Ecological marginalisation in terrestrial mammals

A thesis submitted to the University of Manchester for the degree of Doctor of Philosophy in the Faculty of Science and Engineering

2022

Jake A Britnell

Department of Earth and Environmental Sciences

Table of Contents

Word count: 45474

List of Figures

List of Tables

Table of abbreviations

ACTH	Adrenocorticotrophic hormone
ASV	Assigned sequence variant
CLR	Centred log ratio
CMZ	Cape mountain zebra
CR	Critically Endangered
DNA	Deoxyribonucleic acid
EIA	Enzyme Immunoassay
EN	Endangered
ENMs	Envelope Niche Models
FEC	Faecal egg count
fGCMs	Faecal Glucocorticoid metabolites
fT3	Faecal thyroid hormone
GC	Glucocorticoids
GZ	Grevy's zebra
HPA	Hypothalamus-pituitary-adrenal
IUCN	The International Union for Conservation of Nature
MVE	Minimum Volume Ellipsoid
NDVI	Normalised Difference Vegetation Index
NVH	Niche variation hypothesis
OFT	Optimal foraging theory
OTU	Operational taxonomic unit
PA	Protected areas
PAs	Publicly owned protected areas
PCA	Principal component Analysis
PPA	Protected Private Areas
spp	Species
SVM	Support vector model
TH	Thyroid hormone
TNW	Total Niche Width

Abstract

Humans shape species distributions and niches, causing them to shrink, shift or collapse. Despite increases in the area of land protected globally, population declines and species losses continue. A potential contributing factor to continuing declines is the restriction of species into marginal habitats where they experience poor performance. Range contraction restricts species into a limited subset of historic habitats and niche conditions. Marginalisation occurs when contraction constrains species to geographic or ecological extremes. In this thesis, I ask 1) how widespread is ecological and geographic marginalisation in terrestrial mammals and does it influence extinction risk; 2) what are the physiological and performance consequences of maintaining species in marginal habitats, 3) does marginality influence species in both fragmented and unfragmented landscapes and 4) how does marginalisation influence conservation planning. I begin this thesis with a general introduction and a critical evaluation of methods used in this thesis. In Chapter 3, I use a combination of phylogenetic and niche modelling to evaluate the intrinsic and extrinsic factors associated with range collapse and niche shift across 4785 mammal species. This chapter demonstrates ecological marginalisation is a common consequence of range contraction and increases extinction risk. In Chapter 4, I evaluate whether ecological and demographic factors drive glucocorticoid concentrations in Cape mountain zebra (*Equus zebra zebra,* CMZ). This chapter highlights the importance of using validated assays and links elevated faecal glucocorticoid concentrations to poor population performance. In Chapter 5, I use DNA metabarcoding of diet, microbiome, and nemabiome to investigate the macrophysiological consequences of food limitation across the CMZ species range. This chapter finds dietary composition alters microbiome and nemabiome composition and links low grass diets, and associated physiological consequences, to poor performance. In Chapter 6, I use DNA metabarcoding of diet, microbiome, and nemabiome to investigate seasonal dietary switching and its macrophysiological consequences across an ecological gradient in the Grevy's zebra (*Equus grevyi*). This chapter demonstrates animals in a relatively unfragmented landscape can become restricted to marginal habitats. I conclude by discussing the conservation implications of marginalisation and suggest potential solutions. Published materials available in Appendices 1, 2 and 3 expand on these ideas. This thesis argues ecological marginalisation is an unappreciated conservation threat across terrestrial mammals.

Declaration

No portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification of this or any other university or institution of learning.

Copyright Statement

i. The author of this thesis (including any appendices and/or schedules to this thesis) owns certain copyright or related rights in it (the "Copyright") and s/he has given the University of Manchester certain rights to use such Copyright, including for administrative purposes.

ii. Copies of this thesis, either in full or in extracts and whether in hard or electronic copy, may be made only in accordance with the Copyright, Designs and Patents Act 1988 (as amended) and regulations issued under it or, where appropriate, in accordance with licensing agreements which the University has from time to time. This page must form part of any such copies made.

iii. The ownership of certain Copyright, patents, designs, trademarks and other intellectual property (the "Intellectual Property") and any reproductions of copyright works in the thesis, for example graphs and tables ("Reproductions"), which may be described in this thesis, may not be owned by the author and may be owned by third parties. Such Intellectual Property and Reproductions cannot and must not be made available for use without the prior written permission of the owner(s) of the relevant Intellectual Property and/or Reproductions.

iv. Further information on the conditions under which disclosure, publication and commercialisation of this thesis, the Copyright and any Intellectual Property and/or Reproductions described in it may take place is available in the University IP Policy (see http://documents.manchester.ac.uk/DocuInfo.aspx?DocID=2442 0), in any relevant Thesis restriction declarations deposited in the University Library, the University Library's regulations (see http://www.library.manchester.ac.uk/about/regulations/) and in the University's policy on Presentation of Theses.

To my family, friends, my inspirations and my idols.
Above all, thank you to Fran and Susanne

To all those who suffer the persecution of science
- Joan Roughgarden

The cure for boredom is curiosity. There is no cure for curiosity.
- Dorothy Parker

Normally, we do not so much look at things as overlook them
- Alan Watts

The most erroneous stories are those we think we know best – and therefore never scrutinize or question
- Stephen Jay Gould

Nature itself is a poem that we humans have written
- Richard Rorty

Acknowledgements

A PhD is usually seen as an individualistic pursuit. I cannot speak for others but mine was not. This thesis contains more than just my thought. It is a culmination of all thought, discussions and support I have received doing it. All the academics and non-academics, all the reserve managers and rangers, all the students and the lay-persons, people I have never met in person and those who I have read but are long passed. There are countless people to thank. I will name a few but there will be many more who have had untold influence which I do not have the good fortunate of knowing.

Firstly, I must thank my supervisory team. To Professor Susanne Shultz and Professor Graham Kerley, I cannot express the extent of my gratitude. The influence both of you have had on my thought shapes the way I see the world. I am continually inspired and astonished by the sheer love you both have for your subjects and the amount of work and effort you put into it. Both of you did not give up on me despite the many times which I did not believe I could do it and for that I thank you the most. To Rachael Antwis, Dr. Tyler Kartinzel, Professor Daniel Rubenstein, Professor Rupert Palme, Dr. Danielle Hinchcliffe, I thank you all for your support and the discussions we had.

To Nicholas Harvey for showing me that social science matters. It really matters! To Rebecca Lewis for all her support and strength. To Thomas Richardson, one of the best people I have been fortunate enough to meet. To, someone who showed me the importance of taste, who shares my love of intellect and is a true friend. Importantly, to Elisa Manase for your kindness and your friendship. To Corey Lee, Samuel Purkiss and Kieran Fox, our time in South Africa will not be forgotten. Alice Burton, Reuben Margerison and James Dinsley, it is a pleasure to know you. To my family, Jeff Britnell, Tina Britnell, Billy, Rachael, Albie and Frank, (and many more, I am fortunate enough to have too many to name… but just a few more - Jean Rigby and Wendy Williamson and Raymond Elsner, Deborah Gearing and David Spencer), you are all my foundation. I love you all. Finally, to Frankie, I simply would not be here without you. You gave me the freedom to be who I am and find what I enjoyed. I owe this PhD experience the most for introducing us. To you all, this is for you. Peace, love and onwards.

The author

I received a BSc from the University of Manchester in 2017. Upon graduation, I transition directly onto the National environmental research council (NERC) doctoral training program (DTP) out of which the work of this thesis derives. This thesis represents the major part of my research experience.

Rationale for journal format

This thesis is presented in a journal format. Chapter 3 and 4 of this thesis have been submitted for publication. Chapter 5 is in preparation for publication and Chapter 6 is in preparation for publication as part of a large project. Appendices 1 and 2 include published material. The journal format has allowed me to complete project and submit for dissemination through peer-reviewed journals.

Contributions of candidate and co-authors

Chapters 1,2 and 7 were written in their entirety by myself. Franziska Elsner-Gearing assisted with proofreading and gave me comments.

Chapter 3

The initial draft of Chapter 3 entitled “Conservation in the margins: ecological marginalisation increases extinction risk in mammals” was written by me. Susanne Shultz and I conceptualised the project. I designed and performed niche modelling and data analysis. I wrote the code for the entire chapter and developed methods to evaluate marginalisation. Susanne Shultz provided substantial input into the design of data analysis especially the statistical modelling. Yichun Zu assisted with data generation, curation and land use analysis. Professor Graham Kerley and Susanne Shultz provided substantial comments which improved the manuscript.

Chapter 4

The initial draft of Chapter 4 entitled “The importance of validation: using a validated faecal glucocorticoid metabolite assay links Cape mountain zebra (*Equus zebra zebra*) HPA activity to population performance and sex-specific effects, but not habitat quality or adult-sex ratio” was written by me. Jake Britnell and Susanne Shultz analysed the data for the manuscript. R.P. provided substantial expertise on the assay selection. I performed hormone extractions and sample preparation for reanalysed samples under the supervision of Professor Rupert Palme. Metabolite assays were performed in laboratory of Rupert Palme. I collected samples for biological validation. Rupert Palme performed assays for biological validation experiment except for the DetectX corticosterone EIA which was performed by me. Rupert Palme, Professor Graham Kerley and Professor Susanne Shultz provided substantial input on draft structure and writing. Dr. Jess Lea and Dr. John Jackson contributed to the revisions of the manuscript.

Chapter 5

I collected the dung samples and performed all the field work with the assistance of Corey Lee, Samuel Purkiss and Kieran Fox. I conducted the DNA metabarcoding preparation and Dr Rachael Antwis completed the final MiSeq protocol. I conducted the data analysis, wrote the initial draft of the manuscript. Professor Susanne Shultz provided comments to improve the manuscript. T. de Oliveria and J.Giandhari conducted a subset of the DNA metabarcoding of microbiome (~50 samples). I extracted the environmental data from sources cited in the chapter.

Chapter 6

Dr.Danielle Hinchcliffe conducted field work and faecal collection. I conducted the DNA metabarcoding with Dr Nicholas Harvey at the International Livestock Research Insitute. Regina Njeru, Benedict Karani and Eunice Machuka assisted with carried out sequencing runs on the Illumina Miseq platform. I conducted the data analysis. I wrote the initial draft of the manuscript. Professor Susanne Shultz provided comments on the manuscript.

Chapter 1) Introduction

1.1 Species ranges under human pressure

1.1.1 Human land-use has resulted in widespread range contraction across taxa globally

Human land-use has shaped and transformed the earth's ecology for more than 10,000 years (Ellis et al., 2021). The current intensity, scale and ecological consequences of anthropogenic land-use are unparalleled in earth's history (Ellis, 2011; Ellis et al., 2021). Human land-use has transformed between 75-95% of the earth's ice-free land (Ellis, 2011; Riggio et al., 2020) and is the leading cause of biodiversity loss globally (Newbold et al., 2015; Johnson et al., 2017) . Current biodiversity declines approach the highest rate of faunal extinction in earth's history (Barnosky et al., 2011) with many vertebrates on the brink of extinction (Ceballos et al., 2020).

Human activities disproportionately result in range decline (Pacifici et al. 2020; Chapter 3). Pollution, overexploitation, habitat loss, fragmentation and invasive species have contributed to population declines (Dirzo et al., 2014; Doherty et al., 2016; Ceballos et al., 2017). As human pressures extirpate local populations, a species declines to critically low abundances, occupying less and less of its historic range (di Marco et al., 2014). Widespread range contractions are documented in large carnivores (Wolf & Ripple, 2017), megaherbivores (Ripple et al., 2015), reptiles and amphibians (Towns & Daugherty, 1994), birds (Jersey & Rodríguez, 2002) and large pelagic marine predators (Worm & Tittensor, 2011).

Human land use, especially agriculture or human settlements, disproportionately impacts specific biomes (Boakes et al. 2010; Jacobson et al. 2019; Figure 1). Human pressures extirpate many species from these biomes, fragmenting and contracting their ranges (Haddad et al., 2015). Remaining habitat may be composed of small and isolated patches (Lesica & Allendorf, 1995). As a result, species can become confined to a marginal subset of historic habitats and niche conditions (Kerley et al. 2012; Scheele et al. 2017). As noted by Caughley (1994), the "species end up, not in the habitat most favourable to it, but in the habitat least favourable to the agent of decline [threat]." Here, I term this process: "marginalisation." Marginalisation occurs when threatening pressures displace species from high quality habitats to marginal, low quality habitats, where they experience poor performance. Marginalisation becomes severe when marginal populations outnumber high quality populations (*i.e*. Partial refugee species Lea et al. 2016) or when species become confined to and protected in solely marginal habitats (*i.e.* Refugee species, Kerley et al. 2012).

1.1.2 Populations continue to decline despite increasing protection efforts: a protected area paradox and marginalisation

Following drastic population declines, conservation initiatives began to implement schemes to prevent the extinction of species (Phillips, 2004). A major foundation of conservation efforts is the formation and expansion of protected areas (PAs) (Dinerstein et al., 2019). Since the 1970s, PAs have rapidly expanded, increasing by 4-fold (UNEP-WCMC, IUCN and NGS 2020). PAs have successfully halted biodiversity losses in some instances (Johnson et al., 2017). However, biodiversity has largely continued to decline globally despite increased PA coverage (Pimm et al. 2014; Kerley et al. 2020). For example, over 35% of Africa's monitored bird and fish populations are declining (WWF 2020). Population sizes of mammals, birds, amphibians, reptiles and fish have declined by an average of 68% between 1970 and 2016 (WWF 2020). In the same period, PA coverage has increased to 22 million km^2 of terrestrial land covered by ~250,000 PAs and 28 million km^2 marine space covered by ~20,000 marine PAs (UNEP-WCMC, IUCN and NGS 2020).

The efficacy of PAs varies widely. PAs can protect species abundance and richness at higher levels than unprotected areas (Gray et al., 2016), but this is not guaranteed (Geldmann et al., 2013). Despite long-term status, some PAs fail to stem biodiversity losses (*e.g.* Craigie et al., 2010; Laurance et al., 2012; Habel et al., 2016). When assessing protected area success *via* an indicator species, Lion (*Panthera leo*), 80% of African PAs were in a state of failure or deterioration (Robson et al., 2021).

Many possible explanations exist for why increasing protected area coverage has not stemmed biodiversity losses, such as poor management (Fuller et al., 2010) or poor funding (Barnes et al., 2016; Lindsey et al., 2018). However, one underappreciated explanation for this “protected area paradox” is that protected areas are situated in poor quality habitats protecting marginalised remnant populations (Kerley et al. 2020). Most PAs were established post widespread range contraction, where the last remaining populations exist (Caughley 1994). PAs are biased towards higher elevation, steeper slope “pristine” lands away from dense settlements (Joppa & Pfaff, 2009). These PAs are usually situated in disproportionally less productive ecosystems (Sayre et al., 2020) or areas of reduced economic interest for agriculture (Venter et al., 2018). If high productivity ecosystems are protected, they are often embedded in high intensity agricultural or industrial landscapes (O’Bryan et al., 2021) at compromised sizes (Luck, 2007).

Marginalisation may be a widespread consequence of range contraction. It may also be an underappreciated pressure on protected and unprotected populations. Currently, the extent and scope of the phenomena has not been evaluated. Furthermore, there is little discussion of how marginalisation impacts conservation efforts at various scales. Firstly, this thesis assesses marginalisation across terrestrial mammals. Secondly, it uses Cape mountain zebra (*Equus zebra zebra)* and Grevy’s zebra (*Equus grevyi*) as model species to uncover the impacts of heterogeneous landscapes on these two species’ physiology and dynamics. Ultimately, the work presented evaluates the functional consequences of range decline and how these consequences impact conservation efforts.

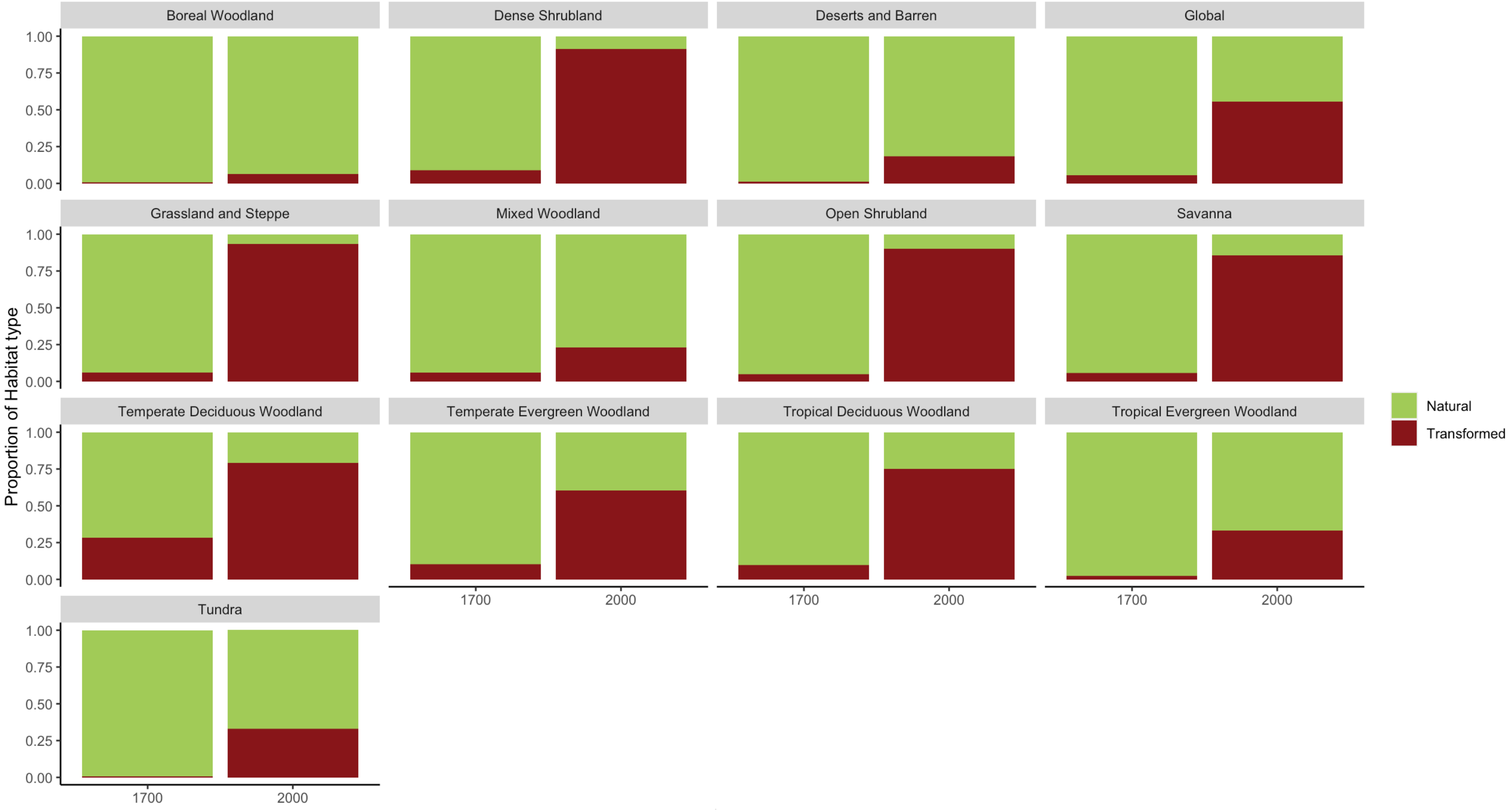


Figure 1.1) Proportion of each biome transformed for human landuse between 1700-2000. Green represents non-transformed “natural” proportions of each biome and red represents transformed proportions of each biome. Adapted from (Ellis et al., 2010)

1.2 Marginality and range loss

1.2.1 Ecological gradients and population dynamics

A population will persist across environmental space where biotic and abiotic conditions promote more births and immigration than deaths and emigration. Across environmental gradients, fitness, as measured by reproduction and survival, will vary due to the quality of conditions (Brown, 1984; Brown et al., 1995; Holt, 2009). Organisms preferentially occupy habitats that maximise quality while minimising potential risks, affording the individuals the greatest available fitness (Fretwell, 1969). While populations are below carrying capacity, high quality or “core” habitats will promote higher birth or reproductive rates (or lower death rates) while marginal populations suffer comparative poor rates (Holt, 2009; Pulliam, 2000). In core habitats, fitness decreases with increasing population density due to density-dependent effects (Clutton-Brock et al., 1987; Guo et al., 2005). As population density increases, the fitness gradient between habitats reaches an equilibrium, and individuals emigrate into more marginal habitats (Fretwell 1969).

