25			-0.22					6	-129.08	271.56	3.91	0.01
(0.33)	(0.34)	(0.19)			(0.11)									
0.57	-0.83	-0.29	-2.20		-0.21			0.10		8	-126.54	271.57	3.92	0.01
(0.47)	(0.34)	(0.30)	(0.92)		(0.11)			(0.14)						
0.63	-1.10	-0.24	-2.23		-0.25		0.47			8	-126.57	271.62	3.98	0.01
(0.42)	(0.47)	(0.27)	(0.83)		(0.11)		(0.70)							
-0.11	-0.59		-0.55							5	-130.47	271.92	4.28	0.01
(0.39)	(0.35)		(0.49)											
0.29	-1.55		-2.00	0.56	-0.31		1.18	0.23		9	-125.49	272.14	4.49	0.01
(0.38)	(0.57)		(0.55)	(0.40)	(0.10)		(0.90)	(0.18)						
0.33		-0.49	-1.70							5	-130.58	272.14	4.50	0.01
(0.54)		(0.32)	(1.01)											
0.29		-0.27	-1.59		-0.17					6	-129.39	272.18	4.53	0.01
(0.51)		(0.33)	(0.97)		(0.11)									
-0.25			-0.28							4	-131.77	272.19	4.55	0.01
(0.41)			(0.49)											
-0.36		0.15			-0.18					5	-130.63	272.24	4.59	0.01
(0.36)		(0.19)			(0.11)									
-0.47				0.20						4	-131.81	272.26	4.61	0.01
(0.43)				(0.41)										
-0.09			-1.01	0.30	-0.21					6	-129.46	272.32	4.67	0.01
(0.42)			(0.55)	(0.40)	(0.10)									
-0.27	-0.75	0.24			-0.21	-0.64		0.39		8	-126.92	272.33	4.68	0.01
(0.35)	(0.34)	(0.19)			(0.11)	(0.31)		(0.20)						
-0.37		-0.04								4	-131.90	272.44	4.79	0.01
(0.38)		(0.16)												
0.83	-1.48	-0.37	-2.62		-0.24		1.23	0.26		9	-125.65	272.46	4.82	0.01
(0.49)	(0.58)	(0.30)	(0.94)		(0.11)		(0.91)	(0.18)						
-0.46	-0.48			0.24						5	-130.82	272.62	4.97	0.01
(0.41)	(0.34)			(0.41)										
-0.47				0.19	-0.12					5	-130.82	272.63	4.98	0.01
(0.42)				(0.41)	(0.09)									
0.37	-0.94	-0.15	-2.08	0.38	-0.26	-0.22				9	-125.80	272.75	5.10	0.01
(0.46)	(0.34)	(0.26)	(0.79)	(0.40)	(0.11)	(0.21)								
-0.36	-0.49				-0.12			0.09		6	-129.71	272.82	5.17	0.01
(0.38)	(0.33)				(0.09)			(0.14)						
-0.46	-0.50			0.23	-0.13					6	-129.73	272.86	5.21	0.01
(0.41)	(0.33)			(0.40)	(0.09)									
-0.34	-0.47	0.01								5	-130.99	272.96	5.31	0.01
(0.36)	(0.35)	(0.16)												
-0.30	-0.66	0.26			-0.23	-0.22				7	-128.58	273.05	5.40	0.01
(0.32)	(0.34)	(0.19)			(0.11)	(0.22)								
0.55	-0.71	-0.50	-2.07			-0.17				7	-128.62	273.13	5.49	0.01
(0.47)	(0.35)	(0.27)	(0.92)			(0.22)								

0.36	-0.89	-0.24	-2.20	0.42	-0.23				0.11	9	-126.01	273.17	5.52	0.01
(0.49)	(0.34)	(0.29)	(0.89)	(0.40)	(0.11)				(0.14)					
0.38	-0.70	-0.49	-2.10	0.29						7	-128.64	273.18	5.53	0.01
(0.52)	(0.35)	(0.28)	(0.92)	(0.40)										
0.42	-1.17	-0.17	-2.23	0.42	-0.27		0.50			9	-126.05	273.26	5.62	0.01
(0.47)	(0.47)	(0.26)	(0.79)	(0.40)	(0.11)		(0.70)							
0.48	-0.77	-0.31	-2.29	0.38	-0.21	-0.86	-0.55		0.48	11	-123.27	273.34	5.69	0.01
(0.52)	(0.69)	(0.30)	(0.95)	(0.38)	(0.10)	(0.45)	(1.31)		(0.20)					
-0.51	-0.65	0.29		0.35	-0.23					7	-128.73	273.35	5.71	0.01
(0.38)	(0.34)	(0.19)		(0.40)	(0.11)									
0.47	-0.58	-0.18	-1.91		-0.23	-0.40	-0.67			9	-126.12	273.39	5.75	0.01
(0.46)	(0.71)	(0.28)	(0.89)		(0.11)	(0.42)	(1.37)							
0.57	-0.92	-0.34	-2.45	0.39	-0.22	-0.74		-0.23	0.48	11	-123.30	273.39	5.75	0.01
(0.50)	(0.45)	(0.29)	(0.89)	(0.38)	(0.10)	(0.31)		(0.68)	(0.20)					
0.24	-1.15		-1.88	0.47	-0.30		0.51	-0.08		9	-126.26	273.67	6.02	0.01
(0.37)	(0.50)		(0.52)	(0.39)	(0.10)		(0.74)	(0.71)						
0.55	-0.71	-0.52	-2.12				0.07			7	-128.89	273.67	6.03	0.01
(0.49)	(0.48)	(0.28)	(0.94)				(0.71)							
-0.24	-0.63		-0.64	0.34						6	-130.14	273.69	6.04	0.01
(0.42)	(0.35)		(0.49)	(0.41)										
0.34	-0.89	-0.18	-2.11	0.40	-0.24			-0.03		9	-126.28	273.72	6.08	0.01
(0.48)	(0.44)	(0.28)	(0.83)	(0.40)	(0.11)			(0.67)						
0.13	-1.00		-1.74	0.49	-0.27			0.18	0.10	9	-126.29	273.74	6.09	0.00
(0.37)	(0.47)		(0.54)	(0.40)	(0.10)			(0.71)	(0.15)					
0.61	-1.61	-0.31	-2.66	0.48	-0.26		1.35		0.29	10	-124.94	273.81	6.16	0.00
(0.50)	(0.58)	(0.28)	(0.90)	(0.39)	(0.11)		(0.91)		(0.19)					
-0.35	-0.62	0.24			-0.21				0.07	7	-128.96	273.82	6.17	0.00
(0.35)	(0.34)	(0.19)			(0.11)				(0.14)					
-0.35			-0.33	0.24						5	-131.61	274.20	6.55	0.00
(0.45)			(0.49)	(0.41)										
0.15		-0.24	-1.58	0.26	-0.18					7	-129.19	274.27	6.62	0.00
(0.55)		(0.33)	(0.96)	(0.40)	(0.11)									
-0.49		0.17		0.25	-0.18					6	-130.44	274.28	6.63	0.00
(0.41)		(0.19)		(0.41)	(0.11)									
-0.44	-0.78	0.27		0.34	-0.21	-0.64			0.40	9	-126.56	274.28	6.64	0.00
(0.40)	(0.34)	(0.19)		(0.39)	(0.11)	(0.31)			(0.20)					
-0.12	-0.52		-0.54				-0.14			6	-130.45	274.31	6.66	0.00
(0.39)	(0.49)		(0.49)				(0.72)							
0.23		-0.48	-1.69	0.20						6	-130.46	274.31	6.67	0.00
(0.57)		(0.31)	(1.01)	(0.41)										
-0.46		-0.03		0.19						5	-131.79	274.57	6.92	0.00
(0.43)		(0.16)		(0.41)										
MEDIUM-TERM (LATE GROWTH, FGMt, N = 85)														
I	FGM	Qcoh	Pop(CH)	Sex(M)	Mass	FGM:Qcoh	FGM:Pop(CH)	FGM:Sex(M)	FGM:Mass	df	Log-lik	AICc	Delta	Weight
-0.54	0.35				-0.15					5	-133.70	278.15	0.00	0.16
(0.23)	(0.18)				(0.05)									

-0.52	0.26				-0.15				-0.08	6	-133.10	279.28	1.12	0.09
(0.23)	(0.20)				(0.05)				(0.08)					
-0.55					-0.15					4	-135.49	279.47	1.32	0.08
(0.24)					(0.05)									
-0.52	0.34			-0.04	-0.15					6	-133.68	280.45	2.29	0.05
(0.26)	(0.18)			(0.26)	(0.05)									
-0.56	0.36		0.04		-0.15					6	-133.69	280.46	2.30	0.05
(0.29)	(0.22)		(0.36)		(0.06)									
-0.54	0.34	0.01			-0.15					6	-133.69	280.46	2.31	0.05
(0.23)	(0.20)	(0.10)			(0.05)									
-0.41			-0.30		-0.17					5	-135.03	280.83	2.67	0.04
(0.27)			(0.31)		(0.06)									
-0.54		0.09			-0.17					5	-135.04	280.84	2.68	0.04
(0.23)		(0.09)			(0.05)									
-0.50				-0.13	-0.14					5	-135.36	281.49	3.33	0.03
(0.26)				(0.26)	(0.05)									
-0.49	0.25			-0.07	-0.14				-0.09	7	-133.07	281.59	3.44	0.03
(0.25)	(0.20)			(0.26)	(0.05)				(0.08)					
-0.55	0.28		0.07		-0.14				-0.09	7	-133.08	281.61	3.46	0.03
(0.28)	(0.23)		(0.36)		(0.06)				(0.08)					
-0.52	0.25	0.01			-0.15				-0.08	7	-133.09	281.64	3.49	0.03
(0.23)	(0.22)	(0.10)			(0.05)				(0.08)					
-0.62	0.52		0.10		-0.14		-0.23			7	-133.56	282.58	4.43	0.02
(0.31)	(0.38)		(0.37)		(0.06)		(0.46)							
-0.67	0.37	0.08	0.29		-0.14					7	-133.62	282.69	4.53	0.02
(0.39)	(0.22)	(0.20)	(0.69)		(0.06)									
-0.54	0.36		0.06	-0.05	-0.14					7	-133.67	282.80	4.65	0.02
(0.30)	(0.22)		(0.36)	(0.27)	(0.06)									
-0.52	0.34	0.01		-0.04	-0.15					7	-133.68	282.82	4.67	0.02
(0.26)	(0.21)	(0.11)		(0.27)	(0.06)									
-0.52	0.34			-0.04	-0.15			0.00		7	-133.68	282.82	4.67	0.02
(0.26)	(0.23)			(0.27)	(0.05)			(0.37)						
-0.53	0.33	0.01			-0.15	-0.02				7	-133.69	282.83	4.67	0.02
(0.25)	(0.22)	(0.11)			(0.05)	(0.14)								
-0.39			-0.28	-0.08	-0.17					6	-135.00	283.07	4.92	0.01
(0.28)			(0.31)	(0.27)	(0.06)									
-0.52		0.08		-0.07	-0.17					6	-135.01	283.10	4.94	0.01
(0.26)		(0.10)		(0.27)	(0.06)									
-0.47		0.05	-0.16		-0.17					6	-135.01	283.10	4.95	0.01
(0.37)		(0.20)	(0.66)		(0.06)									
-0.65	0.52		0.16		-0.12		-0.35		-0.10	8	-132.79	283.48	5.32	0.01
(0.31)	(0.38)		(0.37)		(0.06)		(0.46)		(0.08)					
-0.72	0.29	0.12	0.45		-0.13				-0.09	8	-132.91	283.71	5.56	0.01
(0.39)	(0.22)	(0.20)	(0.70)		(0.06)				(0.08)					
-0.53	0.28		0.09	-0.08	-0.13				-0.09	8	-133.04	283.97	5.82	0.01
(0.29)	(0.23)		(0.36)	(0.27)	(0.06)				(0.08)					

-0.49	0.23			-0.06	-0.14			0.06	-0.09	8	-133.06	284.01	5.85	0.01
(0.25)	(0.25)			(0.27)	(0.05)			(0.37)	(0.08)					
-0.49	0.25	0.01		-0.06	-0.14				-0.09	8	-133.07	284.03	5.87	0.01
(0.25)	(0.22)	(0.11)		(0.27)	(0.06)				(0.08)					
-0.51	0.25	0.01			-0.15	-0.01			-0.08	8	-133.09	284.08	5.92	0.01
(0.24)	(0.23)	(0.10)			(0.05)	(0.14)			(0.08)					
-0.80	0.52		0.56							5	-136.84	284.44	6.28	0.01
(0.30)	(0.21)		(0.31)											
-0.74	0.53	0.08	0.35		-0.13		-0.23			8	-133.49	284.87	6.71	0.01
(0.41)	(0.38)	(0.20)	(0.69)		(0.06)		(0.46)							
-0.61	0.51		0.10	-0.04	-0.13		-0.23			8	-133.55	285.00	6.84	0.01
(0.33)	(0.38)		(0.37)	(0.27)	(0.06)		(0.46)							
-0.66	0.37	0.08	0.29	-0.03	-0.14					8	-133.61	285.11	6.96	0.00
(0.41)	(0.22)	(0.20)	(0.69)	(0.27)	(0.06)									
-0.66	0.36	0.08	0.28		-0.14	-0.01				8	-133.61	285.12	6.97	0.00
(0.41)	(0.24)	(0.20)	(0.70)		(0.06)	(0.15)								

MEDIUM-TERM (LATE GROWTH, mean FGM, N = 63)

I	FGM	Qcoh	Pop(CH)	Sex(M)	Mass	FGM:Qcoh	FGM:Pop(CH)	FGM:Sex(M)	FGM:Mass	df	Log-lik	AICc	Delta	Weight
-0.47					-0.17					4	-97.55	203.78	0.00	0.16
(0.24)					(0.05)									
-0.39	-0.10				-0.12				-0.27	6	-95.24	203.98	0.20	0.14
(0.23)	(0.27)				(0.06)				(0.13)					
-0.46	0.16				-0.17					5	-97.33	205.71	1.93	0.06
(0.24)	(0.25)				(0.05)									
-0.56	0.04		0.35		-0.10				-0.27	7	-94.94	205.92	2.14	0.05
(0.32)	(0.32)		(0.42)		(0.06)				(0.13)					
-0.43				-0.09	-0.16					5	-97.50	206.06	2.28	0.05
(0.27)				(0.31)	(0.05)									
-0.47		0.03			-0.17					5	-97.51	206.08	2.30	0.05
(0.24)		(0.11)			(0.06)									
-0.47			0.01		-0.17					5	-97.54	206.14	2.36	0.05
(0.29)			(0.34)		(0.06)									
-0.39	-0.02	-0.07			-0.11				-0.27	7	-95.13	206.30	2.52	0.04
(0.24)	(0.32)	(0.14)			(0.06)				(0.13)					
-0.36	-0.12			-0.09	-0.12				-0.27	7	-95.20	206.43	2.65	0.04
(0.26)	(0.28)			(0.31)	(0.06)				(0.13)					
-0.60	0.28		0.27		-0.15					6	-97.15	207.81	4.03	0.02
(0.32)	(0.31)		(0.44)		(0.06)									
-0.88		0.28	0.85		-0.16					6	-97.19	207.88	4.10	0.02
(0.52)		(0.31)	(0.95)		(0.06)									
-0.46	0.21	-0.03			-0.17					6	-97.31	208.11	4.33	0.02
(0.24)	(0.32)	(0.14)			(0.06)									
-0.45	0.15			-0.05	-0.17					6	-97.32	208.14	4.36	0.02
(0.26)	(0.25)			(0.32)	(0.05)									
-0.79	0.03	0.16	0.81		-0.10				-0.26	8	-94.81	208.29	4.51	0.02
(0.51)	(0.32)	(0.31)	(0.93)		(0.06)				(0.13)					

-0.53	0.03		0.37	-0.12	-0.09					-0.28	8	-94.87	208.40	4.62	0.02
(0.33)	(0.32)		(0.43)	(0.31)	(0.07)					(0.13)					
-0.30	-0.15	-0.03			-0.12	-0.15				-0.26	8	-94.90	208.46	4.68	0.02
(0.27)	(0.38)	(0.15)			(0.07)	(0.22)				(0.13)					
-0.44		0.02		-0.08	-0.17						6	-97.48	208.47	4.69	0.02
(0.27)		(0.12)		(0.32)	(0.06)										
-0.38	-0.00			-0.14	-0.11				-0.39	-0.28	8	-94.91	208.48	4.70	0.02
(0.26)	(0.32)			(0.31)	(0.06)				(0.50)	(0.13)					
-0.45			0.04	-0.10	-0.16						6	-97.50	208.49	4.71	0.01
(0.30)			(0.35)	(0.32)	(0.06)										
-0.63	0.20		0.41		-0.09			-0.19		-0.28	8	-94.92	208.50	4.72	0.01
(0.43)	(0.77)		(0.52)		(0.07)			(0.85)		(0.13)					
-0.36	-0.03	-0.07		-0.10	-0.10					-0.28	8	-95.07	208.81	5.03	0.01
(0.26)	(0.33)	(0.14)		(0.31)	(0.07)					(0.13)					
-0.32	-0.03	0.01			-0.17	-0.24					7	-96.73	209.50	5.72	0.01
(0.28)	(0.38)	(0.15)			(0.06)	(0.22)									
-0.94	0.25	0.25	0.98		-0.15						7	-96.88	209.80	6.02	0.01
(0.52)	(0.31)	(0.32)	(0.96)		(0.06)										
-0.62	0.25		0.51	-0.20	-0.06				-0.55	-0.30	9	-94.31	210.01	6.23	0.01
(0.34)	(0.38)		(0.44)	(0.31)	(0.07)				(0.51)	(0.13)					
-0.52	0.09		0.20		-0.16			0.22			7	-97.12	210.28	6.49	0.01
(0.44)	(0.80)		(0.53)		(0.06)			(0.86)							
-0.58	0.27		0.29	-0.07	-0.15						7	-97.13	210.29	6.51	0.01
(0.34)	(0.32)		(0.44)	(0.32)	(0.06)										
-0.86		0.28	0.88	-0.10	-0.16						7	-97.14	210.32	6.54	0.01
(0.53)		(0.31)	(0.95)	(0.31)	(0.06)										
-0.46	0.23			-0.07	-0.17				-0.22		7	-97.23	210.49	6.71	0.01
(0.27)	(0.31)			(0.32)	(0.06)				(0.52)						
-0.44	0.20	-0.03		-0.05	-0.16						7	-97.29	210.62	6.84	0.01
(0.27)	(0.32)	(0.15)		(0.32)	(0.06)										
-0.37	0.15	-0.10		-0.17	-0.08				-0.47	-0.30	9	-94.66	210.71	6.93	0.00
(0.26)	(0.38)	(0.14)		(0.31)	(0.07)				(0.51)	(0.13)					

MEDIUM-TERM (PRIME AGE, FGMt, N = 90)

I	FGM	Qcoh	Pop(CH)	Sex(M)	Mass	FGM:Qcoh	FGM:Pop(CH)	FGM:Sex(M)	FGM:Mass	df	Log-lik	AICc	Delta	Weight
-0.19					-0.09					4	-145.49	299.46	0.00	0.12
(0.21)					(0.05)									
-0.19										3	-146.97	300.23	0.77	0.08
(0.21)														
-0.02			-0.37		-0.12					5	-144.96	300.64	1.19	0.07
(0.26)			(0.34)		(0.06)									
-0.19	0.14				-0.09					5	-145.03	300.77	1.31	0.06
(0.21)	(0.14)				(0.05)									
-0.20		0.07			-0.11					5	-145.25	301.22	1.76	0.05
(0.21)		(0.10)			(0.06)									
-0.24				0.12	-0.10					5	-145.40	301.50	2.05	0.04
(0.24)				(0.28)	(0.06)									

-0.19	0.13									4	-146.58	301.63	2.17	0.04
(0.21)	(0.15)													
-0.19		-0.04								4	-146.88	302.24	2.78	0.03
(0.21)		(0.09)												
-0.16				-0.09						4	-146.91	302.28	2.83	0.03
(0.23)				(0.26)										
-0.22			0.05							4	-146.96	302.39	2.93	0.03
(0.26)			(0.28)											
-0.18	0.11				-0.09				-0.04	6	-144.72	302.46	3.00	0.03
(0.22)	(0.15)				(0.05)				(0.05)					
-0.08			-0.41	0.18	-0.14					6	-144.77	302.55	3.09	0.03
(0.27)			(0.34)	(0.28)	(0.07)									
-0.07	0.09		-0.27		-0.11					6	-144.80	302.62	3.16	0.02
(0.27)	(0.16)		(0.37)		(0.06)									
0.11		-0.09	-0.63		-0.12					6	-144.88	302.78	3.32	0.02
(0.41)		(0.21)	(0.69)		(0.06)									
-0.20	0.12	0.04			-0.10					6	-144.97	302.94	3.49	0.02
(0.21)	(0.16)	(0.11)			(0.06)									
-0.22	0.13			0.07	-0.09					6	-145.00	303.01	3.55	0.02
(0.24)	(0.15)			(0.28)	(0.06)									
-0.27		0.09		0.17	-0.13					6	-145.08	303.17	3.71	0.02
(0.24)		(0.10)		(0.28)	(0.07)									
-0.18	0.17	-0.07								5	-146.28	303.28	3.82	0.02
(0.22)	(0.15)	(0.09)												
-0.13	0.15			-0.15						5	-146.42	303.55	4.09	0.02
(0.23)	(0.15)			(0.26)										
-0.27	0.16		0.18							5	-146.43	303.57	4.11	0.02
(0.27)	(0.16)		(0.30)											
0.01		-0.14	-0.40							5	-146.74	304.20	4.74	0.01
(0.42)		(0.21)	(0.70)											
-0.04	0.05		-0.30		-0.12				-0.05	7	-144.44	304.25	4.79	0.01
(0.28)	(0.16)		(0.37)		(0.06)				(0.05)					
-0.15		-0.03		-0.08						5	-146.83	304.38	4.92	0.01
(0.24)		(0.09)		(0.26)										
-0.17			0.04	-0.09						5	-146.90	304.51	5.05	0.01
(0.28)			(0.29)	(0.26)										
-0.20	0.22			0.09	-0.09			-0.27		7	-144.60	304.56	5.11	0.01
(0.25)	(0.18)			(0.28)	(0.05)			(0.30)						
-0.19	0.08	0.06			-0.11				-0.05	7	-144.60	304.57	5.11	0.01
(0.21)	(0.16)	(0.11)			(0.06)				(0.05)					
-0.22	0.09			0.10	-0.10				-0.05	7	-144.66	304.68	5.23	0.01
(0.25)	(0.15)			(0.29)	(0.05)				(0.05)					
-0.00	-0.05		-0.32		-0.12		0.19			7	-144.67	304.71	5.25	0.01
(0.30)	(0.32)		(0.38)		(0.06)		(0.36)							
-0.10	0.07		-0.33	0.14	-0.13					7	-144.70	304.76	5.30	0.01
(0.28)	(0.17)		(0.39)	(0.30)	(0.07)									

0.04		-0.07	-0.63	0.17	-0.14					7	-144.71	304.78	5.32	0.01
(0.42)		(0.21)	(0.69)	(0.29)	(0.07)									
0.06	0.09	-0.09	-0.52		-0.11					7	-144.72	304.81	5.36	0.01
(0.41)	(0.16)	(0.21)	(0.70)		(0.06)									
-0.12	0.24			-0.13					-0.28	6	-146.02	305.05	5.59	0.01
(0.24)	(0.18)			(0.26)					(0.31)					
-0.17	0.08	0.05			-0.10	-0.05				7	-144.86	305.09	5.64	0.01
(0.22)	(0.17)	(0.11)			(0.06)	(0.11)								
-0.24	0.10	0.05		0.11	-0.12					7	-144.90	305.16	5.71	0.01
(0.24)	(0.16)	(0.12)		(0.30)	(0.07)									
-0.12	0.19	-0.07		-0.14						6	-146.14	305.29	5.83	0.01
(0.24)	(0.16)	(0.09)		(0.26)										
-0.15	0.14	-0.06				-0.05				6	-146.19	305.39	5.93	0.01
(0.23)	(0.17)	(0.09)				(0.11)								
-0.05	0.16	-0.14	-0.25							6	-146.23	305.47	6.01	0.01
(0.42)	(0.16)	(0.21)	(0.70)											
-0.21	0.18		0.17	-0.14						6	-146.28	305.57	6.11	0.01
(0.29)	(0.16)		(0.30)	(0.26)										
-0.23	0.08		0.16					0.11		6	-146.38	305.77	6.32	0.01
(0.29)	(0.32)		(0.31)					(0.36)						
-0.08	0.01		-0.39	0.19	-0.14				-0.05	8	-144.24	306.26	6.80	0.00
(0.28)	(0.18)		(0.39)	(0.30)	(0.07)				(0.06)					
0.06		-0.15	-0.42	-0.09						6	-146.67	306.36	6.90	0.00
(0.44)		(0.21)	(0.70)	(0.26)										

MEDIUM-TERM (PRIME AGED, mean FGM, N = 71)

I	FGM	Qcoh	Pop(CH)	Sex(M)	Mass	FGM:Qcoh	FGM:Pop(CH)	FGM:Sex(M)	FGM:Mass	df	Log-lik	AICc	Delta	Weight
-0.28										3	-120.25	246.85	0.00	0.12
(0.22)														
-0.28					-0.08					4	-119.38	247.37	0.52	0.09
(0.23)					(0.06)									
-0.01			-0.55		-0.13					5	-118.52	247.96	1.11	0.07
(0.29)			(0.39)		(0.07)									
-0.30		0.12			-0.12					5	-118.88	248.69	1.84	0.05
(0.22)		(0.12)			(0.07)									
-0.28	-0.09									4	-120.17	248.94	2.09	0.04
(0.22)	(0.23)													
-0.22			-0.13							4	-120.18	248.97	2.12	0.04
(0.28)			(0.34)											
-0.24				-0.11						4	-120.19	248.98	2.13	0.04
(0.26)				(0.31)										
-0.29		0.01								4	-120.24	249.09	2.24	0.04
(0.22)		(0.10)												
0.14	-0.30		-0.82		-0.15					6	-117.89	249.10	2.25	0.04
(0.31)	(0.26)		(0.45)		(0.07)									
-0.34				0.13	-0.09					5	-119.31	249.54	2.69	0.03
(0.27)				(0.35)	(0.07)									