If differences in habitat quality between patches are extreme, within core habitats individuals will have, on average, fitness greater than one (increasing population) and the habitat forms a ‘source’ population (Pulliam, 1988). These source populations produce a higher number of potential emigrants which can disperse to surrounding habitats. Whereas, in marginal habitats, individuals will, on average, have a fitness less than one (decreasing population) and a sink population is formed (Pulliam, 1988). Some marginal populations may be utterly reliant on immigration from source populations for their viability (Pulliam & Danielson, 1991). Alternatively, if differences in habitat quality are not as severe, both habitats may promote, on average, individuals with fitness greater than one. In this balanced dispersal model, marginal habitats have lower carrying capacity than core habitats. If dispersal is not restricted, equal numbers of individuals are predicted to be moving between habitats. As such, there is no net flow of individuals from one habitat patch to another and fitness is equalised across space (McPeek & Holt, 1992). The source-sink (Pulliam & Danielson, 1991) or balanced dispersal dynamics (McPeek & Holt, 1992) of neighbouring populations result in meta-populations, a group of spatially separated populations of the same species which interact (Levins, 1969).

Species range limits form where conditions reach species physiological tolerance limits or where topographic barriers prevent further dispersal (Gaston, 2003, 2009). In the former case, individual fitness (reproduction and survival) and performance metrics (abundance, density, and growth) decline progressively towards range limits. In the latter case, range limits are formed through limited dispersal capacity rather than progressive fitness declines (Gaston 2009).

1.2.2 Ecological vs geographic marginality

Marginality may either be ecological or geographic (Soule, 1973). Ecological marginality occurs when marginal populations form in areas with different environmental conditions from the core (Shreeve et al., 1996; Martínez-Meyer et al., 2013; Abeli et al., 2019). Ecological marginality assumes fitness is driven by environmental conditions. Range limits are therefore predicted to occur where ecological extremes (*e.g.*, resource scarcity or climatic extremes) cause the mortality and emigration rates to exceed the birth rates and immigration rates.

Geographic marginality predicts marginal habitats occur at the edge of the species' geographic range (Brown, 1984). This assumes fitness is linked to the position within geographic space, decreasing towards the geographic range edge (Sagarin et al., 2006). Hence the centre of the geographic range is always assumed to be core niche space, and the geographic periphery is always marginal (Sagarin & Gaines, 2002). However, ecological and geographic marginality gradients are not concordant (Abeli et al., 2014; Pironon et al., 2015, 2017).

Geographic margins have been linked to reduced occurrence, abundance and higher rates of genetic differentiation and inbreeding (Pironon et al., 2017). Geographic margins may also experience greater competition or predation pressures (Legault et al., 2020; Price & Kirkpatrick, 2009). Despite this, empirical evidence suggests demographic and performance trends do not decrease consistently towards geographic edges (Chevalier et al., 2021; Pironon et al., 2017). Therefore, simply because a habitat is at the geographic periphery of a species range does not imply automatic marginality in terms of reproduction or survival, particularly where range limits are formed due to dispersal barriers.

Currently, ecological marginality has been studied in a modelling context, assuming the centre of a species *n*-dimensional hypervolume (Hutchinson, 1957) is the optimal/core conditions for the species. Hence, the distance from the niche centre or distance from niche boundaries are used as an estimate of ecological marginality (Osorio-Olvera et al., 2019, 2020). When species are closer to the niche centroid, they tend to have greater patch occupancy (Burner et al., 2019), abundance (Martínez-Meyer et al., 2013), genetic diversity (Lira-Noriega & Manthey, 2014), population densities (Osorio-Olvera et al., 2020) and are less likely to be declining (Manthey et al., 2015; Martínez-Gutiérrez et al., 2018). However, these general patterns may not always hold across all species or contexts (see Osorio-Olvera et al. 2019; Santini et al. 2019; Holt 2020; Dallas & Santini 2020). Nonetheless, ecological marginality can also be more broadly defined as environmental (*e.g.* resource scarcity or climatic extremes) or landscape factors which decrease the probability of population survival and persistence (Shreeve et al., 1996). Using this broader definition, ecological marginality can be assessed in practice by evaluating the physiological state, demographic rates and resilience of a population. This broader definition also allows the study of ecological marginality to occur at multiple levels, including at the species range level *via* distance to niche centroid, and at the local level.

1.2.3 The dynamics of decline

Marginality and variation in habitat quality are natural phenomena. However, species can no longer utilise the entirety of their historic range's size, breadth, and connectivity. Contemporary species' ranges are determined by species' interactions and environmental gradients as well as human-induced dispersal limitation and habitat destruction (Colwell & Rangel, 2009; Holt, 2009). Human pressures influence species distributions, and the available environmental conditions and resources species have access to (Vitousek et al., 1986; Huston 2005).

How a range declines, both geographically and ecologically, will depend on the intensity of the threatening pressure and the vulnerability of the local populations (Lucas et al., 2016). There are four general ways a species range can decline geographically. Firstly, suppose geographically peripheral populations are extirpated first. In that case, the species range will be made up of only geographically central environments, the so called “demographic hypothesis” (Channell et al., 2000), a “melting range” (Rodríguez, 2002), or “demographic decline” (Hemerik et al., 2006). Secondly, geographically central populations may be extirpated first. In this case, the range declines towards geographic range edges so called “Range collapse” (Hemerik et al. 2006). If a species range collapses towards geographic edges, the species has become geographically marginalised. Thirdly, threatening pressures could occur directionally. In this case, the range declines in the geographic direction away from the threat in a so-called “Eclipse” or “Contagion” model. In mild cases, this can leave both central and peripheral populations. In severe cases, only geographically marginal populations remain (Channell & Lomolino 2000; Hemerik et al. 2006). Lastly, species may lose specific habitats across geographic space leading to fragmentation. In mild cases, both geographically peripheral and central populations are maintained (Figure 1.2).

In terms of ecological marginality, under human pressures species can: 1) lose niche conditions randomly resulting in a fragmented niche but not shifting the niche centre, 2) lose niche conditions due to non-random loss in the niche peripheries or low quality conditions, resulting in contraction to the niche centre or *“*core” habitats, 3) lose niche conditions due to non-random loss in the niche centre or “core” habitat, which can lead to contraction towards niche peripheries or marginal habitats. If the latter occurs, the species has been ecologically marginalised (Figure 1.3).

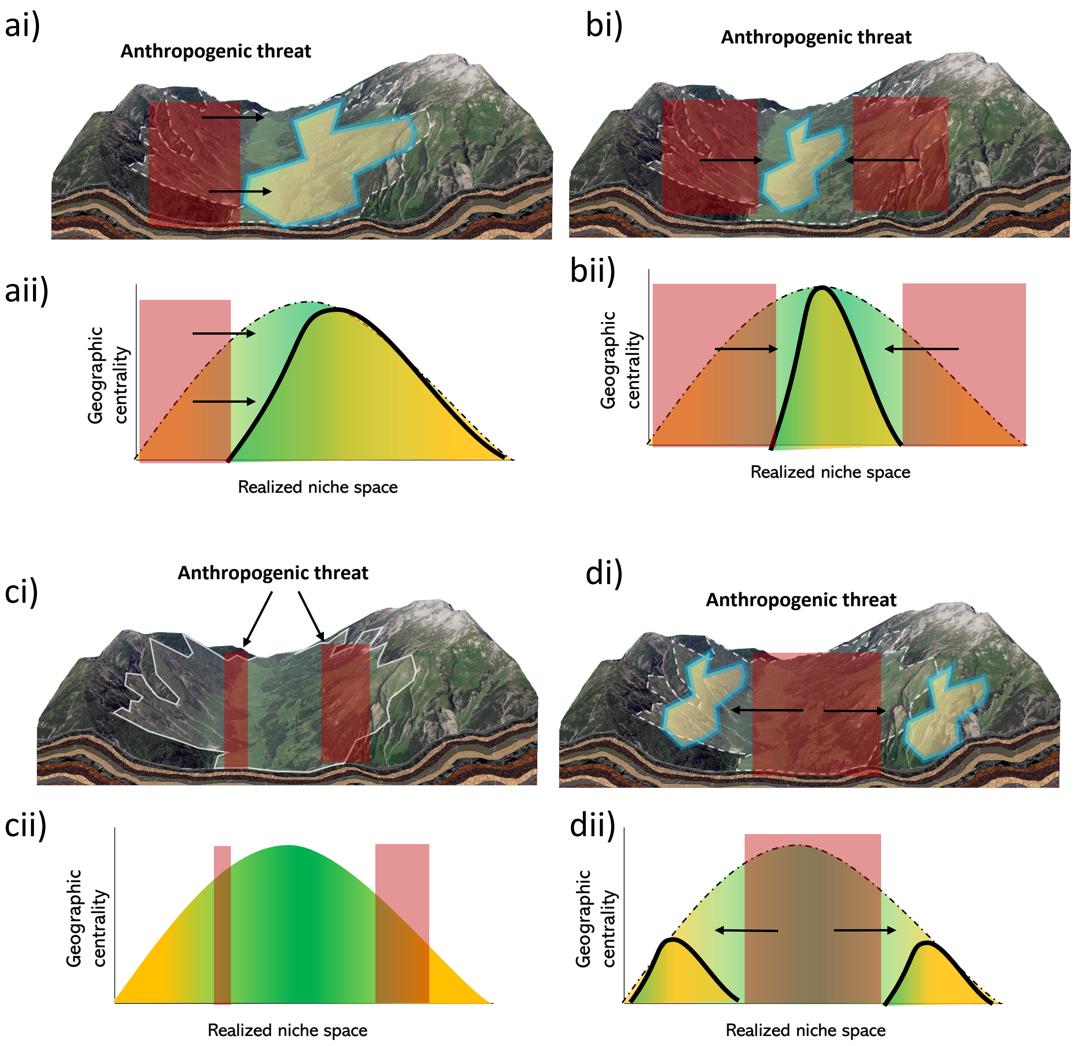


Figure 1.2) Hypothetical geographic range decline dynamics under human pressures. ai) A landscape representation of the contagion model of human transformation pressure with mild anthropogenic pressure. In this model, anthropogenic pressures starts in a single geographic location and spreads aii) Normal distribution of realized niche space being pushed to a geographic extreme under contagion model predictions bi) Demographic decline hypothesis – anthropogenic occurs at range edges and populations contract to geographic centre, bii) Normal distribution of realized niche space being pushed to a geographic centre under demographic decline predictions ci) Fragmentation of landscape leaving portions of the niche space unoccupied cii) Normal distribution of realized niche space under fragmentation . di) Range Collapse – anthropogenic pressures occurs at geographic centre and populations contract to geographic edges dii) Normal distribution of realized niche space being pushed to geographic edges under range collapse predictions. Red represents anthropogenic pressure, green represents geographic centrality and yellow represents geographic extremes (adapted from Hemerik et al 2006).

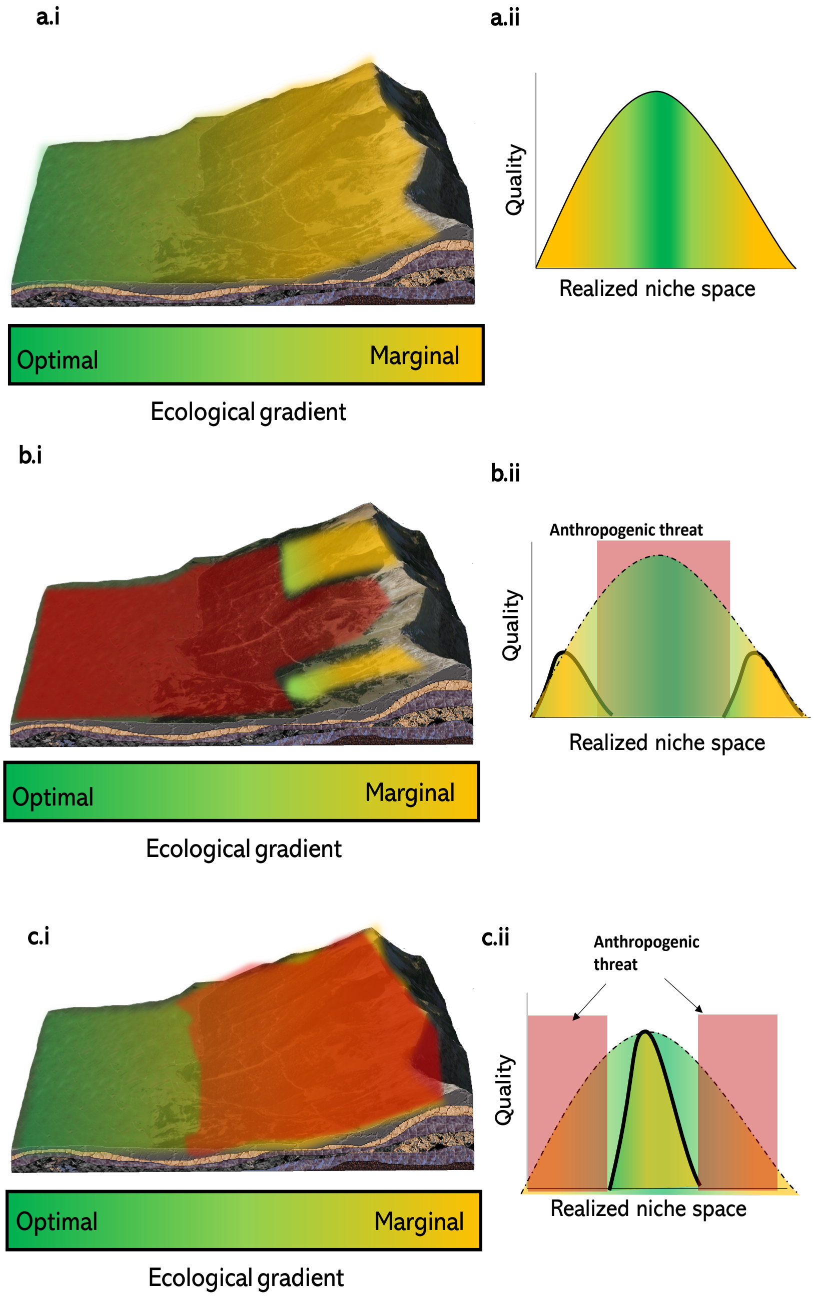


Figure 1.3) Hypothetical niche decline dynamics under human pressures. a.i) Untransformed landscape containing both optimal and marginal environments such that a.ii) species occupies all potential niche space. b.i) Non-random habitat loss in high quality habitat results in b.ii) species niche contracting to ecological margins, c.i) Non-random habitat loss in low quality habitat causes c.ii) species niche contraction to ecological core. Green represents optimal portions of range for the hypothetical species and yellow represents marginal portions of range. Red represents areas experiencing anthropogenic threat which exclude species.

1.2.4 Geographic marginalisation at macroecological levels

Geographic marginalisation has been widely studied. Contraction from the geographic core to the geographic periphery has been found at continental scales across taxa in species which have experienced extensive range contraction (Channell et al., 2000; Channell & Lomolino, 2000). Specifically, geographic marginalisation occurs in mammals and birds (Channell and Lomolino, 2000, see Chapter 3 for terrestrial mammals), European butterfly species (Thomas et al., 2008), Australian marsupials (Fisher et al., 2003), and reptiles and amphibians from New Zealand (Towns & Daugherty, 1994). Interestingly, species believed to be extinct and later rediscovered are more likely to be found at their geographic range edge at low density (Fisher 2011).

However, geographic marginalisation does not occur across all groups and scales. Large mammals (Yackulic et al., 2011) and British birds contract their geographic centre (Donald & Greenwood, 2001). Palaearctic and Indo-Malayan *Galliformes* species contract to their geographic core and show no pattern respectively (Boakes et al., 2018). Channell and Lomolino (2000) noted that island species display contraction to their geographic centre as human pressures were most intense in coastal regions. Overall, geographic marginalisation occurs in approximately 50% of studied groups (Hemerik et al., 2006, Chapter 3).

1.2.5 Ecological marginalisation from non-random land conversion

Ecological marginalisation has not been assessed across species (Chapter 3). However, many case studies of ecological marginalisation exist. These case studies vary in the direct or indirect human pressure that ecologically marginalises the species, but a common factor is human land use. Human land use occurs in areas highly suitable for agricultural or farming purposes (Hooke et al., 2012). Conversion is biased towards low elevation, shallow slope and highly fertile soils (Ellis, 2011; Huston, 2005). For example, forests in mountainous areas of Southeast Asian islands and Tanzania are much less threat of conversion than the adjacent lowlands (Peh, 2007; Hall et al., 2009). Agricultural pressures heavily impact Temperate and Tropical grasslands and Tropical dry, Mediterranean and Temperate broadleaf forests (Jacobson et al., 2019). Arid desert, cold tundra, montane grassland and boreal forest remain largely intact albeit under increasing threat (Riggio et al., 2020). Remaining habitats are inaccessible and unsuitable for human use. Higher elevation, more rugged terrain (Selwood & Zimmer, 2020), isolated or undisturbed islands (Channell et al., 2000), offshore refugia where fishing conditions are precarious (Hilborn et al., 2003) and climatically extreme environments (Nüchel et al. 2018; Sales et al. 2022) act as refugia from human pressures for many species.

Historically, highly productive, resource rich areas were an indispensable source of resources for animal populations (Vitousek et al., 1986, 1997; Huston, 2005). Primary productivity, freshwater, high soil fertility, and marine nutrient-rich waters are crucial to maintaining large populations and trophic diversity (Wright, 1983; Huston, 2005). For example, following the industrial revolution, North American large-bodied mammals experienced extensive range contraction in productive, moderate climatic regions while colder, dryer regions were left largely untransformed (Pineda-Munoz et al., 2021). Extirpation rates in productive midwestern regions in the USA were much higher than those in the lower productivity regions of the western grasslands and mountains (Huston, 2005). For North American large-bodied mammals, the abundance and densities are now lower in the remnant habitats than they were in the midwestern grasslands, savannahs, and forests (Huston 2005). This ecological marginalisation rendered some large species, like the keystone species the plains bison (*Bison bison*), functionally extinct (Freese et al., 2007).

Ecological retreat, the retreat into marginal refugia to escape human pressures, is documented in primates (Crowley et al. 2012), pinnipeds (Newsome et al., 2007), ratites (Miller et al., 2005) and equids (Kaczensky et al., 2017). As a result, range collapse into higher elevation areas and more climatically extreme environments occurs in a variety of species (Table 1.1).

Alongside complete habitat conversion, land use fragments landscapes and reduces connectivity of metapopulations. As a result, animals in contemporary landscapes must move further to disperse while overall dispersal rates have declined (Tucker et al., 2018). Habitat loss and fragmentation are major causes of dispersal limitation in large African mammal species (Harris et al., 2009). Conservation practices and current governance over populations also limit species movement through fencing (Boone & Hobbs, 2004; Woodroffe et al., 2014). Although beneficial for many conservation purposes, fencing deprives populations of opportunities for immigration or emigration without human intervention (Harris et al., 2009). Anthropogenic sources of dispersal limitation prevent vital emigration between source and sink populations or disturb patterns of balancing dispersal. Thus, populations can become confined to low quality marginal conditions of reduced resilience and increased extirpation risk (Kerley et al., 2012).

1.2.6 Ecological marginalisation from dense settlements

Dense settlements are also non-randomly distributed across the landscape (Ellis, 2011). Wetlands, coastal biomes near industrial ports and estuaries are particularly vulnerable to human impacts through accumulated pollution, sedimentation and discharge, industrial and urban waste, harvesting and invasive species (Halpern et al., 2008; Junk et al., 2012). Indeed, human impacts have depleted >90% of formerly important species in estuary and marine coastal water environments (Lotze et al., 2006) and have destroyed >65% of seagrass and wetland habitat (Waycott et al., 2009).

Dense settlements produce novel environments to which organisms are not accustomed (Hobbs et al., 2014; Radeloff et al., 2015). Such environments may act on species' sensory biases and result in selection of a marginal habitat over available high-quality habitat *i.e.* an ecological trap (Schaefer et al., 2002). For example, juvenile African penguins (*Spheniscus demercus)* select habitats of low surface temperature and high chlorophyll-*a* for feeding. These conditions were traditionally associated with high prey abundance. However, due to overfishing and climate change, these conditions are currently associated with depleted prey reserves resulting in a high mortality of juvenile penguins. Importantly, juvenile penguins continue to select these prey-depleted areas even when areas with high prey abundance are available (Sherley et al., 2017). Hale and Swearer (2016) identified 29 different studies, including multiple taxa, displaying an ecological trap. Ecological traps reduce individual fitness (Hale & Swearer, 2016), increase local extinction risk (Battin, 2004) and impact population dynamics (Delibes et al., 2001).

1.2.7 Ecological marginalisation from altered biotic interactions

Humans may also introduce invasive predators or disease or may function as predators through persecution. Invasive predators and human persecution act to confine populations to marginal habitats of reduced predation risk, *i.e.,* predation refugia, instead of ecologically optimal environments (Kinnear et al., 2002). Typically, predation refuge sites are structurally complex and dense habitats lacking adequate food sources (Kinnear et al., 2002; Bilney et al., 2010). For example, Kinnear et al (2002) describe the "niche denial", restriction into structurally complex marginal refugia, of twelve marsupial species due to predation pressure from the invasive red fox (*Vulpes vulpes*). While restricted to marginal refugia*,* all species achieved low population densities due to poor resource availability (Kinnear, Sumner and Onus, 2002). However, following fox-baiting programs, these species reoccupied historical habitats and population densities rapidly increased (Kinnear, Sumner and Onus, 2002).

Humans also introduce invasive wildlife diseases that native species have little resistance to. Species may retreat to, or become extirpated from all but, disease-free refugia. While disease risk is reduced in refugia, environmental factors can be poor. For example, native Hawaiian birds, such as the Common Amakihi (*Hemignathus virens),* Apapane (*Himatione sanguinea),* Liwi (*Vestiaria coccinea),* Japanese White-eye (*Zostero japnoicus)* and Red-billed Leiothrix (*Leiothrix lutea),* do not occupy resource-rich lowland, wet forest due to introduced avian malaria (Atkinson et al., 2013; van Riper et al., 1986; Woodworth et al., 2005). The high elevation environments they are confined to represent a low-quality habitat for the declining Hawaiian birds (van Riper et al., 1986; Atkinson et al., 2013). Similarly, chytrid fungus also restricts amphibian species from high quality habitats (Scheele et al. 2017) (Table 1.1).

Table 1.1) Examples of range contraction from human impacts leading to ecological marginalisation of species.

Species	**Restricted habitat**	**Description**	**References**
Equus ferus	Climatically extreme parts of historic niche	Morphology and paleoecology of feral horses suggest the species today occurs in hotter, less mesic environments compared to its historic niche	(Naundrup and Svenning, 2015)
Snub nosed monkey species (*Rhinopithecus bieti,, Rhinopithecus brelichi, Rhinopithecus roxellana, Rhinopithecus strykeri*, *Rhinopithecus avunculus)*	Climatically extreme parts of historic niche	Species shifted to higher elevation and more climatically extreme areas of their historic niche	(Nüchel et al., 2018)
Bougainville Monkey faced bat (*Pteralopex anceps)*	High elevation	The species is confined solely to its marginal high elevation moss forest. Historically, this species occupied lowland rainforests before they were completely cleared for agriculture	(Fisher, 2011)
Malabar civet (*Viverra civettina*	Habitat shift	Solely occupies dense understorey cashew plantations following conversion of coastal riparian forest valleys	(Fisher, 2011)
European wolf (*Canis lupus)*	High elevation forested habitats	Shift from open landscapes to forested mountains due to human persecution.	(Martínez-Abraín & Jiménez 2016)
Takahe (*Porphyrio hochstetteri)*	Habitat shift to predation refugia	Last population resided in subalpine tussock grasslands where the species was able to avoid high densities of predators. Only occurred at low densities and had poor population performance	(Mills, Lavers and Lee, 1984; Bunin and Jamieson, 1995; Grueber and Jamieson, 2011)
Lord Howe Island Woodhen (*Gallirallus sylvestris)*	High elevation predation refugia	Restricted to an invasive predator-free mountain-top environment. After release from predation pressure, the species recolonised historic low altitude habitats. Population densities increased to higher levels than found on the mountain tops.	(Caughley 1994; Brook et al. 1997; Kerley et al. 2012)

Australian armoured frog (*Litoria lorica)*	Disease free refugia	In 2008, the species was rediscovered in dry eucalypt forest where the threat from invasive chytrid fungus is reduced but appears to be a suboptimal environment for the species	(Scheele et al., 2017)
Australian alpine tree frog (*Litoria verreauxii alpine)*	Disease free refugia	Occupied preferred habitat of ephemeral wetlands until invasive chytrid fungus. Currently, restricted to drought-proof perennial wetlands where threat from invasive chytrid fungus is reduced but species has increased vulnerability to drought.	(Scheele et al., 2016, 2017)
Extant Lemur species: Ring-tailed lemurs, *Lemur catta;* sportive lemurs (*Lepilemur leucopus),* mouse lemurs (*Microcebus griseorufus)* and sifakas (*Propithecus verreauxis)*	Riparian habitats	Shift from dry, more open habitats such as woody savannah or spiny thicket to riparian habitats.	(Crowley et al., 2012)
Giant panda *(Ailuropoda melanoleuca)*	Montane bamboo forest	They are believed to be a specialist bamboo feeder, however, the species historically occupied a wider ecological and dietary niche	(Kerley et al., 2020).
Small Australian marsupial species Black-flanked rock-wallaby (*Petrogale lateralis),* Rothschild's rock-wallaby (*Petrogale xanthopus),* Tammar wallaby (*Macropus eugenii),* Brush-tail bettong or rat kangaroo (*Bettongia penicilata),* Gilberts' potoroo (*Potorous gilbetii),* Western ringtail possum (*Pseudocheirus occidentalis),* Western bushtail possum (*Trichosurus Vulpecula),* Quenda or Southern brown bandicoot (*Isoodon obesulus),* Numbat (*Myrmecobius fasciatus)* Chuditch or Western quoll (*Dasyurus geoffroii)*	Structurally dense predation refugia	Species experienced restriction to predator-free refugia due to invasive red fox (*Lupus lupus*). In predation-refugia all species maintained poor abundance levels. Demographics improved following fox baiting scheme and recolonisation of historic habitat types	(Kinnear, Sumner and Onus, 2000)
Kakapo *(Strigops habroptilus)*	Islands	Fossil evidence suggests species occupied mountain beech and hall's totara or broadleaf forest in areas of high rainfall and moderate winters. Species have been extirpated from these environments due to Invasive species.	(Lentini et al., 2018)

1.3 Refugee species and the partial refugee concept

1.3.1 Refugee species as an extreme form of ecological marginalisation

At the extremes of ecological marginalisation is a refugee species, where marginal habitats make up the entire contemporary species range (Kerley et al. 2012). The first recognised refugee species was the European bison (*Bison bonasus).* The European bison is a vulnerable species that is actively managed in forests and is traditionally believed to be a forest specialist (Kerley et al. 2012). However, the evolutionary background (Shapiro, 2004), dental morphology (Mendoza & Palmqvist, 2008), digestive morphology (Hofmann, 1989), neonatal behaviour (Daleszczyk, 2004) and microhabitat selection (Kuemmerle et al., 2010) are all indicative of a grazing ecology (Kerley et al. 2012). The species are supplementarily fed within forests but remain at low density at the landscape level (Kerley et al. 2012). Historically, European bison occupied open, grass-rich habitats. However, due to a combination of replacement of grassland by forest and increasing human land transformation, the species was pushed to occupy a marginal forest environment (Bocherens et al. 2015; Hofman-Kamińska et al. 2018). Densities in mixed forest environments are higher than those in closed forest (Kerley et al. 2020), and a subsequent reintroduction into open-grassland environments has allowed the species to reach a higher density than seen in forests without any human intervention (Cromsigt et al., 2012). The European bison epitomises a species becoming restricted to a marginal, truncated range through human activities. Since the identification of the European Bison as a refugee species in 2012, further refugees have been identified and more candidates have been suggested (Kerley et al. 2020)

Where ecological marginalisation is less severe, only some populations in the species range are confined to marginal habitat, while others are maintained in high-quality habitats. Such species are termed “Partial refugees " (Lea *et al*, 2016). Partial refugees are formed where threats are not uniform across the whole species range or when a historic sink population becomes isolated and dispersal opportunities limited. For example, Cape mountain zebra (*Equus zebra zebra)* occur in fenced populations across much of its’ historic range (Lea et al., 2016). Fencing confines the species to isolated populations where immigration or emigration between populations cannot occur without human intervention (Hrabar & Kerley, 2013). Some populations are confined to grass poor areas with poor performance and others to grass rich areas with comparative high performance (Lea et al., 2016). This large reproductive variation between populations can leave some populations vulnerable to extirpation (Griffen & Drake, 2008; Griffen & Norelli, 2015). With the current and ever-increasing fragmentation of landscapes (Crooks et al., 2017; Tucker et al., 2018), partial refugees are likely to be a widespread phenomenon.

1.3.2 Common trends in ecologically marginalised populations

Both habitat size and quality determine a species’ population size and growth (Griffen & Drake, 2008). An abundant species may still occupy a marginal habitat, given that the habitat size is large enough (Kerley et al. 2012). Long-lived organisms may show very slow rates of population decline, even with poor recruitment. Hence, ecologically marginalised species and refugees may be difficult to identify. Despite this, refugees show common trends. To illustrate common features of ecologically marginalised species, case studies of candidate and known refugees are discussed below.

One common feature of ecologically marginalised populations is a mismatch between morphology and the environment (Table 2). For example, the Lowland anoa (*Bubalus depressicornis*) occupies closed forest habitat in Sulawesi. Historically, the species is occupied a much wider distribution including many more habitat types (Burton et al., 2005) but historical information on the species is limited. Currently, the species occurs in very low numbers in its current range (Kerley et al. 2012). The species acts as a browser but recent dietary studies on captive anoa suggest the species’ digestive morphology is adapted for significant amounts of grass (Flores-Miyamoto et al., 2005). The species' jaw morphology is also more similar to grazers than browsers (Mendoza & Palmqvist, 2008). The lowland anoa is therefore a candidate refugee species. Other species also display morphological discrepancies with their environment (Table 1.2).

A second feature common in ecologically marginalised populations are behavioural discrepancies with their environment (Table 1.2). The Audouin's gull (*Ichthyaetus audouinii*) is refugee species (Martínez-Abraín & Jiménez 2016; Martínez-Abraín et al. 2019) due to behavioural incompatibilities with their small islet habitat (Parejo et al., 2006; Oro et al., 2011). Audouin's gull historically occupied and evolved in dune fields and on coastal marshes near river deltas (Martínez-Abraín et al., 2003), but due to human conversion of coastal environments, the species retreated to small uninhabited volcanic islet habitats (Martínez-Abraín & Jiménez 2016). On islets, the Audouin's gull elicits rapid vegetation growth by defecating its nitrogen-rich faeces directly onto vegetation (Vidal et al., 1998). Eventually, this vegetation growth leads to the islets becoming unsuitable for breeding (Martínez-Abraín & Jiménez 2016). There is no fossil record of the Audouin's gull on these small islets (Alcover et al., 1992). The species' high nitrogen faeces does not produce the same rapid vegetation growth in high salt content soils, found in areas this species historically bred (Martínez-Abraín & Jiménez 2016). Furthermore, when Audouin's gull can recolonise such environments, rapid growth of colonies occurs (Oro & Ruxton, 2001).

Importantly, the Kittiwake (*Rissa tridactyla),* the only historical island-nesting gull species in Europe, defecates on islet cliffs rather than vegetation and does not elicit unsustainable vegetation growth (Martínez-Abraín & Jiménez 2016). All European large ground nesting gull species who display the same behavioural maladaptation, including the Yellow-legged Gull (*Larus michahellis)*, Caspian Gull (*Larus cachinnanas)*, Herring Gull (*Larus argentatus*) and Black-backed Gull (*Larus fuscus)*, also qualify as candidate refugee species (Martínez-Abraín & Jiménez 2016).

A third common feature of ecologically marginalised populations is confinement to marginal habitats relatively recently in evolutionary or historical time. Historic information can reveal inconsistencies in species ecologies before and after widespread human impact (Table 1.1). Refugee species display shifts in ecological traits essential to individual fitness and population performance. For example, diet is a major ecological trait that impacts organisms' fitness (Kitaysky et al., 2006). Prior to its extinction in the wild, Przewalski's horse (*Equus ferus przewalskii)* was heavily hunted. Hunting pressures force the species to shift its diet from a grass-dominated diet to a grass-browse diet in the winter (Kaczensky et al., 2017). Equids are morphologically suited to grazing and only include browse in their diet when limited food sources are available (Mendoza & Palmqvist 2008). This dietary change to low quality food sources could have led to reduced performance, as seen in other equids, and therefore increased extinction rate (Lea et al., 2016).

A fourth feature is habitat shifts over time (Table 1.1). These shifts can be broad scale shifts or shifts away from specific habitats vital to the species' life-cycle. The Mediterranean monk seal (*Monachus monachus)* is a Critically Endangered ecologically marginalised pinniped (Karamanlidis & Dendrinos, 2015). Historically, this species was remarkably abundant and is often the most common vertebrate species in many paleo-archaeological sites (Aura Tortosa et al., 2002; Stringer et al., 2008). The species currently breeds in sea caves (González, 2015), but historical references document the species bred on open beaches. The species' biological characteristics are more typical of an open beach breeder, such as the dark color of pups, which protects them from direct sunlight (Martínez-Abraín et al., 2019; Martínez-Abraín & Jiménez, 2016a). Pup mortality inside caves is higher than on beaches (Martínez-Jauregui et al., 2012) and Monk seals breeding in sea caves have lower pup survival rates than other open beach breeding pinnipeds (Gazo et al., 2000). If threatening pressures are alleviated, the species recolonises and breeds on open beaches and breeding success largely increases (Martínez-Abraín et al., 2019). Other species also display habitat shifts through time due to human impact and therefore can be considered candidate refugees (Table 1.1).

A fifth feature of ecologically marginalised species is physiological challenge. Marginalised populations should display comparative physiological extremes representing chronic physiological state or an accumulation of deleterious health biomarkers (Shultz et al. 2021; Figure 1.6). Comparative endocrine analysis provides an opportunity to assess whether populations have been ecologically marginalised (Chapter 4) (Table 1.2).

A sixth feature of refugee species is poor performance. Comparative reproductive, survival or density data can estimate the ecological marginality experienced by a population or species. For example, the Knysna elephant (Loxodonta africana) population displays very low reproductive rates compared to elephants in Savannah. The species is restricted to Afromontane forests and suffers from dietary limits to reproduction (Seydack et al., 2000).

Despite protected status for over a century, the population has declined to a single individual (Moolman, Ferreira, et al., 2019; Moolman, Morney, et al., 2019). Likewise, European otters (*Lutra lutra*) were believed to exclusively occupy unpolluted river heads (Mason and MacDonald, 1986). However, when sections of the river were restored from pollution, the otters recolonised middle and lower sections (Martínez-Abraín & Jiménez, 2016a). Performance was greatest in the middle stretches of rivers which contained higher amounts of preferred prey items (Remonti et al., 2009; Ruiz-Olmo et al., 2011). Recolonisation of historic habitat types and subsequent increased performance is a post-hoc identification strategy, however, it provides a new baseline to assess whether other populations of the same species are confined to marginal habitats (Table 1.2).

Table 1.2) Identification of ecologically marginalised species from mismatches with their environments

Species	Mismatch	Description	References
Iberian brown bear (*Ursus arctos)*	Morphological	Species is not adapted to locomotion on steep slopes which it currently inhabits.	(Martínez-Abraín & Jiménez, 2016).
Mainland serow (*Capricornis milneedwardsii)*	Habitat and dietary shift	Historically fed on C4 grasses in open grasslands. Currently feeding on C3 browse in deep canopy forests.	(Pushkina et al., 2010)
Aurochs (*Bos primigenius)*	Habitat and dietary shift	Fossil record found in open grasslands but species last found in forests before extinction and displays dietary shift from grasslands to forest habitats	(Noe-Nygaard, Price and Hede, 2005; Hall, 2008; Kerley, Kowalczyk and Cromsigt, 2012; Bocherens et al., 2015)
Northern fur seal (*Callorhinus ursinus)*	Habitat and dietary shift	Loss of breeding sites, change in reproductive strategy and performance declines for unknown reasons	(Newsome et al., 2007)
Alligator (*Alligator mississippiensis)*	Recolonisation	Recolonisation of Salt marshes, mangroves, oyster reefs and subsequent increase in performance	(Silliman *et al.*, 2018)
Sea otters (*Enhydra lutris)*	Recolonisation	Recolonisation of kelp forest and subsequent increase in performance	(Silliman *et al.*, 2018)
River otters (*Lontra canadensis)*	Recolonisation	Recolonisation of estuaries, coastal marshes and subsequent increase in performance	(Silliman *et al.*, 2018)
Harbour seals (*Phoca vitulina)*	Recolonisation	Recolonisation of Elkhorn Slough, California and subsequent increase in performance	(Silliman *et al.*, 2018)
Grey whales (*Eschrichtius robustus)*	Recolonisation	Recolonisation of lagoons and subsequent increase in performance	(Silliman *et al.*, 2018)
Mountain lion (*Puma concolor)*	Recolonisation	Recolonisation of grasslands and subsequent increase in performance	(Silliman *et al.*, 2018)
Savannah Chimpanzees (*Pan troglodytes)*	Comparative endocrine analysis	Physiological urinary markers for chronic dehydration and elevated cortisol levels suggest chronic heat stress compared to forest dwelling chimpanzees	(Wessling et al., 2018)

Hawaiian monk seal (*Monachus schauinslandi)*	Comparative endocrine analysis	Population growth rates vary widely, and physiological evidence suggests that poorly performing populations are resource limited	(Gobush, Booth and Wasser, 2014)
Grizzly bears (*Ursus arctos)* in the Mackenzie Delta	Behavioural	Due to poor habitat quality, the population began to prefer resource searching behaviours over mate searching behaviours decreasing reproduction and performance.	(Edwards and Derocher, 2015)
Hirola (*Beatragus hunter)*	Habitat shift	Tree encroachment and loss of ecosystem engineers led to temporal habitat change and loss of large proportion of optimal habitat. Females found to have reduced survival due to rangeland quality.	(Ali et al., 2017, 2018)
Brown teal (*Anas cholortis)*	Historical reference and dietary shift	Fossil sites show brown teal occupied habitats away from lakes and rivers, near the coast and in areas covered by wet and seasonally dry forests, and lower montane forests. Dietary analysis supports this constriction of niche	(Worthy, 2002; Kerley, Kowalczyk and Cromsigt, 2012; Holdaway, Williams and Hawke, 2013)
Australian emu (*Dromaius novaeollhollandie*	Habitat shift	Preferred drought-adapted mosaic of trees, shrubs and nutritious grasslands have been converted to modern fire-adapted desert scrub. Food-sources are limited in current habitats.	(Miller et al., 2005)
Wombat species (*Vombatidae spp)*	Habitat shift	Preferred drought-adapted mosaic of trees, shrubs and nutritious grasslands have been converted to modern fire-adapted desert scrub. Food-sources are limited in current habitats.	(Miller et al., 2005)
American bison (*Bison bison*)	Poor demographics in protected areas	Following widespread displacement from productive habitats post-industrial revolution, the species is confined to protected areas where density varies largely.	(Plumb & McMullen, 2018)
Cape mountain zebra (*Equus zebra zebra)*	Habitat shift, poor demographic between protected areas and see Chapter 4 and Chapter 5)	Cape mountain zebra is believed to occupy only mountain habitats which they are now restricted to and protected in. However, historically the species would have altitudinally migrated following food availability..	(Faith, 2012; Lea et al., 2016)
Huemul (*Hippocamelus bisulcus)*	Morphological discrepancies and poor diet quality	The Patagonian Huemul deer has morphological incompatibilities with its habitat and experiences micronutrient deficiencies leading to bone problems	(Flueck, 2021)

1.3.3 The demographic consequences of ecological marginalisation

The consequences of marginality can be extremely deleterious to population viability, resilience and conservation efforts. Organisms experience reduced fitness in the form of reduced survival and reproductive rates compared to populations in high quality habitats (Kerley et al. 2012). For example, the Cape mountain zebra (*Equus zebra zebra*) displays reduced fecundity rates in habitats with poor grass availability (Lea *et al*, 2016). Additionally, Huemul (*Hippocamelus bisulcus*) have been restricted to high elevation habitats in the Andean mountains which it is not morphologically adapted to moving within. Populations continue to decline as many individuals die from micronutrient deficiencies leading to osteopathy and death (Flueck, 2021).