-0.28	-0.07				-0.08					5	-119.33	249.59	2.74	0.03
(0.23)	(0.23)				(0.06)									
-0.08			-0.60	0.23	-0.16					6	-118.32	249.95	3.10	0.03
(0.31)			(0.40)	(0.36)	(0.08)									
0.12		-0.08	-0.78		-0.13					6	-118.47	250.25	3.40	0.02
(0.49)		(0.24)	(0.80)		(0.07)									
-0.31	-0.23	0.18			-0.13					6	-118.49	250.29	3.44	0.02
(0.21)	(0.26)	(0.14)			(0.07)									
0.06	-0.37		-0.97	0.36	-0.20					7	-117.40	250.58	3.73	0.02
(0.32)	(0.27)		(0.46)	(0.37)	(0.09)									
-0.40		0.14		0.21	-0.15					6	-118.71	250.72	3.88	0.02
(0.27)		(0.12)		(0.36)	(0.08)									
-0.16	-0.17		-0.25							5	-119.98	250.88	4.04	0.02
(0.29)	(0.26)		(0.37)											
0.05		-0.16	-0.63							5	-120.00	250.92	4.07	0.02
(0.50)		(0.25)	(0.81)											
-0.16			-0.15	-0.12						5	-120.10	251.13	4.28	0.01
(0.32)			(0.34)	(0.31)										
-0.25	-0.08			-0.09						5	-120.13	251.18	4.33	0.01
(0.26)	(0.24)			(0.31)										
-0.29	-0.13	0.03								5	-120.13	251.18	4.33	0.01
(0.22)	(0.26)	(0.11)												
-0.24		0.01		-0.11						5	-120.18	251.28	4.43	0.01
(0.26)		(0.10)		(0.31)										
0.17	-0.32		-0.86		-0.16				-0.03	7	-117.84	251.45	4.60	0.01
(0.32)	(0.27)		(0.46)		(0.07)				(0.08)					
0.08	-0.17		-0.77		-0.15		-0.16			7	-117.86	251.50	4.65	0.01
(0.38)	(0.59)		(0.48)		(0.07)		(0.65)							
0.22	-0.29	-0.05	-0.97		-0.15					7	-117.87	251.52	4.67	0.01
(0.49)	(0.26)	(0.24)	(0.80)		(0.07)									
-0.34	-0.08			0.15	-0.09					6	-119.25	251.80	4.95	0.01
(0.28)	(0.23)			(0.35)	(0.07)									
-0.28	-0.07				-0.08				0.01	6	-119.33	251.98	5.13	0.01
(0.23)	(0.24)				(0.06)				(0.08)					
-0.46	-0.30	0.22		0.32	-0.18					7	-118.11	251.99	5.14	0.01
(0.27)	(0.27)	(0.14)		(0.37)	(0.09)									
-0.38	-0.17	0.16			-0.13	0.11				7	-118.27	252.32	5.47	0.01
(0.23)	(0.28)	(0.14)			(0.07)	(0.17)								
0.03		-0.06	-0.79	0.22	-0.15					7	-118.28	252.35	5.50	0.01
(0.51)		(0.25)	(0.79)	(0.36)	(0.08)									
-0.31	-0.26	0.19			-0.14				-0.02	7	-118.45	252.67	5.82	0.01
(0.22)	(0.27)	(0.14)			(0.08)				(0.09)					
0.10	-0.43		-1.06	0.41	-0.21				-0.05	8	-117.24	252.80	5.95	0.01
(0.33)	(0.29)		(0.49)	(0.37)	(0.09)				(0.09)					
0.10	-0.16	-0.15	-0.72							6	-119.81	252.93	6.08	0.01
(0.50)	(0.26)	(0.25)	(0.81)											

0.05	-0.43		-0.97	0.36	-0.20			0.17		8	-117.34	253.00	6.15	0.01
(0.32)	(0.31)		(0.46)	(0.37)	(0.09)			(0.48)						
0.01	-0.25		-0.92	0.36	-0.20		-0.15			8	-117.37	253.07	6.22	0.01
(0.38)	(0.59)		(0.50)	(0.37)	(0.09)		(0.65)							
-0.25	0.05		-0.17				-0.27			6	-119.90	253.10	6.26	0.01
(0.36)	(0.60)		(0.41)				(0.66)							
0.10	-0.37	-0.02	-1.04	0.36	-0.20					8	-117.40	253.12	6.27	0.01
(0.51)	(0.27)	(0.25)	(0.80)	(0.37)	(0.09)									
-0.36	-0.06	0.01					0.11			6	-119.91	253.13	6.28	0.01
(0.24)	(0.28)	(0.12)					(0.17)							
0.11		-0.16	-0.64	-0.12						6	-119.92	253.15	6.30	0.01
(0.52)		(0.25)	(0.81)	(0.31)										
-0.11	-0.16		-0.26	-0.11						6	-119.92	253.16	6.31	0.01
(0.33)	(0.26)		(0.38)	(0.31)										
-0.25	-0.12	0.04		-0.10						6	-120.08	253.47	6.62	0.00
(0.26)	(0.26)	(0.11)		(0.31)										
-0.25	-0.11			-0.09				0.10		6	-120.11	253.53	6.68	0.00
(0.26)	(0.28)			(0.31)				(0.49)						
0.13	-0.24	-0.05	-0.89		-0.15	0.08				8	-117.77	253.85	7.00	0.00
(0.53)	(0.28)	(0.24)	(0.81)		(0.07)	(0.17)								

Table S4. Linear mixed effects model (LMM) selection table for the long-term relationship between adult body mass and faecal glucocorticoid metabolites ('FGM') measured as juvenile. We accounted for cohort quality ('Qcoh'), population (reference level is Chizé: CH, and is compared to Trois-Fontaines: TF), and sex (reference level is male: M, and is compared to females: F). Values give the parameter coefficient and values between brackets in grey rows are the standard-errors. 'I' is the Intercept, 'df' is the number of parameters, 'Log-lik' is the log-likelihood, 'delta' is the difference of AICc between the candidate model and the model having the lowest AICc, and 'weight' the AIC weight of each model. Retained model is in bold. Only models up to delta AICc = 7 are displayed.

LONG-TERM (N = 345 obs., n = 159 ind.)												
I	FGM	Qcoh	Pop(CH)	Sex(M)	FGM:Qcoh	FGM:Pop(CH)	FGM:Sex(M)	df	Log-lik	AICc	Delta	Weight
-0.04		0.40						5	-652.34	1314.85	0.00	0.20
(0.28)		(0.20)										
-0.20		0.41	0.33					6	-651.82	1315.90	1.05	0.12
(0.32)		(0.20)	(0.32)									
0.04		0.39		-0.17				6	-652.20	1316.65	1.80	0.08
(0.32)		(0.20)		(0.32)								
-0.06								4	-654.27	1316.66	1.81	0.08
(0.29)												
-0.04	-0.04	0.40						6	-652.32	1316.88	2.03	0.07
(0.28)	(0.19)	(0.20)										
-0.10		0.40	0.35	-0.20				7	-651.64	1317.61	2.76	0.05
(0.35)		(0.20)	(0.33)	(0.32)								
-0.21			0.32					5	-653.80	1317.77	2.92	0.05
(0.33)			(0.33)									
-0.20	0.02	0.41	0.34					7	-651.82	1317.97	3.12	0.04

(0.33)	(0.20)	(0.20)	(0.34)									
0.05				-0.23				5	-654.03	1318.23	3.37	0.04
(0.33)				(0.32)								
-0.06	-0.04							5	-654.25	1318.68	3.82	0.03
(0.29)	(0.19)											
0.05	-0.04	0.39		-0.17				7	-652.18	1318.70	3.85	0.03
(0.32)	(0.19)	(0.20)		(0.32)								
-0.04	-0.04	0.41			-0.09			7	-652.26	1318.85	4.00	0.03
(0.28)	(0.19)	(0.20)			(0.26)							
-0.09			0.34	-0.26				6	-653.49	1319.22	4.37	0.02
(0.36)			(0.33)	(0.33)								
-0.11	0.03	0.40	0.36	-0.20				8	-651.63	1319.69	4.84	0.02
(0.36)	(0.20)	(0.20)	(0.34)	(0.32)								
-0.22	0.02		0.33					6	-653.80	1319.84	4.99	0.02
(0.33)	(0.20)		(0.34)									
-0.20	0.02	0.42	0.34		-0.10			8	-651.75	1319.94	5.08	0.02
(0.33)	(0.20)	(0.20)	(0.34)		(0.26)							
-0.19	-0.03	0.41	0.33			0.07		8	-651.81	1320.04	5.19	0.01
(0.34)	(0.35)	(0.20)	(0.35)			(0.42)						
0.04	0.11	0.40		-0.14			-0.29	8	-651.89	1320.21	5.36	0.01
(0.32)	(0.27)	(0.20)		(0.32)			(0.37)					
0.06	-0.04			-0.23				6	-654.01	1320.26	5.41	0.01
(0.33)	(0.19)			(0.33)								
0.04	-0.04	0.40		-0.16	-0.08			8	-652.14	1320.71	5.85	0.01
(0.32)	(0.19)	(0.21)		(0.32)	(0.26)							
-0.13	0.19	0.41	0.38	-0.17			-0.31	9	-651.28	1321.10	6.24	0.01
(0.36)	(0.28)	(0.20)	(0.34)	(0.32)			(0.37)					
-0.10	0.03		0.36	-0.26				7	-653.48	1321.29	6.44	0.01
(0.37)	(0.20)		(0.35)	(0.33)								
-0.12	0.02	0.40	0.36	-0.19	-0.08			9	-651.58	1321.70	6.84	0.01
(0.36)	(0.20)	(0.21)	(0.34)	(0.32)	(0.26)							
-0.09	-0.03	0.39	0.34	-0.20		0.08		9	-651.61	1321.76	6.91	0.01
(0.37)	(0.35)	(0.20)	(0.35)	(0.32)		(0.42)						

***Appendix C – Supplementary information: N:L ratios and
body mass***

Supporting Information to:

*Neutrophil:Lymphocyte ratio reflects body condition and growth rates
rather than stress response in a wild ungulate (in preparation)*

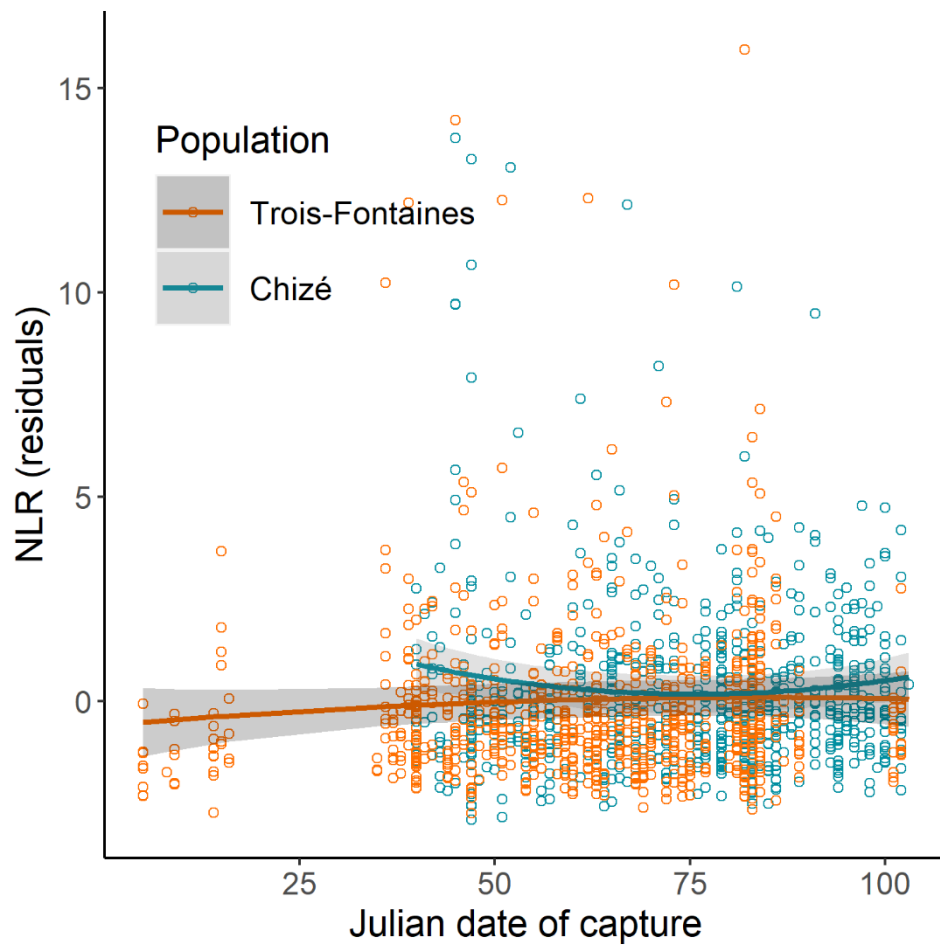


Figure S1. Seasonal variation of Neutrophil:Lymphocyte (N:L) ratio according to Julian date of capture (starting early December, ending early March) and the population (Trois-Fontaines: orange, Chizé: blue). N:L ratio increase across the capture season in TF and decreased down to mid-season and increased afterwards in CH. Points are residuals of the N:L ratios over the time between capture and sampling, and lines are predictions from the selected model with 95% confidence intervals.

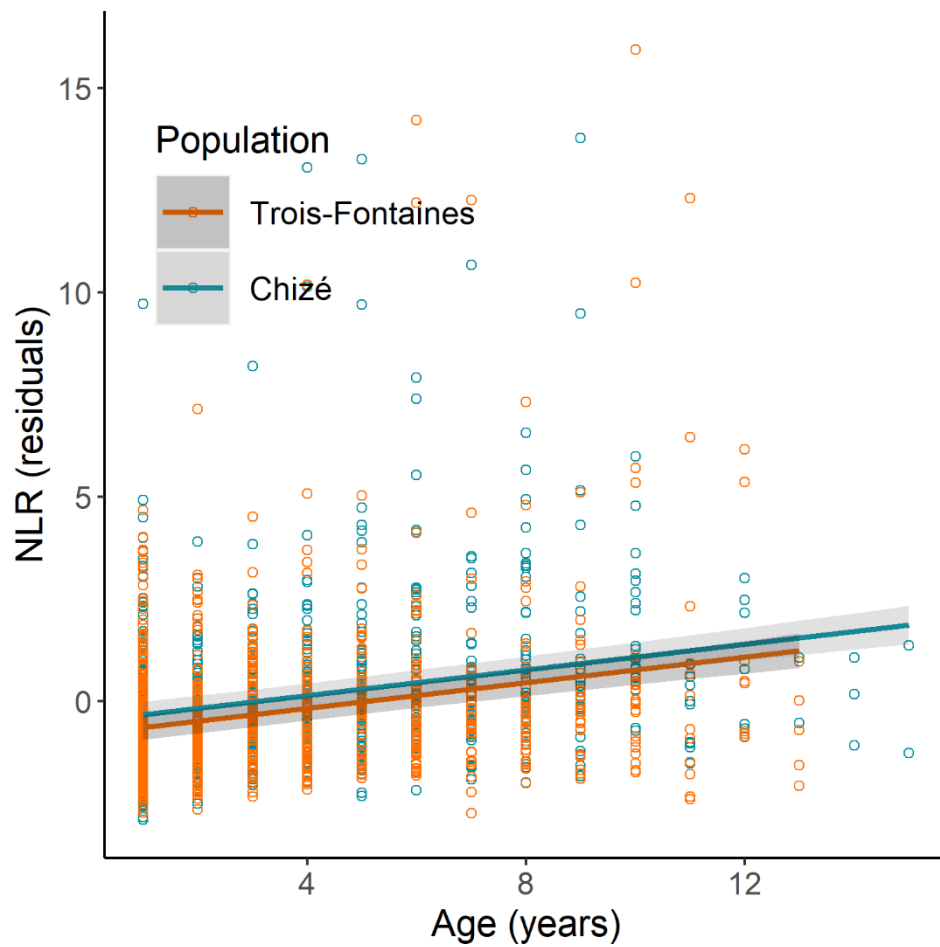


Figure S2. Age variation of Neutrophil:Lymphocyte (N:L) ratio in the two populations of Trois-Fontaines (TF, orange) and Chizé (CH, blue). Points are residuals of the N:L ratios over the time between capture and sampling, and lines are predictions from the selected model with 95% confidence intervals.

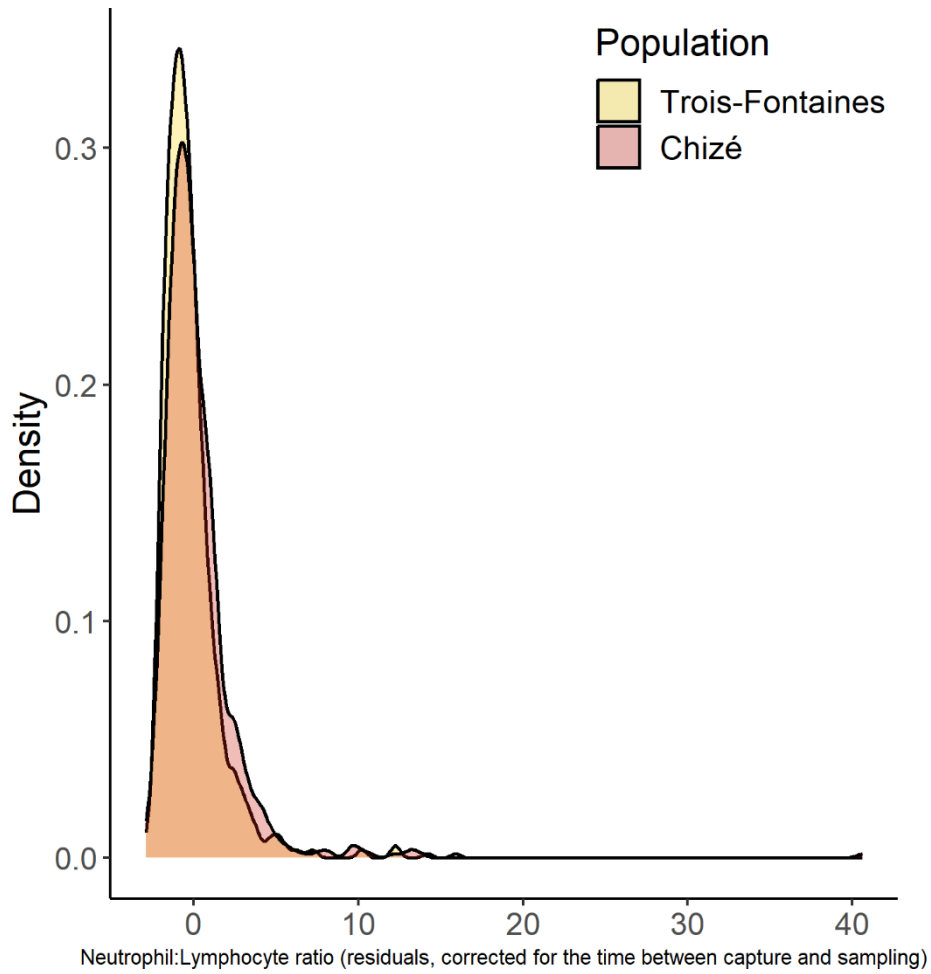


Figure S3. Density plot of the residuals of Neutrophil:Lymphocyte (N:L) ratio over the time between capture and blood sampling, according to population (Trois-Fontaines TF in yellow, Chizé CH in orange), evidencing a particularly high N:L ratio.

Table S1. Linear mixed effect model selected for the short-term relationship between relative body mass and Neutrophil:Lymphocyte (N:L) ratio, including one high N:L ratio measured on a 3 years old female from Chizé. Models accounted for sex, population and for cohort quality. Models were selected through model selection based on AICc and parameters estimated through model averaging. 95%CI: 95% confidence interval, V: variance, SD: standard-deviation.

Short-term		
Adults		
Random effects	V	SD
Individual ID	3.44	1.86
Year of capture	0.30	0.55
Fixed effects	Estimate	95% CI
Intercept	-0.07	[-0.42, 0.29]
Marginal R ²	-	
Conditional R ²	0.80	

Table S2. Linear mixed effects model (LMM) selection table for the short-term relationship between relative body mass for juveniles and adults (*i.e.* aged 2 years old and older) and concomitant Neutrophil:Lymphocyte ratio ('NLR'). We accounted for cohort quality ('Qcoh'), population (reference level is Chizé: CH, and is compared to Trois-Fontaines: TF), and sex (reference level is male: M, and is compared to females: F). Values give the parameter coefficient and values between brackets are the standard-errors. '1' is the Intercept, 'df' is the number of parameters, 'Log-lik' is the log-likelihood, 'Delta' is the difference of AICc between the candidate model and the model having the lowest AICc, and 'w' the AIC weight of each model. Retained model is in bold. Only models up to delta AICc = 7 are displayed.

SHORT-TERM (Juveniles, N = 470 observations and individuals)												
l	NLR	Qcoh	Pop(CH)	Sex(M)	NLR:Qcoh	NLR:pop(CH)	NLR:sex(M)	df	Log-lik	AICc	Delta	w
-0.04 (0.13)	0.27 (0.10)	0.95 (0.11)	0.11 (0.20)			-0.25 (0.12)		6	-1016.64	2045.45	0.00	0.16
0.03 (0.10)	0.10 (0.06)	0.97 (0.11)						4	-1019.05	2046.19	0.74	0.11
-0.04 (0.13)	0.25 (0.10)	0.94 (0.11)	0.13 (0.20)		0.09 (0.08)	-0.25 (0.12)		7	-1015.98	2046.21	0.76	0.11
0.03 (0.10)	0.09 (0.06)	0.96 (0.11)			0.09 (0.08)			5	-1018.45	2047.03	1.58	0.07
0.03 (0.10)		0.97 (0.11)						3	-1020.55	2047.15	1.70	0.07
-0.09 (0.16)	0.26 (0.10)	0.95 (0.11)	0.11 (0.20)	0.10 (0.20)		-0.25 (0.12)		7	-1016.52	2047.28	1.83	0.06
-0.02 (0.13)	0.10 (0.06)	0.97 (0.11)	0.11 (0.20)					5	-1018.91	2047.94	2.49	0.04
-0.03 (0.15)	0.10 (0.06)	0.97 (0.11)		0.10 (0.20)				5	-1018.92	2047.97	2.52	0.04
-0.10 (0.16)	0.25 (0.10)	0.94 (0.12)	0.13 (0.20)	0.10 (0.20)	0.09 (0.08)	-0.25 (0.12)		8	-1015.84	2048.00	2.55	0.04
-0.09 (0.16)	0.33 (0.11)	0.95 (0.11)	0.11 (0.20)	0.09 (0.20)		-0.25 (0.12)	-0.12 (0.11)	8	-1015.89	2048.10	2.65	0.04
-0.02 (0.13)	0.09 (0.06)	0.96 (0.11)	0.13 (0.20)		0.09 (0.08)			6	-1018.25	2048.68	3.23	0.03
-0.03 (0.15)	0.17 (0.08)	0.97 (0.11)		0.10 (0.20)			-0.13 (0.11)	6	-1018.27	2048.72	3.26	0.03
-0.03 (0.15)	0.09 (0.06)	0.96 (0.12)		0.11 (0.20)	0.09 (0.08)			6	-1018.29	2048.77	3.31	0.03
-0.03 (0.15)		0.97 (0.11)		0.11 (0.20)				4	-1020.40	2048.89	3.43	0.03
-0.10 (0.16)	0.32 (0.11)	0.94 (0.12)	0.13 (0.20)	0.10 (0.20)	0.09 (0.08)	-0.25 (0.12)	-0.12 (0.11)	9	-1015.25	2048.90	3.45	0.03
-0.00 (0.13)		0.97 (0.11)	0.08 (0.20)					4	-1020.48	2049.04	3.59	0.03
-0.03 (0.15)	0.15 (0.08)	0.96 (0.12)		0.11 (0.20)	0.09 (0.08)		-0.12 (0.11)	7	-1017.68	2049.59	4.14	0.02
-0.07 (0.17)	0.10 (0.06)	0.97 (0.11)	0.10 (0.20)	0.10 (0.20)				6	-1018.79	2049.76	4.30	0.02
-0.08 (0.17)	0.09 (0.06)	0.96 (0.12)	0.12 (0.20)	0.10 (0.20)	0.09 (0.08)			7	-1018.11	2050.46	5.01	0.01
-0.07 (0.16)	0.17 (0.08)	0.97 (0.11)	0.10 (0.20)	0.10 (0.20)			-0.13 (0.11)	7	-1018.13	2050.50	5.05	0.01
-0.06 (0.17)		0.97 (0.11)	0.07 (0.20)	0.10 (0.20)				5	-1020.34	2050.81	5.35	0.01

	0.16 (0.08)	0.96 (0.12)	0.12 (0.20)	0.10 (0.20)	0.09 (0.08)		-0.12 (0.11)	8	-1017.49	2051.29	5.84	0.01
SHORT-TERM (Adults, N = 1033 observations, n = 488 individuals)												
I	NLR	Qcoh	Pop(CH)	Sex(M)	NLR:Qcoh	NLR:pop(CH)	NLR:sex(M)	df	Log-lik	AICc	Delta	w
-0.05 (0.18)		0.24 (0.11)						5	-1939.74	3889.53	0.00	0.21
-0.06 (0.18)	-0.00 (0.02)	0.22 (0.11)			-0.04 (0.02)			7	-1938.49	3891.09	1.57	0.09
-0.07 (0.20)		0.24 (0.11)	0.04 (0.18)					6	-1939.71	3891.51	1.98	0.08
-0.06 (0.19)		0.24 (0.11)		0.01 (0.18)				6	-1939.73	3891.55	2.02	0.08
-0.05 (0.18)	-0.00 (0.02)	0.24 (0.11)						6	-1939.73	3891.55	2.02	0.07
-0.06 (0.18)								4	-1942.08	3892.20	2.67	0.05
-0.06 (0.20)	0.03 (0.03)	0.22 (0.11)		0.01 (0.18)	-0.05 (0.02)		-0.06 (0.04)	9	-1937.24	3892.65	3.12	0.04
-0.07 (0.20)	-0.00 (0.02)	0.22 (0.11)	0.02 (0.18)		-0.04 (0.02)			8	-1938.48	3893.11	3.58	0.03
-0.06 (0.20)	-0.00 (0.02)	0.22 (0.11)		0.01 (0.18)	-0.04 (0.02)			8	-1938.49	3893.12	3.59	0.03
-0.08 (0.21)		0.24 (0.11)	0.04 (0.18)	0.01 (0.18)				7	-1939.71	3893.53	4.00	0.03
-0.07 (0.20)	-0.00 (0.02)	0.24 (0.11)	0.04 (0.18)					7	-1939.71	3893.53	4.01	0.03
-0.06 (0.19)	-0.00 (0.02)	0.24 (0.11)		0.01 (0.18)				7	-1939.73	3893.57	4.04	0.03
-0.09 (0.20)			0.06 (0.18)					5	-1942.03	3894.12	4.59	0.02
-0.06 (0.18)	-0.00 (0.02)							5	-1942.08	3894.22	4.69	0.02
-0.07 (0.20)				0.00 (0.18)				5	-1942.08	3894.22	4.69	0.02
-0.06 (0.20)	0.02 (0.03)	0.22 (0.11)	0.02 (0.18)		-0.03 (0.02)	-0.03 (0.04)		9	-1938.09	3894.35	4.82	0.02
-0.06 (0.19)	0.02 (0.03)	0.24 (0.11)		0.01 (0.18)			-0.04 (0.04)	8	-1939.14	3894.43	4.90	0.02
-0.07 (0.20)	0.02 (0.03)	0.24 (0.11)	0.04 (0.18)			-0.04 (0.04)		8	-1939.17	3894.48	4.95	0.02
-0.07 (0.22)	0.03 (0.03)	0.22 (0.11)	0.02 (0.18)	0.01 (0.18)	-0.05 (0.02)		-0.06 (0.04)	10	-1937.23	3894.68	5.15	0.02
-0.07 (0.22)	-0.00 (0.02)	0.22 (0.11)	0.02 (0.18)	0.01 (0.18)	-0.04 (0.02)			9	-1938.48	3895.14	5.61	0.01
-0.08 (0.21)	-0.00 (0.02)	0.24 (0.11)	0.04 (0.18)	0.01 (0.18)				8	-1939.71	3895.56	6.03	0.01
-0.06 (0.21)	0.05 (0.03)	0.22 (0.11)	0.02 (0.18)	0.01 (0.18)	-0.05 (0.02)	-0.04 (0.04)	-0.06 (0.04)	11	-1936.75	3895.75	6.22	0.01
-0.09 (0.20)	-0.00 (0.02)		0.06 (0.18)					6	-1942.03	3896.14	6.61	0.01
-0.09 (0.22)			0.06 (0.18)	0.00 (0.18)				6	-1942.03	3896.14	6.62	0.01
-0.07 (0.20)	-0.00 (0.02)			0.00 (0.18)				6	-1942.08	3896.24	6.71	0.01
-0.07 (0.21)	0.02 (0.03)	0.22 (0.11)	0.02 (0.18)	0.01 (0.18)	-0.03 (0.02)	-0.03 (0.04)		1	-1938.09	3896.39	6.86	0.01
-0.08 (0.21)	0.02 (0.03)	0.24 (0.11)	0.04 (0.18)	0.01 (0.18)			-0.04 (0.04)	9	-1939.12	3896.42	6.89	0.01

-0.07 (0.21)	0.02 (0.03)	0.24 (0.11)	0.04 (0.18)	0.02 (0.18)	-0.04 (0.04)	9	-1939.16	3896.50	6.98	0.01
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Table S3. Linear mixed effects model (LMM) selection table for the medium-term relationship between the change of body mass between two consecutive years for early-growing (*i.e.* between the first and second years of life), late-growing (*i.e.* between 2 and 4 years old) and prime age (*i.e.* between 4 and 10 years old) individuals, and Neutrophil:Lymphocyte ratio ('NLR') measured age t ('NLR t ') or NLR considered as the mean NLR value of the NLR measured at age t and age $t+1$ ('mean NLR'). We accounted for cohort quality ('Qcoh'), population (reference level is Chizé: CH, and is compared to Trois-Fontaines: TF), and sex (reference level is male: M, and is compared to females: F) and the initial body mass (*i.e.* measured year t , 'Mass'). Values give the parameter coefficient and values between brackets are the standard-errors. 'I' is the Intercept, 'df' is the number of parameters, 'Log-lik' is the log-likelihood, 'Delta' is the difference of AICc between the candidate model and the model having the lowest AICc, and 'w' the AIC weight of each model. Retained model is in bold. Only models up to delta AICc = 7 are displayed.