Due to these poor demographic rates, organisms confined to marginal habitats have low abundance, density (Figure 1.3) and intrinsically low or declining population growth rates (Figure 1.4). Poor demographic rates maintain low numbers reducing demographic viability and resilience to stochastic effects (Scheele et al. 2017). Importantly, animals in marginal habitat can decline despite strict protection. The Knysna elephant (*Loxodonta africana*) population became confined to Afromontane forest following widespread landuse change and persecution (Kerley et al. 2012). Despite a century of official protection, the population has declined to a single individual (Moolman et al., 2019) due to poor reproductive rates from dietary limitation (Seydack, 2000).

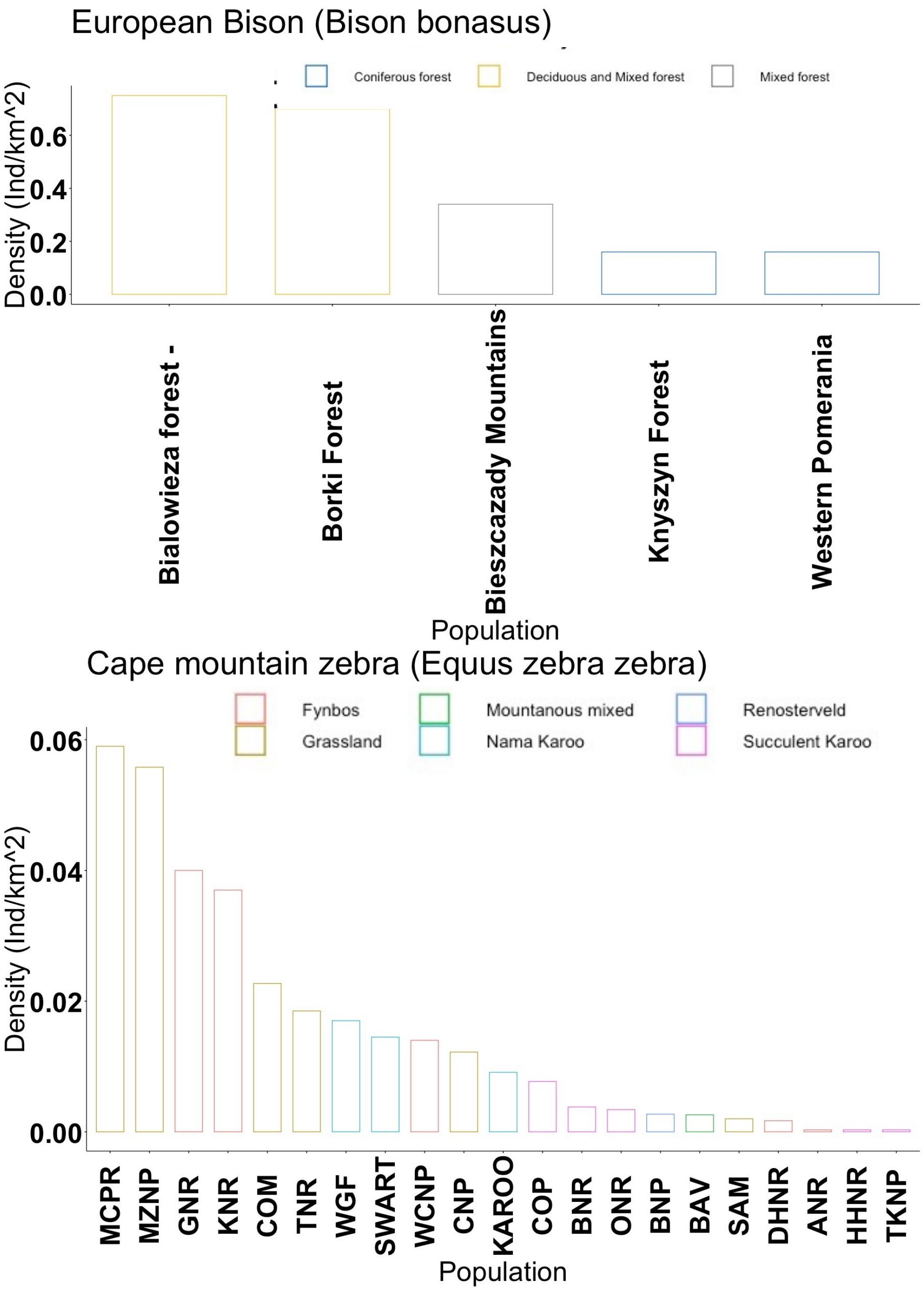


Figure 1.4) Demographic differences across protected areas in confirmed ecological marginalised species. (Top) Average density (individuals per km^2) across protected habitat types in European Bison (*Bison bonasus*) (data from (Kerley et al. 2020), Bottom) density (individuals per km^2), across protected habitat types in Cape mountain zebra (*Equus zebra zebra*) (data from (Lea et al., 2016). For Cape mountain zebra populations abbreviations of population depict the following: ANR: Anysberg Nature Reserve, BNR: Bakkrans Nature Reserve, BAV: Baviaanskloof Wilderness area, BNP: Bontebok National Park, CNP: Camdeboo National Park, COM: Commandodrift Nature Reserve, COP: Coppermoon Private Reserve, DHNR: De Hoop Nature Reserve, GNR: Gamkaberg Nature Reserve, HHNR: Hottentots Holland Nature Reserve, KNR: Kammannasie Nature Reserve, KNP: Karoo National Park, MCPR: Mount Camdeboo Private Reserve, MZNP: Mountain zebra National Park, ONR: Oorogskloof Nature Reserve SWART: Swartberg Private Game Reserve, SAM: Samara Private Reserve, TKNR: Tankwa-Karoo Nature Park, TNR: Tsolwana Nature Reserve, WGF: Welgevonden Garm Farm, WCNP: West Coast National Park. Populations are occurred by major habitat type.

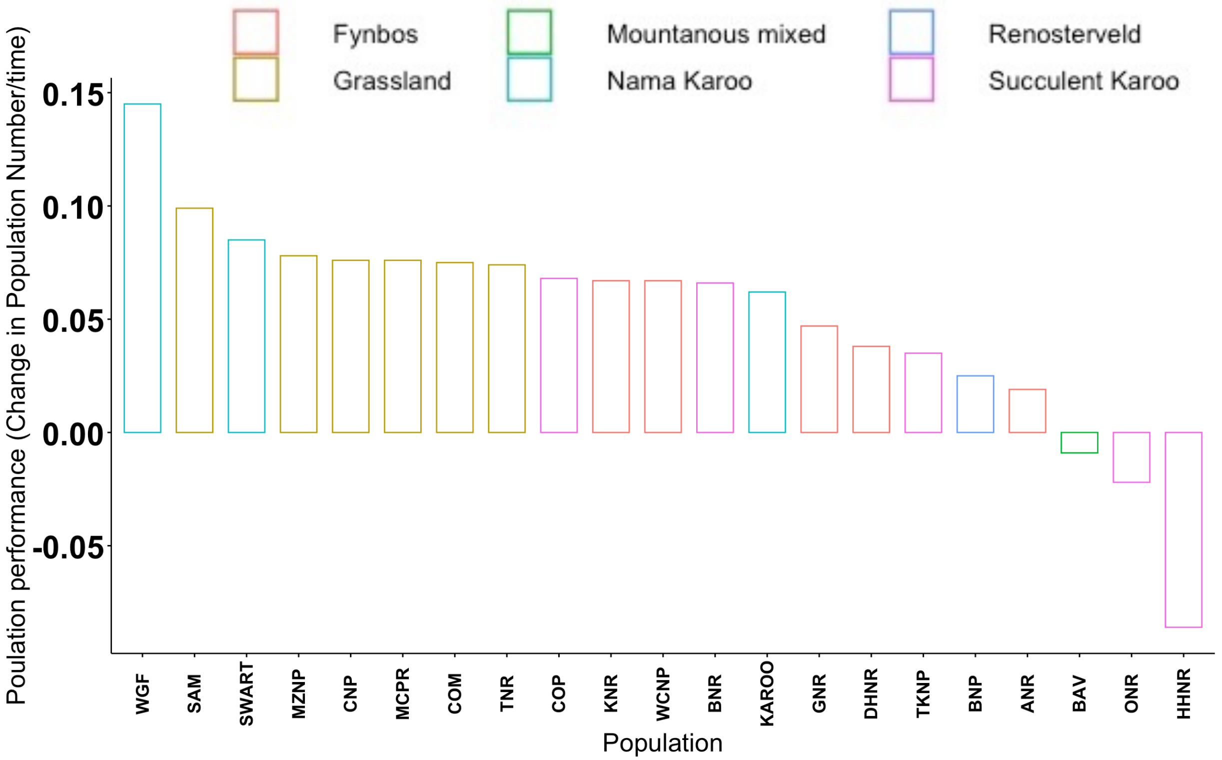


Figure 1.5) Variation in conservation success and population performance in the Cape mountain zebra (*Equus zebra zebra*). Population growth rates across protected habitat types (coloured) (data from (Lea et al., 2016). Populations abbreviations are: ANR: Anysberg Nature Reserve, BNR: Bakkrans Nature Reserve, BAV: Baviaanskloof Wilderness area, BNP: Bontebok National Park, CNP: Camdeboo National Park, COM: Commandodrift Nature Reserve, COP: Coppermoon Private Reserve, DHNR: De Hoop Nature Reserve, GNR: Gamkaberg Nature Reserve, HHNR: Hottentots Holland Nature Reserve, KNR: Kammannasie Nature Reserve, KNP: Karoo National Park, MCPR: Mount Camdeboo Private Reserve, MZNP: Mountain zebra National Park, ONR: Oorogskloof Nature Reserve SWART: Swartberg Private Game Reserve, SAM: Samara Private Reserve, TKNR: Tankwa-Karoo Nature Park, TNR: Tsolwana Nature Reserve, WGF: Welgevonden Garm Farm, WCNP: West Coast National Park. Populations are occurred by major habitat type.

1.4 Assessing ecological marginalisation at local scales in wild populations

1.4.1 The functional marginality framework

Functional indicators, such as physiological and behavioural biomarkers, can be used to evaluate patterns of range contraction and ecological marginality. Under the functional marginality hypothesis, populations with a high intrinsic growth rate or high densities at carrying capacity are predicted to have improved physiological and behavioural states (Shultz et al, 2021). As species approach ecological or range limits, they may approach their physiological tolerance limits (Lee et al., 2009). As species approach ecological extremes or tolerance limits, we would predict an increased load from negative biomarkers and poor physiological conditions. During range shift, we would predict populations in decline or at the retreating edge should show declines in positive indicators and the increasing burden of negative indicators (Figure 1.6). Moreover, suppose range contraction is characterised by retreat from anthropogenic threats into ecologically marginal populations (Scheele et al. 2017). In that case, remaining populations should have poor functional condition compared to populations in core or high-quality habitats (Figure 1.6). On the other hand, if species retreat into core habitats (Channell and Lomolino 2000) then functional traits in the remaining habitats should be comparable to other source populations or high-quality conditions.

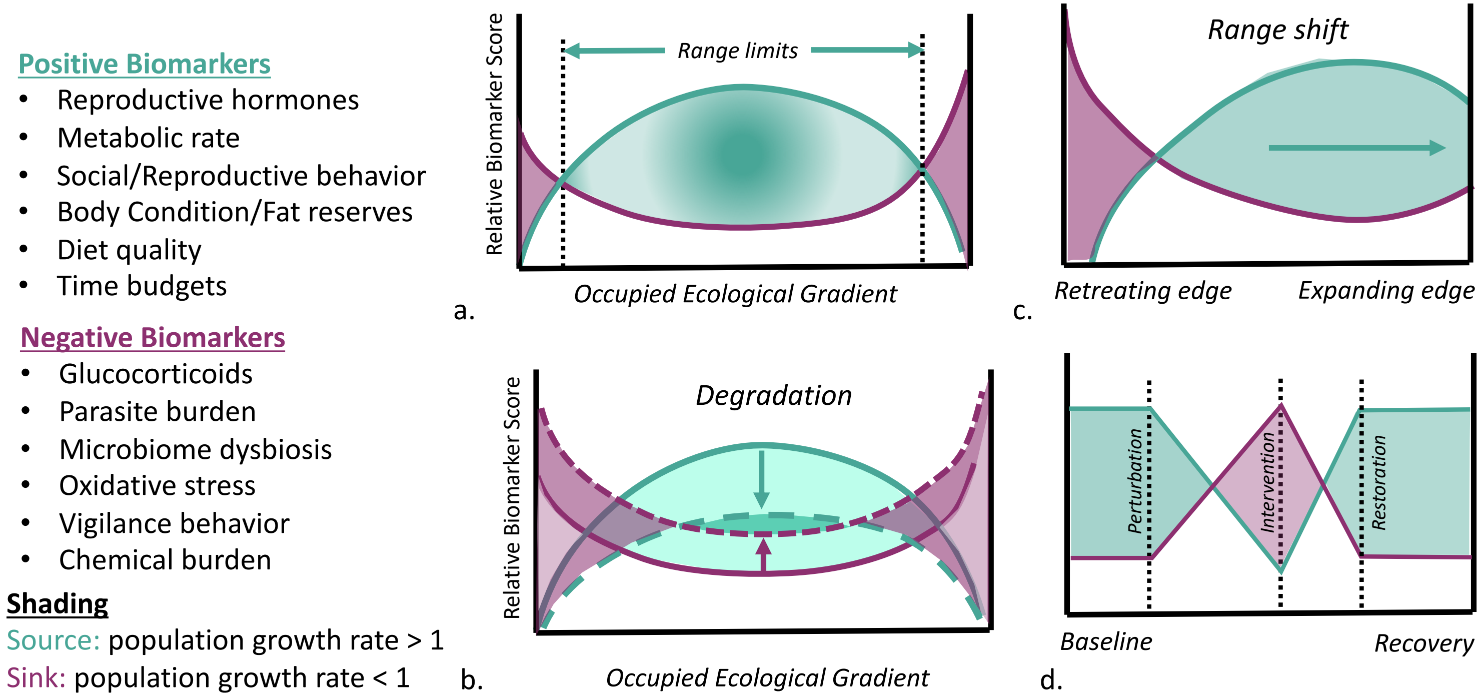


Figure 1.6) Conceptual diagram of the *Functional Marginality* Framework. a) Viable populations are determined by good physiological condition which leads to increased birth and reduced death rates. Range limits occur where increased load from negative biomarkers overwhelm physiological tolerance limits b) The expanding edge of a species range will experience a higher proportion of positive than negative biomarkers, c) Habitat degradation leads to a net decline in functional condition d) Physiological perturbations can be tracked by deviation from a baseline value and recovery following perturbation. Blue depicts when positive biomarkers are in higher proportion than negative biomarkers (purple) Taken from (Shultz et al., 2021).

1.4.2 Functional markers to assess ecological marginalisation

1.4.2.1 Glucocorticoids

Stressors from abiotic and biotic factors impact multiple aspects of physiology, leading to declining population dynamics (Boonstra et al., 1998; Kitaysky et al., 2010). Environmental or psychological challenges activate the hypothalamus-pituitary-adrenal (HPA) axis (Palme, 2019) resulting in the release of glucocorticoids (GCs) (McEwen & Wingfield, 2003). GCs are a class of steroid hormones with a range of biological functions including metabolism, immunity, behavioural regulation, fluid balance and cognition (Möstl & Palme, 2002; Sapolsky et al., 2000).

Responses to stress can be acute (Busch, 2010), long-lasting but transient (Viljoen et al., 2009) or chronic (Linklater & Gedir, 2011). Prolonged stress can result in chronically elevated or depressed HPA axis activity. Chronic altered GC concentrations can cause anti-inflammatory responses and immunosuppression (Hing et al., 2016; Munck & Naray-Fejes-Toth, 1994) and suppress reproductive hormones (Cameron, 1997). Both can have deleterious effects on reproduction and survival (Dantzer et al., 2014). Glucocorticoids can also alter behaviour, modulating further release in a feedback loop (Baugh et al., 2017). Although glucocorticoids are not analogous to “stress” levels (MacDougall-Shackleton et al., 2019), they do consistently show physiological responses to acute stressors (Shultz et al. 2021). Therefore, under the functional marginality hypothesis we would predict greater amounts of HPA axis activity in ecologically marginalised populations and in populations displaying poor performance.

1.4.2.2 Diet

In biological networks modulated by bottom-up control, habitat quality is driven by resource availability *i.e.* access to nutritionally rich food items (Griffen & Drake, 2008). Variation in abiotic conditions results in differences in vegetation communities across ecological space. Under the optimality theory, organisms occupy habitats which maximize access to high quality food items (MacArthur & Pianka, 1966). Within areas or periods of resource scarcity, organisms cannot access highly palatable food items, risking reduced diet quality and poor nutritional state.

Nutritional and energetic stresses are major factors leading to the decline of many populations (*e.g.* du Dot et al., 2009; Wasser et al., 2017). Food shortages result in population fluctuations through increased mortality by starvation (Mduma et al., 1999; Nagy & Holmes, 2005) and reduced reproductive rates (Nagy & Holmes, 2005; Parker et al., 2009). Higher quality diets promote better body condition (Parker et al., 2009) and improved pregnancy rates (Wasser et al., 2017). In addition, dietary shifts have been recorded following habitat conversion (Tecot et al., 2019) . There are strong links that resource availability is a major factor in population limitation (reviewed in White 2008). If food availability drives population regulation, we would predict ecologically marginal populations would have poor resource availability and poor diet quality.

1.4.2.3 Microbiome

The gut microbiome system performs key to digestive activities within the vertebrate gastrointestinal system. The microbiome is essential in breaking down and assimilating nutrients from food items and conferring immunity (Sommer & Bäckhed, 2013). The microbiome assists in extracting energy from food items (Dearing & Kohl, 2017). A composition change in the microbial community may reduce the communities' ability to break down food resources or confer immunity against pathogens. Microbiome composition therefore likely impacting host fitness (Suzuki, 2017; Trevelline et al., 2019).

Various factors such as habitat, diet, social network properties and climatic conditions influence microbiome composition (Antwis et al., 2018; Hicks et al., 2018; Trevelline et al., 2019). However, diet seems to be an especially important factor (Hicks et al., 2018; Kartzinel et al., 2019). Changes in dietary composition from habitat degradation alter microbiome composition and structure. For example, in the endangered black howler monkey (*Alouatta pigra*), diet diversity and microbial diversity were higher in untransformed continuous forest compared to fragmented forests (Amato et al., 2013). The implications for health from the direct and indirect effects of low-quality habitat may converge to increase the likelihood of extirpation (Clayton et al., 2018). Stress, leading to endocrinological changes, such as the release of glucocorticoids during HPA axis activation, modulates the microbiome composition (Avramenko et al., 2015; Noguera et al., 2018). Therefore, the microbiome may be an important aspect of the physiology influenced by ecological marginality.

1.4.2.4 Nemabiome

The nemabiome refers to the communities of helminths within an animal's gastrointestinal system (Avramenko et al., 2015). Gastrointestinal helminths are a source of negative health outcomes in many species. For example, at the population level, large burdens are associated with declines in fecundity and survival (Moss & Camin, 1970; Anderson & May, 1979; Oppliger, 1993; Hudson et al., 1998; Patterson et al., 2013). However, some helminth species may play in important role in animal health (McKay, 2009; Ezenwa & Jolles, 2015). Parasitic helminths can influence host immunological responses affecting resistance to, or coinfection of, species (Supali et al., 2010). Moreover, the presence of gastrointestinal helminths impacts the gastrointestinal immune system. Removal of helminths induces strong inflammatory responses (Betancourt et al., 2015; Walshe et al., 2019) and can trigger autoimmune diseases (Cooper et al., 2012; McKay, 2009).

There are multiple aspects of parasite infections, *i.e.* diversity, abundance, burden, and host physiological condition. Parasite species richness and composition negatively impact survival (Cooper et al., 2012) and determine infection success and host fitness (Johnson & Hoverman, 2012) . Individuals in compromised physiological conditions have reduced resilience to parasitic infection (Beldomenico & Begon, 2010). These combined effects of parasites with other physiological responses to marginal conditions may create a positive feedback system of compromised immunity and greater susceptibility to further infection (Beldomenico & Begon, 2010). These positive feedback loops may explain population-level crashes associated with helminth populations (Hudson et al., 1998). Variation in parasitic infection has been found to increase towards a species range limit (Chapman et al., 2006). Under the functional marginality hypothesis, we predict ecologically marginal populations should display higher parasite burdens and more virulent parasite communities.