MEDIUM-TERM (Early growth, NLRt, N = 105 observations and individuals)														
I	NLR	Qcoh	Pop(CH)	Sex(M)	Mass	NLR:Qcoh	NLR:pop(CH)	NLR:sex(M)	NLR:Mass	df	Log lik	AICc	Delta	w
0.21 (0.27)	0.09 (0.09)		-1.25 (0.38)		-0.14 (0.08)				-0.09 (0.04)	7	-199.44	414.04	0.00	0.13
0.26 (0.26)			-1.34 (0.39)		-0.19 (0.08)					5	-201.87	414.35	0.31	0.11
0.26 (0.27)	0.08 (0.09)		-1.35 (0.39)		-0.19 (0.08)					6	-201.48	415.81	1.77	0.05
0.21 (0.27)	0.20 (0.20)		-1.24 (0.38)		-0.14 (0.08)		-0.16 (0.25)		-0.11 (0.05)	8	-199.24	415.98	1.94	0.05
0.27 (0.31)	0.08 (0.09)		-1.24 (0.39)	-0.12 (0.31)	-0.14 (0.08)				-0.09 (0.04)	8	-199.36	416.23	2.19	0.04
0.35 (0.30)			-1.31 (0.39)	-0.19 (0.32)	-0.19 (0.08)					6	-201.70	416.27	2.23	0.04
0.21 (0.38)	0.09 (0.09)	0.00 (0.25)	-1.25 (0.78)		-0.14 (0.09)				-0.09 (0.04)	8	-199.44	416.38	2.35	0.04
-0.30 (0.23)	0.10 (0.09)	0.35 (0.13)			-0.15 (0.09)				-0.10 (0.04)	7	-200.69	416.53	2.50	0.04
0.32 (0.37)		-0.06 (0.25)	-1.48 (0.76)		-0.18 (0.09)					6	-201.85	416.55	2.51	0.04
0.27 (0.38)	0.17 (0.11)	-0.04 (0.25)	-1.31 (0.77)		-0.13 (0.09)	0.11 (0.08)			-0.13 (0.05)	9	-198.50	416.90	2.86	0.03
-0.27 (0.23)	0.18 (0.11)	0.33 (0.13)			-0.14 (0.09)	0.10 (0.08)			-0.14 (0.05)	8	-199.92	417.34	3.30	0.02
0.04 (0.27)			-0.84 (0.34)							4	-204.50	417.40	3.36	0.02
0.25 (0.27)	-0.01 (0.18)		-1.34 (0.39)		-0.19 (0.08)		0.14 (0.21)			7	-201.27	417.70	3.66	0.02
-0.29 (0.23)		0.35 (0.13)			-0.19 (0.09)					5	-203.64	417.88	3.84	0.02
0.33 (0.31)	0.08 (0.09)		-1.33 (0.39)	-0.15 (0.32)	-0.19 (0.08)					7	-201.37	417.89	3.85	0.02
0.29 (0.38)	0.08 (0.09)	-0.03 (0.25)	-1.43 (0.77)		-0.19 (0.09)					7	-201.47	418.10	4.06	0.02

0.27 (0.31)	0.19 (0.20)		-1.23 (0.39)	-0.12 (0.31)	-0.14 (0.08)				-0.16 (0.25)				-0.11 (0.05)	9	-199.17	418.24	4.20	0.02
0.20 (0.38)	0.20 (0.20)	0.01 (0.25)	-1.21 (0.78)		-0.14 (0.09)				-0.16 (0.25)				-0.11 (0.05)	9	-199.24	418.37	4.34	0.01
0.44 (0.42)		-0.08 (0.25)	-1.52 (0.77)	-0.20 (0.32)	-0.18 (0.09)									7	-201.65	418.46	4.42	0.01
0.26 (0.31)	0.10 (0.13)		-1.24 (0.39)	-0.12 (0.31)	-0.14 (0.08)							-0.04 (0.18)	-0.09 (0.04)	9	-199.34	418.58	4.54	0.01
0.33 (0.39)		-0.25 (0.24)	-1.57 (0.80)											5	-204.00	418.60	4.56	0.01
0.29 (0.43)	0.08 (0.09)	-0.02 (0.26)	-1.28 (0.78)	-0.13 (0.32)	-0.14 (0.09)								-0.09 (0.04)	9	-199.36	418.62	4.58	0.01
-0.26 (0.29)	0.10 (0.09)	0.35 (0.13)		-0.07 (0.32)	-0.14 (0.09)								-0.10 (0.04)	8	-200.66	418.82	4.79	0.01
0.33 (0.38)	0.03 (0.24)	-0.07 (0.25)	-1.42 (0.78)		-0.13 (0.09)	0.17 (0.12)	0.28 (0.40)						-0.13 (0.05)	10	-198.26	418.86	4.82	0.01
0.04 (0.27)	0.07 (0.10)		-0.85 (0.34)											5	-204.21	419.03	4.99	0.01
0.17 (0.31)			-0.83 (0.34)	-0.25 (0.32)										5	-204.21	419.03	5.00	0.01
-0.30 (0.23)	0.10 (0.10)	0.37 (0.13)			-0.20 (0.09)									6	-203.11	419.08	5.04	0.01
0.34 (0.43)	0.17 (0.11)	-0.05 (0.25)	-1.34 (0.77)	-0.12 (0.32)	-0.13 (0.09)	0.10 (0.08)							-0.13 (0.05)	10	-198.44	419.21	5.17	0.01
-0.24 (0.29)	0.17 (0.11)	0.33 (0.13)		-0.06 (0.32)	-0.13 (0.09)	0.10 (0.08)							-0.14 (0.05)	9	-199.90	419.70	5.66	0.01
0.33 (0.31)	-0.02 (0.18)		-1.32 (0.39)	-0.15 (0.32)	-0.19 (0.08)				0.14 (0.21)					8	-201.16	419.82	5.78	0.01
-0.22 (0.29)		0.34 (0.13)		-0.15 (0.33)	-0.18 (0.09)									6	-203.53	419.93	5.89	0.01
0.28 (0.38)	-0.01 (0.18)	-0.03 (0.25)	-1.42 (0.77)		-0.19 (0.09)				0.14 (0.21)					8	-201.27	420.03	5.99	0.01
0.51 (0.44)		-0.27 (0.24)	-1.63 (0.80)	-0.29 (0.32)										6	-203.61	420.07	6.04	0.01
-0.31 (0.25)		0.19 (0.11)												4	-205.84	420.09	6.05	0.01
0.32 (0.31)	0.11 (0.13)		-1.33 (0.39)	-0.15 (0.32)	-0.19 (0.08)							-0.06 (0.19)		8	-201.31	420.12	6.09	0.01
0.39 (0.43)	0.08 (0.10)	-0.05 (0.25)	-1.46 (0.77)	-0.16 (0.33)	-0.18 (0.09)									8	-201.34	420.19	6.15	0.01
0.31 (0.40)	0.06 (0.10)	-0.23 (0.25)	-1.54 (0.81)											6	-203.77	420.41	6.37	0.01
0.28 (0.38)	0.08 (0.11)	-0.03 (0.25)	-1.42 (0.78)		-0.19 (0.09)	-0.01 (0.06)								8	-201.46	420.43	6.39	0.01
0.26 (0.31)	0.22 (0.23)		-1.23 (0.39)	-0.11 (0.31)	-0.14 (0.08)				-0.16 (0.25)				-0.11 (0.05)	10	-199.14	420.62	6.58	0.00
0.04 (0.27)	-0.05 (0.18)		-0.85 (0.34)						0.17 (0.22)					6	-203.91	420.67	6.64	0.00
0.27 (0.43)	0.19 (0.20)	-0.01 (0.26)	-1.24 (0.78)	-0.12 (0.32)	-0.14 (0.09)				-0.16 (0.25)				-0.11 (0.05)	10	-199.17	420.68	6.65	0.00
0.15 (0.32)	0.07 (0.10)		-0.84 (0.34)	-0.22 (0.33)										6	-203.98	420.82	6.79	0.00
-0.32 (0.25)														3	-207.39	421.02	6.98	0.00
0.29 (0.43)	0.10 (0.13)	-0.02 (0.26)	-1.29 (0.78)	-0.13 (0.32)	-0.14 (0.09)							-0.04 (0.18)	-0.09 (0.04)	10	-199.34	421.02	6.98	0.00

MEDIUM-TERM (Early growth, mean NLR, N = 93 observations and individuals)

I	NLR	Pop(CH)	Qcoh	Sex(M)	Mass	NLR:Qcoh	NLR:pop(CH)	NLR:sex(M)	NLR:mass	df	Log lik	AICc	Delta	w
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I	NLR	Qcoh	Pop(CH)	Sex(M)	Mass	NLR:Qcoh	NLR:pop(CH)	NLR:sex(M)	NLR:mass	df	Log-lik	AICc	Delta	w
-0.34 (0.14)					-0.18 (0.05)					4	-189.00	386.37	0.00	0.12
-0.28 (0.17)	-0.25 (0.11)			-0.24 (0.25)	-0.17 (0.05)			0.26 (0.13)		7	-185.90	386.87	0.50	0.09
-0.34 (0.14)	-0.08 (0.06)				-0.18 (0.05)					5	-188.27	387.10	0.73	0.08
-0.24 (0.17)				-0.27 (0.26)	-0.17 (0.05)					5	-188.44	387.45	1.08	0.07
-0.26 (0.19)			-0.17 (0.29)		-0.19 (0.05)					5	-188.84	388.25	1.88	0.05
-0.34 (0.14)		0.02 (0.09)			-0.18 (0.05)					5	-188.97	388.50	2.13	0.04
-0.25 (0.17)	-0.07 (0.06)			-0.24 (0.26)	-0.17 (0.05)					6	-187.85	388.51	2.13	0.04
-0.26 (0.17)	-0.26 (0.11)	-0.06 (0.10)		-0.29 (0.27)	-0.15 (0.06)			0.28 (0.13)		8	-185.72	388.84	2.47	0.04
-0.31 (0.20)	-0.25 (0.11)		0.08 (0.30)	-0.26 (0.26)	-0.16 (0.06)			0.27 (0.13)		8	-185.87	389.13	2.76	0.03
-0.28 (0.19)	-0.07 (0.06)		-0.13 (0.29)		-0.20 (0.05)					6	-188.17	389.15	2.78	0.03
-0.28 (0.17)	-0.24 (0.11)			-0.24 (0.26)	-0.17 (0.05)			0.26 (0.13)	0.00 (0.02)	8	-185.88	389.15	2.78	0.03
-0.34 (0.14)	-0.07 (0.06)				-0.18 (0.05)				0.01 (0.02)	6	-188.22	389.24	2.87	0.03
-0.34 (0.14)	-0.08 (0.06)	0.02 (0.09)			-0.19 (0.05)					6	-188.25	389.30	2.93	0.03
-0.21 (0.20)			-0.09 (0.30)	-0.25 (0.27)	-0.18 (0.06)					6	-188.40	389.61	3.23	0.02
-0.24 (0.18)		-0.01 (0.10)		-0.28 (0.27)	-0.16 (0.06)					6	-188.44	389.68	3.31	0.02
-0.13 (0.30)		-0.10 (0.19)	-0.47 (0.61)		-0.19 (0.05)					6	-188.71	390.22	3.85	0.02
-0.25 (0.17)	-0.07 (0.06)			-0.23 (0.26)	-0.17 (0.05)				0.01 (0.02)	7	-187.83	390.73	4.36	0.01
-0.23 (0.20)	-0.07 (0.06)		-0.06 (0.30)	-0.22 (0.27)	-0.18 (0.06)					7	-187.83	390.74	4.37	0.01
-0.25 (0.17)	-0.07 (0.06)	-0.01 (0.10)		-0.25 (0.27)	-0.17 (0.06)					7	-187.85	390.77	4.40	0.01
-0.25 (0.17)	-0.24 (0.11)	-0.06 (0.10)		-0.32 (0.27)	-0.15 (0.06)	0.03 (0.05)		0.28 (0.13)		9	-185.54	390.85	4.48	0.01
-0.11 (0.32)	-0.25 (0.11)	-0.15 (0.19)	-0.35 (0.61)	-0.30 (0.27)	-0.15 (0.06)			0.28 (0.13)		9	-185.57	390.91	4.54	0.01
-0.26 (0.17)	-0.26 (0.11)	-0.06 (0.10)		-0.29 (0.27)	-0.15 (0.06)			0.28 (0.13)	0.01 (0.02)	9	-185.68	391.13	4.76	0.01
-0.17 (0.30)	-0.07 (0.06)	-0.08 (0.19)	-0.37 (0.61)		-0.20 (0.05)					7	-188.09	391.25	4.88	0.01
-0.28 (0.19)	-0.07 (0.06)		-0.12 (0.30)		-0.20 (0.05)				0.01 (0.02)	7	-188.14	391.36	4.99	0.01
-0.02 (0.32)		-0.14 (0.19)	-0.49 (0.61)	-0.29 (0.27)	-0.18 (0.06)					7	-188.16	391.40	5.03	0.01
-0.28 (0.19)	-0.09 (0.11)		-0.13 (0.30)		-0.19 (0.05)		0.02 (0.14)			7	-188.16	391.40	5.03	0.01
-0.31 (0.20)	-0.25 (0.11)		0.09 (0.31)	-0.25 (0.26)	-0.16 (0.06)			0.27 (0.13)	0.01 (0.02)	9	-185.84	391.45	5.07	0.01
-0.33 (0.14)	-0.06 (0.07)	0.02 (0.09)			-0.19 (0.05)	0.02 (0.05)				7	-188.19	391.46	5.09	0.01
-0.30 (0.21)	-0.24 (0.14)		0.08 (0.31)	-0.26 (0.26)	-0.16 (0.06)		-0.01 (0.14)	0.27 (0.13)		9	-185.86	391.49	5.12	0.01

-0.03 (0.37)	-0.25 (0.17)	-0.25 (0.23)	-0.38 (0.73)		-0.14 (0.06)		0.03 (0.24)		0.11 (0.04)	9	-148.37	316.94	5.01	0.01
0.36 (0.40)	-0.69 (0.26)	-0.45 (0.24)	-0.95 (0.73)		-0.14 (0.06)	0.41 (0.18)	1.07 (0.53)		0.09 (0.05)	11	-145.86	317.03	5.10	0.01
0.30 (0.38)	-0.63 (0.26)	-0.42 (0.24)	-1.04 (0.74)		-0.17 (0.06)	0.45 (0.19)	0.94 (0.54)			9	-148.58	317.35	5.43	0.01
-0.07 (0.21)	-0.20 (0.11)	-0.13 (0.10)			-0.15 (0.06)	0.15 (0.07)				8	-149.94	317.62	5.69	0.01
-0.32 (0.23)	-0.19 (0.13)		0.33 (0.32)		-0.15 (0.06)				-0.09 (0.19)	9	-148.74	317.68	5.75	0.01
0.16 (0.39)	-0.20 (0.11)	-0.30 (0.24)	-0.72 (0.75)		-0.17 (0.06)	0.15 (0.07)				8	-150.01	317.75	5.82	0.01
-0.34 (0.24)	-0.23 (0.17)		0.37 (0.32)		-0.14 (0.06)		0.01 (0.24)		0.11 (0.05)	9	-148.84	317.88	5.95	0.01
-0.27 (0.16)	-0.30 (0.10)	-0.09 (0.10)			-0.16 (0.06)					6	-152.48	317.96	6.03	0.01
0.18 (0.41)	-0.20 (0.10)	-0.32 (0.24)	-0.59 (0.75)		-0.14 (0.06)	0.08 (0.08)			0.08 (0.05)	10	-147.79	318.29	6.36	0.01
0.52 (0.40)	-0.66 (0.26)	-0.50 (0.24)	-1.20 (0.73)		-0.15 (0.06)	0.48 (0.19)	1.00 (0.54)			10	-147.79	318.29	6.37	0.01
-0.35 (0.22)	-0.29 (0.10)		0.18 (0.32)		-0.17 (0.06)					6	-152.73	318.45	6.52	0.01
-0.22 (0.19)	-0.27 (0.10)				-0.19 (0.05)					6	-152.79	318.56	6.63	0.01
-0.11 (0.21)	-0.19 (0.13)	-0.15			-0.13 (0.06)	0.06 (0.08)			-0.03 (0.20)	10	-148.05	318.81	6.88	0.01

MEDIUM-TERM (Prime age, NLRt, N = 127 observations and individuals)

I	NLR	Qcoh	Pop(CH)	Sex(M)	Mass	NLR:Qcoh	NLR:pop(CH)	NLR:sex(M)	NLR:mass	df	Log-lik	AICc	Delta	w
0.50 (0.32)		-0.28 (0.16)	-1.24 (0.54)		-0.14 (0.05)					6	-205.53	423.75	0.00	0.13
0.11 (0.23)			-0.45 (0.27)		-0.16 (0.05)					5	-206.95	424.39	0.64	0.09
-0.10 (0.20)					-0.11 (0.04)					4	-208.27	424.87	1.11	0.07
0.42 (0.34)		-0.27 (0.16)	-1.23 (0.53)	0.18 (0.24)	-0.16 (0.06)					7	-205.25	425.43	1.68	0.06
0.48 (0.32)	0.03 (0.04)	-0.26 (0.17)	-1.21 (0.53)		-0.14 (0.05)					7	-205.26	425.45	1.70	0.06
0.13 (0.23)	0.04 (0.04)		-0.49 (0.28)		-0.16 (0.05)					6	-206.47	425.64	1.89	0.05
0.03 (0.25)			-0.48 (0.27)	0.21 (0.24)	-0.18 (0.06)					6	-206.57	425.83	2.08	0.05
-0.10 (0.20)	0.03 (0.04)			0.16 (0.24)	-0.10 (0.04)					5	-208.00	426.49	2.74	0.03
-0.17 (0.23)					-0.12 (0.05)					5	-208.04	426.58	2.82	0.03
-0.10 (0.20)		0.05 (0.08)			-0.13 (0.05)					5	-208.12	426.73	2.98	0.03
0.41 (0.34)	0.03 (0.04)	-0.25 (0.17)	-1.21 (0.53)	0.17 (0.24)	-0.16 (0.06)					8	-205.01	427.24	3.49	0.02
0.06 (0.25)	0.04 (0.04)		-0.51 (0.28)	0.19 (0.24)	-0.18 (0.06)					7	-206.15	427.24	3.49	0.02
0.49 (0.32)	0.03 (0.04)	-0.27 (0.17)	-1.24 (0.54)		-0.14 (0.05)				-0.01 (0.01)	8	-205.02	427.27	3.51	0.02
0.12 (0.23)	0.04 (0.04)		-0.48 (0.28)		-0.16 (0.05)				-0.01 (0.01)	7	-206.34	427.61	3.86	0.02
0.13 (0.23)	0.02 (0.06)		-0.50 (0.28)		-0.16 (0.05)		0.04 (0.08)			7	-206.35	427.64	3.89	0.02

0.13 (0.25)	0.05 (0.08)		-0.48 (0.27)	0.07 (0.24)	-0.18 (0.06)		-0.00 (0.13)		8	-191.35	400.01	6.49	0.01
-0.09 (0.19)	0.05 (0.06)	0.07 (0.09)			-0.15 (0.05)	-0.02 (0.04)			7	-192.54	400.08	6.56	0.01
-0.06 (0.19)	0.04 (0.06)	0.07 (0.09)			-0.14 (0.05)			0.01 (0.02)	7	-192.57	400.13	6.61	0.01
-0.10 (0.22)	0.04 (0.06)	0.06 (0.09)		0.05 (0.25)	-0.15 (0.06)				7	-192.64	400.28	6.76	0.01

Table S4. Linear mixed effects model (LMM) selection table for the long-term relationship between adult body mass and Neutrophil:Lymphocyte ratio ('NLR') measured as juvenile. We accounted for cohort quality ('Qcoh'), population (reference level is Chizé: CH, and is compared to Trois-Fontaines: TF), and sex (reference level is male: M, and is compared to females: F). Values give the parameter coefficient and values between brackets are the standard-errors. 'I' is the Intercept, 'df' is the number of parameters, 'Log-lik' is the log-likelihood, 'Delta' is the difference of AICc between the candidate model and the model having the lowest AICc, and 'wt' the AIC weight of each model. Retained model is in bold. Only models up to delta AICc = 7 are displayed.

LONG-TERM (N = 331 observations, n = 159 individuals)													
I	NLR	Qcoh	Pop(CH)	Sex(M)	NLR:Qcoh	NLR:pop(CH)	NLR:sex(M)	df	Log-lik	AICc	Delta	w	
-0.01 (0.29)		0.42 (0.21)						5	-627.85	1265.88	0.00	0.11	
-0.00 (0.28)	0.12 (0.08)	0.45 (0.21)						6	-626.83	1265.92	0.04	0.11	
-0.22 (0.32)	0.12 (0.08)	0.47 (0.21)	0.46 (0.31)					7	-625.79	1265.92	0.04	0.11	
-0.22 (0.32)		0.44 (0.21)	0.45 (0.32)					6	-626.85	1265.97	0.09	0.10	
-0.04 (0.29)								4	-629.77	1267.66	1.78	0.04	
0.01 (0.33)		0.42 (0.21)		-0.03 (0.32)				6	-627.84	1267.94	2.06	0.04	
-0.18 (0.35)	0.12 (0.08)	0.47 (0.21)	0.46 (0.32)	-0.08 (0.32)				8	-625.75	1267.95	2.07	0.04	
-0.18 (0.35)		0.43 (0.21)	0.46 (0.32)	-0.08 (0.32)				7	-626.82	1267.99	2.11	0.04	
0.01 (0.33)	0.12 (0.08)	0.45 (0.21)		-0.03 (0.32)				7	-626.82	1267.99	2.11	0.04	
-0.01 (0.29)	0.12 (0.08)	0.45 (0.22)			-0.01 (0.13)			7	-626.82	1268.00	2.12	0.04	
-0.22 (0.32)	0.14 (0.17)	0.47 (0.21)	0.46 (0.31)			-0.03 (0.19)		8	-625.78	1268.00	2.12	0.04	
-0.22 (0.32)	0.12 (0.08)	0.47 (0.22)	0.46 (0.32)		0.00 (0.13)			8	-625.79	1268.02	2.14	0.04	
-0.23 (0.33)			0.41 (0.32)					5	-628.97	1268.12	2.24	0.04	
-0.03 (0.29)	0.10 (0.08)							5	-629.04	1268.26	2.39	0.03	
-0.23 (0.33)	0.10 (0.08)		0.41 (0.32)					6	-628.22	1268.69	2.81	0.03	

***Appendix D – Supplementary information: Glucocorticoids
and physiological condition***

Supporting Information to:

*Glucocorticoids covary positively with indices of body condition, but
do not modulate senescence patterns in a long-lived free-ranging
mammal (in preparation)*

Table S1. Linear mixed effects model (LMM) selection table for the correction of early-life faecal glucocorticoid metabolites (FGM, log-transformed, N = 162 observations on 162 individuals) according to Julian date of capture (linear and quadratic), the time delay between capture and sampling ('Delay' in minutes) and whether faecal samples were immediately frozen at -80°C or frozen within 24 hours after collection ('Freezing'). Values give the parameter coefficient and values between brackets are the standard-errors. 'I' is the Intercept, 'df' is the number of parameters, 'Log-lik' is the log-likelihood, 'Delta' is the difference of AICc between the candidate model and the model having the lowest AICc, and 'w' the AIC weight of each model. Retained model is in bold. Dashed line separate models below 2 Δ AICc and models above.

I	Julian	Julian ²	Delay	Freezing(Immediate)	Log-lik	AICc	Delta	w
6.39 (0.05)					-156.25	316.57	0.00	0.19
6.18 (0.17)	0.03.10 ⁻¹ (0.02.10 ⁻¹)				-155.41	316.97	0.40	0.15
6.28 (0.10)		0.02.10 ⁻³ (0.02.10 ⁻³)			-155.52	317.18	0.62	0.14
6.35 (0.09)				0.06 (0.11)	-156.10	318.36	1.79	0.08
6.35 (0.15)			0.01.10 ⁻² (0.05.10 ⁻²)		-156.21	318.58	2.01	0.07
6.17 (0.17)	0.03.10 ⁻¹ (0.02.10 ⁻¹)			0.03 (0.11)	-155.38	319.01	2.45	0.06
6.12 (0.33)	0.01 (0.01)	-0.02.10 ⁻³ (0.01.10 ⁻²)			-155.38	319.01	2.45	0.06
6.15 (0.21)	0.03.10 ⁻¹ (0.02.10 ⁻¹)		0.01.10 ⁻² (0.05.10 ⁻²)		-155.39	319.02	2.46	0.05
6.26 (0.12)		0.02.10 ⁻³ (0.02.10 ⁻³)		0.03 (0.11)	-155.48	319.22	2.66	0.05
6.25 (0.17)		0.02.10 ⁻³ (0.02.10 ⁻³)	0.01.10 ⁻² (0.05.10 ⁻²)		-155.50	319.25	2.69	0.05
6.29 (0.18)			0.02.10 ⁻² (0.05.10 ⁻²)	0.06 (0.11)	-156.04	320.34	3.77	0.03
6.14 (0.23)	0.03.10 ⁻¹ (0.02.10 ⁻¹)		0.01.10 ⁻² (0.05.10 ⁻²)	0.03 (0.11)	-155.34	321.07	4.51	0.02
6.10 (0.33)	0.01 (0.01)	-0.02.10 ⁻³ (0.01.10 ⁻²)		0.03 (0.11)	-155.35	321.08	4.51	0.02
6.07 (0.37)	0.01 (0.01)	-0.02.10 ⁻³ (0.01.10 ⁻²)	0.01.10 ⁻² (0.05.10 ⁻²)		-155.35	321.08	4.51	0.02
6.23 (0.19)		0.02.10 ⁻³ (0.02.10 ⁻³)	0.01.10 ⁻² (0.05.10 ⁻²)	0.03 (0.11)	-155.45	321.29	4.73	0.02
6.05 (0.38)	0.01 (0.01)	-0.02.10 ⁻³ (0.01.10 ⁻²)	0.02.10 ⁻² (0.05.10 ⁻²)	0.03 (0.11)	-155.30	323.14	6.58	0.01

Table S2. Linear mixed effects model (LMM) selection table for the correction of faecal glucocorticoid metabolites (FGM, log-transformed, N = 775 observations on 437 individuals) measured during adulthood, according to Julian date of capture (linear and quadratic), the time delay between capture and sampling ('Delay' in minutes) and whether faecal samples were immediately frozen at -80°C or frozen within 24 hours after collection ('Freezing'). Values give the parameter coefficient and values between brackets are the standard-errors. 'I' is the Intercept, 'df' is the number of parameters, 'Log-lik' is the log-likelihood, 'Delta' is the difference of AICc between the candidate model and the model having the lowest AICc, and 'w' the AIC weight of each model. Retained model is in bold. Dashed line separate models below 2 Δ AICc and models above. Results including one high FGM value (> 5000 ng/g) were similar.

I	Julian	Julian ²	Delay	Freezing(Immediate)	Log lik	AICc	Delta	w
5.97 (0.11)	0.07.10⁻¹ (0.01.10⁻¹)				-715.71	1441.49	0.00	0.81
5.87 (0.16)	0.07.10 ⁻¹ (0.01.10 ⁻¹)			0.15 (0.17)	-716.15	1444.41	2.92	0.19
6.45 (0.08)					-723.63	1455.31	13.82	0.00
6.19 (0.09)		0.05.10 ⁻³ (0.01.10 ⁻³)			-722.70	1455.49	14.00	0.00
6.22 (0.15)				0.30 (0.17)	-722.98	1456.04	14.55	0.00
5.91 (0.12)	0.07.10 ⁻¹ (0.01.10 ⁻¹)		0.03.10 ⁻² (0.02.10 ⁻²)		-722.21	1456.52	15.03	0.00
6.07 (0.15)		0.05.10 ⁻³ (0.01.10 ⁻³)		0.18 (0.16)	-723.00	1458.11	16.62	0.00
5.79 (0.17)	0.06.10 ⁻¹ (0.01.10 ⁻¹)		0.03.10 ⁻² (0.02.10 ⁻²)	0.17 (0.17)	-722.54	1459.22	17.73	0.00
5.74 (0.20)	0.02 (0.01)	-0.07.10 ⁻³ (0.05.10 ⁻³)			-723.77	1459.65	18.16	0.00
5.65 (0.23)	0.01 (0.01)	-0.06.10 ⁻³ (0.05.10 ⁻³)		0.14 (0.17)	-724.28	1462.70	21.21	0.00
6.33 (0.10)			0.05.10 ⁻² (0.02.10 ⁻²)		-729.09	1468.25	26.76	0.00
6.08 (0.17)			0.05.10 ⁻² (0.02.10 ⁻²)	0.32 (0.17)	-728.26	1468.63	27.14	0.00
6.12 (0.10)		0.05.10 ⁻³ (0.01.10 ⁻³)	0.03.10 ⁻² (0.02.10 ⁻²)		-729.17	1470.45	28.96	0.00
5.97 (0.16)		0.04.10 ⁻³ (0.01.10 ⁻³)	0.03.10 ⁻² (0.02.10 ⁻²)	0.20 (0.16)	-729.34	1472.82	31.34	0.00
5.65 (0.21)	0.02 (0.01)	-0.07.10 ⁻³ (0.05.10 ⁻³)	0.03.10 ⁻² (0.02.10 ⁻²)		-730.18	1474.51	33.02	0.00
5.55 (0.24)	0.01 (0.01)	-0.07.10 ⁻³ (0.05.10 ⁻³)	0.04.10 ⁻² (0.02.10 ⁻²)	0.16 (0.17)	-730.59	1477.36	35.87	0.00

FGM levels according to julian date of capture

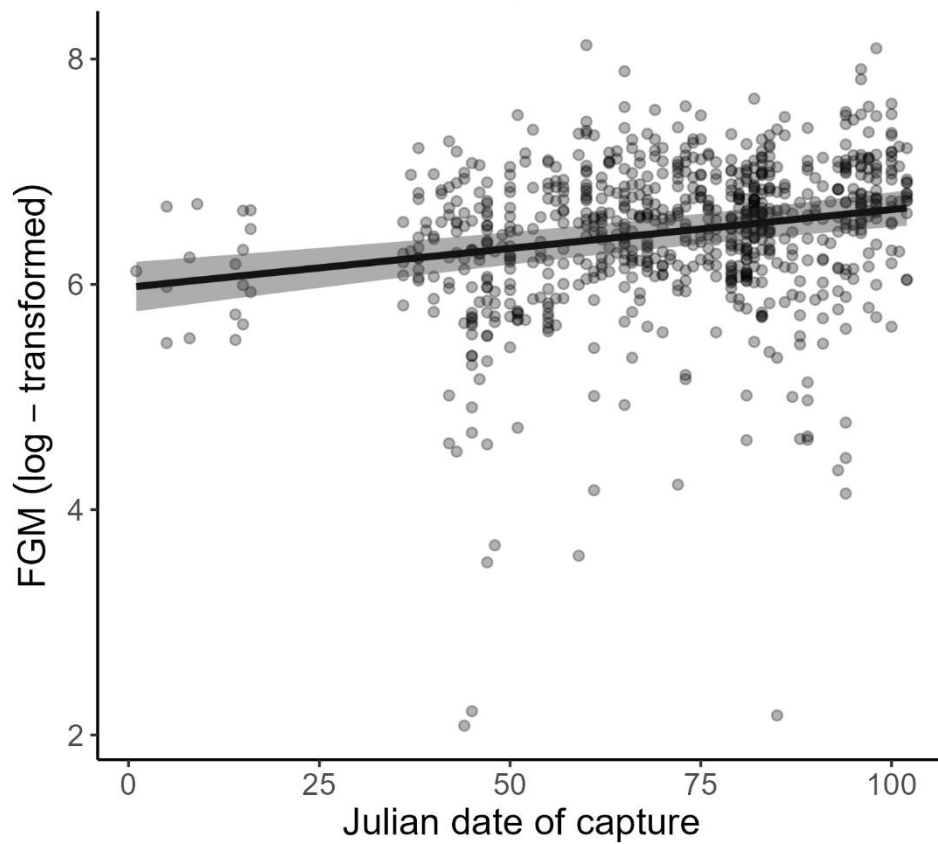


Figure S1. Seasonal variation of faecal glucocorticoid metabolites (FGM, log-transformed) according to Julian date of capture (starting early December, ending early March). FGMs increase linearly across the capture season. Points are raw data and the line is the prediction from the selected model with 95% confidence intervals.

Table S3. Linear mixed effects model (LMM) selection table for the short-term relationship between faecal glucocorticoid metabolites (FGM, log-transformed) and albumin concentrations measured at the same capture event, when excluding two low FGM values. Model selection was based on the senescence trajectories previously found and included the additive effect of FGMs and the two-way interactions between FGMs and all age terms retained in the senescence trajectories, population, sex, cohort quality and mass. M: Males, Qcoh(P): cohort environmental quality (Poor, as opposed to 'good cohort quality'), CH: population of Chizé, Age-last: age at last observation, Delay: time between capture and sampling (in minutes). Values give the parameter coefficient and values between brackets are the standard-errors. 'i' is the intercept, 'df' is the number of parameters, 'Log-lik' is the log-likelihood, 'Delta' is the difference of AICc between the candidate model and the model having the lowest AICc, and 'w' the AIC weight of each model. Retained model is in bold. Only models up to delta AICc = 2 are displayed.

Albumin (N = 608 observations, n = 360 individuals)																		
i	Age	Age²	Sex(M)	Age-last	Delay	Mass	FGM	Pop(CH)	Qcoh(P)	Sex(M): FGM	Mass: FGM	Pop(CH): FGM	Qcoh(P): FGM	df	Log-lik	AICc	Delta	w
56.14 (16.90)	-0.67 (0.34)	-0.01 (0.03)	-2.98 (0.46)	0.33 (0.12)	0.01 (0.00)	-1.24 (0.77)	-4.87 (2.55)	2.92 (1.93)			0.26 (0.12)			13	-1818.46	3663.53	0.00	0.03
58.06 (16.85)	-0.65 (0.34)	-0.01 (0.03)	-2.96 (0.46)	0.33 (0.12)	0.01 (0.00)	-1.26 (0.77)	-4.92 (2.55)				0.26 (0.12)			12	-1819.52	3663.57	0.04	0.03
54.18 (16.99)	-0.70 (0.34)	-0.01 (0.03)	-7.42 (4.15)	0.32 (0.12)	0.01 (0.00)	-1.06 (0.79)	-4.57 (2.57)	2.94 (1.92)		0.69 (0.64)	0.24 (0.12)			14	-1817.88	3664.47	0.94	0.02
56.15 (16.94)	-0.67 (0.34)	-0.01 (0.03)	-7.30 (4.15)	0.32 (0.12)	0.01 (0.00)	-1.08 (0.79)	-4.62 (2.57)			0.67 (0.64)	0.24 (0.12)			13	-1818.97	3664.55	1.02	0.02
55.62 (16.90)	-0.64 (0.34)	-0.01 (0.03)	-2.94 (0.46)	0.33 (0.12)	0.01 (0.00)	-1.21 (0.77)	-4.75 (2.55)	2.93 (1.92)	-0.43 (0.46)		0.26 (0.12)			14	-1818.01	3664.73	1.20	0.02
47.71 (19.15)	-0.69 (0.34)	-0.01 (0.03)	-2.97 (0.46)	0.33 (0.12)	0.01 (0.00)	-0.98 (0.82)	-3.58 (2.90)	7.47 (5.24)			0.22 (0.12)	-0.70 (0.75)		14	-1818.02	3664.75	1.22	0.02
57.56 (16.85)	-0.61 (0.34)	-0.01 (0.03)	-2.92 (0.46)	0.33 (0.12)	0.01 (0.00)	-1.23 (0.77)	-4.80 (2.55)		-0.42 (0.46)		0.26 (0.12)			13	-1819.09	3664.79	1.26	0.02
60.70 (17.26)	-0.65 (0.34)	-0.01 (0.03)	-2.94 (0.46)	0.33 (0.12)	0.01 (0.00)	-1.27 (0.77)	-5.58 (2.62)	2.99 (1.93)	-6.36 (4.21)		0.27 (0.12)		0.92 (0.65)	15	-1817.01	3664.84	1.31	0.02
62.60 (17.21)	-0.62 (0.34)	-0.01 (0.03)	-2.92 (0.46)	0.33 (0.12)	0.01 (0.00)	-1.29 (0.77)	-5.62 (2.62)		-6.26 (4.21)		0.27 (0.12)		0.90 (0.65)	14	-1818.12	3664.95	1.42	0.02
58.52 (16.90)		-0.06 (0.01)	-2.93 (0.46)	0.30 (0.12)	0.01 (0.00)	-1.33 (0.77)	-5.00 (2.56)				0.26 (0.12)			11	-1821.33	3665.11	1.58	0.01
56.74 (16.95)		-0.06 (0.01)	-2.95 (0.46)	0.30 (0.12)	0.01 (0.00)	-1.32 (0.77)	-4.97 (2.56)	2.72 (1.92)			0.26 (0.12)			12	-1820.40	3665.32	1.79	0.01

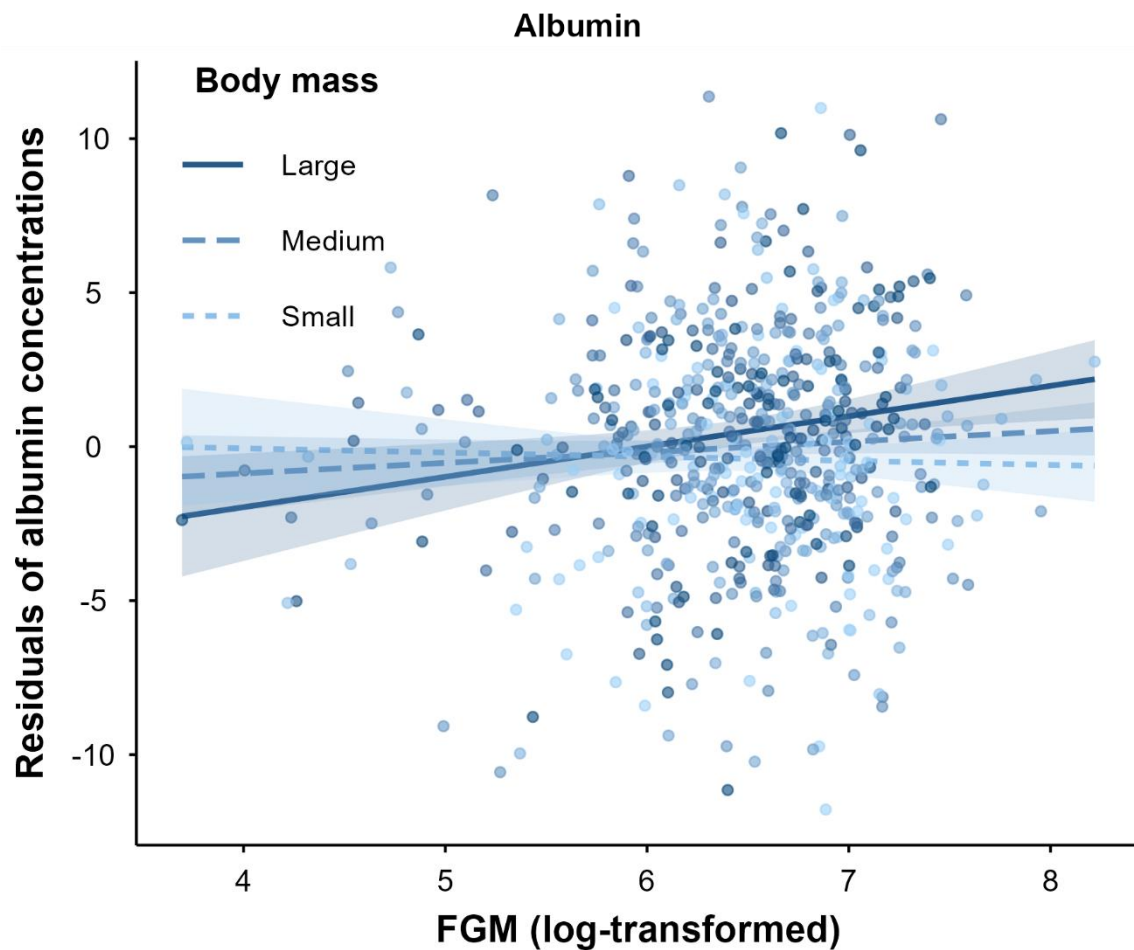


Figure S2. Predicted roe deer albumin concentrations (residuals of the retained senescence trajectories accounting for age, sex, age at last observation and delay between capture and sampling) according to faecal glucocorticoid metabolites (FGM, log-transformed) measured at the same capture events. Data excluded two low FGM values that were suspected to drive patterns found in the manuscript (Figure 2a). Indeed, the interaction between sex and FGM we found in the manuscript disappeared and is replaced here by a positive interaction between body mass and FGM. Body mass is a normally distributed continuous variable, and for graphical representation we split it in three mass categories based on terciles (Median of each tercile: Small = 19.1 kg, light blue, dashed-line; Medium = 21.4 kg ; blue, dashed-line; Large = 24.5 kg, dark blue, solid line). Lines are model predictions and shaded areas are 95% CIs.

***Appendix E – Supplementary information: Glucocorticoids
and immunosenescence***

Supporting Information to:

*Glucocorticoids and immunosenescence patterns in a wild ungulate
(in preparation)*

Table S1. Linear mixed effects model (LMM) selection table for the correction of early-life faecal glucocorticoid metabolites (FGM, log-transformed, N = 162 observations on 162 individuals) according to Julian date of capture (linear and quadratic), the time delay between capture and sampling ('Delay' in minutes) and whether faecal samples were immediately frozen at -80°C or frozen within 24 hours after collection ('Freezing'). Values give the parameter coefficient and values between brackets are the standard-errors. 'I' is the Intercept, 'df' is the number of parameters, 'Log-lik' is the log-likelihood, 'Delta' is the difference of AICc between the candidate model and the model having the lowest AICc, and 'w' the AIC weight of each model. Retained model is in bold. Dashed line separate models below 2 Δ AICc and models above.

I	Julian	Julian ²	Delay	Freezing(Immediate)	df	Log-lik	AICc	Delta	w
6.39 (0.05)					2	-156.25	316.57	0.00	0.19
6.18 (0.17)	0.03.10 ⁻¹ (0.02.10 ⁻¹)				3	-155.41	316.97	0.40	0.15
6.28 (0.10)		0.02.10 ⁻³ (0.02.10 ⁻³)			3	-155.52	317.18	0.62	0.14
6.35 (0.09)				0.06 (0.11)	3	-156.10	318.36	1.79	0.08
6.35 (0.15)			0.01.10 ⁻² (0.05.10 ⁻²)		3	-156.21	318.58	2.01	0.07
6.17 (0.17)	0.03.10 ⁻¹ (0.02.10 ⁻¹)			0.03 (0.11)	4	-155.38	319.01	2.45	0.06
6.12 (0.33)	0.06.10 ⁻¹ (0.01)	-0.02.10 ⁻³ (0.09.10 ⁻³)			4	-155.38	319.01	2.45	0.06
6.15 (0.21)	0.03.10 ⁻¹ (0.02.10 ⁻¹)		0.01.10 ⁻² (0.05.10 ⁻²)		4	-155.39	319.02	2.46	0.05
6.26 (0.12)		0.02.10 ⁻³ (0.02.10 ⁻³)		0.03 (0.11)	4	-155.48	319.22	2.66	0.05
6.25 (0.17)		0.02.10 ⁻³ (0.02.10 ⁻³)	0.01.10 ⁻² (0.05.10 ⁻²)		4	-155.50	319.25	2.69	0.05
6.29 (0.18)			0.01.10 ⁻² (0.05.10 ⁻²)	0.06 (0.11)	4	-156.04	320.34	3.77	0.03
6.14 (0.23)	0.03.10 ⁻¹ (0.02.10 ⁻¹)		0.01.10 ⁻² (0.05.10 ⁻²)	0.03 (0.11)	5	-155.34	321.07	4.51	0.02
6.10 (0.33)	0.06.10 ⁻¹ (0.01)	-0.02.10 ⁻³ (0.09.10 ⁻³)		0.03 (0.11)	5	-155.35	321.08	4.51	0.02
6.07 (0.37)	0.06.10 ⁻¹ (0.01)	-0.02.10 ⁻³ (0.09.10 ⁻³)	0.01.10 ⁻² (0.05.10 ⁻²)		5	-155.35	321.08	4.51	0.02
6.23 (0.19)		0.02.10 ⁻³ (0.02.10 ⁻³)	0.01.10 ⁻² (0.05.10 ⁻²)	0.03 (0.11)	5	-155.45	321.29	4.73	0.02
6.05 (0.38)	0.06.10 ⁻¹ (0.01)	-0.03.10 ⁻³ (0.09.10 ⁻³)	0.02.10 ⁻² (0.06.10 ⁻²)	0.03 (0.11)	6	-155.30	323.14	6.58	0.01

Table S2. Linear mixed effects model (LMM) selection table for the early-late relationship between early-life faecal glucocorticoid metabolites (FGM, log-transformed) and immunosenescence trajectories. Model selection was based on the immunosenescence trajectories previously found and included the additive effect of FGMs and the interaction between FGMs and all age terms retained in the senescence trajectories. ‘M’: Males, ‘CH’: population of Chizé, ‘Age-last’: age at last observation, ‘Delay’: time between capture and sampling (in minutes), ‘Julian’: Julian date of capture. Values give the parameter coefficient and values between brackets are the standard-errors. ‘I’ is the Intercept, ‘df’ is the number of parameters, ‘Log-lik’ is the log-likelihood, ‘Delta’ is the difference of AICc between the candidate model and the model having the lowest AICc, and ‘w’ the AIC weight of each model. Retained model is in bold. Dashed lines separate models below 2 Δ AICc and models above.

Neutrophil (N=267 observations, n=142 individuals)											
	I	Age ²	Pop(CH)	FGM	Age ² :FGM	Delay	df	Log-lik	AICc	Delta	w
M1	4.91 (0.39)	0.01 (0.01)	-0.82 (0.28)			0.04.10⁻¹ (0.01.10⁻¹)	7	-547.47	1109.38	0.00	0.45
M2	2.87 (1.52)	0.01 (0.01)	-0.74 (0.29)	0.31 (0.23)		0.04.10 ⁻¹ (0.01.10 ⁻¹)	8	-546.52	1109.60	0.22	0.40
M3	3.16 (1.85)	-0.01 (0.08)	-0.74 (0.29)	0.27 (0.28)	0.04.10 ⁻¹ (0.01)	0.04.10 ⁻¹ (0.01.10 ⁻¹)	9	-546.48	1111.67	2.29	0.14
Monocyte (N=267 observations, n=142 individuals)											
	I	Age.1	FGM	Age.1:FGM	df	Log-lik	AICc	Delta	w		
M1	0.23 (0.05)	-0.04 (0.01)			5	-26.57	63.38	0.00	0.44		
M2	0.47 (0.18)	-0.04 (0.01)	-0.04 (0.03)		6	-25.66	63.64	0.26	0.39		
M3	0.63 (0.29)	0.05 (0.13)	-0.07 (0.05)	-0.02 (0.02)	7	-25.41	65.25	1.87	0.17		
Basophil (N=267 observations, n=142 individuals)											
	I	FGM	Delay	df	Log-lik	AICc	Delta	w			
M1	0.11 (0.02)		-0.02.10⁻² (0.06.10⁻³)	5	260.03	-509.84	0.00	0.73			
M2	0.13 (0.06)	-0.03.10 ⁻¹ (0.01)	-0.02.10 ⁻² (0.06.10 ⁻³)	6	260.07	-507.82	2.01	0.27			
Eosinophil (N=267 observations, n=142 individuals)											
	I	FGM	Sex(M)	Delay	df	Log-lik	AICc	Delta	w		
M1	0.31 (0.03)		-0.03 (0.02)	-0.06.10⁻² (0.09.10⁻³)	6	124.15	-235.98	0.00	0.74		
M2	0.33 (0.11)	-0.03.10 ⁻¹ (0.02)	-0.03 (0.02)	-0.06.10 ⁻² (0.09.10 ⁻³)	7	124.17	-233.90	2.08	0.26		
Hemagglutination (N=332 observations, n=159 individuals)											
	I	FGM	FGM	df	Log-lik	AICc	Delta	w			
M1		3.84 (0.18)		4	-529.20	1066.53	0.00	0.68			
M2		4.33 (0.70)	-0.08 (0.11)	5	-528.93	1068.05	1.52	0.32			