1.5 Understanding ecological marginality in different landscapes

1.5.1 South Africa: a fragmented landscape - Private land ownership and fencing conservation regime

In South Africa, wildlife is found on three categories of land: Publicly owned protected areas (PAs), Protected Private Areas (PPA) and private land. Publicly owned protected areas are those owned and operated by state infrastructure. Protected Private Areas are defined as PAs under private governance. Protected Private Areas can include many formal agreements with the state over the rights or ownership of animals or land (e.g., stewardship agreements). Finally, an alternative, commercial approach is the use of wildlife on land not declared as PPAs, where the primary goal of keeping wildlife is profit, although conservation gains may be an inadvertent outcome.

Each of these categories forms difficulties for conservation. PA are largely underfunded (Lindsey et al., 2018), and may be formed for alternative reasons than biodiversity protection (Joppa & Pfaff, 2009). For example, state-owned PAs in the Cape Floristic Region of South Africa were formed in upland catchments to ensure water supplies or in areas with the lowest socioeconomic conflicts and costs (Rouget et al., 2003).

PPAs and Private lands are restricted by many legislations, but most importantly the Game Theft Act of 1991 (No. 105 of 1991). This act gave protections and ownership rights over wild animals on private land to private landowners assuming the animals were maintained “within adequately enclosed areas”. Ownership rights depend on landowners having adequate game-proof fencing to prevent escape of species (Blackmore, 2020). Game fencing restricts the movement of large bodied species (Boone & Hobbs, 2004) and leads to poor gene flow between populations (Woodroffe et al., 2014). As much as 85% of private land has game fences and an estimated one-sixth of all land in South Africa is ‘game fenced’, producing an extremely fragmented landscape (Snijders, 2014). As purchasing large amounts of land is expensive, PPAs and private lands tend to be small but occupy high quality areas (Clements et al., 2019)

In summary, South African conservation is a mixture of large fenced Public PA usually situated in areas of poor productivity and small privately-owned fenced land usually in potentially productive areas (Clements et al., 2019). The variation in lands being protected results in an ecological gradient. Meanwhile, the fencing results in discrete populations which all experience unique climatic conditions, biotic interactions and resource availability.

1.5.1.1 Cape mountain zebra as a model species to understand ecological marginality in fragmented landscapes

The Cape mountain zebra (*Equus zebra zebra,* CMZ) is endemic to the Northern, Eastern and Western Capes of South Africa. The species historically occurred in Nama Karoo, Succulent Karoo and Grassland Biomes (Boshoff, Landman and Kerley, 2016). Historically, CMZ occupied a much larger ecological niche and may have undertaken altitudinal migration (Faith, 2012). Due to over-hunting and widespread agricultural transformation, CMZ populations were reduced to ~30 individuals distributed across three relict populations: Kammanassie Nature Reserve, Gamkaberg Nature Reserve and Mountain Zebra National Park (MZNP) (Watson and Chadwick, 2007). Currently, an estimated 1714-3247 mature CMZ individuals remain across at least 75 fenced populations (Hrabar and Kerley, 2015). The CMZ population has been increasing between 8.6%-9.6% since 1985 (Hrabar and Kerley, 2015). However, MZNP and Karoo National Park contain 25% and 18% of the global CMZ population respectively. Subpopulation census estimates range from 4-1191 individuals and population growth rate varies hugely between reserves (between -0.086 and 0.145) due to habitat characteristics (Watson et al., 2005; Lea et al., 2016).

CMZ provides an opportunity to understand how abiotic conditions impact population performance. The CMZ is an example of a partial refugee species (Lea *et al*, 2016). Some populations are confined to a marginal environment (Figure 1.7) *i.e.* showing reduced performance. In contrast, others perform well in more optimal environments (Lea *et al.*, 2016, Figure 1.5). The grazing ecology of the species is simplistic relying on 4 major potential impacts to determine population trends: predation, water availability, grass availability and parasites. As CMZ exist in predominately predator-free fenced environments, population performance variation due to predation is negligible. Furthermore, as migration is impossible without human intervention, CMZ can be located easily for sampling. Identification of individuals is also possible due to individual stripe pattern variation. CMZ have a limited historical distribution, allowing sampling of the entirety of the species range, which may be impossible for more abundant or widespread species. Accurate records of historical sightings, founder dates, population growth rates, foal and adult sex ratios, hormonal profiles and demographic changes exist and are freely available (Boshoff, Landman and Kerley, 2016; Lea *et al.*, 2016, 2017).

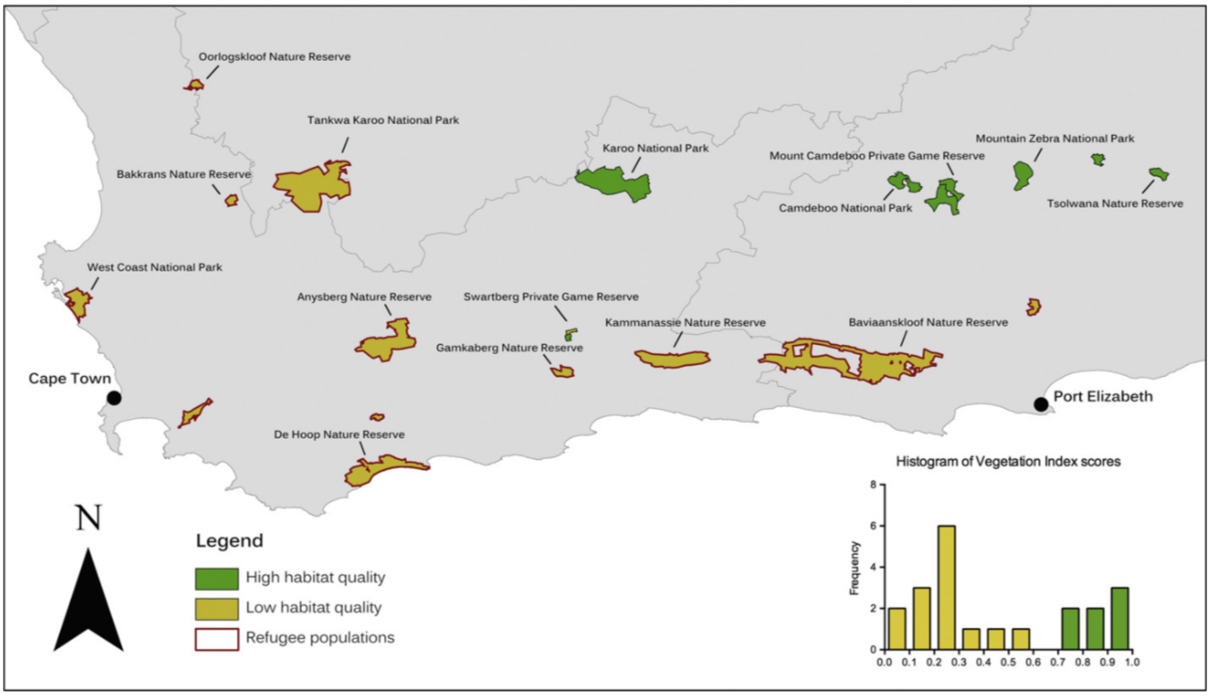


Figure 1.7) Distribution and variability of habitat quality of Cape mountain zebra populations. The Cape mountain zebra are endemic to the eastern and western cape of South Africa. Currently, the species occupies habitats of varying qualities such that some habitats have low availability of grass (coloured). Green represents high quality populations with high vegetation index scores and tallow represent low quality population with relatively poor vegetation index scores. Vegetation index scores are a proxy of grassiness of the habitat. Refugee populations are outlined in red. Bar chart display the distribution of vegetation index scores found across the range. These low-quality habitats result in reduced population performance *via* reduced reproduction or survival and therefore constitute refugee populations. (Taken from Lea *et al*, 2016)

1.5.2 Kenya: a patchwork landscape – Non-fenced conservation regime

Wildlife conservation spaces in Kenya are different to those in South Africa. Instead of having discrete PAs, Kenyan conservation areas usually encompass formally protected areas, communal and private lands and land used and owned by traditional pastoral communities (Weldemichel & Lein, 2019). Large areas of the subdivided land have been consolidated and set aside for nature conservancies (a sanctuary for wildlife established by any person or a community who owns land that is inhabited by wildlife). Alongside this change, pastoralism has continued. Pastoralists have been resistant to fencing. Mobility is key for pastoral livelihoods as they track climatic variations and forage availability for their livestock. Although fencing has increased throughout Kenya (Løvschal et al., 2017), conservation landscapes such as Laikipia-Samburu are largely unfragmented and allow species to predominantly move freely (Kirathe et al., 2021).

In summary, Kenyan Conservation is a mixture of Publicly owned protected areas interacting with privately owned conservancies and mobile pastoralist communities. This has resulted in relatively reduced fencing of the landscape compared to South Africa. Wildlife (and livestock) can move more freely and better track preferred conditions.

1.5.2.1 Grevy's zebra as a model species to understand ecological marginality in Kenya

Grevy's zebra (*Equus grevyi*) are large grazing equids native to semiarid habitats throughout the horn of Africa (Rubenstein et al, 2016). However, following a 75% decline since the 1970s, the species is now restricted mainly to Kenya especially the Laikipia–Samburu Ecosystems of central Kenya (Rubenstein et al, 2016). A vital concern for the effective protection of Grevy's zebra is identifying high quality areas in terms of survival and reproduction (Low et al., 2009). Across the Laikipia–Samburu Ecosystems, Grevy's zebra population sizes and recruitment rates vary (Figure 1.8). Across the Laikipia–Samburu Ecosystems, ecosystems vary in overall habitat quality which may vary due to gradients in rainfall (400-1000mm) and land-use type (mosaic of conservancies, commercial livestock ranches and community rangelands). Most Grevy's zebra are found on the community rangelands, properties occupied and managed by traditional pastoralists.

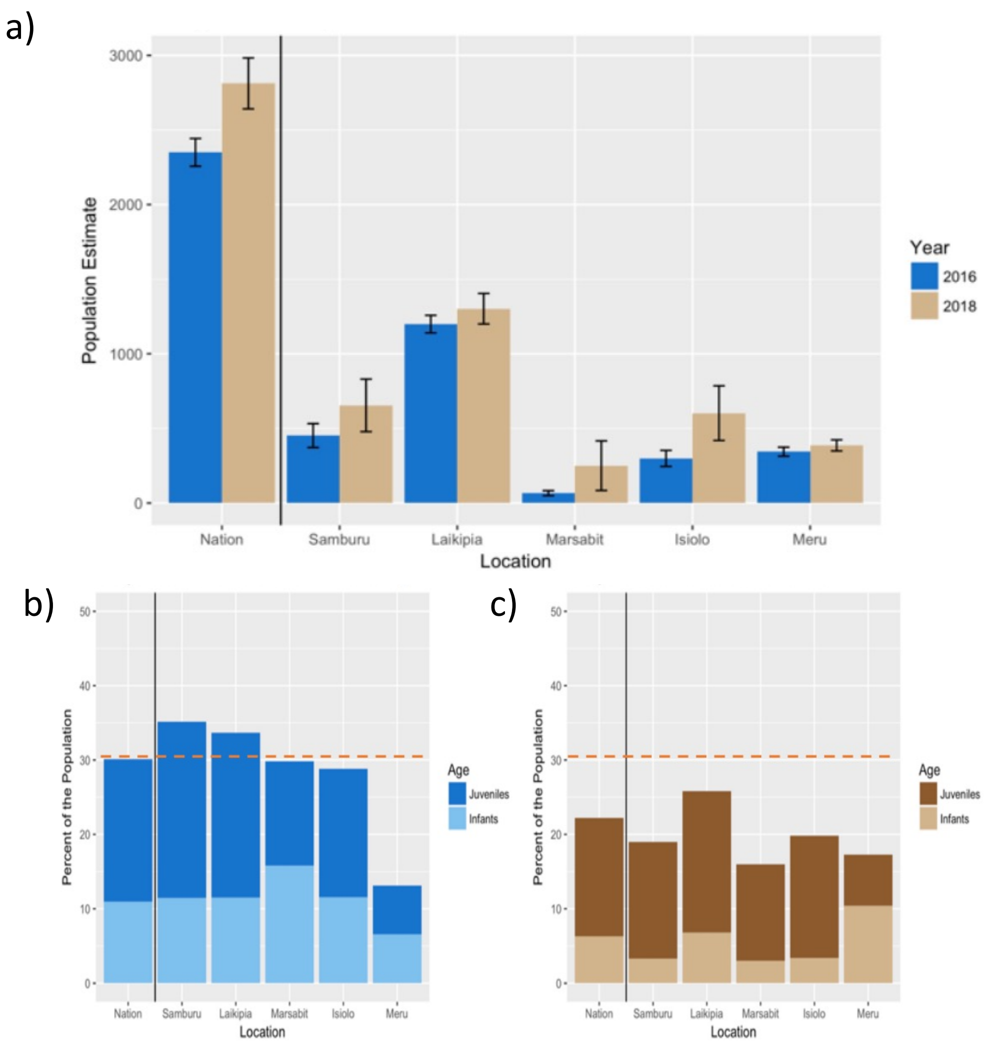


Figure 1.8) Grevy's zebra performance rate differences across the metapopulation. a) Population estimates vary across the metapopulation and estimates vary in rate of growth. Blue represents population estimates in 2016 and beige in 2018. Standard error bars of estimate for each population are displayed. Bottom) Recruitment rates vary across the metapopulation with some areas having greater birth or survival rates of infants and juveniles b) Juvenile age distribution in 2016. Juveniles are in dark blue and infants in light blue. c) Juvenile age distribution in 2018. Juveniles are in dark brown and infants in light brown (Taken from (Rubenstein et al., 2018).

1.6 Objectives and Chapters

The first objective of this thesis is to establish the scope of ecological and geographic marginalisation and whether it impacts extinction. Following a critical evaluation of methods in Chapter 2, I evaluate the scope of ecological and geographic marginalisation in Chapter 3. Chapter 3 models the historic and contemporary niche spaces of terrestrial mammals and evaluates the extrinsic and intrinsic factors associated with marginalisation and extinction risk.

The second objective of this thesis is to investigate the physiological consequences of marginality. Here I use two model species across two management regimes varying in fragmentation. I investigate how marginality impacts diet, physiology and performance of Cape mountain zebra across nine fenced protected areas spanning most of its species range in South Africa. I also investigate Grevy's Zebra diet and physiology across an ecological gradient in a relative unfragmented landscape spanning Laikipia, Samburu and Meru Counties, Kenya. I do this in Chapters 4 and 5 and 6. Chapter 4 revisits the ecological and demographic drivers of faecal glucocorticoid concentrations from *Lea* et al, 2017. Here I validate a faecal metabolite glucocorticoid assay for CMZ and compare results to an unvalidated assay used in *Lea et al* , 2017. Chapter 5 uses DNA metabarcoding of diet, microbiome and nemabiome to evaluate how diet and gastrointestinal health impact on fecundity across the CMZ species range. Finally, Chapter 6 uses DNA metabarcoding of diet, microbiome and nemabiome in Grevy's zebra across Laikipia, Kenya to evaluate the seasonal drivers of marginality across an ecological gradient.

Appendices 1, 2 and 3 include my published works. The manuscript in Appendix 1 outlines a framework of hypotheses to evaluate ecological marginality across species. The manuscript in Appendix 2 discusses the conservation implications of widespread range contraction and marginality. The manuscript in Appendix 3 provides anecdotal evidence of infanticide in Cape mountain zebra within a resource rich and resource poor environments.

1.7 References

Abeli, T., Gentili, R., Mondoni, A., Orsenigo, S., & Rossi, G. (2014). Effects of marginality on plant population performance. *Journal of Biogeography*, *41*(2), 239–249.

Abeli, T., Ghitti, M., & Sacchi, R. (2019). Does ecological marginality reflect physiological marginality in plants? *Plant Biosystems*, *154*(2), 149–157.

Alcover, J. A. (1992). The avifaunas of the isolated Mediterranean Islands during the middle and upper Pleistocene. Papers in Avian Ornithology. Honoring Pierce Brodkorb. *Natural History Museum of Los Angeles County*, *36*, 273-283.

Ali, A. H., Ford, A. T., Evans, J. S., Mallon, D. P., Hayes, M. M., King, J., Amin, R., & Goheen, J. R. (2017). Resource selection and landscape change reveal mechanisms suppressing population recovery for the world's most endangered antelope. *Journal of Applied Ecology*, *54*(6), 1720–1729.

Ali, A.H., Kauffman, M.J., Amin, R., Kibara, A., King, J., Mallon, D., Musyoki, C. and Goheen, J.R., (2018).Demographic drivers of a refugee species: large-scale experiments guide strategies for reintroductions of hirola. *Ecological Applications*, *28*(2), 275-283.

Amato, K. R., Yeoman, C. J., Kent, A., Righini, N., Carbonero, F., Estrada, A., Rex Gaskins, H., Stumpf, R. M., Yildirim, S., Torralba, M., Gillis, M., Wilson, B. A., Nelson, K. E., White, B. A., & Leigh, S. R. (2013). Habitat degradation impacts black howler monkey (*Alouatta pigra*) gastrointestinal microbiomes. *The ISME Journal*, *7*(7), 1344–1353.

Anderson, R. M., & May, R. M. (1979). Population biology of infectious diseases: Part I. *Nature*, *280*.

Antwis, R. E., Lea, J. M. D. D., Unwin, B., & Shultz, S. (2018). Gut microbiome composition is associated with spatial structuring and social interactions in semi-feral Welsh Mountain ponies. *Microbiome*, *6*(1), 207.

Atkinson, C. T., Saili, K. S., Utzurrum, R. B., & Jarvi, S. I. (2013). Experimental evidence for evolved tolerance to avian malaria in a wild population of low elevation Hawai'i 'Amakihi (*Hemignathus virens*). *EcoHealth*, *10*(4), 366–375.

Aura Tortosa, J. E., Jordá Pardo, J. F., Badal García, E., Guillem Calatayud, P., Perez Ripoll, M., & Rodrigo García, M. J. (2002). The far south: the Pleistocene–Holocene transition in Nerja Cave (Andalucia, Spain). *Quaternary International*, *94–94*, 19–30.

Avramenko, R. W., Redman, E. M., Lewis, R., Yazwinski, T. A., Wasmuth, J. D., & Gilleard, J. S. (2015). Exploring the gastrointestinal "nemabiome": Deep amplicon sequencing to quantify the species composition of parasitic nematode communities. *PLoS ONE*, *10*(12), e0143559.

Barnes, M. D., Craigie, I. D., Harrison, L. B., Geldmann, J., Collen, B., Whitmee, S., Balmford, A., Burgess, N. D., Brooks, T., Hockings, M., & Woodley, S. (2016). Wildlife population trends in protected areas predicted by national socio-economic metrics and body size. *Nature Communications 7*(1), 1–9.

Barnosky, A. D., Matzke, N., Tomiya, S., Wogan, G. O. U., Swartz, B., Quental, T. B., Marshall, C., McGuire, J. L., Lindsey, E. L., Maguire, K. C., Mersey, B., & Ferrer, E. A. (2011). Has the Earth's sixth mass extinction already arrived? *Nature*, *471*(7336), 51–57.

Battin, J. (2004). When good animals love bad habitats: Ecological traps and the conservation of animal populations. *Conservation Biology*, *18*(6), 1482–1491.

Baugh, A. T., Witonsky, K. R., Davidson, S. C., Hyder, L., Hau, M., & van Oers, K. (2017). Novelty induces behavioural and glucocorticoid responses in a songbird artificially selected for divergent personalities. *Animal Behaviour*, *130*, 221–231.

Beldomenico, P. M., & Begon, M. (2010). Disease spread, susceptibility and infection intensity: vicious circles? *Trends in Ecology and Evolution*, *25*(1), 21–27.

Betancourt, A., Lyons, E. T., & Horohov, D. W. (2015). Characterisation of the inflammatory cytokine response to anthelmintic treatment in ponies. *Equine Veterinary Journal*, *47*(2), 240–244.

Bilney, R. J., Cooke, R., & White, J. G. (2010). Underestimated and severe: Small mammal decline from the forests of south-eastern Australia since European settlement, as revealed by a top-order predator. *Biological Conservation*, *143*(1), 52–59.

Blackmore, A. (2020). Climate change and the ownership of game: A concern for fenced wildlife areas. *Koedoe*, *62*(1), 5.

Boakes, E. H., Isaac, N. J. B., Fuller, R. A., Mace, G. M., & McGowan, P. J. K. (2018). Examining the relationship between local extinction risk and position in range. *Conservation Biology*, *32*(1), 229–239.

Boakes, E. H., Mace, G. M., McGowan, P. J. K., & Fuller, R. A. (2010). Extreme contagion in global habitat clearance. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1684), 1081–1085.