Hemolysis (N=328 observations, n=159 individuals)															
	I	Age ²	Sex(M)	FGM	Age ² :sex(M)	Age ² :FGM	Julian	Delay	Mass	df	Log-lik	AICc	Delta	w	
M1	0.03 (0.23)							0.07.10 ⁻² (0.06.10 ⁻²)		5	-483.90	977.99	0.00	0.51	
M2	0.91 (0.66)							0.07.10 ⁻² (0.06.10 ⁻²)		6	-482.90	978.06	0.07	0.49	
Alpha1-globulin (N=136 observations and individuals)															
	I	Age ²	Sex(M)	FGM	Age ² :sex(M)	Age ² :FGM	Julian	Delay	Mass	df	Log-lik	AICc	Delta	w	
M1	3.92 (0.46)	0.01 (0.01)	0.35 (0.13)		-0.01 (0.01)		0.02.10 ⁻¹ (0.03.10 ⁻¹)	0.05.10 ⁻² (0.04.10 ⁻²)	-0.06 (0.02)	9	-106.62	232.67	0.00	0.71	
M2	3.92 (0.64)	0.01 (0.01)	0.35 (0.13)	-0.03.10 ⁻³ (0.07)	-0.01 (0.01)		0.02.10 ⁻¹ (0.03.10 ⁻¹)	0.05.10 ⁻² (0.04.10 ⁻²)	-0.06 (0.02)	10	-106.62	235.00	2.33	0.22	
M3	3.79 (0.80)	0.03 (0.06)	0.35 (0.13)	0.02 (0.10)	-0.01 (0.01)	-0.03.10 ⁻¹ (0.01)	0.02.10 ⁻¹ (0.03.10 ⁻¹)	0.05.10 ⁻² (0.04.10 ⁻²)	-0.06 (0.02)	11	-106.58	237.30	4.63	0.07	
Alpha2-globulin (N=136 observations and individuals)															
	I	Age ²	Sex(M)	FGM	Age ² :sex(M)	Age ² :FGM	Age:FGM	Age ² :FGM	Mass	df	Log-lik	AICc	Delta	w	
M1	6.54 (0.91)	-0.25 (0.51)	0.01 (0.06)		-0.36 (0.25)					6	-246.11	504.88	0.00	0.47	
M2	7.42 (1.59)	-0.27 (0.51)	0.01 (0.06)		-0.36 (0.25)	-0.13 (0.20)				7	-245.90	506.68	1.80	0.19	
M3	3.51 (3.58)	1.19 (1.31)	0.01 (0.06)		-0.34 (0.24)	0.49 (0.55)	-0.23 (0.19)			8	-245.19	507.51	2.63	0.13	
M4	-11.36 (10.43)	10.89 (6.54)	-1.33 (0.89)		-0.32 (0.24)	2.79 (1.62)	-1.74 (1.01)	0.21 (0.14)		9	-244.07	507.57	2.69	0.12	
M5	6.24 (2.04)	-0.29 (0.51)	0.17 (0.18)		-0.35 (0.25)	0.06 (0.29)		-0.02 (0.03)		8	-245.51	508.15	3.27	0.09	
Betaglobulin (N=249 observations, n=136 individuals)															
	I	Age	Sex(M)	FGM	Age:FGM	Age ² :FGM	Mass	Mass	df	Log-lik	AICc	Delta	w		
M1	7.64 (0.86)	0.25 (0.06)	0.36 (0.20)				-0.10 (0.04)		7	-462.01	938.49	0.00	0.68		
M2	7.77 (1.43)	0.25 (0.06)	0.36 (0.20)		-0.02 (0.17)		-0.10 (0.04)		8	-462.01	940.62	2.12	0.23		
M3	6.91 (2.62)	0.50 (0.64)	0.35 (0.20)		0.11 (0.38)		-0.09 (0.04)		9	-461.93	942.61	4.12	0.09		
Haptoglobin (N=254 observations, n=138 individuals)															
	I	Age.1	Age.2	Sex(M)	FGM	Age.1:sex(M)	Age.2:sex(M)	Age.1:FGM	Age.2:FGM	Mass	df	Log-lik	AICc	Delta	w
M1	1.82 (0.76)	0.00 (0.15)	0.04 (0.11)	-0.70 (0.62)		0.37 (0.21)	0.02 (0.15)			-0.07 (0.03)	10	-421.90	864.70	0.00	0.59
M2	1.63 (1.21)	0.01 (0.15)	0.04 (0.11)	-0.70 (0.62)	0.03	0.37 (0.21)	0.03 (0.15)			-0.07 (0.03)	11	-421.88	866.85	2.14	0.20
M3	-0.77 (2.89)	0.83 (0.11)	0.04 (0.11)	-0.72 (0.61)	0.41 (0.44)	0.38 (0.21)	0.02 (0.15)	-0.13 (0.15)		-0.06 (0.04)	12	-421.46	868.21	3.51	0.10
M4	1.63 (1.28)	0.01 (0.15)	0.04 (0.11)	-0.70 (0.62)	0.03 (0.16)	0.37 (0.21)	0.03 (0.15)			-0.04.10 ⁻² (0.16)	12	-421.88	869.05	4.35	0.07

	I	Age	Age ²	Pop(CH)	Sex(M)	FGM	Age:FGM	Age ² :FGM	Age ² :Pop(CH)	Age ² :FGM	Delay	df	Log-lik	AICc	Delta	w
M5	-1.49 (3.13)	1.18 (1.08)	-0.67 (1.20)	-0.73 (0.61)	0.53 (0.48)	0.39 (0.21)	0.03 (0.15)	-0.19 (0.17)	0.12 (0.20)	-0.06 (0.04)	13	-421.28	870.08	5.38	0.04	
Lymphocyte (N=267 observations, n=142 individuals)																
M1	2.30 (1.00)	-0.51 (0.14)	0.14 (0.04)	-0.17 (0.25)	-0.19 (0.12)	0.27 (0.14)	-0.02 (0.01)	-0.01 (0.01)	-0.02 (0.01)	-0.02.10 ⁻¹ (0.06.10 ⁻²)	12	-356.79	738.80	0.00	0.29	
M2	1.08 (1.49)	0.20 (0.41)	0.06 (0.01)	-0.17 (0.25)	-0.18 (0.12)	0.47 (0.22)	-0.11 (0.06)	-0.02 (0.01)	-0.02 (0.01)	-0.02.10 ⁻¹ (0.06.10 ⁻²)	12	-356.92	739.06	0.26	0.25	
M3	4.13 (0.38)	-0.56 (0.14)	0.07 (0.01)	-0.19 (0.26)	-0.17 (0.12)	0.09 (0.10)	-0.02 (0.01)	-0.02 (0.01)	-0.02 (0.01)	-0.02.10⁻¹ (0.06.10⁻²)	10	-359.15	739.16	0.36	0.24	
M4	3.54 (0.79)	-0.55 (0.14)	0.06 (0.01)	-0.18 (0.25)	-0.17 (0.12)	0.09 (0.10)	-0.02 (0.01)	-0.02 (0.01)	-0.02 (0.01)	-0.02.10 ⁻¹ (0.06.10 ⁻²)	11	-358.79	740.62	1.81	0.12	
M5	2.46 (3.07)	-0.60 (1.59)	0.15 (0.18)	-0.17 (0.25)	-0.19 (0.12)	0.25 (0.49)	0.01 (0.26)	-0.02 (0.01)	-0.02 (0.03)	-0.02.10 ⁻¹ (0.06.10 ⁻²)	13	-356.79	741.01	2.21	0.10	
Gammaglobulin (N=249 observations, n=136 individuals)																
	I	Age ²	Pop(CH)	FGM	Age ² :FGM	Mass	df	Log-lik	AICc	Delta	w					
M1	20.25 (2.20)	0.04 (0.01)	3.31 (1.30)	-0.16 (0.39)	-0.27 (0.09)	7	-646.13	1306.73	0.00	0.66						
M2	21.36 (3.46)	0.04 (0.01)	3.26 (1.31)	-0.16 (0.39)	-0.27 (0.09)	8	-646.05	1308.70	1.97	0.25						
M3	20.23 (4.04)	0.12 (0.15)	3.27 (1.31)	-0.00 (0.49)	-0.26 (0.09)	9	-645.91	1310.57	3.83	0.10						
Gastro-intestinal strongyles (N=274 observations, n=140 individuals)																
	I	Age ²	Sex(M)	FGM	Age ² :FGM	Age-last	Mass	df	Log-lik	AICc	Delta	w				
M1	3.29 (0.83)	0.02 (0.01)	0.54 (0.21)	0.09 (0.17)	-0.12 (0.06)	-0.06 (0.04)	8	-488.03	992.61	0.00	0.63					
M2	2.68 (1.39)	0.02 (0.01)	0.54 (0.21)	0.09 (0.17)	-0.11 (0.06)	-0.06 (0.04)	9	-487.88	994.45	1.84	0.25					
M3	3.36 (1.67)	-0.03 (0.06)	0.54 (0.21)	-0.02.10 ⁻¹ (0.22)	0.01 (0.01)	-0.06 (0.04)	10	-487.62	996.08	3.47	0.11					
Coccidia (N=273 observations, n=140 individuals)																
	I	Age ²	FGM	Age:FGM	Age ² :FGM	Mass	df	Log-lik	AICc	Delta	w					
M1	1.26 (0.12)	0.02 (0.01)	0.02 (0.09)	0.02 (0.01)	0.02 (0.01)	-0.05 (0.04)	4	-570.07	1148.29	0.00	0.51					
M2	-0.47 (1.23)	0.02 (0.09)	0.02 (0.01)	0.02 (0.09)	0.02 (0.01)	-0.05 (0.04)	5	-569.08	1148.38	0.09	0.49					
Trichuris sp. (N=252 observations, n=129 individuals)																
	I	Age	Age ²	Pop(CH)	Sex(M)	FGM	Age:Sex(M)	Age ² :Pop(CH)	Age:FGM	Age ² :FGM	Mass	df	Log-lik	AICc	Delta	w
M1	2.21 (0.82)	-0.55 (0.23)	0.06 (0.02)	0.67 (0.27)	0.41 (0.37)	0.02 (0.01)	0.02 (0.09)	0.02 (0.01)	0.02 (0.01)	-0.05 (0.04)	11	-421.52	866.14	0.00	0.53	
M2	2.64 (1.30)	-0.55 (0.23)	0.06 (0.02)	0.66 (0.27)	0.42 (0.37)	0.02 (0.01)	0.02 (0.09)	0.02 (0.01)	0.02 (0.01)	-0.05 (0.04)	12	-421.43	868.16	2.02	0.19	
M3	3.60 (1.63)	-0.58 (0.23)	-0.01 (0.07)	0.64 (0.27)	0.40 (0.37)	0.02 (0.01)	0.03 (0.09)	0.02 (0.01)	0.01 (0.01)	-0.05 (0.04)	13	-420.97	869.47	3.33	0.10	

Appendix F – Side project: SARS-CoV-2 in free-ranging roe deer

Likely circulation of a coronavirus in roe deer (Capreolus capreolus) populations suggested by SARS-CoV-2 serological investigation in France (in preparation)

Likely circulation of a coronavirus in roe deer (*Capreolus capreolus*) populations suggested by SARS-CoV-2 serological investigation in France

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Abstract

The potential for viral transmissions from domestic and wildlife animals to human is of high concern for human health. On the other hand, human can also transmit viral infection back to captive, domestic and wild animals, which can act as reservoir for the maintenance of viruses with the potential for the emergence of new epidemic diseases. The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), causing the COVID-19, likely originating from wildlife has already been evidenced to be transmitted from human to captive, domestic and wild animals. In particular, white-tailed deer (*Odocoileus virginianus*) show high-prevalence of SARS-CoV-2 following human contamination. In turn, cases of transmission from newly infected white-tailed deer to human have been identified, suggesting that white-tailed deer could be an emerging virus reservoir. Here, we investigated whether SARS-CoV-2 could have emerged in longitudinally-monitored populations of roe deer (*Capreolus capreolus*) in direct contact with human. We also investigated the exposure and prevalence of the virus in other cervid species. We performed indirect tests (*i.e.* serological ELISAs and seroneutralisation) on serum collected pre- and post-emergence of the virus in human populations. We also performed direct test (nasal swabs with RT-PCR) to detect the presence of SARS-CoV-2 in those populations in 2022. ELISA tests were positive for 2.20 % of the sera tested, both pre- and post-emergence of the virus, but all positive samples were negative following seroneutralisation. Direct testing showed no positive samples in 2022. Our results suggest that SARS-CoV-2 has not emerged in those populations, and that cross-reactions with another circulating virus (possibly a bovine coronavirus), which remains to be identified, is likely.

Keywords: COVID 19, SARS-CoV-2, roe deer, ungulates, spill-over, spill-back, zoonoses

Introduction

The potential spill-over (*i.e.* the transmission of a parasite from a reservoir species to a naïve species; Ellwanger and Chies 2021) of viral infections from livestock or wildlife to humans is of high concern for human health worldwide, as vertebrates constitute the main source of human emerging diseases (Jones et al. 2008). Conversely, human pathogens can spill-back (*i.e.* the transmission of a parasite from the spill-over host back to a maintenance host) to domestic or wild animal species, potentially inducing new epidemics in these species (*e.g.* Terzian et al. 2018). Hence, there is a need to better understand the characteristics (*e.g.* mutation rate, transmission mode) of pathogens that can transmit across species, and to identify possible or existing contacts between species.

Coronaviruses, hosted by many mammal species, are among the viruses most able to be transmitted among them, including to humans (Monchatre-Leroy et al. 2017, Alluwaimi et al. 2020, Delaune et al. 2021). The COVID-19 pandemic, due to the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), most likely originating in wildlife (Lytras et al. 2021, Domingo 2022). Early after the beginning of the COVID-19 pandemic, spill-back events from human to domestic, farmed or captive animals have been observed, for example to farmed minks (*Neovison vison*; Oreshkova et al. 2020, Oude Munnink et al. 2021), domestic cats *Felis catus* and dogs *Canis lupus familiaris* (Hobbs and Reid 2021, Krafft et al. 2021) or wild felids kept in zoos (McAloose et al. 2020). These first observed spill-back events occurred in domestic, captive or intensive farming contexts and thus could be explained by elevated rates of direct contacts between humans and animals. More recently, however, multiple events of transmission of SARS-CoV-2 from human to free-living white-tailed deer (*Odocoileus virginianus*) wildlife were observed in North America. These were followed by transmission within local deer population, resulting in deer populations displaying high prevalence levels (Chandler et al. 2021, Hale et al. 2022, Kuchipudi et al. 2022, Feng et al. 2023). Finally, three

cases of transmission from the newly infected white-tailed deer populations to humans were identified, suggesting that the white-tailed deer may become an emerging virus reservoir in North America (Feng et al. 2023).

Similar patterns of contamination could be expected in Europe, as several species of cervid are highly abundant. First cases of seropositivity have been recently observed in fallow deer *Dama dama*, in a free-living urban population in Ireland, suggesting that the host tropism of SARS-CoV-2 may change as new variants emerge (Purves et al. 2023). Several cervid species are present in Europe and exposed to viral transmission. Among them, the roe deer (*Capreolus capreolus*) is particularly interesting because the species occupies almost the same ecological niche than the white-tailed deer (Borkowski et al. 2021), can live close to human habitations, and thus might be similarly exposed to human contaminants (Gerard et al. 1992). The species may also display a gregarious behaviour in winter (depending on landscape openness; Lorenzini et al. 2022), favouring disease transmission. Furthermore, the hunting regulations and the absence of large predator during the last 50 years resulted in high abundance all over Europe. In addition, sampling of roe deer is facilitated by the large number of individual hunted (Burbaite and Csanyi 2009). Finally, hunting represents a frequent occasion of contact between hunters and roe deer, exposing hunters and handlers to potential viral transmission.

Benefiting from a longitudinal follow-up on four wild-living and one captive populations of wild roe deer subject to hunting and/or direct manipulations by scientists within the framework of capture-marking-recapture programs, we investigated whether SARS-CoV-2 emerged recently in these intensively monitored wild-living roe deer populations. Because of annual captures, deer from these populations are particularly exposed to viral transmission from humans, particularly in 2020 when few information was available on the virus. Moreover, the continuous follow-up of the same individuals before and after 2020 was an occasion to detect virus arrival in individuals. The virus was searched for by means of either direct testing (on

recently captured individuals) and by indirect testing to assess exposure before and after 2020. We further investigated exposure and presence of the virus in other cervid species (fallow deer *Dama dama*, sika deer *Cervus nippon* and hybrids of red deer and sika deer *Cervus elaphus* × *Cervus nippon*).

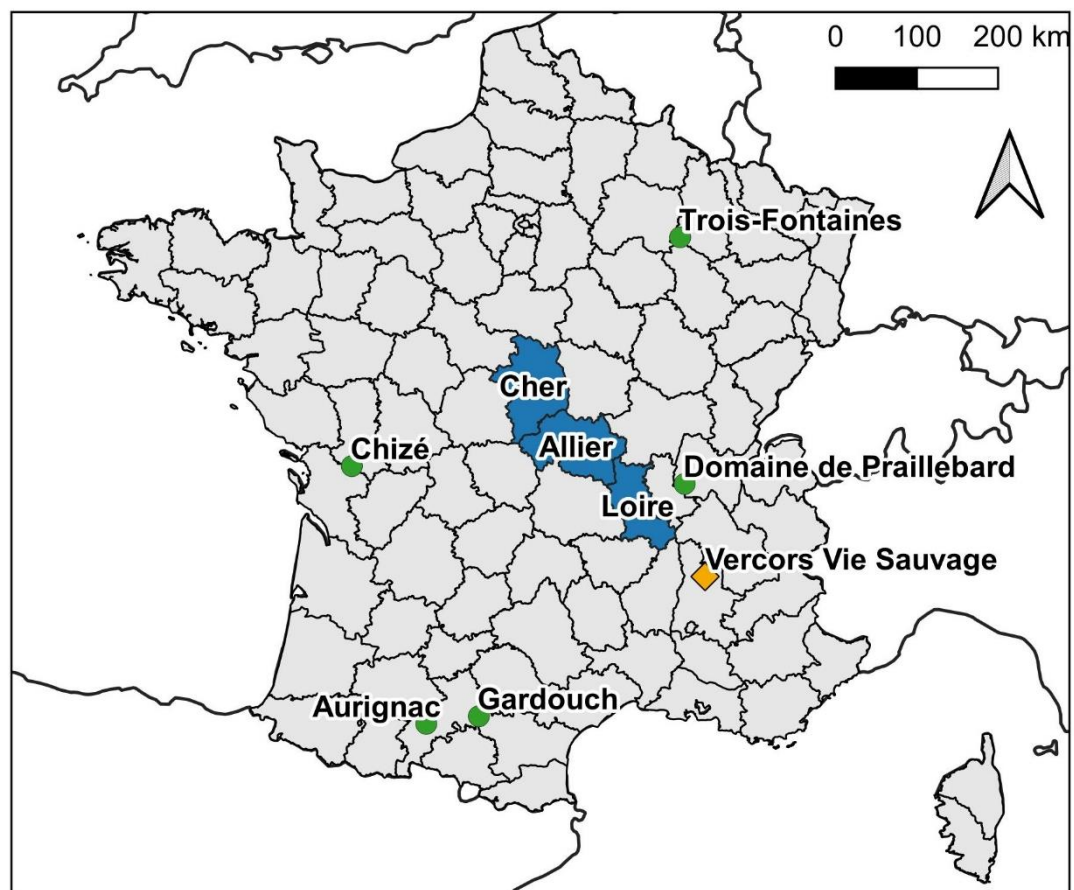
Material and methods

Sampling

Sera were collected from 2,134 captures, concerning 1,289 different individuals. Sampling occurred before COVID-19 emergence in France (2010-2020, n = 1,752) and post-COVID-19 emergence (2021-2022, n = 382) periods. The year 2020 was included in the pre-pandemic period because sampling occurred in winter (December to March), before the first declared human case in France (Paireau et al. 2022). Pre-pandemic samples were collected each year between December (year *t-1*) and March (year *t*) and were used as controls to test for potential cross-reaction with other coronaviruses circulating in these populations. The roe deer sampled for their sera were from five populations (Fig. 1): Trois-Fontaines (TF), Chizé (CH), Aurignac (AU), the Domaine of Praillebard (DP) and Gardouch (GA; Fig. 1). TF is a 1,360 ha fenced forest in north-eastern France (48°43'N, 4°55'E) hosting an enclosed wild roe deer population; CH is a 2,614 ha fenced Integral Biological Reserve (unmanaged forest) since 2006 in western France (46°05'N, 0°25'W) hosting also a closed wild roe deer population; AU is an open study area covering about 7,500 ha in southern France (43°16'N, 0°53'E) including forests and agricultural landscapes with free-ranging roe deer; DP is a private fenced area of 148 ha in eastern France (45°57'N, 4°55'E) hosting a small roe deer population introduced from TF and CH. GA is an experimental station in southern France (43°22', 1°40'E) hosting captive roe deer in small 0.5 ha enclosures or semi-captive roe deer in a large enclosure composed of 5 ha of meadows and 9 ha of forest. Additionally, 30 sera from other cervid species sampled from the

Vercors Vie Sauvage (VVS) reserve located in eastern France (44°57'N, 5°14'E; Fig. 1) were included in the study. These included 16 European fallow deer (*Dama dama*), five sika deer (*Cervus nippon*) and nine red deer × sika deer hybrids (*Cervus elaphus* × *Cervus nippon*), all obtained in 2022. Sera sampling is summarized in Table 1.

For direct virus detection, nasal swabs were collected in 2022 during the roe deer capture-mark-recapture program from TF (n = 49), CH (n = 95), and AU (n = 61). Samples were also collected on hunted roe deer in three departments of central France (Allier: n = 3, Cher: n = 29, and Loire: n = 4), and on red deer in one department (Cher: n = 3; Fig. 1).



Legend

- Departements with roe deer and/or red deer tested by RT-PCR in 2022
- Sampling locations of roe deer populations included in the serological study from 2009 to 2022 and tested by RT-PCR in 2022
- Sampling location of red deer, sika deer, and fallow deer included in the serological study in 2022

Figure 1. Locations of deer (Cervidae) populations sampled for the sera used in the SARS-CoV-2 serological study and the nasal swabs used in the SARS-CoV-2 RNA RT-PCR screening.

Table 1. Summary of the sampling scheme and the SARS-CoV-2 serological results for roe deer (*Capreolus capreolus*) and other cervids for pre- and post-COVID-19 emergence.

Location (sampling period)	Species	Number of distinct individuals	Number of samples (pre/post)	Number positive results (pre/post)	Seroprevalence % (pre/post)
TF (2010-2022)	Roe deer	539	942 (832/110)	17 (16/1)	1.80 (1.92/0.91)
CH (2010-2022)	Roe deer	557	965 (817/148)	23 (15/8)	2.38 (1.84/5.41)
AU (2019-2022)	Roe deer	180	205 (97/108)	7 (4/3)	3.41 (4.12/2.78)
GA (2019-2022)	Roe deer	9	18 (6/12)	0 (0/0)	N/A
DP (2021)	Roe deer	4	4 (0/4)	0 (0/0)	N/A
VWS (2022)	Fallow deer	16	16 (0/16)	1 (0/1)	N/A
	Red × sika deer	9	9 (0/9)	0 (0/0)	N/A
	Sika deer	5	5 (0/5)	1 (0/1)	N/A
Total	Roe deer	1,289	2,134 (1,752/382)	47 (35/12)	2.20 (2.00/3.14)
	All species	1,319	2,164 (1,752/412)	49 (35/14)	2.26 (2.00/3.40)

Exposure assessment by ELISA tests and confirmation tests by seroneutralisation

Serum samples were analysed using the double-antigen ID Screen® SARS-CoV-2 Double Antigen Multi-species ELISA kit (ID Vet, France) following the manufacturer's instructions. The sensitivity and specificity of the assay were 93.3% [95%CI: 78.8–98.2] and 99.9% [99.6–100], respectively. To confirm the serological results and to test for the possibility of cross-reaction of the ID Vet ELISA-test with other coronaviruses, positive samples along with a subset of negative samples from 2019 to 2022 were further analysed by seroneutralisation (sVNT) assay based on the detection of anti-SARS-CoV-2 antibodies for the S-protein.

Direct detection

Nasal swabs were analysed using RT-PCR (reverse transcriptase polymerase chain reaction) technic and allowed to determine whether an individual was currently infected with SARS-CoV-2.

Data analyses

Seroprevalences observed in roe deer during pre- and post-COVID-19 emergence (all populations together) were compared with a Chi-square test. Serological results were further analysed to identify individual and populational factors related to seropositivity. In this aim, we used 901 samples from TF, 865 from CH, and 202 from AU, corresponding to individuals for which age and sex was known. For AU, the exact age was not known and age was estimated using 5 age classes (< 1, 2, 3, 4-5 and ≥ 6 years old). In this case, the youngest possible age was considered (*e.g.* for an individual of the 4-5 years age class, it was considered as a 4 year-old individual). A binomial Generalised Linear Model (GLM) was used to model the individual serological status as a function of COVID-19 pandemic periods (pre and post-emergence, 2010-2020 and 2021-2022 respectively), age (1-16 years old), sex, population, sampling year (2010-2022) and age:population, age:year, age:sex and population:year interactions. An AICc-based selection model procedure was then performed to evaluate model credibility. All statistics were

done with R version 4.2.2 (R Core Team 2023) in Rstudio 2023.03.0 (Posit Team 2023), and with the *car* 3.1-2 (Fox and Weisberg 2019), *MuMIn* 1.47.5 (Bartoń 2023), *readr* 2.1.4 (Wickham et al. 2023) and *visreg* 2.7.0 (Breheny and Burchett 2020) packages.

Results

ELISA test

A total of 47 out of 2,134 ELISA tests (2.20 % [1.62-2.92]) displayed positive results for anti-SARS-CoV-2 antibodies against the N-protein (positivity threshold: S/P \geq 60 %), including samples collected during both the pre- and post-COVID-19 pandemic periods (2.00 % [1.40-2.77], and 3.14 % [1.63-5.42], respectively; Table 1). S/P were overall largely above the positivity threshold (41 sera with a range of 71.9–824.5 %), except for 6 sera for which positivity was close to threshold (range: 60.8–68.5 %). One individual tested three weeks apart in the same year was positive for each sample. S/P declined from 328.83 to 119.66. When considering all roe deer populations together, the difference in seroprevalence between the two periods was not statistically significant (Chi² test: $\chi^2=1.41$, df=1, p=0.235). Among the sera from other cervids inhabiting the VVS reserve, ELISA tests performed on one fallow deer out of 16 and one sika deer out of five gave seropositive results (Table 1).

sVNT test

sVNT assays were performed on nine positive and 15 negative roe deer samples of the post- emergence period, and on four positive and 15 negative roe deer samples of the pre-emergence period. All sVNT assays produced negative results.

Direct test – RT-PCR

Using nasal swabs, RT-PCR results did not reveal any positive result during the capture and hunting period of winter 2022, on 205 captured roe deer, 36 hunted roe deer and three hunted red deer.

Detailed results for roe deer

The 47 sera that gave positive results originate from 46 individuals from CH, TF, and AU (see Table 1). Among these 46 individuals, 27 were blood sampled and tested only once, and 19 were blood sampled and tested multiple times: eight were tested twice, six tested thrice, four tested four times and one was tested five times. During the follow up of the 19 positive individuals tested multiple times, 13 seronegative individuals were found seropositive in the following years (six on the next year), while 12 seropositive individuals were found seronegative in a following year (four on the next year). Only one individual was tested positive twice during two different years (2016 and 2018) but no sample was available in-between (2017). Nineteen positive results were from individuals in their first year. The probability that an individual was positive two different years is not different from what is expected by chance only based on the observed prevalence (binomial test: p -value = 1). So, seropositivity events can be considered as independent in the following analyses.

According to the AICc-based selection procedure, the best model of seroprevalence includes only the sampling year ($\beta = 0.09 \pm \text{SE: } 0.05, p = 0.04$). The modelled seroprevalence probability increased from 1.11 % [0.52–2.34] to 3.25 % [2.03–5.16] during the 12 years study period (Figure 2).

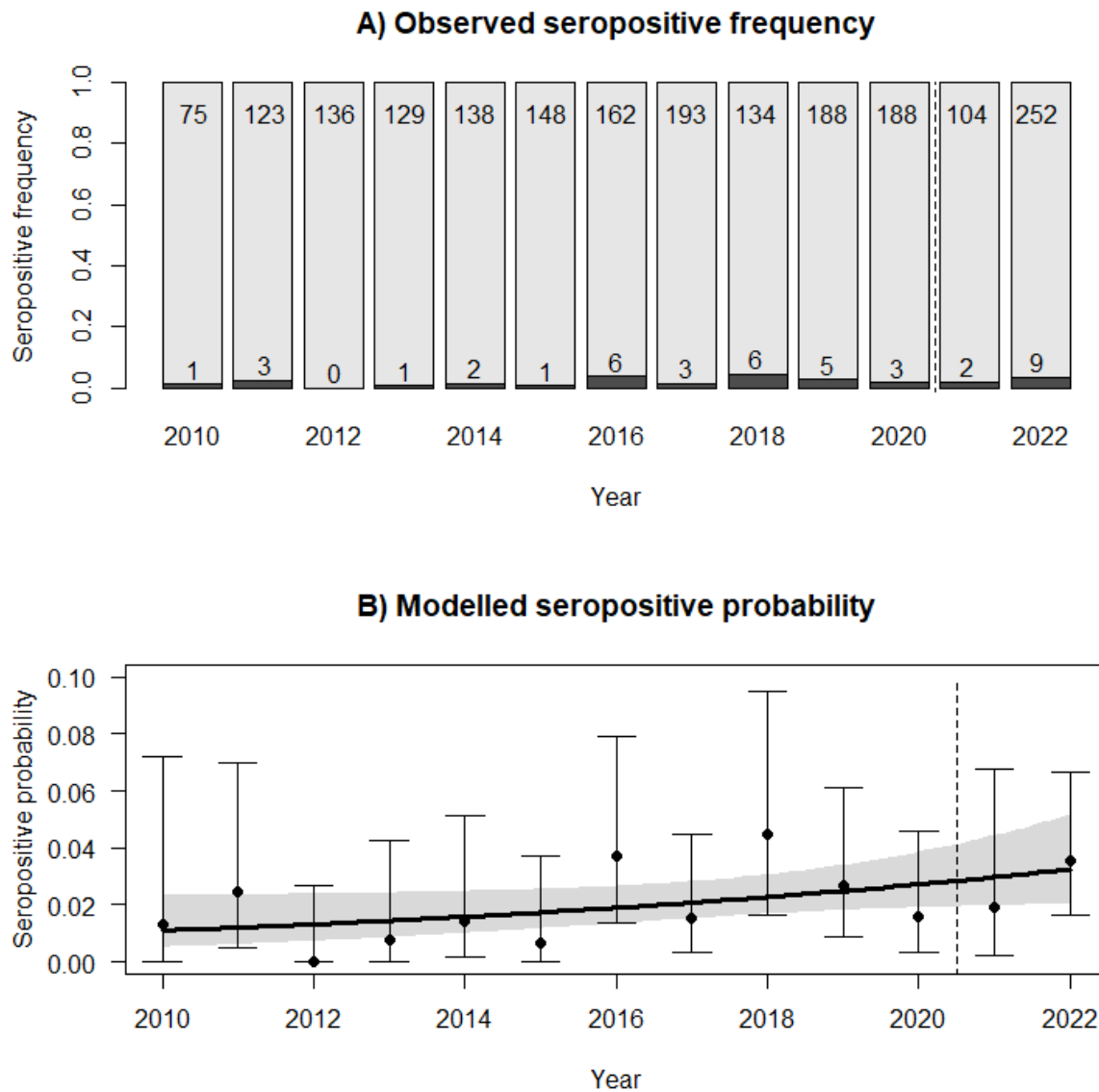


Figure 2. SARS-CoV-2 seroprevalence of roe deer (*Capreolus capreolus*) as a function of sampling year. Observed (dots) and modelled (curve) seroprevalence, both with 95% confidence interval (B), for individuals from AU, CH, and TF populations together as a function of sampling year. The vertical dot line marks the emergence of COVID-19 in France.

Discussion

This study is the first long-term individual monitoring of serological status of wild-living cervid for SARS-CoV-2. Our survey intended to detect, at the individual or populational levels, an increase in seropositivity or seroprevalence following the emergence of SARS-CoV-2 in France.

Interpreting serological results in terms of viral infection is not straightforward. SARS-CoV-2 ELISA tests indicated an overall seroprevalence of 2.20% in roe deer. However, the sera, including 13 positive ones, submitted to the sVNT assay and all swabs tested for direct detection of the virus by RT-PCR in 2022 produced negative results, indicating that SARS-CoV-2 was unlikely to circulate in the studied populations of roe deer.

The absence of detection of SARS-CoV-2 with sVNT assay is consistent with other studies conducted in Germany and Austria on roe deer, red deer and fallow deer (Moreira-Soto et al. 2022). Negative sVNT assays despite positive ELISA tests were also reported in wild boars *Sus scrofa*, red foxes *Vulpes vulpes* and jackals *Canis aureus moreoticus* (Jemeršić et al. 2021). It can be explained by an inadequate specificity of the ELISA test allowing cross-reaction with antibodies directed to other closely-related viruses (Krüttgen et al. 2021). Such results underline the importance of not relying on ELISA tests alone before drawing any conclusions. Nonetheless, these positive results suggest that the ELISA test used likely cross-reacts with one or several other coronaviruses circulating in the studied roe deer populations. Bovine like coronaviruses (BcoVs), which have been already demonstrated in red deer and sika deer (Amer 2018), are potential candidates that could have been detected by our ELISA analyses. The circulation of another unknown coronavirus in the roe deer populations is also possible. More investigations are required before drawing any firm conclusion, for instance by using more specific tests to confirm or infirm the presence of BCoVs antibodies in ELISA positive roe deer sera (Ulrich et al. 2020).

When studying more closely the spatio-temporal and individual factors of seroprevalence, a significant general increase of the seroprevalence was observed over the sampling period. Without formally identifying responsible virus or viruses, observed patterns are difficult to explain. We did not detect any significant statistical difference in seroprevalence

between the pre- and post-emergence periods, but we cannot exclude that the virus started to transmit among individuals in 2021 and that the prevalence increased only from 2022.

The seroprevalence do not seem to increase with age. Furthermore, among thirteen positive individuals that were tested on more than one year, only one was found seropositive twice. Other individuals tested the following years were seronegative, including four individuals tested only one year later. Additionally, one individual tested three weeks apart in the same year was positive for each sample with a decline from 328.83 to 119.66 in observed S/P absorbance densities (but a single datum was kept in the presented data). Together, these results are consistent with a short persistence time of the antibodies (less than one year), but for at least several weeks. Antibodies after natural SARS-CoV-2 infection in humans can persist for more than a year (Townsend et al. 2022, 2023, Sarjomaa et al. 2022), but persistence in other species is not well known.

Positive serologies were observed in all populations of roe deer, except DP and GA for which only four and 18 sera were tested, respectively. We also had positive results in other cervids from VVS despite a low sample size ($n = 1$ out of 16 fallow deer and $n = 1$ out of 5 sika deer). When comparing seroprevalence between the three roe deer populations with a sufficient sample size (CH, TF and AU), no significant statistical difference was observed. If a virus of bovine origin is involved, one would expect a lower seroprevalence in the two isolated roe deer populations of CH and TF, compared to the free roaming roe deer population of AU exposed to cattle. These results are not in favour of the BCoV hypothesis in CH and TF but could be in AU. Further investigations are required to *i*) confirm or infirm the hypothesis of a BCoV in AU, and to *ii*) test for the presence of a circulating virus and to identify it if so.

Spill-over of the SARS-CoV-2 virus from human to roe deer is thus unlikely to have happened to date in our populations, contrary to what has been observed among white-tailed deer populations in North America (Chandler et al. 2021, Hale et al. 2022, Kuchipudi et al.

2022). Our results confirm the results of a previous study evidencing an absence of contact between the most common European cervids and the SARS-CoV-2 virus (Moreira-Soto et al. 2022). Indeed, roe deer is not as peri-urban as white-tailed deer or as close to human as reindeer. Therefore, contact rate between roe deer and human is expected to be lower than for white-tailed deer and reindeer. Roe deer is also not as close to white-tailed deer relative to their ACE2 receptor (with which the SARS-CoV-2 virus spike protein interacts) than reindeer is, nor as gregarious as reindeer, and likely less susceptible to SARS-CoV-2 infection (Damas et al. 2020). Despite the low probability of roe deer to serve as rescue host (*i.e.* host favouring the maintenance of a parasite) for SARS-CoV-2, the circulation of another coronavirus, maybe BCoVs, in roe deer populations might raise the question of a possible virus cross-recombination leading to a new virus variant. Further studies should be done to identify coronaviruses circulating in wild cervids and domestic ruminants and assess the possibility of spill-over to human and/or domestic or wild ruminants.

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***Appendix G – Previous publication: Body mass senescence
in Asian elephants***

*Sex-specific body mass ageing trajectories in adult Asian elephants
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RESEARCH ARTICLE

Sex-specific body mass ageing trajectories in adult Asian elephants

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Abstract

In species with marked sexual dimorphism, the classic prediction is that the sex which undergoes stronger intrasexual competition ages earlier or quicker. However, more recently, alternative hypotheses have been put forward, showing that this association can be disrupted. Here, we utilize a unique, longitudinal data set of a semi-captive population of Asian elephants (*Elephas maximus*), a species with marked male-biased intrasexual competition, with males being larger and having shorter lifespans, and investigate whether males show earlier and/or faster body mass ageing than females. We found evidence of sex-specific body mass ageing trajectories: adult males gained weight up to the age of 48 years old, followed by a decrease in body mass until natural death. In contrast, adult females gained body mass with age until a body mass decline in the last year of life. Our study shows sex-specific ageing patterns, with an earlier onset of body mass declines in males than females, which is consistent with the predictions of the classical theory of ageing.

KEYWORDS

Elephas maximus, long-term data, senescence, sex differences, terminal decline

1 | INTRODUCTION

Ageing—a decline in organismal functioning with age (Monaghan et al., 2008)—has been observed in many species (Jones et al., 2014). However, the onset and rates of ageing differ both between (Jones et al., 2014) and within species (Nussey et al., 2007) and between sexes (Clutton-Brock & Isvaran, 2007; Lema tre et al., 2020; Tidi ere et al., 2015). A main challenge in ageing research is to quantify and

explain such differences in the onset and rates of ageing (Rando & Wyss-Coray, 2021).

In species with sex-specific intrasexual competition, the classical theory of ageing predicts that the sex with the highest intrasexual competition has a shorter lifespan and an earlier onset and/or higher rate of ageing (Williams, 1957). The rationale is that high intrasexual selection often results in one sex showing conspicuous displays or aggressive intrasexual behaviours, leading to increased

mortality and a *live fast, die young* pace of life (Bonduriansky et al., 2008; Clutton-Brock & Isvaran, 2007; Maklakov & Lummaa, 2013). For that sex, antagonistically pleiotropic genes or deleterious mutations are not counter selected due to a weakened force of selection in late life (Williams, 1957). Accordingly, in polygynous species with male-biased intrasexual competition, males often die earlier (Lemaître et al., 2020) and age earlier or faster than females (Beirne et al., 2015; Clutton-Brock & Isvaran, 2007; Douhard et al., 2017; Nussey et al., 2009; Tidière et al., 2015). However, recent conceptual developments have shown that this association can be disrupted. This can occur for example because of condition-dependent extrinsic mortality selecting particularly high-performing individuals in the population (Chen & Maklakov, 2014) or canalization (i.e. the more a trait contributes to fitness, the less it should deviate from optimal trait value, with respect to environmental variation (Flatt, 2005)), thereby contradicting the theoretically expected earlier or faster ageing in males. The extent to which such phenomena occur in nature remains unknown.

Here, we used a unique long-term data set to describe sex-specific body mass ageing trajectories in a nutritionally unsupplemented semi-captive population of Asian elephants (*Elephas maximus*) employed in timber harvesting and living in their natural forest environment in Myanmar. Body mass is of interest in the study of ageing because it is positively associated with key life-history traits such as reproduction and lifespan in many non-human species (Briga et al., 2019; Gaillard et al., 2000; Hämäläinen et al., 2014; Pelletier et al., 2007). Therefore, the study of body mass ageing fits into the evolutionary framework of ageing. Accordingly, in Asian elephants, seasonal variation in body mass was positively associated with survival the following month (e.g. low body mass was associated with low survival during dry season) (Mumby et al., 2015). Moreover, male Asian elephants benefit from being heavy during intrasexual competition for dominance and mating (Sukumar, 2003).

However, we know almost nothing about body mass ageing in elephants despite the interest in studying ageing in a long-lived, social and sexually dimorphic non-human species. Whilst females live in kin groups, adult males often roam solitarily, undergo a more intense intrasexual competition for dominance and mating (Sukumar, 2003) and hence are bigger, heavier (Mumby et al., 2015), more aggressive, less sociable (Seltmann et al., 2019) and shorter-lived than females (respective median lifespans in this population: 30.8 and 44.7 years) (Lahdenperä et al., 2018). Based on this male-biased intrasexual competition and shorter lifespan and following the classical theory of ageing (Williams, 1957), we tested the prediction that males experience an earlier and/or faster body mass loss than females (Bonduriansky et al., 2008; Maklakov & Lummaa, 2013).

2 | MATERIALS AND METHODS

2.1 | Study population

We studied the world's largest semi-captive Asian elephant population consisting of around 3000 individually marked elephants owned by the government-run Myanma Timber Enterprise (MTE)

(Leimgruber et al., 2008). Their birth, death, maternal-lineage pedigree and morphological measurements have been recorded for almost a century by local veterinarians. These elephants are distributed across Myanmar in forest camps and used as riding, transport and drafting animals. Elephants work during the day but socialize, mate and forage freely and unsupervised in forests at night (Oo, 2010; Zaw, 1997). There are no husbandry procedures and timber elephants are never culled. Calves born in captivity are cared for and nursed by their biological mother and allomothers (Lahdenperä et al., 2016; Lynch et al., 2019). Therefore, breeding rates are natural with no reproductive management. Moreover, there is minimal food provisioning, but elephants benefit from veterinary care that consists of the treatment of simple injuries and monitoring of working conditions.

Both males and females are used in the workforce, and each working group of six elephants is composed of both sexes. Males and females follow the same government set limitations on taming age, working and retirement age, working days per year, hours of work per day and tonnage pulled annually apply to both sexes, although it is possible that males might be used for somewhat different working tasks at times (e.g. when use of tusks is required; only males can possess long tusks in Asian elephants). Pregnant females are given a rest period from mid-pregnancy (around 11 months into gestation) until the calf is one year old (Toke Gale, 1974), whilst they and their calf are being monitored by their mahouts (individual caretakers and riders) throughout this period. Following this break, mothers are used for light work but are kept with calves at heel and able to suckle on demand until the calf is four or five years old (Oo, 2010) at which point calves are assigned a rider, name, logbook and registration number. After the training period, elephants are used for light work duties until the age of 17, when they enter the full workforce until their retirement at the age of 50 years old. The MTE maintains their care and logbooks until death.

2.2 | Data collection and selection

Our analyses focused on age- and sex-specific variation in adult body mass from age 18 onwards to omit the phase during which elephants grow in height (Mumby et al., 2015) and to focus only on adult body mass age-specific variations. We compiled a total of 3886 body mass measurements on 493 individuals (2570 body masses on 322 females, and 1316 body masses on 171 males). These data came from two sources: (i) body masses were either measured on elephants on the field or (ii) estimated using height to the shoulder and chest girth (method in Supplementary Information 1 and following Chapman et al., 2016). For the first source, we collected 1901 body masses of 347 elephants (1297 measurements on 230 females and 604 measurements on 117 males) and for the second source, we estimated 1,985 body masses on 342 individuals (1273 estimations on 226 females and 712 estimations on 116 males—a same individual can have both measured and estimated body masses). For all elephants, we knew

their working localities ("township"), sex, year of birth ("YOB"), alive or dead status at the moment of the study, origin (captive-born or wild-caught) and measurement season (hot: Feb-May, monsoon: Jun-Sep, cold: Oct-Jan (Mumby et al., 2015)). The alive or dead status was used to test for a potential terminal decline. Of the 493 individuals considered, 5 males (63 observations) and 18 females (185 observations) died during the study period. We had measurements during the last year of life for 2 males (7 observations) and 10 females (54 observations). The elephants in our study had an age range between 18 and 72 years (mean = 39.3) and were born between 1941 and 1999. Age and cohort information were comparable between sexes, with 171 males ($n = 1316$ observations) born between 1954 and 1999 and aged between 18 and 64 years (mean = 37.4), and 322 females ($n = 2570$ observations) born between 1941 and 1999 and aged between 18 and 72 years (mean = 40.2).

Most elephants of this semi-captive population are at least occasionally measured for height and chest girth by local veterinarians using tape measures in inches or centimetres, depending on location, with no selection with respect to their age, sex or condition. Body mass is measured to the nearest kg only in camps provided with measurement scales such as Eziweigh 3000 used in this study. All elephants within the reach of those camps get weighed, again without any bias regarding their age, sex or condition. The log-books containing these measurements have thus far been translated from Burmese to English mainly from the Sagaing region for logistic reasons but again without any bias or pre-selection of certain individuals.

In total, we used a median of 4.0 measurements/individual (2.5–97.5th percentiles: [1.0; 36.4]), followed for a median period of 2.8 years (2.5–97.5th: [0.0; 36.6] on 493 elephants ($n = 3886$ observations)). Two influential observations of one male measured at age 18 and 23 were removed because of particularly low Δ age (Cook's distance = 0.61 and 0.25, relative to a mean of 0.001 for all males). Other observations for this male, all after age 50, were included and showed no particular Cook's distance.

2.3 | Statistical analyses

We investigated the age- and sex-specific variation in body mass in R version 4.1.1 (R Core Team, 2021), using the body mass (log-transformed to reach normality of the variable and because of the allometric relationship between body mass and size) as a dependent variable with a normal error distribution. First, we tested in a single model, whether there were sex-specific ageing trajectories using an interaction term (Table S2). Given that this interaction was statistically significant, we compared the sex-specific ageing trajectories for both sexes separately. We did these analyses using both general linear mixed models (GLMMs) and general additive mixed models (GAMMs) with cubic regression splines (but note that other spline functions gave similar conclusions as those shown here) using the functions "lmer" of the package "lme4" (v.

1.1-27.1, Bates et al., 2015) and "gam" of the package "mgcv" (v. 1.8-36, Wood, 2011), respectively. GAMMs allow more flexible ageing trajectories than GLMMs, but the more constrained ageing trajectories in GLMMs allow a more precise identification of differences in ageing trajectories (Figure S1), and both approaches gave consistent conclusions (see results section). We identified the best-fitting models using the model selection approach based on the second-order Akaike Information Criterion (AICc) as implemented in the package "MuMIn" (v. 1.43.17, Bartoń, 2021). In brief, the best-fitting model has the lowest AICc value, with other models within 7 Δ AICc being plausible and models becoming increasingly equivocal up to 14 Δ AICc, after which they become implausible (Burnham et al., 2011). Thus, we performed model averaging on ageing trajectories within 7 Δ AICc to calculate averaged model estimates with confidence intervals. Visual inspection of model residuals confirmed that all assumptions of distribution and homogeneity were fulfilled without any influential data points or outliers (see above).

2.3.1 | Within- vs. between-individual change

In all models, we accounted for non-independence of data due to repeated measurements from the same individual by including elephant identity ("ID") as a random intercept. The composition of the population can change with age, for example due to selective disappearance of certain (e.g. lighter or heavier) individuals, which can affect the age trajectory. To alleviate this problem as much as possible, we disentangled body mass changes with age into between- and within-individual changes following the approach developed by van de Pol and Verhulst, (2006) and van de Pol and Wright, (2009) using two terms: (i) the age at last measurement for each individual, which captures the between-individual variations and (ii) a " Δ age" term (age at measurement minus the individual's mean age for all measurements) capturing the within-individual changes with age. We mean-centred and standardized " Δ age" so that (i) individuals measured once all get a Δ age = 0 and hence contribute to the variance of the Δ age intercept but not to its slope and (ii) to avoid collinearity and to have comparable variance for Δ age and Δ age² (Bolker, 2008; Zuur et al., 2009). Among the 493 individuals of our data set, 105 individuals had only one measurement. We included these individuals by giving them Δ age = 0 (i.e. mean-centred), so they do not contribute to the coefficient but contribute to the variance along the Y axis on Δ age = 0, diminishing the likelihood of a false positive and contribute to the coefficient of the age at last measurement term, thereby avoiding a bias in the dataset from selecting only the longer-lived or most monitored individuals.

2.3.2 | Testing ageing trajectories

We tested several within-individual ageing trajectories, first using GAMMs, which can provide curvilinear relationships and allow to describe trends, and using GLMMs, able to detect breaking points

if necessary, by testing linear, quadratic, threshold and terminal models (Figure S1), and we selected the ageing trajectory with the lowest AICc. For GAMMs, we identified the age at which maxima occurred based on the first-order derivative ($= 0$) using the function “fderiv” of the package “gratia” (v. 0.6.0, Simpson & Singmann, 2021). For threshold models (Figure S1C), we followed the approaches previously developed in Briga et al., (2019) and Douhard et al., (2017). In brief, we first identified the best-fitting threshold age in a series of models, varying the threshold in the “ Δ age” term between -35 and 22 years with intervals of one Δ age (a unit of the mean-centred Δ age = 4.4 and 4.5 years for males and females, respectively) and estimated the threshold and its confidence intervals using ± 4 Δ AIC age range. Note that threshold models give separate coefficients for the pre-peak and post-peak slopes and thus allow to discriminate between a post-peak asymptote, decline or increase in body mass. However, if a threshold ageing trajectory is retained as the best-fitting ageing trajectory, we tested the statistical significance of the post-peak slope using only post-peak data, with the intercept offset to the peak. Then we compared the best-fitting threshold model with the other ageing trajectories. Sometimes, declines in trait value appear shortly before death (terminal decline). We coded a “terminal” change (Figure S1D) as a binomial factor for whether an individual died during the year following the measurement. We used a one-year window to avoid a possible seasonal covariation in weight and because it was the best-fitting time-window, but note that models with other time-windows gave consistent conclusions (Figure S3).

2.3.3 | Accounting for temporal and spatial variation in body mass

As body mass variation can be influenced by seasonal, spatial and within-individual factors, we tested whether body mass values were affected by (i) whether they were measured or estimated, (ii) whether individuals were alive or dead at the time of the analyses, (iii) whether individuals were captive- or wild-born, and (iv) the season during measurement. To this end, we used a model selection approach, using the function “dredge” of the package “MuMIn” (v. 1.43.17, Bartoń, 2021) on the best-fitting ageing trajectories for each sex to test for confounding factors (Table S3). In our models, we included as random intercepts individual identity to account for the repeated measurement of the same individual. We also included “township” to account for the spatial clustering of individuals across Myanmar, although actually adding township worsened the model fit in most cases (male GLMM: Δ AICc = $+1.8$; male GAMM: Δ AICc = $+1.5$; female GLMM: Δ AICc = -21.2 ; female GAMM: Δ AICc = $+0.6$).

3 | RESULTS

At the measurements' starting age of 18 years, males were on average 235 kg heavier than females, weighing, respectively, 2541 kg

[95% CI: 2,406; 2,683] and 2306 kg [95%CI: 2258; 2355], and this difference was statistically significant (Δ AICc = -122.6 in a GLMM with vs without sex as a fixed effect).

We identified the elephant's body mass ageing trajectories using general linear mixed models (GLMMs) and general additive mixed models (GAMMs) and both approaches gave consistent results. Both analyses showed that sexes have different body mass ageing trajectories (interaction term, GLMM: Δ AICc = -47.0 , Figure 1, Table S2; GAMM: Δ AICc = -65.7 , Figure S4, Table S2), and hence, we identified the ageing trajectories for both sexes separately.

For males, both GLMMs and GAMMs indicated a body mass gain from age 18 years until their late forties or early fifties (GLMM maximum: 48.2 years [4 AICc CI: 47.1; 51.6], Figure 1a; GAMM maximum: 54 years [95%CI: 53; 56], Figure S5), followed by a decline until death (Figure 1A, Figure S4A, Table 1, Table S4). In GLMMs, this maximum was shown through the best fit of a threshold model (Δ AICc = -30.4 compared to a linear trajectory): males gained mass at a rate of 22 kg/year [95%CI: 19.4 23.7] or 1% [95%CI: 0.9; 1.1] of males' mean body mass. Following the peak, males lost mass at a rate of 29 kg/year [95%CI: 14.9; 41.9], that is 1.3% [95%CI: 0.7; 1.9] of males' mean body mass (Figure 1A, Table 2) or an effect size (Cohen's d) of -0.59 [95%CI: -0.87 ; -0.30] based on 47 observations of 14 individuals. An analysis with only the post-peak data confirmed this decline with males losing mass at a rate of 22 kg/year [95%CI: 6.3; 37.1], that is 1.0% [95%CI: 0.3; 1.7] of males' mean mass or an effect size of -0.40 [95%CI: -0.68 ; -0.11].

For females, both GLMMs and GAMMs indicated a body mass gain throughout their lives until a terminal decline during their last year of life (Figure 1B, Figure S4B, Table 1, Table S4). GLMMs indicated a mass gain of 9 kg/year [95% CI: 7.5; 10.4] or 0.35% [95% CI: 0.3; 0.4] of females' mean body mass (Δ AICc = -6.7 , Table 1, Table 2). Loss of body mass occurred in the last year of life (GLMM: Δ AICc = -4.9 , Table 1, Figure 1B, Figure S3B; GAMM: Δ AICc = -12.2 , Table S4, Figure S4B) and consisted of 173 kg [95% CI: 80; 263], that is 6.8% [95% CI: 3.2; 10.4] of their mean body mass (Table 2) or an effect size of -0.071 [95% CI: -0.11 ; -0.03] based on 185 observations of 18 females.

In comparison with females, in males, neither GLMMs nor GAMMs showed statistical support that the decline was terminal rather than mid-age: in GLMMs replacing the mid-age decline by a terminal decline worsened the model fit (Δ AICc = $+35.6$, Table 1) and there was no statistical support for the same trajectory in males and females (Δ AICc = $+42.1$, Table 1). For males, in GLMMs, adding a terminal term on top of the mid-age decline also worsened the model fit (Δ AICc = $+5.0$, Table 1, Figure S3A), but in GAMMs, models with and without the terminal term were almost equivalent (Δ AICc = -0.01 , Table S4). To better understand the possibility of terminal declines in both sexes, we compared the extent and effect size of the terminal decline in both sexes. In males the terminal decline, if any, is just over half that in females at 96 kg [95%CI: -19 ; 205], that is 4.3% [95%CI: -0.9 ; 9.2] of males' mean body mass or an effect size of -0.05 [95%CI: -0.10 ; 0.01] based on 1,316 observations on 171 males.