Bocherens, H., Hofman-Kamińska, E., Drucker, D. G., Schmölcke, U., & Kowalczyk, R. (2015). European bison as a refugee species? Evidence from isotopic data on Early Holocene bison and other large herbivores in northern Europe. *PLoS ONE*, *10*(2), 1–19.

Boone, R. B., & Hobbs, N. T. (2004). Lines around fragments: Effects of fencing on large herbivores. *African Journal of Range and Forage Science*, *21*(3), 147–158.

Boonstra, R., Hik, D., Singleton, G. R., & Tinnikov, A. (1998). The Impact of Predator-Induced Stress on the Snowshoe Hare Cycle. *Ecological Monographs*, *68*(3), 371.

Brook, B. W., Lim, L., Harden, R., & Frankham, R. (1997). How Secure Is the Lord Howe Island Woodhen? a Population Viability Analysis Using Vortex. *Pacific Conservation Biology*, *3*, 125–133.

Brown, J. H. (1984). On the Relationship between Abundance and Distribution of Species. *The American Naturalist*, *124*(2), 255–279.

Brown, J. H., Mehlman, D. W., & Stevens, G. C. (1995). Spatial variation in abundance. *Ecology*, *76*(7), 2028–2043.

Bunin, J. S., & Jamieson, I. G. (1995). New Approaches Toward a Better Understanding of the Decline of Takahe (*Porphyrio mantelli*) in New Zealand. *Conservation Biology*, *9*(1), 100–106.

Burner, R. C., Styring, A. R., Rahman, M. A., & Sheldon, F. H. (2019). Occupancy patterns and upper range limits of lowland Bornean birds along an elevational gradient. *Journal of Biogeography*, *46*(11), 2583–2596

Burton, J. A., Hedges, S., & Mustari, A. H. (2005). The taxonomic status, distribution and conservation of the lowland anoa *Bubalus depressicornis* and mountain anoa *Bubalus quarlesi*. *Mammal Review*, *35*(1), 25–50.

Busch, D. S. (2010). Measuring stress in conservation settings: A reply to Linklater. *Biological Conservation*, *5*(143), 1039-1040.

Cameron, J. L. (1997). Stress and behaviorally induced reproductive dysfunction in primates. *Seminars in Reproductive Endocrinology*, *15*(1), 37–45.

Caughley, G. (1994). Directions in Conservation Biology. *The Journal of Animal Ecology*, *63*(2), 215.

Ceballos, G., Ehrlich, P. R., & Dirzo, R. (2017). Biological annihilation via the ongoing sixth mass extinction signaled by vertebrate population losses and declines. *Proceedings of the National Academy of Sciences*, 201704949.

Ceballos, G., Ehrlich, P. R., & Raven, P. H. (2020). Vertebrates on the brink as indicators of biological annihilation and the sixth mass extinction. *Proceedings of the National Academy of Sciences of the United States of America*.

Channell, R., & Lomolino, M. V. (2000). Trajectories to extinction: spatial dynamics of the contraction of geographic ranges of endangered species. *Journal of Biogeography*, *27*, 169–180.

Channell, R., Lomolino, M. v., & Lomollno, M. v. (2000). Dynamic biogeography and conservation of endangered species. *Nature*, *403*(6765), 84–86.

Chapman, C. A., Speirs, M. L., Gillespie, T. R., Timothy., H., & Austad, K. M. (2006). Life on the Edge: Gastrointestinal Parasites from the Forest Edge and Interior Primate Groups. *American Journal of Primatology*, *1222*(68), 397–409.

Chevalier, M., Broennimann, O., & Guisan, A. (2021). Using a robust multi-settings inference framework on published datasets still reveals limited support for the abundant centre hypothesis: More testing needed on other datasets. *Global Ecology and Biogeography*, *30*(11),

Ciuti, S., Muhly, T. B., Paton, D. G., McDevitt, A. D., Musiani, M., & Boyce, M. S. (2012). Human selection of elk behavioural traits in a landscape of fear. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1746), 4407–4416.

Clayton, J. B., Al-Ghalith, G. A., Long, H. T., Tuan, B. van, Cabana, F., Huang, H., Vangay, P., Ward, T., Minh, V. van, Tam, N. A., Dat, N. T., Travis, D. A., Murtaugh, M. P., Covert, H., Glander, K. E., Nadler, T., Toddes, B., Sha, J. C. M., Singer, R., … Johnson, T. J. (2018). Associations Between Nutrition, Gut Microbiome, and Health in A Novel Nonhuman Primate Model. *Scientific Reports* *8*(1), 1–16.

Clements, H. S., Kerley, G. I. H., Cumming, G. S., de Vos, A., & Cook, C. N. (2019). Privately protected areas provide key opportunities for the regional persistence of large- and medium-sized mammals. *Journal of Applied Ecology*, *56*(3), 537–546.

Clutton-Brock, T. H., Major, M., Albon, S. D., & Guinness, F. E. (1987). Early Development and Population Dynamics in Red Deer. I. Density-Dependent Effects on Juvenile Survival. *The Journal of Animal Ecology*, *56*(1), 53.

Colwell, R. K., & Rangel, T. F. (2009). Hutchinson's duality: the once and future niche. *Proceedings of the National Academy of Sciences of the United States of America*, *106,* 19651–19658.

Cooper, N., Kamilar, J. M., & Nunn, C. L. (2012). Host longevity and parasite species richness in mammals. *PLoS ONE*, *7*(8), e42190.

Craigie, I. D., Baillie, J. E. M., Balmford, A., Carbone, C., Collen, B., Green, R. E., & Hutton, J. M. (2010). Large mammal population declines in Africa's protected areas. *Biological Conservation*, *143*(9), 2221–2228.

Cromsigt, J. P. G. M., Kerley, G. I. H., & Kowalczyk, R. (2012). The difficulty of using species distribution modelling for the conservation of refugee species - the example of European bison. *Diversity and Distributions*, *18*(12), 1253–1257.

Crooks, K.R., Burdett, C.L., Theobald, D.M., King, S.R., Di Marco, M., Rondinini, C. and Boitani, L., 2017. Quantification of habitat fragmentation reveals extinction risk in terrestrial mammals. *Proceedings of the national Academy of Sciences*, *114*(29), 7635-7640.

Crowley, B. E., Godfrey, L. R., Guilderson, T. P., Zermeño, P., Koch, P. L., & Dominy, N. J. (2012). Extinction and ecological retreat in a community of primates. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1742), 3597–3605.

Daleszczyk, K. (2004). Mother-calf relationships and maternal investment in European *bison Bison bonasus*. *Acta Theriologica*, *49*(4), 555–566.

Dallas, T. A., & Santini, L. (2020). The influence of stochasticity, landscape structure and species traits on abundant–centre relationships. *Ecography*, *43*(9), 1341–1351.

Dantzer, B., Fletcher, Q. E., Boonstra, R., & Sheriff, M. J. (2014). Measures of physiological stress: a transparent or opaque window into the status, management and conservation of species? *Conservation Physiology*, *2*(1).

Dearing, M. D., & Kohl, K. D. (2017). Beyond Fermentation: Other Important Services Provided to Endothermic Herbivores by their Gut Microbiota. *Integrative and Comparative Biology*, *57*(4), 723–731.

Delibes, M., Ferreras, P., & Gaona, P. (2001). Attractive sinks, or how individual behavioural decisions determine source-sink dynamics. *Ecology Letters*, *4*(5), 401–403.

di Marco, M., Boitani, L., Mallon, D., Hoffmann, M., Iacucci, A., Meijaard, E., Visconti, P., Schipper, J., & Rondinini, C. (2014). A Retrospective evaluation of the global decline of carnivores and ungulates. *Conservation Biology*, *28*(4), 1109–1118.

Dinerstein, E., Vynne, C., Sala, E., Joshi, A. R., Fernando, S., Lovejoy, T. E., Mayorga, J., Olson, D., Asner, G. P., Baillie, J. E. M., Burgess, N. D., Burkart, K., Noss, R. F., Zhang, Y. P., Baccini, A., Birch, T., Hahn, N., Joppa, L. N., & Wikramanayake, E. (2019). A Global Deal for Nature: Guiding principles, milestones, and targets. *Science Advances*, *5*(4).

Dirzo, R., Young, H. S., Galetti, M., Ceballos, G., Isaac, N. J. B., & Collen, B. (2014). Defaunation in the Anthropocene. *Science* 345(6195), 401–406.

Doherty, T. S., Glen, A. S., Nimmo, D. G., Ritchie, E. G., & Dickman, C. R. (2016). Invasive predators and global biodiversity loss. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(40), 11261–11265.

Donald, P. F., & Greenwood, J. J. D. (2001). Spatial patterns of range contraction in British breeding birds. *Ibis*, *143*(3), 593–601.

Edwards, M. A., & Derocher, A. E. (2015). Mating-related behaviour of grizzly bears inhabiting marginal habitat at the periphery of their North American range. *Behavioural Processes*, *111*(1), 75–83.

Ellis, E. C. (2011). Anthropogenic transformation of the terrestrial biosphere. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, *369*(1938), 1010–1035.

Ellis, E. C., Gauthier, N., Goldewijk, K. K., Bird, R. B., Boivin, N., Díaz, S., Fuller, D. Q., Gill, J. L., Kaplan, J. O., Kingston, N., Locke, H., McMichael, C. N. H., Ranco, D., Rick, T. C., Rebecca Shaw, M., Stephens, L., Svenning, J. C., & Watson, J. E. M. (2021). People have shaped most of terrestrial nature for at least 12,000 years. *Proceedings of the National Academy of Sciences of the United States of America*, *118*(17), 2023483118.

Ellis, E. C., Goldewijk, K. K., Siebert, S., Lightman, D., & Ramankutty, N. (2010). Anthropogenic transformation of the biomes, 1700 to 2000. *Global Ecology and Biogeography*, *19*(5), 589–606.

Ezenwa, V. O., & Jolles, A. E. (2015). Opposite effects of anthelmintic treatment on microbial infection at individual versus population scales. *Science*, *347*(6218), 175–177.

Faith, J. T. (2012). Palaeozoological insights into management options for a threatened mammal: Southern Africa's Cape mountain zebra (*Equus zebra zebra*). *Diversity and Distributions*, *18*(5), 438–447.

Fisher, D. O. (2011). Trajectories from extinction: Where are missing mammals rediscovered? *Global Ecology and Biogeography*, *20*(3), 415–425.

Fisher, D. O., Blomberg, S. P., & Owens, I. P. F. (2003). Extrinsic versus intrinsic factors in the decline and extinction of Australian marsupials. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(1526), 1801–1808.

Flores-Miyamoto, K., Clauss, M., Ortmann, S., & Sainsbury, A. W. (2005). Nutrition of captive lowland anoa (*Bubalus depressicornis*): a study on ingesta passage, intake, digestibility, and a diet survey. *Zoo Biology*, *24*(2), 125-134.

Flueck, W. T. (2021). Functional limb anatomy in a refugee species: The endangered Patagonian huemul deer (*Hippocamelus bisulcus*). *Anatomia, Histologia, Embryologia*, *50*(2), 411–416.

Freese, C. H., Aune, K. E., Boyd, D. P., Derr, J. N., Forrest, S. C., Cormack Gates, C., Gogan, P. J. P., Grassel, S. M., Halbert, N. D., Kunkel, K., & Redford, K. H. (2007). Second chance for the plains bison. *Biological Conservation*, *136*(2), 175–184.

Fretwell, S. D. (1969). On territorial behavior and other factors influencing habitat distribution in birds. *Acta Biotheoretica*, *19*(1), 45–52.

Fuller, R. A., McDonald-Madden, E., Wilson, K. A., Carwardine, J., Grantham, H. S., Watson, J. E. M., Klein, C. J., Green, D. C., & Possingham, H. P. (2010). Replacing underperforming protected areas achieves better conservation outcomes. *Nature*, *466*(7304), 365–367.

Gaston, K. J. (2003). The Structure and Dynamics of Geographic Ranges. *Oxford Series in Ecology and Evolution*. Oxford University Press.

Gaston, K. J. (2009). Geographic range limits of species. *Proceedings of the Royal Society B: Biological Sciences*, *276*(1661), 1391–1393.

Gazo, M., Aparicio, F., Cedenilla, M. A., Layna, J. F., & Gonzalez, L. M. (2000). Pup Survival in the Mediterranean Monk Seal (*Monachus Monachus*) Colony At Cabo Blanco Peninsula (Western Sahara-Mauritania). *Marine Mammal Science*, *16*(1), 158–168.

Geldmann, J., Barnes, M., Coad, L., Craigie, I. D., Hockings, M., & Burgess, N. D. (2013). Effectiveness of terrestrial protected areas in reducing habitat loss and population declines. *Biological Conservation*, *161*, 230–238.

Gobush, K. S., Booth, R. K., & Wasser, S. K. (2014). Validation and application of noninvasive glucocorticoid and thyroid hormone measures in free-ranging Hawaiian monk seals. *General and Comparative Endocrinology*, *195*, 174–182.

González, L. M. (2015). Prehistoric and historic distributions of the critically endangered Mediterranean monk seal (*Monachus monachus*) in the eastern Atlantic. *Marine Mammal Science*, *31*(3), 1168–1192.

Gray, C. L., Hill, S. L. L., Newbold, T., Hudson, L. N., Boïrger, L., Contu, S., Hoskins, A. J., Ferrier, S., Purvis, A., & Scharlemann, J. P. W. (2016). Local biodiversity is higher inside than outside terrestrial protected areas worldwide. *Nature Communications* *7*(1), 1–7.

Griffen, B. D., & Drake, J. M. (2008). Effects of habitat quality and size on extinction in experimental populations. *Proceedings of the Royal Society B: Biological Sciences*, *275*(1648), 2251–2256.

Griffen, B. D., & Norelli, A. P. (2015). Spatially variable habitat quality contributes to within-population variation in reproductive success. *Ecology and Evolution*, *5*(7), 1474–1483.

Grueber, C. E., & Jamieson, I. G. (2011). Low genetic diversity and small population size of Takahe Porphyrio hochstetteri on European arrival in New Zealand. *Ibis*, *153*(2), 384–394.

Guo, Q., Taper, M., Schoenberger, M., & Brandle, J. (2005). Spatial-temporal population dynamics across species range: from centre to margin. *Oikos*, *108*(1), 47–57.

Habel, J. C., Segerer, A., Ulrich, W., Torchyk, O., Weisser, W. W., & Schmitt, T. (2016). Butterfly community shifts over two centuries. *Conservation Biology*, *30*(4), 754–762.

Haddad, N. M., Brudvig, L. A., Clobert, J., Davies, K. F., Gonzalez, A., Holt, R. D., Lovejoy, T. E., Sexton, J. O., Austin, M. P., Collins, C. D., Cook, W. M., Damschen, E. I., Ewers, R. M., Foster, B. L., Jenkins, C. N., King, A. J., Laurance, W. F., Levey, D. J., Margules, C. R., … Townshend, J. R. (2015). Habitat fragmentation and its lasting impact on Earth ' s ecosystems. *Applied Ecology*, 1–9.

Hale, R., & Swearer, S. E. (2016). Ecological traps: current evidence and future directions. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1824), 20152647.

Hall, J., Burgess, N. D., Lovett, J., Mbilinyi, B., & Gereau, R. E. (2009). Conservation implications of deforestation across an elevational gradient in the Eastern Arc Mountains, Tanzania. *Biological Conservation*, *142*(11), 2510–2521.

Hall, S. J. G. (2008). A comparative analysis of the habitat of the extict aurochs and other prehistoric mammals in Britain. *Ecography*, *31*, 187–190.

Halpern, B. S., Walbridge, S., Selkoe, K. A., Kappel, C. v., Micheli, F., D'Agrosa, C., Bruno, J. F., Casey, K. S., Ebert, C., Fox, H. E., Fujita, R., Heinemann, D., Lenihan, H. S., Madin, E. M. P., Perry, M. T., Selig, E. R., Spalding, M., Steneck, R., & Watson, R. (2008). A Global Map of Human Impact on Marine Ecosystems. *Science*, *319*(5865), 948–952.

Harris, G., Thirgood, S., Hopcraft, J. G. C., Cromsigt, J. P. G. M., & Berger, J. (2009). Global decline in aggregated migrations of large terrestrial mammals. *Endangered Species Research*, *7*(1), 55–76.

Hemerik, L., Hengeveld, R., & Lippe, E. (2006). The eclipse of species ranges. *Acta Biotheoretica*, *54*(4), 255–266.

Hicks, A. L., Lee, K. J., Couto-Rodriguez, M., Patel, J., Sinha, R., Guo, C., Olson, S. H., Seimon, A., Seimon, T. A., Ondzie, A. U., Karesh, W. B., Reed, P., Cameron, K. N., Lipkin, W. I., & Williams, B. L. (2018). Gut microbiomes of wild great apes fluctuate seasonally in response to diet. *Nature Communications*, *9*(1).

Hilborn, R., Branch, T. A., Ernst, B., Magnusson, A., Minte-Vera, C. V., Scheuerell, M. D., & Valero, J. L. (2003). State of the world's fisheries. *Annual review of Environment and Resources*, *28*(1), 359-399.

Hing, S., Narayan, E. J., Thompson, R. C. A., Godfrey, S. S., Hing, S., Narayan, E. J., Thompson, R. C. A., & Godfrey, S. S. (2016). The relationship between physiological stress and wildlife disease: consequences for health and conservation. *Wildlife Research*, *43*(1), 51–60.

Hobbs, R. J., Higgs, E., Hall, C. M., Bridgewater, P., Chapin, F. S., Ellis, E. C., Ewel, J. J., Hallett, L. M., Harris, J., Hulvey, K. B., Jackson, S. T., Kennedy, P. L., Kueffer, C., Lach, L., Lantz, T. C., Lugo, A. E., Mascaro, J., Murphy, S. D., Nelson, C. R., … Yung, L. (2014). Managing the whole landscape: Historical, hybrid, and novel ecosystems. *Frontiers in Ecology and the Environment*, *12*(10), 557–564.

Hofman-Kamińska, E., Bocherens, H., Borowik, T., Drucker, D. G., & Kowalczyk, R. (2018). Stable isotope signatures of large herbivore foraging habitats across Europe. *PLoS ONE*, *13*(1).

Hofmann, R. R. (1989). Evolutionary steps of ecophysiological adaptation and diversification of ruminants: a comparative view of their digestive system. *Oecologia*, *78*(4), 443–457.

Holdaway, R. N., Williams, M., & Hawke, D. J. (2013). A comparison of the pre-human and present isotopic niches of brown teal (Anas chlorotis): Implications for conservation. *Notornis*, *60*(3), 233–244.

Holt, R. D. (2009). Bringing the Hutchinsonian niche into the 21st century: Ecological and evolutionary perspectives. *Proceedings of the National Academy of Sciences*, *106*,19659–19665.

Holt, R. D. (2020). Reflections on niches and numbers. *Ecography*, *43*(3), 387–390.

Hooke, R. L. B., Martín-Duque, J. F., & Pedraza, J. (2012). Land transformation by humans: A review. *GSA Today*, *22*(12), 4–10.

Hrabar, H., & Kerley, G. I. H. (2013). Conservation goals for the Cape mountain zebra Equus zebra zebra - Security in numbers? *Oryx*, *47*(3), 403–409.

Hudson, P., Dobson, A., & Newborn, D. (1998). Prevention of population cycles by parasites removal. *Science*, *282*, 2256–2258.

Huston, M. A. (2005). The three phases of land-use change: Implications for biodiversity. *Ecological Applications* 15(6), 1864–1878).

Hutchinson, G. E. (1957). Concluding Remarks. *Cold Spring Harbor Symposia on Quantitative Biology*, *22*, 415–427.

Jacobson, A. P., Riggio, J., M. Tait, A., & E. M. Baillie, J. (2019). Global areas of low human impact (‘Low Impact Areas’) and fragmentation of the natural world. *Scientific Reports*, *9*(1), 1–13.

Jeanniard du Dot, T., Rosen, D. A. S., Richmond, J. P., Kitaysky, A. S., Zinn, S. A., & Trites, A. W. (2009). Changes in glucocorticoids, IGF-I and thyroid hormones as indicators of nutritional stress and subsequent refeeding in Steller sea lions (*Eumetopias jubatus*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *152*(4), 524–534.

Jersey, N., & Rodríguez, J. P. (2002). Range contraction in Declining North American Bird Populations. *Ecological Applications*, *12*(1), 238–248.