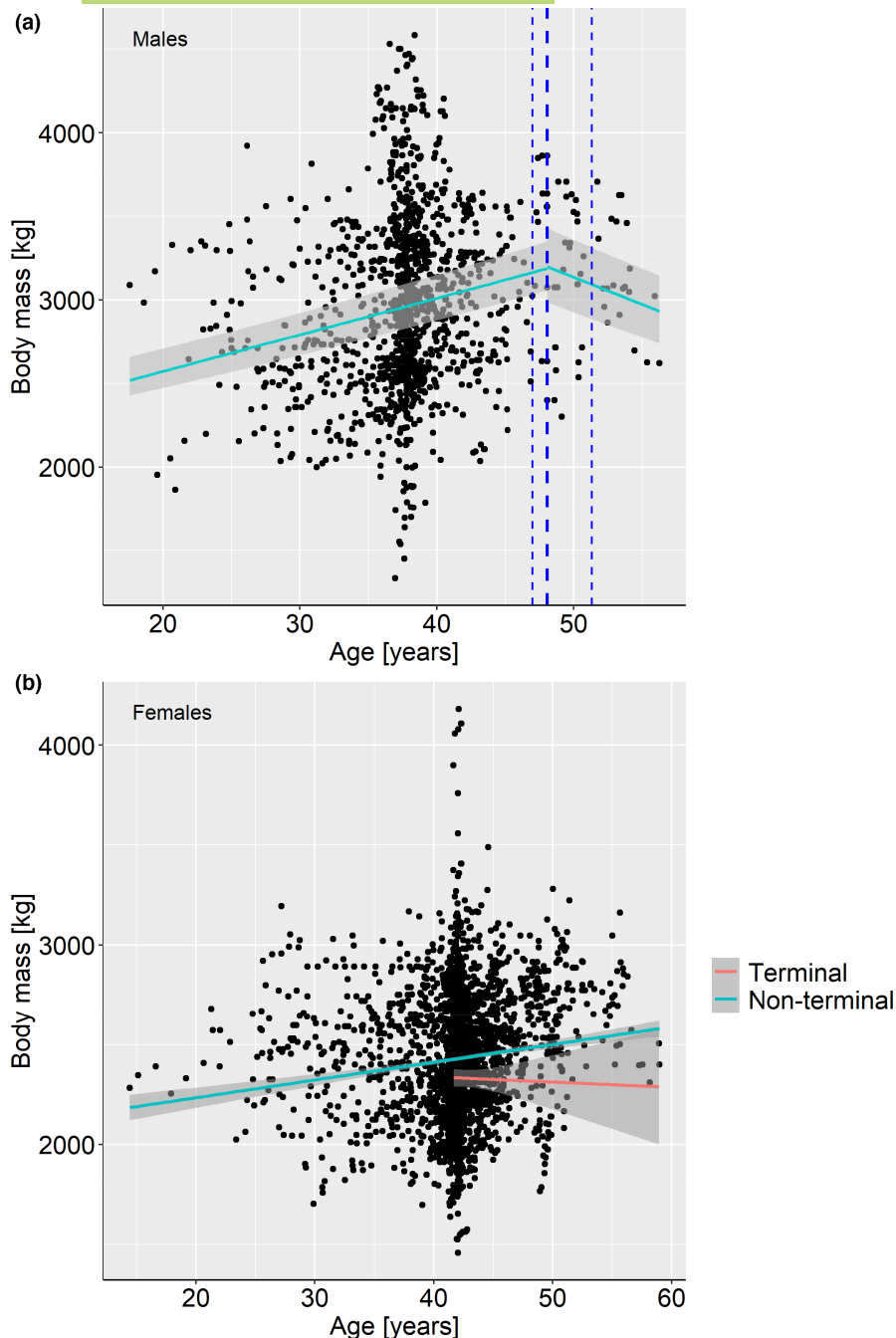


FIGURE 1 Body mass ageing trajectories of (a) males ($n = 1316$ measurements on 171 individuals) and (b) females ($n = 2570$ measurements on 322 individuals) with predictions of the best-fitting GLMMs (Table 1) with grey areas 95% CI. For males, the thick dashed line shows the threshold age at onset of the body mass decline (1.9 or 48.3 years) with thin dashed lines the 4 Δ AICc-Cl [46.6, 52.3]. For females, measurements in the terminal year (red) are significantly lower (intercept) than measurements at other ages (blue). Note 1: the terminal slope is for illustration purposes only and was not statistically tested. Note 2: the original x-axis is Δ age, but for simplicity, we presented here the x-axis as age. For the original figure, please see Figure S2

Note that for females and for both GLMMs and GAMMs, the second best model confirms the linear age trajectory but excludes the terminal effect (GLMM: Δ AICc = +4.9, Table 1; GAMM: Δ AICc = +12.2, Table S4). For males, the second best model (for GLMMs and GAMMs) conserves the same threshold trajectory but includes the terminal effect (GLMM: Δ AICc = +5.0, Table 1; GAMM: Δ AICc = -0.01, Table S4). Model averaging on ageing trajectories within 7 Δ AICc (Burnham et al., 2011) confirmed the ageing trajectories found, that is a threshold trajectories for males with a significant decline of body mass from 48 years old onwards (GLMM: $\beta = -0.06$ [95%CI: -0.09; -0.03]) and a non-significant terminal decline (GLMM: $\beta = -0.04$ [95%CI: -0.10; 0.01], GAMM: $\beta = -0.04$ [95%CI: -0.09; 0.01]). Similarly, model averaging performed on models

within 7 Δ AICc for females confirmed the ageing trajectory found, that is a body mass gain throughout life (GLMM: $\beta = 0.015$ [95%CI: 0.01; 0.02]) with a significant decline starting in the last year of life (GLMM: $\beta = -0.07$ [95%CI: -0.11; -0.03]). Also, we found no effect of the temporal and spatial confounding variables tested (Table S3).

4 | DISCUSSION

We tested whether in a species with marked male-biased intra-sexual competition, males showed an earlier and/or faster rate of body mass ageing than females. Both sexes gained mass during early adulthood. However, the onset of body mass ageing

differed between the sexes: whilst males began to lose mass from 48.3 years old onwards, females lost body mass generally at an older age, namely in their last year of life. Compared with a previous study on growth curves of this population (Mumby et al., 2015), we are now using a larger dataset, including older and a larger number of retired individuals. This allowed us to evidence body mass ageing in this species, which was not possible until now. Here, we discuss the implications of our results in the light of the classical theory of ageing (Williams, 1957) and of the management of Asian elephants.

To the best of our knowledge, we describe for the first time a sex-specific pattern of body mass ageing in this species. Body mass ageing is often used in mammals as it may underpin actuarial and reproductive ageing (Beirne et al., 2015; Bérubé et al., 1999; Nussey et al., 2011). In our population, sex-specific actuarial senescence has already been shown (Lahdenperä et al., 2018) and males display higher mortality than females at all ages. However, reproductive ageing has only been described for females of this population, which is in part due to the difficulty of recording paternity in male elephants (Hayward et al., 2014; Mumby et al., 2015; Robinson et al., 2012). Our results in this long-lived and highly social species provide valuable insights on how body condition declines with age and offer a broader perspective on the multifaceted ageing, often referred to as a mosaic ageing (Walker & Herndon, 2010).

Asian elephants show male-biased intrasexual competition, with males being more aggressive (Seltmann et al., 2019), fighting more for dominance and showing higher rates of mortality at all ages than females, including during early development, as calves and during adulthood (Lahdenperä et al., 2018). In such species, the classical theory of ageing predicts that males should show an earlier onset or accelerated ageing (Williams, 1957). Indeed, in several polygynous mammals, males display an earlier onset or higher rates of ageing than females, suggested to be due to their stronger intrasexual competition (Clutton-Brock & Isvaran, 2007 but see also Camus et al., 2012; Tower, 2006). For example, in European badgers (*Meles meles*, Beirne et al., 2015) and Soay sheep (*Ovis aries*, Hayward et al., 2015), males systematically showed stronger or earlier body mass ageing compared with females. Conversely, in monogamous species, the onsets and rates of ageing tend to be similar in males and females (Bronikowski et al., 2011; Clutton-Brock & Isvaran, 2007; Thorley et al., 2020). Our results are consistent with those studies and with the prediction of the classical theory of ageing, but we agree less with later alternatives proposing that the prediction of the classical theory of ageing can be disrupted by high early-life condition-dependent mortality in males (Chen & Maklakov, 2014) or by canalization (Flatt, 2005).

Previous work on this population showed age-related declines in the survival and reproduction of females (Hayward et al., 2014; Robinson et al., 2012). Hence, the ageing trajectories do not synchronize between traits in females. Empirically, this heterogeneity of ageing patterns is more the rule than the exception as found in other species (Briga & Verhulst, 2021; Hayward et al., 2015; Walker

& Herndon, 2010). In our population, this mismatch can be explained by the fact that body mass is a poor predictor of reproductive success, number of offspring produced or raised up to independence (5 years old), and that no relationship between height and survival has been found in females (Crawley et al., 2017). Our results that females do not show age-dependent body mass decline combined with previous results are at odds those on other vertebrates. Asian elephants reproduce all year around (Brown, 2014), despite living in a seasonal environment, meaning that females finance reproduction through energy stored before reproduction. This is contrary to, say roe deer (*Capreolus capreolus*), an income breeder energetically financing reproduction concurrently to gestation as this ungulate does not store reserves (Andersen et al., 2000). In the latter case, reproductive success therefore depends on body condition and available resources, whilst in the former case, elephants reproduce when they have stored sufficient resources to finance gestation. This might in part explain the absence of relation between female body mass ageing and reproductive senescence, contrary to males, which benefit more than females from being heavy during intrasexual competition (Sukumar, 2003).

Our study is subject to a number of limitations when it comes down to identifying why the sexes may differ in their ageing trajectories. First, it is possible that male elephants in our timber elephant population are used more for tasks requiring strength or tusks, thereby causing an earlier onset of body mass declines in males than in females. However, both sexes fall under the same government set workload, care and retirement regulation, except for females' maternity leave. One substantial difference between the sexes is that parental care is concentrated on females, with for example only females being given "parental leave" following reproduction (Toke Gale, 1974). However, since maternity is energetically expensive and no more favourable than timber working, this is unlikely to lead to the delayed onset of body mass declines in females. An ideal test would be to analyse the effect of timber work and maternity leave on body mass dynamics, but it has not been conducted to date.

Second, elephants have a specific dentition that consists of molar teeth that eventually wear down at the end of their lives, and pathologic malocclusions or lack of molars can lead to weight loss and death by starvation. In sexually dimorphic species of ungulates, males generally display smaller molar teeth size compared with females, relative to body size. This results in teeth wearing down faster and depleting earlier in males than in females (Carranza & Pérez-Barbería, 2007), with potential consequences for sex-specific senescence. In Asian elephants, although both sexes have the same molar dental anatomy, it is possible that the earlier onset of body mass declines in males reflects sex-specific differences in tooth wear. Indeed, in captive species, dental problems have been described well before the last year of life (Gaillard et al., 2015) and, if there is sex-specific tooth wear, this could be associated with the earlier onset of body mass ageing in males.

Third, male elephants have recurring periods of physiological "musth" throughout their adult lives, which can temporarily but

TABLE 1 Best-fitting body mass ageing trajectories (**bold**) for males and females, using GLMMs for each model ageing trajectories ranked from the least to the most complex. AICc: second-order Akaike Information Criterion; Δ AICc: change in AICc relative to the best-fitting model; k : degrees of freedom. Δ age1 and Δ age2 refer, respectively, to the pre- and post-peak changes of body mass with age in the threshold models

Model type	Model	Males			Females		
		k	AICc	Δ AICc	k	AICc	Δ AICc
null	$\log(\text{bm}) \sim 1$	4.0	-2828.3	364.2	4.0	-5442.9	160.7
linear	$\log(\text{bm}) \sim \Delta\text{age} + \text{age-last}$	6.0	-3162.0	30.4	6.0	-5586.1	17.5
+terminal	$\log(\text{bm}) \sim \Delta\text{age} + \text{age-last} + \text{terminal}$	7.0	-3156.9	35.6	7.0	-5592.3	11.3
age-last ²	$\log(\text{bm}) \sim \Delta\text{age} + \text{age-last} + \text{age-last}^2$	7.0	-3155.5	37.0	7.0	-5598.7	4.9
+terminal	$\log(\text{bm}) \sim \Delta\text{age} + \text{age-last} + \text{age-last}^2 + \text{terminal}$	8.0	-3150.4	42.1	8.0	-5603.6	0.0
Δage^2	$\log(\text{bm}) \sim \Delta\text{age} + \Delta\text{age}^2 + \text{age-last}$	7.0	-3176.8	15.7	7.0	-5571.6	32.0
+terminal	$\log(\text{bm}) \sim \Delta\text{age} + \Delta\text{age}^2 + \text{age-last} + \text{terminal}$	8.0	-3171.2	21.3	8.0	-5577.9	25.7
quadratic	$\log(\text{bm}) \sim \Delta\text{age} + \Delta\text{age}^2 + \text{age-last} + \text{age-last}^2$	8.0	-3170.1	22.3	8.0	-5584.3	19.3
+terminal	$\log(\text{bm}) \sim \Delta\text{age} + \Delta\text{age}^2 + \text{age-last} + \text{age-last}^2 + \text{terminal}$	9.0	-3164.6	27.9	9.0	-5589.3	14.3
threshold	$\log(\text{bm}) \sim \Delta\text{age1} + \Delta\text{age2} + \text{age-last}$	8.0	-3192.5	0.0	8.0	-5580.5	23.1
+terminal	$\log(\text{bm}) \sim \Delta\text{age1} + \Delta\text{age2} + \text{age-last} + \text{terminal}$	9.0	-3187.5	5.0	9.0	-5586.0	17.6
threshold (age-last ²)	$\log(\text{bm}) \sim \Delta\text{age1} + \Delta\text{age2} + \text{age-last} + \text{age-last}^2$	9.0	-3185.8	6.6	9.0	-5592.6	11.0
terminal	$\log(\text{bm}) \sim \Delta\text{age1} + \Delta\text{age2} + \text{age-last} + \text{age-last}^2 + \text{terminal}$	10.0	-3180.9	11.6	10.0	-5596.9	6.7

TABLE 2 Parameter estimates of linear mixed-effect models including individual body mass beyond 18 years of age as the response variable (in kg, log-transformed) for male and female Asian elephants

Males			Females		
Random effects	V	SD	Random effects	V	SD
Individual identity	0.019	0.137	Individual identity	0.011	0.103
Township	0.0005	0.023	Township	0.003	0.056
Fixed effects	Estimate	SE	Fixed effects	Estimate	SE
Intercept	7.707	0.021	Intercept	7.840	0.019
Age at last measurement	0.087	0.012	Age at last measurement	0.025	0.007
Δage1	0.041	0.002	Age at last measurement ²	-0.028	0.006
Δage2	-0.057	0.014	Δage	0.016	0.001
			Terminal (1)	-0.071	0.020
Marginal R^2	0.24		Marginal R^2	0.10	
Conditional R^2	0.89		Conditional R^2	0.77	

Note: Marginal and conditional R^2 give the variance explained by fixed effects, and both fixed and random effects, respectively. Δage1 and Δage2 refer, respectively, to the pre- and post-peak changes of body mass with age in the threshold models.

Abbreviations: SD, standard deviation; SE, standard error; V, variance.

profoundly impact the body mass of individual males (Eisenberg et al., 1971) thereby affecting the body mass ageing trajectory. Although accounting for musth would improve future analyses, recording morphological measurements is unfortunately difficult during the musth period when males display highly aggressive behaviours.

Fourth, in our study, we did not find any evidence for body mass-based selective disappearance, but, as it is often the case in long-lived species, the average longitudinal individual monitoring is short relative to the lifespan of this species (e.g. Global BMI Mortality Collaboration et al., 2016; Prospective Studies Collaboration, 2009),

and hence we only have limited power to detect such an association. It is possible that there are sex-specific dynamics of selective disappearance, but whether that is the case in Asian elephants remains to be shown. An analysis with more longitudinal data would be useful to tackle this question.

Fifth, for both sexes, it is possible that maximum body mass is set by physiological and ecological constraints as indicated by the weight growth curves found earlier in this population (Mumby et al., 2015). These constraints could to some extent be sex-specific, although, at this point, we can only speculate as to why these constraints may drive sex-specific ageing trajectories. Finally, we found

a maximum body mass in males but not in females. This sex-specific difference could be driven by the fact that male elephants benefit more than females from being heavy during intrasexual competition (Sukumar, 2003).

Another factor to take into account is that retirement occurs at around 50 years in both sexes, which likely diminishes physical exercise and allows more time for foraging, thereby continuing the weight gain. The reduced intrasexual competition in females relative to males, together with this retirement, could lead to the continued mass gain of females. One way of disentangling the effect of senescence and retirement on body mass trajectories would be to know whether muscle is lost over fat. Unfortunately, we do not have such data. However, a recent study in this semi-captive population did not detect declines in muscle function with age in either sex, as measured by the activity of the creatinine kinase (Reichert et al., 2022). On the contrary, fat storage as measured by levels of circulating triglycerides remained constant up to adulthood, decreasing from retirement onwards in both sexes (Reichert et al., 2022). Also, all elephants officially retire at age 55, but most elephants enter a form of pre-retirement or reduced labour around the age of 50. These points, taken together with the onset of body mass decline we found in males (*i.e.* 48 years old), suggest that retired individuals lose fat in both sexes rather than muscle and that body mass ageing is a cause rather than a consequence of retirement in males. Nevertheless, given that elephants in the wild do not experience timber labour and retirement, we cannot exclude that the sex-specific body mass ageing trajectories could be different in a wild (non-working) population of Asian elephants compared with those found in our study.

We found that females experienced a terminal body mass decline in the last year of life. Our data contain both males and females among the oldest ages (>50), hence sex-specific terminal decline is unlikely to emerge from differences in lifespan. In European badgers, a species in which females outlive males, both sexes displayed terminal body mass declines (Beirne et al., 2015). We evaluated the possibility that sex-specific ageing trajectories result from differences in sample sizes. For males, our post-peak data (starting at $\Delta\text{age} = 1.9$, equivalent to an age of 48 years old) contain 47 observations on 14 individuals. For females, the dataset after a peak at the same age ($\Delta\text{age} = 1.4$) contains 191 observations on 35 individuals. Thus, as is often the case in ageing studies, the sample sizes at older ages are relatively small, which is a limitation. Note, however, that the three best ageing trajectories for males include a mid-age decline in body mass and that we found a medium effect size (Cohen, 1988) of -0.59 [95%CI: -0.87 ; -0.30] for the post-peak decline, which makes, we believe, our results trustworthy. For the terminal decline, our data contain only 5 dead males (63 observations) and 18 dead females (185 observations), and among them, we had measurements during the last year of life for 2 males (7 observations) and 10 females (54 observations). Hence, sample sizes are small and it is possible that the sex-specific declines in our study resulted from differences in power. Indeed, for both males and females, the coefficient and effect size of the terminal terms were negative, although the effect size in males

remained about half of that in females (Cohen's $d_{\text{males}} = -0.045$ [95% CI: -0.10 ; 0.01] = a decline of 96 kg [95% CI: -19 ; 205], Cohen's $d_{\text{females}} = -0.071$ [95% CI: -0.11 ; -0.03] = a decline of 173 kg [95% CI: 80 ; 263]). Noteworthy, these effect sizes are below 0.20 and hence remain small following the conventional benchmarks (Cohen, 1988; Nakagawa & Cuthill, 2007). Hence, it is possible that the sex-specific terminal effect is driven by power issues, and we look forward to testing that with several more years of monitoring.

Terminal declines emphasize that the chronological age is rarely a perfect estimation of the biological age which can better describe the “true biological state” of an organism (Klemera & Doubal, 2006). In that sense, a terminal decline is a biomarker of health and remaining lifespan. The “terminal illness” hypothesis refers to the age-independent decrease of a trait value, related to the imminent death of the individual (Coulson & Fairweather, 2001). Such terminal effects were shown for example for body mass in European badgers, with larger effects in males than females (Beirne et al., 2015), in both sexes in Soay sheep (Hayward et al., 2015) and in male but not female Alpine marmots (Tafari et al., 2013) and for sexual signals in birds (Simons et al., 2016). For which traits or under which conditions to expect terminal declines remain yet poorly understood but our study highlights the importance of studying sex-specific differences in ageing and illustrates the need to improve our understanding of the mechanisms driving the diversity of ageing patterns in the wild.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHORS' CONTRIBUTION

HHA, WH and UKN performed field work and data collection. VL, VB and MB designed the study and LL selected, extracted and translated data. LL carried out all statistical analyses with contributions from VB and MB. LL wrote the manuscript and all authors critically reviewed it. All authors approved the manuscript for publication and agree to be held accountable for the content therein and approve the final version of the manuscript.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in Dryad at <https://doi.org/10.5061/dryad.5dv41ns59>.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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Supplementary information to:
Sex-specific body mass ageing trajectories in adult Asian elephants

Table of content

Supplementary Information 1. Body mass estimation	2
Equation (1) and (2), Table S1	
Supplementary Information 2. Illustration of body mass ageing trajectories using GLMMs	4
Figure S1	
Supplementary Information 3. Sex-specific age trajectories.....	5
Table S2	
Supplementary Information 4. Seasonal and spatial confounding variables	6
Table S3	
Supplementary Information 5. Body mass ageing trajectories using GLMMs	7
Figure S2	
Supplementary Information 6. Testing terminal decline windows.....	8
Figure S3	
Supplementary Information 7. Body mass ageing trajectories using GAMMs.....	9
Table S4, Table S5, Figure S4, Figure S5	
References	12

Supplementary Information 1. Body mass estimation

Because of the high correlation of chest girth (CG) with body mass, compared to other morphological metrics (Chapman et al., 2016), we performed a model selection according to AICc values (Akaike Information Criteria corrected) to choose the best way of estimating body masses from CG for males and females separately, as they have different weight growth curves (Chapman et al., 2016; Mumby, Chapman, et al., 2015). In addition to CG being a good predictor of body mass, we also included height to shoulders (H) in the models as indicative of structural size in Asian elephants. Our data indicate a robust correlation between CG and body mass for individuals with both measurements ($r_{\text{males}}=0.80$; $r_{\text{females}}=0.71$). The correlation between CG and body mass is higher than the correlation between CG and H (*i.e.* structural size ($r_{\text{males}}=0.64$; $r_{\text{females}}=0.51$). Body mass estimations and equations were estimated comparing $n = 1,470$ ($n_{\text{male}} = 491$, $n_{\text{female}} = 979$) known body mass measurements to the same number of both CG and H measurements.

For females, the full model, including both CG and H and their quadratic effects, showed lower AICc and the best correlation between estimations and measurements, while it was the model including the linear and quadratic effect of CG, and the linear effect of H which was retained for males (table S1). We would rather use the most accurate equations to predict body mass from other metrics, although CG and H were correlated for both sexes ($r_{\text{males}} = 0.65$, $t = 18.7$, $p < 0.0001$; $r_{\text{females}} = 0.51$, $t = 18.5$, $p < 0.0001$). From the coefficients of the selected model, we formulated equations (1) and (2) for males and females respectively. The last term of both equations was added to correct for the tendency of the equations to overestimate body masses. Correlations between estimated body masses from equations (1) and (2) and known body masses was $r = 0.90$ [95%CI: 0.89; 0.91].

Males

$$BM = 2,829 - 32.17 \times CG + 0.06 \times CG^2 + 17.57 \times H - 63.26 \quad (1)$$

Females

$$BM = 7,697 - 16.40 \times CG + 0.04 \times CG^2 - 48.77 \times H + 0.14 \times H^2 - 21.26 \quad (2)$$

Table S1. Best predictors (in bold) of body mass estimation based on morphometric measures based on the second-order Akaike information criterion (AICc). CG: chest girth, H: height, r: spearman correlation coefficient, k: degrees of freedom.

Response	Model	r	Male			Female		
			AICc	k	r	AICc	k	r
Body mass	CG	0.851	7,116.7	3	0.800	13,596.5	3	0.711
	CG + CG ²	0.858	7,096.8	4	0.810	13,559.0	4	0.725
	CG + H	0.895	6,849.4	4	0.890	13,434.9	4	0.763
	CG + CG² + H	0.902	6,802.2	5	0.901	13,404.2	5	0.772
	CG + H + H ²	0.898	6,841.5	5	0.892	13,406.0	5	0.771
	CG + CG² + H + H²	-0.461	6,803.4	6	0.496	13,389.8	6	0.776

Supplementary Information 2. Illustration of body mass ageing trajectories using GLMMs

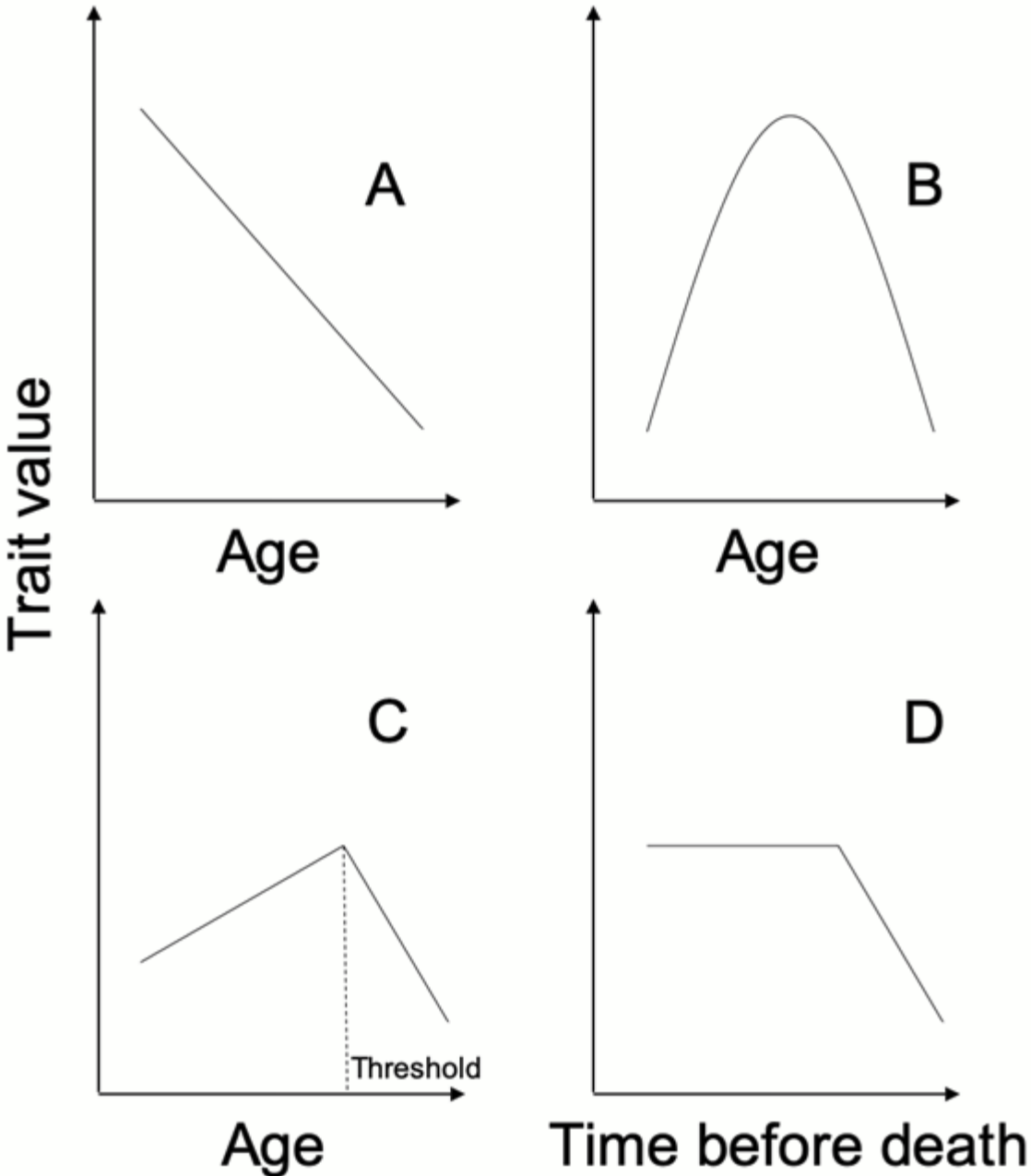


Figure S1. Schematic representation of the four ageing trajectories tested. Using the individual’s chronological age, ageing trajectory can be (A) linear, (B) quadratic, or (C) with a threshold. However, using the time before death, ageing can correspond to (D) a terminal decline. The slopes here are illustrative only and can, in practice, take any value. Figure adapted from Briga et al., 2019.