Johnson, C. N., Balmford, A., Brook, B. W., Buettel, J. C., Galetti, M., Guangchun, L., & Wilmshurst, J. M. (2017). Biodiversity losses and conservation responses in the Anthropocene. *Science*, *356*(6335), 270–275.

Johnson, P. T. J., & Hoverman, J. T. (2012). Parasite diversity and coinfection determine pathogen infection success and host fitness. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(23), 9006–9011.

Joppa, L. N., & Pfaff, A. (2009). High and far: Biases in the location of protected areas. *PLoS ONE*, *4*(12), e8273.

Junk, W. J., An, S., Finlayson, C. M., Gopal, B., Květ, J., Mitchell, S. A., Mitsch, W. J., & Robarts, R. D. (2012). Current state of knowledge regarding the world's wetlands and their future under global climate change: a synthesis. *Aquatic Sciences 2012 75:1*, *75*(1), 151–167.

Jürgensen, J., Drucker, D. G., Stuart, A. J., Schneider, M., Buuveibaatar, B., & Bocherens, H. (2017). Diet and habitat of the saiga antelope during the late Quaternary using stable carbon and nitrogen isotope ratios. *Quaternary Science Reviews*, *160*, 150–161.

Kaczensky, P., Burnik Šturm, M., Sablin, M. v., Voigt, C. C., Smith, S., Ganbaatar, O., Balint, B., Walzer, C., & Spasskaya, N. N. (2017). Stable isotopes reveal diet shift from pre-extinction to reintroduced Przewalski's horses. *Scientific Reports*, *7*(1), 1–9.

Karamanlidis, A. & Dendrinos, P. 2015. *Monachus monachus*. The IUCN Red List of Threatened Species 2015: e.T13653A117647375.

Kartzinel, T. R., Hsing, J. C., Musili, P. M., Brown, B. R. P., & Pringle, R. M. (2019). Covariation of diet and gut microbiome in African megafauna. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(47), 23588–23593.

Kerley, G. I. H., Beest, M., Cromsigt, J. P. G. M., Pauly, D., & Shultz, S. (2020). The Protected Area Paradox and refugee species: The giant panda and baselines shifted towards conserving species in marginal habitats. *Conservation Science and Practice*, *2*(5).

Kerley, G. I. H., Kowalczyk, R., & Cromsigt, J. P. G. M. (2012). Conservation implications of the refugee species concept and the European bison: King of the forest or refugee in a marginal habitat? *Ecography*, *35*(6), 519–529.

Kinnear, J. E., Sumner, N. R., & Onus, M. L. (2002). The red fox in Australia—an exotic predator turned biocontrol agent. *Biological Conservation*, *108*(3), 335–359.

Kirathe, J. N., Githaiga, J. M., Chira, R. M., & Rubenstein, D. (2021). Land use influence on distribution and abundance of herbivores in Samburu-Laikipia, Kenya. *Journal of Sustainability, Environment and Peace*, *4*(1), 21–29.

Kitaysky, A. S., Piatt, J. F., Hatch, S. A., Kitaiskaia, E. v., Benowitz-Fredericks, Z. M., Shultz, M. T., & Wingfield, J. C. (2010). Food availability and population processes: severity of nutritional stress during reproduction predicts survival of long-lived seabirds. *Functional Ecology*, *24*(3), 625–637.

Kuemmerle, T., Perzanowski, K., Chaskovskyy, O., Ostapowicz, K., Halada, L., Bashta, A. T., Kruhlov, I., Hostert, P., Waller, D. M., & Radeloff, V. C. (2010). European Bison habitat in the Carpathian Mountains. *Biological Conservation*, *143*(4), 908–916.

Laurance, W. F., Carolina Useche, D., Rendeiro, J., Kalka, M., Bradshaw, C. J. A., Sloan, S. P., Laurance, S. G., Campbell, M., Abernethy, K., Alvarez, P., Arroyo-Rodriguez, V., Ashton, P., Benítez-

Malvido, J., Blom, A., Bobo, K. S., Cannon, C. H., Cao, M., Carroll, R., Chapman, C., … Zamzani, F. (2012). Averting biodiversity collapse in tropical forest protected areas. *Nature 489*(7415), 290–294.

Lea, J. M., Kerley, G. I., Hrabar, H., Barry, T. J., & Shultz, S. (2016). Recognition and management of ecological refugees: A case study of the Cape mountain zebra. *Biological Conservation*, *203*, 207–215.

Lee, J. E., Janion, C., Marais, E., van Vuuren, B. J., & Chown, S. L. (2009). Physiological tolerances account for range limits and abundance structure in an invasive slug. *Proceedings of the Royal Society B: Biological Sciences*, *276*(1661), 1459.

Legault, G., Bitters, M. E., Hastings, A., & Melbourne, B. A. (2020). Interspecific competition slows range expansion and shapes range boundaries. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(43), 26854–26860.

Lentini, P. E., Stirnemann, I. A., Stojanovic, D., Worthy, T. H., & Stein, J. A. (2018). Using fossil records to inform reintroduction of the kakapo as a refugee species. *Biological Conservation*, *217*, 157–165.

Lesica, P., & Allendorf, F. W. (1995). When Are Peripheral Populations Valuable for Conservation? When Are Peripheral Populations Valuable for Conservation? *Conservation Biology*, *9*(4), 753–760.

Levins, R. (1969). Some Demographic and Genetic Consequences of Environmental Heterogeneity for Biological Control. *Bulletin of the Entomological Society of America*, *15*(3), 237–240.

Lindsey, P. A., Miller, J. R. B., Petracca, L. S., Coad, L., DIckman, A. J., Fitzgerald, K. H., Flyman, M. v., Funston, P. J., Henschel, P., Kasiki, S., Knights, K., Loveridge, A. J., MacDonald, D. W., Mandisodza-Chikerema, R. L., Nazerali, S., Plumptre, A. J., Stevens, R., van Zyl, H. W., & Hunter, L. T. B. (2018). More than $1 billion needed annually to secure Africa's protected areas with lions. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(45), E10788–E10796.

Linklater, W. L., & Gedir, J. v. (2011). Distress unites animal conservation and welfare towards synthesis and collaboration. *Animal Conservation* 14(1), 25–27).

Lira-Noriega, A., & Manthey, J. D. (2014). Relationship Of Genetic Diversity And Niche Centrality: A Survey And Analysis. *Evolution*, *68*(4), 1082–1093.

Lotze, H. K., Lenihan, H. S., Bourque, B. J., Bradbury, R. H., Cooke, R. G., Kay, M. C., Kidwell, S. M., Kirby, M. X., Peterson, C. H., & Jackson, J. B. C. (2006). Depletion, Degradation, and Recovery Potential of Estuaries and Coastal Seas. *Science*, *312*(5781), 1806–1809.

Løvschal, M., Bøcher, P. K., Pilgaard, J., Amoke, I., Odingo, A., Thuo, A., & Svenning, J. C. (2017). Fencing bodes a rapid collapse of the unique Greater Mara ecosystem. *Scientific Reports 7*(1), 1–7.

Low, B., Sundaresan, S. R., Fischhoff, I. R., & Rubenstein, D. I. (2009). Partnering with local communities to identify conservation priorities for endangered Grevy's zebra. *Biological Conservation*, *142*(7), 1548–1555.

Lucas, P. M., González-Suárez, M., & Revilla, E. (2016). Toward multifactorial null models of range contraction in terrestrial vertebrates. *Ecography*, *39*(11), 1100–1108.

Luck, G. W. (2007). The relationships between net primary productivity, human population density and species conservation. *Journal of Biogeography*, *34*(2), 201–212.

MacArthur, R. H., & Pianka, E. R. (1966). On Optimal Use of a Patchy Environment. *The American Naturalist*, *100*(916), 603–609.

MacDougall-Shackleton, S. A., Bonier, F., Romero, L. M., & Moore, I. T. (2019). Glucocorticoids and "Stress" Are Not Synonymous. *Integrative Organismal Biology*, *1*(1).

Manthey, J. D., Campbell, L. P., Saupe, E. E., Soberón, J., Hensz, C. M., Myers, C. E., Owens, H. L., Ingenloff, K., Townsend Peterson, A., Barve, N., Lira-Noriega, A., & Barve, V. (2015). A test of niche centrality as a determinant of population trends and conservation status in threatened and endangered North American birds. *Endangered Species Research*, *26*(3), 201–208.

Martínez-Abraín, A., & Jiménez, J. (2016). Anthropogenic areas as incidental substitutes for original habitat. *Conservation Biology*, *30*(3), 593–598.

Martínez-Abraín, A., Jiménez, J., & Oro, D. (2019). Pax Romana: 'refuge abandonment' and spread of fearless behavior in a reconciling world. I*Animal Conservation* 22(1), 3–13)

Martínez-Abraín, A., Oro, D., Forero, M. G., & Conesa, D. (2003). Modeling temporal and spatial colony-site dynamics in a long-lived seabird. *Population Ecology*, *45*(2), 133–139.

Martínez-Gutiérrez, P. G., Martínez-Meyer, E., Palomares, F., & Fernández, N. (2018). Niche centrality and human influence predict rangewide variation in population abundance of a widespread mammal: The collared peccary (*Pecari tajacu*). *Diversity and Distributions*, *24*(1), 103–115.

Martínez-Jauregui, M., Tavecchia, G., Cedenilla, M. A., Coulson, T., Fernández De Larrinoa, P., Muñoz, M., & González, L. M. (2012). Population resilience of the Mediterranean monk seal Monachus monachus at Cabo Blanco peninsula. *Marine Ecology Progress Series*, *461*, 273–281.

Martínez-Meyer, E., Díaz-Porras, D., Peterson, A. T., & Yáñez-Arenas, C. (2013). Ecological niche structure and rangewide abundance patterns of species. *Biology Letters*, *9*(1).

Mason, C.F & MacDonald, S.M. (1986). Otters: ecology and conservation. Cambridge University Press, New York.

McEwen, B. S., & Wingfield, J. C. (2003). The concept of allostasis in biology and biomedicine. *Hormones and Behavior*, *43*(1), 2–15.

McKay, D. M. (2009). The therapeutic helminth? *Trends in Parasitology*, *25*(3), 109–114.

McPeek, M. A., & Holt, R. D. (1992). The evolution of dispersal in spatially and temporally varying environments. *American Naturalist*, *140*(6), 1010–1027.

Mduma, S. A. R., Sinclair, A. R. E., & Hilborn, R. (1999). Food regulates the Serengeti wildebeest: A 40-year record. *Journal of Animal Ecology*, *68*(6), 1101–1122.

Mendoza, M., & Palmqvist, P. (2008). Hypsodonty in ungulates: an adaptation for grass consumption or for foraging in open habitat?. *Journal of Zoology*, *274*(2), 134-142.

Miller, G. H., Fogel, M. L., Magee, J. W., Gagan, M. K., Clarke, S. J., & Johnson, B. J. (2005). Ecosystem Collapse in Pleistocene Australia and a Human Role in Megafaunal Extinction. *Science*, *309*, 287–290.

Mills, J. A., Lavers, R. B., & Lee, W. G. (1984). The takahe - a relict of the Pleistocene grassland avifauna of New Zealand. *New Zealand Journal of Ecology*, *7*(1984), 57–70.

Moolman, L., Ferreira, S. M., Gaylard, A., Zimmerman, D., & Kerley, G. I. H. (2019). The decline of the Knysna elephants: Pattern and hypotheses. *South African Journal of Science*, *115*(3/4), 1–7.

Moolman, L., Morney, M. A. de, Ferreira, S. M., Ganswindt, A., Poole, J. H., & Kerley, G. I. H. (2019). And Then There was One: A Camera Trap Survey of the Declining Population of African Elephants in Knysna, South Africa. *African Journal of Wildlife Research*, *49*(1), 16–26.

Moss, W., & Camin, J. H. (1970). Nest Parasitism, Productivity and Clutch Size in Purple Martins. *Science*, *168*(3934), 1000–1003.

Möstl, E., & Palme, R. (2002). Hormones as indicators of stress. *Domestic Animal Endocrinology*, *23*(1–2), 67–74.

Munck, A., & Naray-Fejes-Toth, A. (1994). Glucocorticoids and Stress: Permissive and Suppressive Actions. *Annals of the New York Academy of Sciences*, *746*(1), 115–130.

Nagy, L. R., & Holmes, R. T. (2005). Food limits annual fecundity of a migratory songbird: An experimental study. *Ecology*, *86*(3), 675–681.

Naundrup, P. J., & Svenning, J. C. (2015). A geographic assessment of the global scope for rewilding with wild-living horses (*Equus ferus*). *PLoS ONE*, *10*(7), 1–26.

Newbold, T., Hudson, L. N., Hill, S. L. L., Contu, S., Lysenko, I., Senior, R. A., Börger, L., Bennett, D. J., Choimes, A., Collen, B., Day, J., de Palma, A., Díaz, S., Echeverria-Londoño, S., Edgar, M. J., Feldman, A., Garon, M., Harrison, M. L. K., Alhusseini, T., … Purvis, A. (2015). Global effects of land use on local terrestrial biodiversity. *Nature 520*(7545), 45–50.

Newsome, S. D., Etnier, M. A., Gifford-Gonzalez, D., Phillips, D. L., van Tuinen, M., Hadly, E. A., Costa, D. P., Kennett, D. J., Guilderson, T. P., & Koch, P. L. (2007). The shifting baseline of northern fur seal ecology in the northeast Pacific Ocean. *Proceedings of the National Academy of Sciences*, *104*(23), 9709–9714.

Noe-Nygaard, N., Price, T. D., & Hede, S. U. (2005). Diet of aurochs and early cattle in southern Scandinavia: evidence from 15N and 13C stable isotopes. *Journal of Archaeological Science*, *32*(6), 855–871.

Noguera, J. C., Aira, M., Pérez-Losada, M., Domínguez, J., & Velando, A. (2018). Glucocorticoids modulate gastrointestinal microbiome in a wild bird. *Royal Society Open Science*, *5*(4), 171743.

Nüchel, J., Bøcher, P. K., Xiao, W., Zhu, A. X., & Svenning, J. C. (2018). Snub-nosed monkeys (Rhinopithecus): potential distribution and its implication for conservation. *Biodiversity and Conservation*, *27*(6), 1517–1538.

O'Bryan, C. J., Garnett, S. T., Fa, J. E., Leiper, I., Rehbein, J. A., Fernández-Llamazares, Á., Jackson, M. v., Jonas, H. D., Brondizio, E. S., Burgess, N. D., Robinson, C. J., Zander, K. K., Molnár, Z., Venter, O., & Watson, J. E. M. (2021). The importance of Indigenous Peoples' lands for the conservation of terrestrial mammals. *Conservation Biology 35*(3), 1002–1008.

Oppliger, A. (1993). nest-site choice , desertion , and hatching success in the great tit (Pants major). *Behavioral Ecology*, *5*(2), 130–134.

Oro, D., & Ruxton, G. D. (2001). The Formation and Growth of Seabird Colonies : Audouin ' s Gull as a Case Study. *Journal of Animal Ecology*, *70*(3), 527–535.

Oro, D., Tavecchia, G., & Genovart, M. (2011). Comparing demographic parameters for philopatric and immigrant individuals in a long-lived bird adapted to unstable habitats. *Oecologia*, *165*(4), 935–945.

Osorio-Olvera, L., Soberón, J., & Falconi, M. (2019). On population abundance and niche structure. *Ecography*, *42*(8), 1415–1425.

Osorio-Olvera, L., Yañez-Arenas, C., Martínez-Meyer, E., & Peterson, A. T. (2020). Relationships between population densities and niche-centroid distances in North American birds. *Ecology Letters*, *23*(3), 555–564.

Pacifici, M., di Marco, M., & Watson, J. E. M. (2020). Protected areas are now the last strongholds for many imperiled mammal species. *Conservation Letters*, *13*(6), e12748.

Palme, R. (2019). Non-invasive measurement of glucocorticoids: Advances and problems. In *Physiology and Behavior* 199, 229–243.

Parejo, D., Oro, D., & Danchin, E. (2006). Testing habitat copying in breeding habitat selection in a species adapted to variable environments. *Ibis*, *148*(1), 146–154.

Parker, K. L., Barboza, P. S., & Gillingham, M. P. (2009). Nutrition integrates environmental responses of ungulates. *Functional Ecology*, *23*(1), 57–69.

Patterson, J. E. H., Neuhaus, P., Kutz, S. J., & Ruckstuhl, K. E. (2013). Parasite Removal Improves Reproductive Success of Female North American Red Squirrels (*Tamiasciurus hudsonicus*). *PLoS ONE*, *8*(2), 1–5.

Peh, K. S. H. (2007). Potential Effects of Climate Change on Elevational Distributions of Tropical Birds in Southeast Asia. *American Ornithological Society*, *109*(2), 437–441.

Phillips, A. (2004). The history of the international system of protected area management categories. *Parks*, *14*(3), 4-14.

Pimm, S. L., Jenkins, C. N., Abell, R., Brooks, T. M., Gittleman, J. L., Joppa, L. N., Raven, P. H., Roberts, C. M., & Sexton, J. O. (2014). The biodiversity of species and their rates of extinction, distribution, and protection. *Science*, *344*(6187).

Pineda-Munoz, S., Wang, Y., Lyons, S. K., Tóth, A. B., & McGuire, J. L. (2021). Mammal species occupy different climates following the expansion of human impacts. *Proceedings of the National Academy of Sciences*, *118*(2).

Pironon, S., Papuga, G., Villellas, J., Angert, A. L., García, M. B., & Thompson, J. D. (2017). Geographic variation in genetic and demographic performance: new insights from an old biogeographical paradigm. *Biological Reviews*, *92*(4), 1877–1909.

Pironon, S., Villellas, J., Morris, W. F., Doak, D. F., & García, M. B. (2015). Do geographic, climatic or historical ranges differentiate the performance of central versus peripheral populations? *Global Ecology and Biogeography*, *24*(6), 611–620.

Plumb, G., & McMullen, C. (2018). Bison on the Southwest Colorado Plateau: Conservation at the Edge. *The Southwestern Naturalist*, *63*(1), 42–48.

Price, T. D., & Kirkpatrick, M. (2009). Evolutionarily stable range limits set by interspecific competition. *Proceedings of the Royal Society B: Biological Sciences*, *276*(1661), 1429–1434.

Pulliam, H. R. (1988). Sources, Sinks, and Population Regulation. *The American Naturalist*, *132*(5), 652–661.

Pulliam, H. R. (2000). On the relationship between niche and distribution. *Ecology Letters*, *3*(4), 349–361.

Pulliam, H. R., & Danielson, B. J. (1991). Sources, Sinks, and Habitat Selection: A Landscape Perspective on Population Dynamics. *The American Naturalist*, *137*, S50–S66.

Pushkina, D., Bocherens, H., Chaimanee, Y., & Jaeger, J. J. (2010). Stable carbon isotope reconstructions of diet and paleoenvironment from the late Middle Pleistocene Snake Cave in Northeastern Thailand. *Naturwissenschaften*, *97*(3), 299–309.

Radeloff, V. C., Williams, J. W., Bateman, B. L., Burke, K. D., Carter, S. K., Childress, E. S., Cromwell, K. J., Gratton, C., Hasley, A. O., Kraemer, B. M., Latzka, A. W., Marin-Spiotta, E., Meine, C. D., Munoz, S. E., Neeson, T. M., Pidgeon, A. M., Rissman, A. R., Rivera, R. J., Szymanski, L. M., & Usinowicz, J. (2015). The rise of novelty in ecosystems. *Ecological Applications*, *25*(8), 2051–2068.

Remonti, L., Balestrieri, A., & Prigioni, C. (2009). Altitudinal gradient of Eurasian otter (Lutra lutra) food niche in Mediterranean habitats. *Canadian Journal of Zoology*, *87*(4), 285–291.

Riggio, J., Baillie, J. E. M., Brumby, S., Ellis, E., Kennedy, C. M., Oakleaf, J. R., Tait, A., Tepe, T., Theobald, D. M., Venter, O., Watson, J. E. M., & Jacobson, A. P. (2020). Global human influence maps reveal clear opportunities in conserving Earth’s remaining intact terrestrial ecosystems. *Global Change Biology*, *26*(8), 4344–4356.

Ripple, W.J., Newsome, T.M., Wolf, C., Dirzo, R., Everatt, K.T., Galetti, M., Hayward, M.W., Kerley, G.I., Levi, T., Lindsey, P.A. and Macdonald, D.W., (2015). Collapse of the world’s largest herbivores. *Science advances*, *1*(4), p.e1400103.

Robson, A., Trimble, M., Bauer, D., Loveridge, A., Thomson, P., Western, G., & Lindsey, P. (2021). Over 80% of Africa’s savannah conservation land is failing or deteriorating according to lions as an indicator species. *Conservation Letters*, e12844.