Supplementary Information 3. Sex-specific age trajectories

Table S2. Evidence for sex-specific body mass ageing trajectories both in GAM and GLM models based on model selection approaches. The models are ranked from the best-fitting (lowest AICc, on the top) to poorer-fitting ones (higher AICc, at the bottom). Best-fitting in bold. AICc: second-order Akaike Information Criteria, Δ AICc: difference in AICc relative to the best model, k: degrees of freedom, w: model weight, s=smooth. All models contain the random intercepts individual identity ($1/ID$) and township ($1/township$).

Model	Model	k	AICc	Δ AICc	w
GAMM	$\log(\text{bm}) \sim \text{terminal} + \text{sex} + \text{s}(\Delta\text{age}) + \text{age-last} + \text{age-last}^2 + \text{s}(\Delta\text{age}, \text{by}=\text{sex})$	17.9	-8,809.1	0.0	1.0
	$\log(\text{bm}) \sim \text{terminal} + \text{sex} + \text{s}(\Delta\text{age}) + \Delta\text{age}^2 + \text{age-last} + \text{age-last}^2$	10.3	-8,743.4	65.7	0.0
GLMM	$\log(\text{bm}) \sim \text{terminal} + \text{sex} + \Delta\text{age} + \text{age-last} + \text{age-last}^2 + \text{sex}:\Delta\text{age}$	10.0	-8,730.6	0.0	1.0
	$\log(\text{bm}) \sim \text{terminal} + \text{sex} + \Delta\text{age} + \text{age-last} + \text{age-last}^2$	9.0	-8,683.6	47.0	0.0

Supplementary Information 4. Seasonal and spatial confounding variables

Table S3. Model selection of covariates based on the best fitting models in Table 1 describing body mass ageing trajectories for both sexes. The column ‘Model’ refers to the model names as given in Table 1. AICc: Akaike Information Criteria (corrected) of the selected models, k: degrees of freedom, ‘season’: Δ AICc (*i.e.* AICc differential compared to the selected model) when including the season of the body mass measurement, ‘alive’: Δ AICc when including whether individuals were dead or alive at the moment of the analysis, ‘cw’: Δ AICc when including whether the individuals were captive-born or wild-caught, ‘measure’: Δ AICc when including whether the body masses were estimated or measured.

Sex	Model selected	AICc	Δ AICc	k	w
Males GAMMs	$\log(\text{bm}) \sim s(\Delta\text{age}) + \text{age-last} + \text{cw}$	-3,222.8	0.0	9.1	0.234
	$\log(\text{bm}) \sim s(\Delta\text{age}) + \text{age-last}$	-3,221.9	0.9	8.1	0.148
	$\log(\text{bm}) \sim s(\Delta\text{age}) + \text{age-last} + \text{cw} + \text{season}$	-3,221.2	1.6	11.1	0.104
	$\log(\text{bm}) \sim s(\Delta\text{age}) + \text{age-last} + \text{cw} + \text{measure}$	-3,220.8	2.0	10.1	0.085
	$\log(\text{bm}) \sim s(\Delta\text{age}) + \text{age-last} + \text{cw} + \text{alive}$	-3,220.4	2.4	11.1	0.071
	$\log(\text{bm}) \sim s(\Delta\text{age}) + \text{age-last} + \text{season}$	-3,220.2	2.6	10.1	0.066
	$\log(\text{bm}) \sim s(\Delta\text{age}) + \text{age-last} + \text{alive}$	-3,219.9	2.9	10.1	0.055
	$\log(\text{bm}) \sim s(\Delta\text{age}) + \text{age-last} + \text{measure}$	-3,219.8	2.9	9.1	0.054
	$\log(\text{bm}) \sim s(\Delta\text{age}) + \text{age-last} + \text{cw} + \text{measure} + \text{season}$	-3,219.1	3.6	12.1	0.038
$\log(\text{bm}) \sim s(\Delta\text{age}) + \text{age-last} + \text{alive} + \text{measure} + \text{season}$	-3,218.7	4.1	13.1	0.031	
Females GAMMs	$\log(\text{bm}) \sim \text{terminal} + s(\Delta\text{age}) + \text{age-last} + \text{age-last}^2 + \text{season}$	-5,641.5	0.0	12.8	0.316
	$\log(\text{bm}) \sim \text{terminal} + s(\Delta\text{age}) + \text{age-last} + \text{age-last}^2 + \text{season} + \text{measure}$	-5,640.2	1.4	13.7	0.160
	$\log(\text{bm}) \sim \text{terminal} + s(\Delta\text{age}) + \text{age-last} + \text{age-last}^2$	-5,639.7	1.8	10.9	0.127
	$\log(\text{bm}) \sim \text{terminal} + s(\Delta\text{age}) + \text{age-last} + \text{age-last}^2 + \text{season} + \text{cw}$	-5,638.8	2.7	14.8	0.082
	$\log(\text{bm}) \sim \text{terminal} + s(\Delta\text{age}) + \text{age-last} + \text{age-last}^2 + \text{measure}$	-5,638.4	3.1	11.7	0.066
	$\log(\text{bm}) \sim \text{terminal} + s(\Delta\text{age}) + \text{age-last} + \text{age-last}^2 + \text{season} + \text{alive}$	-5,638.1	3.4	14.8	0.057
	$\log(\text{bm}) \sim \text{terminal} + s(\Delta\text{age}) + \text{age-last} + \text{age-last}^2 + \text{season} + \text{measure} + \text{cw}$	-5,637.5	4.1	15.9	0.042
$\log(\text{bm}) \sim \text{terminal} + s(\Delta\text{age}) + \text{age-last} + \text{age-last}^2 + \text{cw}$	-5,637.0	4.5	12.9	0.033	
Males GLMMs	$\log(\text{bm}) \sim \Delta\text{age1} + \Delta\text{age2} + \text{age-last}$	-3,192.5	0.0	8.0	0.889
	$\log(\text{bm}) \sim \Delta\text{age1} + \Delta\text{age2} + \text{age-last} + \text{cw}$	-3,187.9	4.6	9.0	0.089
	$\log(\text{bm}) \sim \Delta\text{age1} + \Delta\text{age2} + \text{age-last} + \text{alive}$	-3,184.4	8.1	10.0	0.016
Females GLMMs	$\log(\text{bm}) \sim \text{terminal} + \Delta\text{age} + \text{age-last} + \text{age-last}^2$	-5,603.6	0.0	8.0	0.983
	$\log(\text{bm}) \sim \text{terminal} + \Delta\text{age} + \text{age-last} + \text{age-last}^2 + \text{measure}$	-5,594.6	9.0	9.0	0.011

Supplementary Information 5. Body mass ageing trajectories using GLMMs

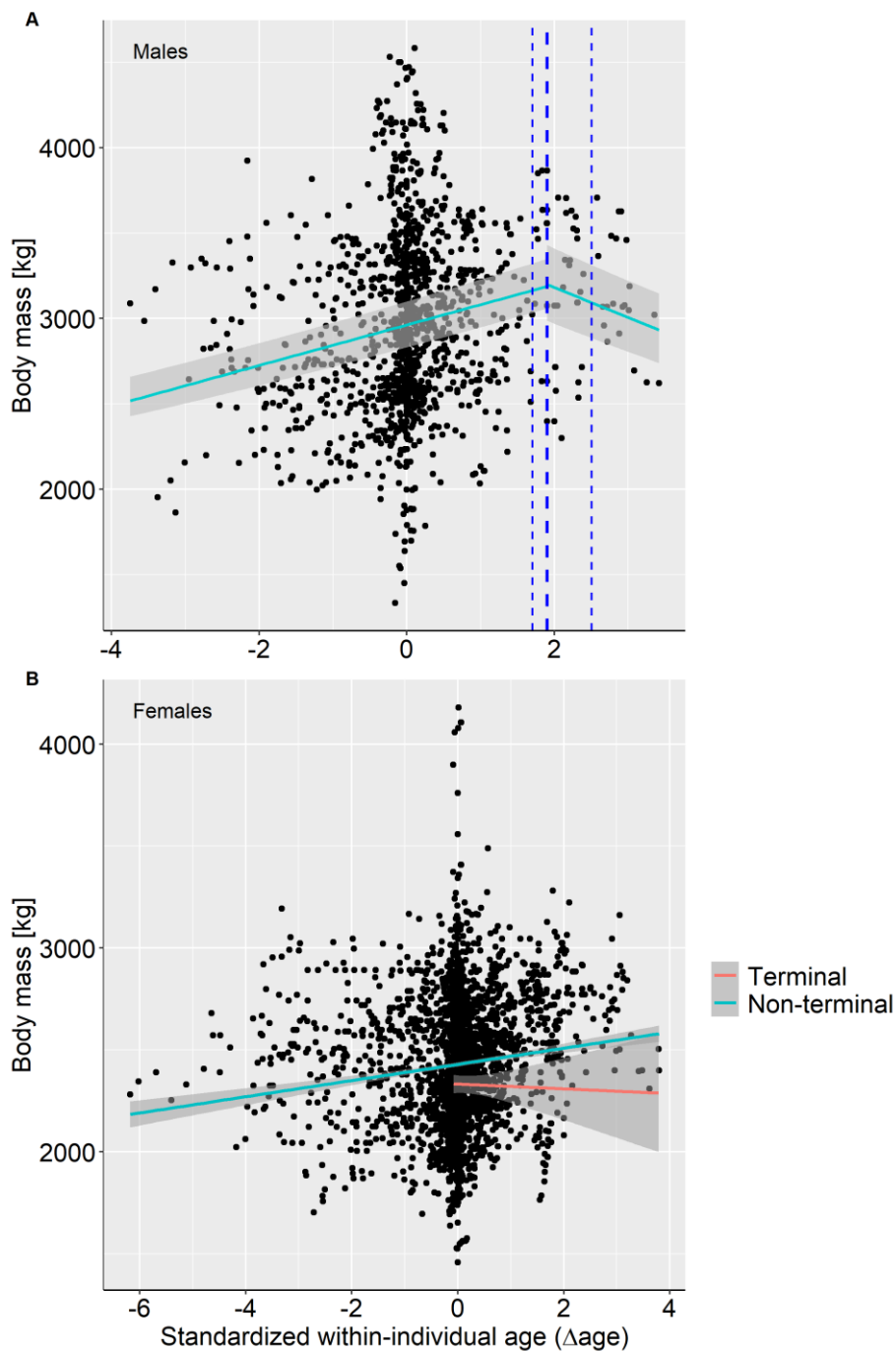


Figure S2. Body mass ageing trajectories of (A) males ($n = 1,316$ measurements on 171 individuals) and (B) females ($n = 2,570$ measurements on 322 individuals) with predictions of the best-fitting GLMMs (Table 1) with grey areas 95%CI. For males, the thick dashed-line shows the threshold age at onset of the body mass decline (1.9 or 48.3 years) with thin dashed-lines the 4 Δ AICc-CI [46.6, 52.3]. For females, measurements in the terminal year (red) are significantly lower (intercept) than measurements at other ages (blue). Note that the terminal slope is for illustration purposes only and was not statistically tested.

Supplementary Information 6. Testing terminal decline windows

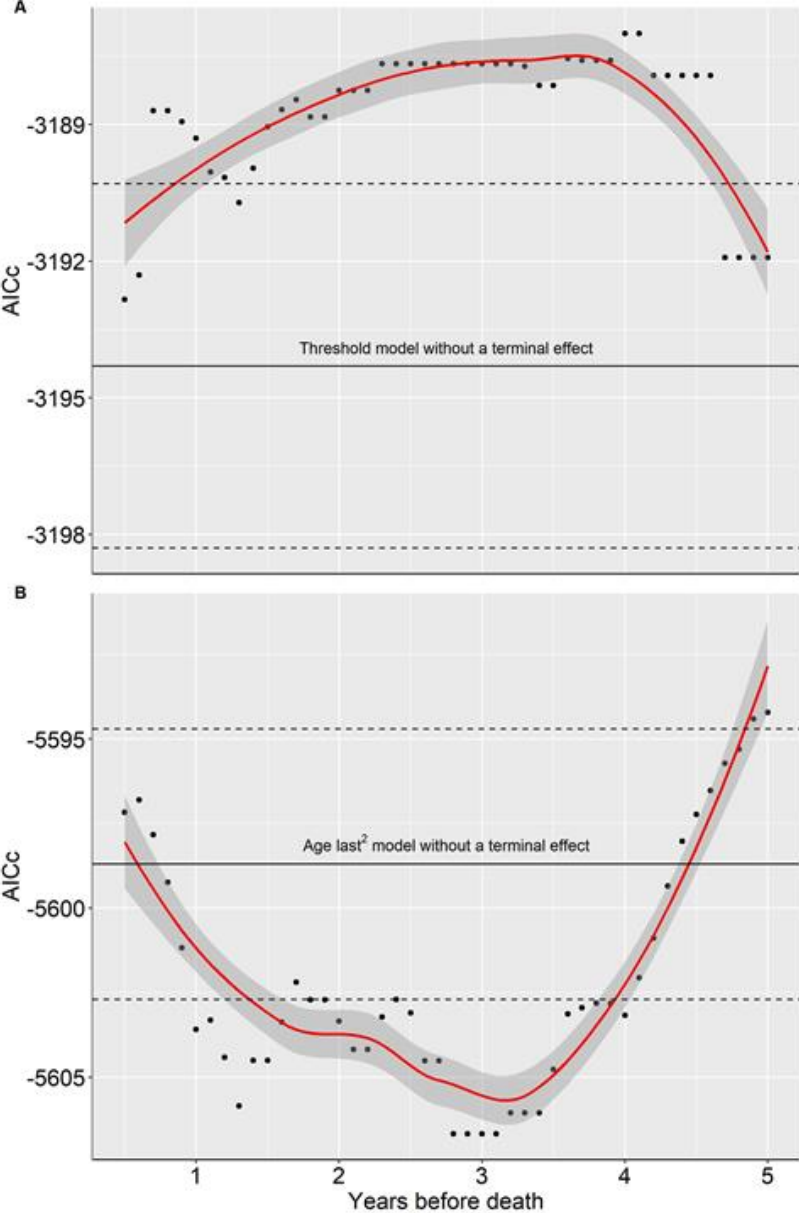


Figure S3. Testing variation in windows for the age at onset of the terminal decline for A) males and B) females. The black solid line represents the fit of the model selected without a terminal effect, and the dashed lines represent the confidence interval of this model ($\pm 4AICc$). A) In males, terminal declines were not statistically supported (best fitting model in table 1), irrespective of the window used. B) In females, a statistically significant terminal decline occurs between 1 and 4 years before death and the statistical support through this windows is equivalent. Hence, we chose to illustrate models with terminal windows of one year.

Supplementary Information 7. Body mass ageing trajectories using GAMMs

Table S4. Model selection of body mass ageing trajectories (bold) for males and females, using GAMMs for each model ageing trajectories ranked from the least to the most complex. AICc: second-order Akaike Information Criterion; Δ AICc: change in AICc relative to the best fitting model; k: degrees of freedom.

Model type	Model	Males			Females		
		k	AICc	Δ AICc	k	AICc	Δ AICc
null	$\log(\text{bm}) \sim 1$	4.0	-2,835.8	386.1	4.0	-5,422.6	217.1
smooth1	$\log(\text{bm}) \sim s(\Delta\text{age}) + \text{age-last}$	8.1	-3,221.9	0.01	8.7	-5,613.9	25.8
+terminal	$\log(\text{bm}) \sim s(\Delta\text{age}) + \text{age-last} + \text{terminal}$	9.1	-3,221.9	0.0	10.0	-5,627.3	12.4
smooth2	$\log(\text{bm}) \sim s(\Delta\text{age}) + \text{age-last} + \text{age-last}^2$	9.1	-3,221.2	0.7	9.7	-5,627.5	12.2
+terminal	$\log(\text{bm}) \sim s(\Delta\text{age}) + \text{age-last} + \text{age-last}^2 + \text{terminal}$	10.1	-3,221.3	0.6	10.9	-5,639.7	0.0

Table S5. Estimates of general additive mixed models (GAMMs) including individual body mass beyond 18 years of age as the response variable (in kg, log-transformed) for male and female Asian elephants. V: variance, SD: standard deviation, SE: standard-error, Df: degrees of freedom, F: Fisher value. Marginal and conditional R^2 give the variance explained by fixed effects, and both fixed and random effects, respectively.

Males			Females		
Random effects	V	SD	Random effects	V	SD
Individual identity	0.018	0.136	Individual identity	0.009	0.095
Township	0.0004	0.021	Township	0.004	0.060
Fixed effects	Estimate	SE	Fixed effects	Estimate	SE
Intercept	7.997	0.013	Intercept	7.832	0.009
Age at last measurement	0.089	0.012	Age at last measurement	0.034	0.007
			Age at last measurement ²	-0.023	0.006
			Terminal (1)	-0.075	0.020
Smooth term	Df	F	Smooth term	Df	F
Δ age	6.096	70.4	Δ age	6.899	27.9
Marginal R^2	0.23		Marginal R^2	0.10	
Conditional R^2	0.89		Conditional R^2	0.80	

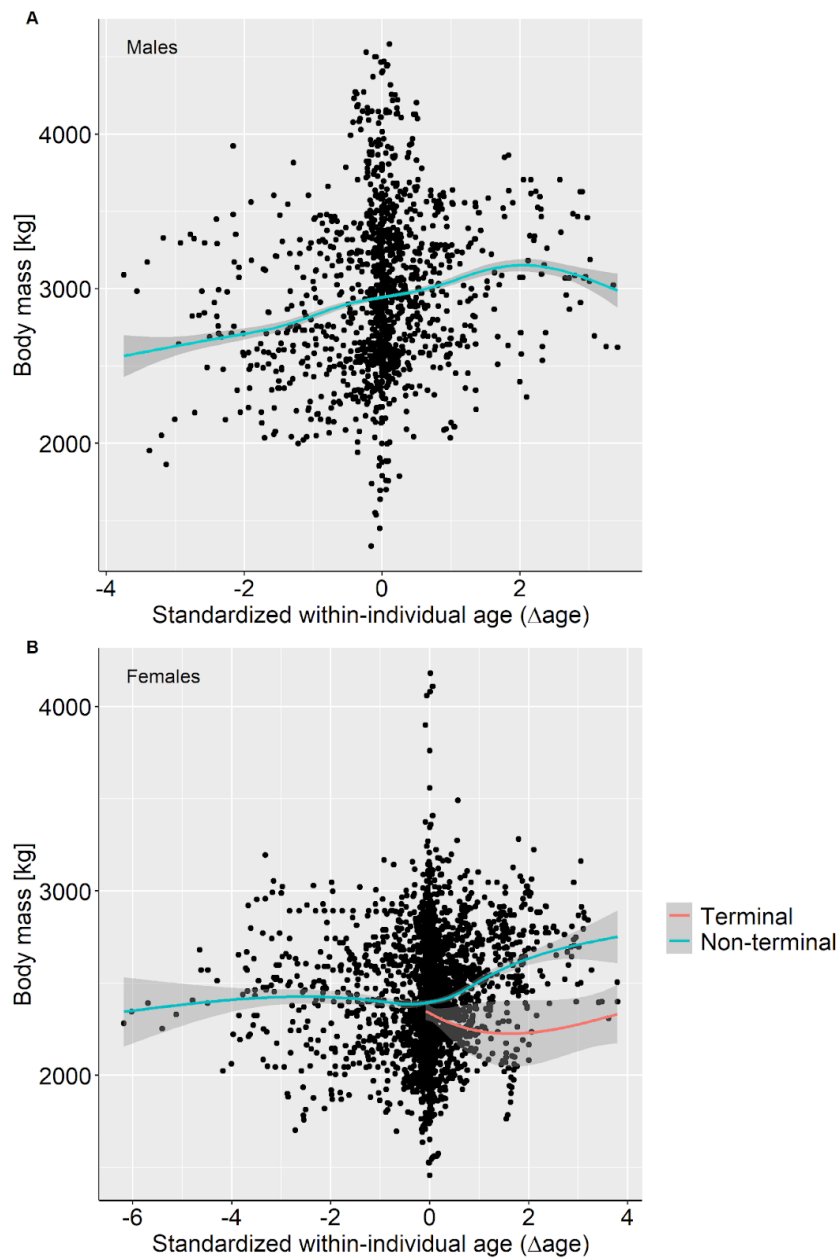


Figure S4. Body mass ageing trajectories of (A) males ($n=1,316$ measurements on 171 individuals) and (B) females ($n=2,570$ measurements on 322 individuals) with solid lines showing predictions of the best-fitting GAM models (Table S4) and grey areas 95%CI. For females, measurements in the terminal year (red) are significantly lower than measurements at other ages (grey), but note that the association (slope) with Δ age is for illustration purposes only and was not statistically tested.

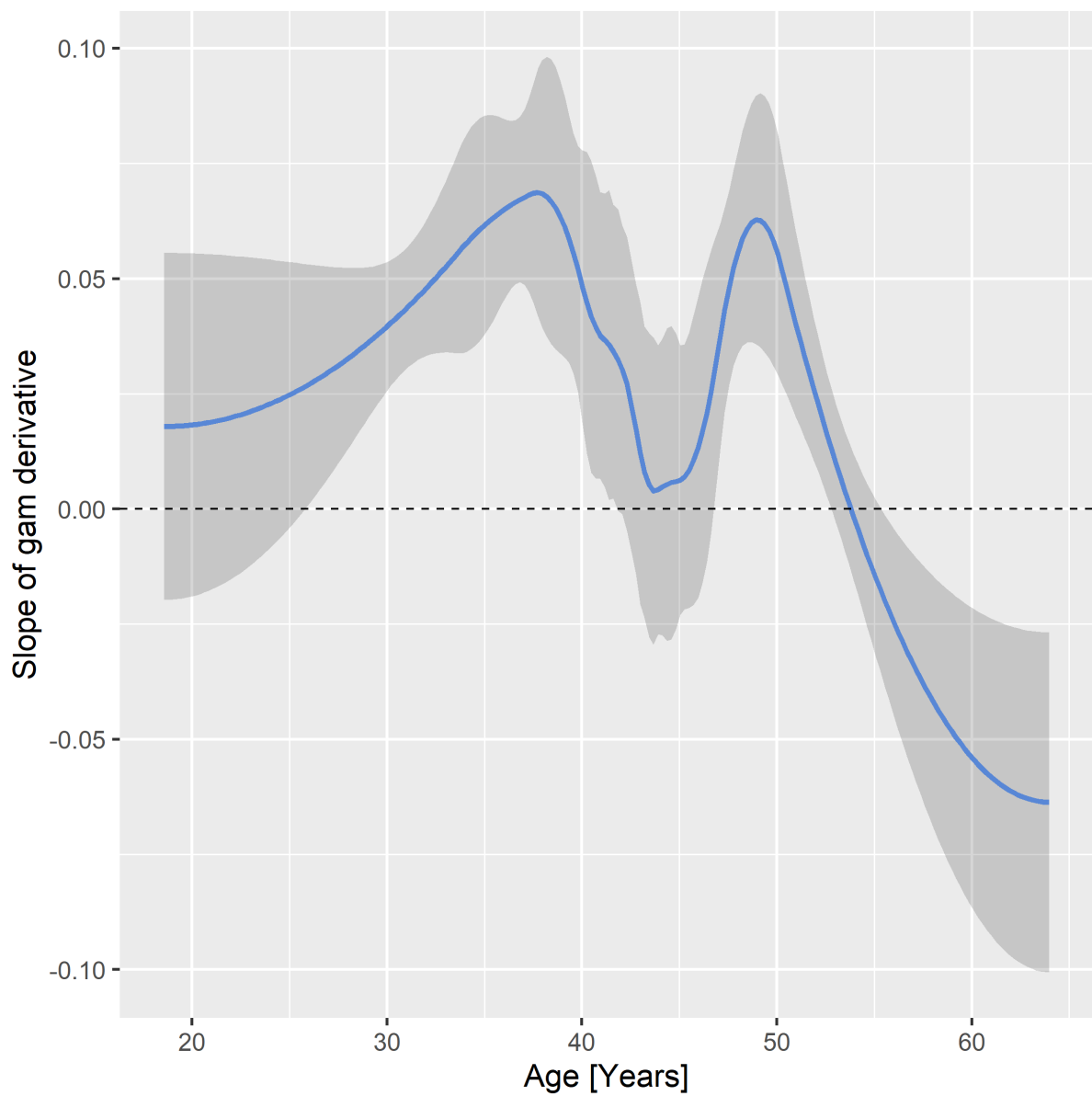


Figure S5. Derivative of the best fitting GAM model in males (Table S4, smooth 1) to identify the age at which maximum body mass is reached, *i.e.* when the derivative (blue line) is zero. Grey zones indicate the 95% confidence interval around the age at maximum.

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