Rodríguez, J. P. (2002). Range contraction in declining North American Bird populations. *Ecological Applications*, *12*(1), 238–248.

Rouget, M., Richardson, D. M., & Cowling, R. M. (2003). The current configuration of protected areas in the Cape Floristic Region, South Africa—reservation bias and representation of biodiversity patterns and processes. *Biological Conservation*, *112*(1–2), 129–145.

Rubenstein, D., Low Mackey, B., Davidson, ZD, Kebede, F. & King, S.R.B. (2016). *Equus grevyi*. The IUCN Red List of Threatened Species 2016: e.T7950A89624491.

Rubenstein, D., Parham, J., Stewart, C., Berger-Wolf, T., Holmberg, J., Crall, J., Mackey, B. L., Funnel, S., Cockerill, K., Davidson, Z., Mate, L., Nzomo, C., Warungu, R., Martins, D., Ontita, V., Omulupi, J., Weston, J., Anyona, G., Chege, G., … Rubenstein, N. (2018). The State of Kenya’s Grevy’s Zebras and Reticulated Giraffes: Results of the Great Grevy’s Rally 2018. *Kenya Wildlife Service, Internal Report*, *5*, 12.

Ruiz-Olmo, J., Batet, A., Mañas, F., & Martínez-Vidal, R. (2011). Factors affecting otter (Lutra lutra) abundance and breeding success in freshwater habitats of the northeastern Iberian Peninsula. *European Journal of Wildlife Research*, *57*(4), 827–842.

Sagarin, R. D., & Gaines, S. D. (2002). The “abundant centre” distribution: To what extent is it a biogeographical rule? *Ecology Letters*, *5*(1), 137–147.

Sagarin, R. D., Gaines, S. D., & Gaylord, B. (2006). Moving beyond assumptions to understand abundance distributions across the ranges of species. *Trends in Ecology & Evolution*, *21*(9), 524–530.

Sales, L. P., Galetti, M., Carnaval, A., Monsarrat, S., Svenning, J.-C., & Pires, M. M. (2022). The effect of past defaunation on ranges, niches, and future biodiversity forecasts. *Global Change Biology*.

Santini, L., Pironon, S., Maiorano, L., & Thuiller, W. (2019). Addressing common pitfalls does not provide more support to geographical and ecological abundant-centre hypotheses. *Ecography*, *42*(4), 696–705.

Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews* 21(1), 55–89

Sayre, R., Karagulle, D., Frye, C., Boucher, T., Wolff, N. H., Breyer, S., Wright, D., Martin, M., Butler, K., van Graafeiland, K., Touval, J., Sotomayor, L., McGowan, J., Game, E. T., & Possingham, H. (2020). An assessment of the representation of ecosystems in global protected areas using new maps of World Climate Regions and World Ecosystems. *Global Ecology and Conservation*, *21*, e00860.

Schaefer, H. M., Runge, M. C., & Sherman, P. W. (2002). Ecological and evolutionary traps. *Trends in Ecology & Evolution*, *17*(1), 22–27.

Scheele, B. C., Foster, C. N., Banks, S. C., & Lindenmayer, D. B. (2017). Niche Contractions in Declining Species: Mechanisms and Consequences. *Trends in Ecology and Evolution*, *32*(5), 346–355.

Scheele, B. C., Hunter, D. A., Banks, S. C., Pierson, J. C., Skerratt, L. F., Webb, R., & Driscoll, D. A. (2016). High adult mortality in disease-challenged frog populations increases vulnerability to drought. *Journal of Animal Ecology*, *85*(6), 1453–1460.

Selwood, K. E., & Zimmer, H. C. (2020). Refuges for biodiversity conservation: A review of the evidence. In *Biological Conservation* 245, 108502.

Seydack, A., Vermeulen, C., & Huisamen, H. (2000). Habitat quality and the decline of an African elephant population: implications for conservation. *South African Journal of Wildlife Research* 30(1) 34–42.

Shapiro, B. (2004). Rise and Fall of the Beringian Steppe Bison. *Science*, *306*(5701), 1561–1565.

Sherley, R. B., Ludynia, K., Dyer, B. M., Lamont, T., Makhado, A. B., Roux, J. P., Scales, K. L., Underhill, L. G., & Votier, S. C. (2017). Metapopulation Tracking Juvenile Penguins Reveals an Ecosystem-wide Ecological Trap. *Current Biology*, *27*(4), 563–568.

Shreeve, T. G., Dennis, R. L. H., & Pullin, A. S. (1996). Marginality: scale determined processes and the conservation of the British butterfly fauna. *Biodiversity & Conservation 1996 5:10*, *5*(10), 1131–1141.

Shultz, S., Britnell, J. A., & Harvey, N. (2021). Untapped potential of physiology, behaviour and immune markers to predict range dynamics and marginality. *Ecology and Evolution*, *11*(23), 16446–16461.

Snijders, D. (2014). Wildlife policy matters: Inclusion and exclusion by means of organisational and discursive boundaries. *Journal of Contemporary African Studies*, *32*(2), 173–189.

Sommer, F., & Bäckhed, F. (2013). The gut microbiota-masters of host development and physiology. *Nature Reviews Microbiology* 11(4), 227–238.

Soule, M. (1973). The Epistasis Cycle: A Theory of Marginal Populations. *Annual Review of Ecology and Systematics*, *4*, 165–187.

Stringer, C. B., Finlayson, J. C., Barton, R. N. E., Fernandez-Jalvo, Y., Caceres, I., Sabin, R. C., Rhodes, E. J., Currant, A. P., Rodriguez-Vidal, J., Giles-Pacheco, F., & Riquelme-Cantal, J. A. (2008). Neanderthal exploitation of marine mammals in Gibraltar. *Proceedings of the National Academy of Sciences*, *105*(38), 14319–14324.

Supali, T., Verweij, J. J., Wiria, A. E., Djuardi, Y., Hamid, F., Kaisar, M. M. M., Wammes, L. J., Lieshout, L. van, Luty, A. J. F., Sartono, E., & Yazdanbakhsh, M. (2010). Polyparasitism and its impact on the immune system. *International Journal for Parasitology 40*(10), 1171–1176.

Suzuki, T. A. (2017). Links between Natural Variation in the Microbiome and Host Fitness in Wild Mammals. *Integrative and Comparative Biology*, *57*(4), 756–769.

Tecot, S. R., Irwin, M. T., & Raharison, J. L. (2019). Faecal glucocorticoid metabolite profiles in diademed sifakas increase during seasonal fruit scarcity with interactive effects of age/sex class and habitat degradation. *Conservation Physiology*, *7*(1), coz001.

Thomas, C. D., Bulman, C. R., & Wilson, R. J. (2008). Where within a geographical range do species survive best? A matter of scale. *Insect Conservation and Diversity*, *1*(1), 2–8.

Towns, D. R., & Daugherty, C. H. (1994). Patterns of range contractions and extinctions in the New Zealand herpetofauna following human colonisation. *New Zealand Journal of Zoology*, *21*(4), 325–339.

Trevelline, B. K., Fontaine, S. S., Hartup, B. K., & Kohl, K. D. (2019). Conservation biology needs a microbial renaissance: a call for the consideration of host-associated microbiota in wildlife management practices. *Proceedings of the Royal Society B*, *286*(1895).

Tucker, M. A., Böhning-gaese, K., Fagan, W. F., Fryxell, J. M., Moorter, B. van, Alberts, S. C., Ali, A. H., Allen, A. M., Attias, N., Avgar, T., Bartlam-brooks, H., Bayarbaatar, B., Belant, J. L., Bertassoni, A., Beyer, D., Bidner, L., Beest, F. M. van, Blake, S., Blaum, N., … Rimmler, M. (2018). Moving in the Anthropocene: Global reductions in terrestrial mammalian movements. *Science*, *469*, 466–469.

UNEP-WCMC and IUCN 2020. Protected Planet: The World Database on Protected Areas (WDPA) and World Database on Other Effective Area-based Conservation Measures (WD-OECM), November 2020. UNEP-WCMC and IUCN, Cambridge.

van Riper, C., van Riper, S. G., Goff, M. L., & Laird, M. (1986). The Epizootiology and Ecological Significance of Malaria in Hawaiian Land Birds. *Ecological Monographs*, *56*(4), 327–344.

Venter, O., Magrach, A., Outram, N., Klein, C. J., Possingham, H. P., Marco, M. di, Watson, J. E. M., di Marco, M., & Watson, J. E. M. (2018). Bias in protected-area location and its effects on long-term aspirations of biodiversity conventions. *Conservation Biology*, *32*(1), 127–134.

Vidal, E., Medail, F., & Tatoni, T. (1998). Is the yellow-legged gull a super abundant bird species in the Mediterranean? Impact on fauna and flora, conservation measures and research priorities. *Biodiversity & Conservation*, *1026*(8), 1013–1026.

Viljoen, J. J., Ganswindt, A., du Toit, J. T., & Langbauer, W. R. (2009). Translocation stress and faecal glucocorticoid metabolite levels in free-ranging African savanna elephants. *South African Journal of Wildlife Research*, *38*(2), 146–152.

Vitousek, P. M., Ehrlich, P. R., Ehrlich, A. H., & Matson, P. A. (1986). Human Appropriation of the Products of Photosynthesis. *BioScience*, *36*(6), 368–373.

Vitousek, P. M., Mooney, H. a, Lubchenco, J., & Melillo, J. M. (1997). Human Domination of Earth’ s Ecosystems. *Science*, *277*(5325), 494–499.

Walshe, N., Duggan, V., Cabrera-Rubio, R., Crispie, F., Cotter, P., Feehan, O., & Mulcahy, G. (2019). Removal of adult cyathostomins alters faecal microbiota and promotes an inflammatory phenotype in horses. *International Journal for Parasitology*, *49*(6), 489–500.

Wasser, S. K., Lundin, J. I., Ayres, K., Seely, E., Giles, D., Balcomb, K., Hempelmann, J., Parsons, K., & Booth, R. (2017). Population growth is limited by nutritional impacts on pregnancy success in endangered Southern Resident killer whales (Orcinus orca). *PLOS ONE*, *12*(6), e0179824.

Waycott, M., Duarte, C. M., Carruthers, T. J. B., Orth, R. J., Dennison, W. C., Olyarnik, S., Calladine, A., Fourqurean, J. W., Heck, K. L., Hughes, A. R., Kendrick, G. A., Kenworthy, W. J., Short, F. T., &

Williams, S. L. (2009). Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the National Academy of Sciences*, *106*(30), 12377–12381.

Weldemichel, T. G., & Lein, H. (2019). Fencing is our last stronghold before we lose it all. A political ecology of fencing around the maasai Mara national reserve, Kenya. *Land Use Policy*, *87*, 104075.

Wessling, E. G., Deschner, T., Mundry, R., Pruetz, J. D., Wittig, R. M., & Kühl, H. S. (2018). Seasonal variation in physiology challenges the notion of chimpanzees (*Pan troglodytes verus*) as a forest-adapted species. *Frontiers in Ecology and Evolution*, *6*, 60.

White, T. C. R. (2008). The role of food, weather and climate in limiting the abundance of animals. *Biological Reviews*, *83*(3), 227–248.

Wolf, C., & Ripple, W. J. (2017). Range contractions of the world's large carnivores. *Royal Society Open Science*, *4*(7), 170052.

Woodroffe, R., Hedges, S., & Durant, S. M. (2014). To Fence or Not to Fence. *Science*, *344*(6179), 46–48.

Woodworth, B. L., Atkinson, C. T., LaPointe, D. A., Hart, P. J., Spiegel, C. S., Tweed, E. J., Henneman, C., LeBrun, J., Denette, T., DeMots, R., Kozar, K. L., Triglia, D., Lease, D., Gregor, A., Smith, T., & Duffy, D. (2005). Host population persistence in the face of introduced vector-borne diseases: Hawaii amakihi and avian malaria. *Proceedings of the National Academy of Sciences*, *102*(5), 1531–1536.

Worm, B., & Tittensor, D. P. (2011). Range contraction in large pelagic predators. *Proceedings of the National Academy of Sciences*, *108*(29), 11942–11947.

Worthy, T. H. (2002). *Fossil distribution of brown teal (Anas chlorotis) in New Zealand*. Wellington, UK: Department of Conservation.

Wright, D. H. (1983). Species-Energy Theory: An Extension of Species-Area Theory. *Oikos*, *41*(3), 496.

WWF (2020) Living Planet Report 2020 -Bending the curve of biodiversity loss. Almond, R.E.A., Grooten M. and Petersen, T. (Eds). WWF, Gland, Switzerland

Yackulic, C. B., Sanderson, E. W., & Uriarte, M. (2011). Anthropogenic and environmental drivers of modern range loss in large mammals. *Proceedings of the National Academy of Sciences*, *108*(10), 4024–4029.

Ylönen, H., Pech, R., & Davis, S. (2003). Heterogeneous landscapes and the role of refuge on the population dynamics of a specialist predator and its prey. *Evolutionary Ecology*, *17*(4), 349–369.

Chapter 2) Critical evaluation of methods and data sources

This thesis uses a range of data sources and methodological techniques to evaluate the scope of ecological marginalisation and the physiological impacts it has on animals. Here, I critically evaluate the sources and methods by providing a brief background into their prior use and the rationale for their use in the work presented. I justify the use of each method or data source and outline potential limitations.

2.1 Chapter 3: Modelling ecological marginalisation across terrestrial mammals

2.1.1 Rationale

Envelope Niche Models (ENMs) are based on the differences between occurrences and a defined background. Species' distributions are traditionally predicted by curating all observations for a species (historical and/or contemporary) then cleaning the data to prevent spatial bias and overfitting. The conditions with greater occurrence relative to the background conditions are deemed to be more "suitable" areas (Merow et al., 2013). However, most historic species' occurrences are incomplete or biased, such that they may not accurately reflect potential occupancy or distribution. Instead, biased data may reflect biases in human preference for areas or species such as areas with good accessibility or infrastructure (Monsarrat et al., 2019), more charismatic species (Monsarrat & Kerley, 2018) or economically important species. This amounts to environmental non-equilibrium in the historic range *i.e.* the sampling of the species likely does not account for occupancy across the entire spatial extent of their potential climatic niche (Araújo & Pearson, 2005). Environmental non-equilibrium invalidates many assumptions required for ENMs (Pili et al., 2020). Using opportunistically collected presence data can greatly underestimate niche breadth and bias the estimation of the species niche centre (Santini et al., 2019).

These ENM approaches also assume the species' contemporary range, or opportunistically collected historic observations/records, encompasses the species optima *i.e.* the conditions under which the species' vital rates and performance will be maximised (Braunisch et al., 2008; Hirzel & Lay, 2008). Ecologically marginalised species invalidate this assumption (Cromsigt et al., 2012). By producing model inferences from an already altered system, we form shifted models *i.e* attempts to model species' ecology with human influenced niche parameters (Kerley & Monserrat, 2022). MAXENT outputs and other correlative ecological niche models do not measure suitability or fitness but are instead a measure of the similarity between background cells and cells of species occurrences or records (Merow et al., 2013). If a species was historically marginalised (*e.g.* Aurochs (*Bos primigenius*) (Kerley et al., 2012) or Przewalski's horse (*Equus ferus przewalskii*) (Kaczensky et al., 2017), we may make the assumption that occurrences in ecologically marginal habitats are in suitable environmental space. Hence, we may form erroneous inferences for species which have experienced range contraction or human influence for a long time period.

In Chapter 3, I can take an alternative approach by investigating the set of potential habitat and niche conditions *available* to each species in their contemporary and historic ranges (similar to Di Marco et al., 2021). By modelling *available* environmental space, I capture all possible conditions which the species historically occupied. Thus, this method covers the full range of ecological conditions where the species is able to survive and therefore the species optima. This approach also relaxes the assumption that species climatic niches are conserved over time (Wiens & Graham, 2005). A limitation of this approach is that the species polygons may encompass low density areas or areas which were only temporarily occupied (Yackulic et al., 2011). I accounted for this by only including biomes which the species are known to occupy and trimming ranges to known elevational limits for each of the species. Although one caveat of this approach is that biome occupancy or elevational limits may be more accurate for certain species due to biases in study effort.

2.1.2 Datasets used

2.1.2.1 PHYLACINE database for species distributions

For species distributions, I used 'present-natural range' *i.e.* estimated distribution of the species without any human pressures, and 'contemporary ranges' from the PHYLACINE dataset (Faurby et al., 2018). Present-natural refers to the inferred distributions before large scale human impacts whereas contemporary ranges refers to species current ranges. All cells within a species' range polygon were assumed to be an occurrence point to account for all potential conditions available to the species across the two timepoints. Usually, these inferred distributions prior to large scale human impacts are assumed to equate to historic ranges and are used interchangeably (*e.g.* Di Marco *et al*, 2021). Nonetheless, this assumption may not hold for all species. The PHYLACINE datasets are macroecological estimates of species ranges and the accuracy of these may vary due to study biases. Species may have occupied biomes transiently and historic ranges may have changed through time with climatic change. Despite this, the PHYLACINE dataset is frequently used in the macroecology literature to model mammalian distributional changes (Davis et al., 2018; Berti & Svenning, 2020; Marco et al., 2021; Monsarrat & Svenning, 2021).

2.1.2.2 Anthropogenic Biomes Version 2 and Resolve2017

To evaluate land-use within historic species range, we used the Anthropogenic Biomes Version 2 dataset (Ellis et al., 2010). The spatial datasets of anthropogenic biome (anthrome) classification are a set of simplified 12-class ‘potential vegetation’ biome datasets, adapted from the 15 classes in (Ramankutty & Foley, 1999). To categorise anthromes of interest, I classified them into four major groups: Dense settlements (urban villages and rural settlements), Agricultural and rangelands (croplands and rangelands with substantial/significant human populations), Seminatural and wild woodlands (woodlands with little human impacts) and remote landscapes without natural tree cover - grasslands, shrublands, tundra, desert and barren lands). The proportion of historic range converted to high intensity agricultural lands, rangelands and dense settlements was summed as a proxy of the proportion of human transformation across the historic range for each species. A limitation of this dataset is that it only assesses human land-use change between 1700-2000. Although large-scale land use changes have likely occurred in the past 22 years, the 1700-2000 time period aligns with the timescale of climatic variables used. To estimate habitat diversity, we calculated the number of ecoregions a species occupied from the Resolve2017 database (Dinerstein et al., 2017). Habitat diversity loss was calculated as the corresponding loss of ecoregions between historic and contemporary ranges. Ecoregions are geographically or ecologically distinct areas. Although, habitat diversity could be estimated from other forms of habitat categorisation, these would be expected to correlate highly. Both anthrome maps and Resolve2017 are frequently used to assess human landuse and biome occupancy respectively (Ellis et al., 2010, 2021; Martin et al., 2012).

2.1.2.3 General circulation models for climatic variables

For present climate conditions, I assessed the period 1970–2000 using the Worldclim version 2 dataset (Fick & Hijmans, 2017). There is a possibility that contemporary climatic change in last 20 years may have exacerbated or altered some results. However, this change will likely be minimal compared to the effect of widespread range contraction and climatic change since the Holocene period. For past climatic conditions, I chose the Holocene (ca. 6000 BC). This period likely reflects a time before widespread intensification of human land use and range contraction. Although humans have been shaping earth environments for more than 10,000 years, early agricultural changes were less intensive (Ellis et al., 2021). To estimate Holocene climate, I used Worldclim version 1 (Hijmans et al., 2005) and averaged three general circulation models (GCMs) (CNRM_CM5, IPSL-CM5A-LR and the MPI-ESM-P). I averaged three models as there is uncertainty as to the exact climatic conditions during the Holocene period (Knutti et al., 2013; Di Marco et al., 2021). While more general circulation models exist, these three models encompass extreme estimates which should average to a more representative set of climatic conditions (Di Marco et al., 2021). The resolution of the maps may be too coarse to pick up microhabitat climatic differences, however the spatial extent of the analysis (across countries and continents) means this should not influence overall findings.

2.1.3 Modelling ecological marginalisation

Considering a single metric for changes in ecological niche gradients may be misleading (Pironon et al., 2015) as some variables are only partially correlated (Santini et al., 2019). I, therefore, analysed two metrics for ecological marginalisation: 1) average Mahalanobis distance to historic niche centroid from all contemporary and historic niche points, and 2) the distance between niche centroids, modelled as three dimensional hypervolumes. Mahalanobis distances were chosen as they are calculated on the observed distribution only and account for the covariance of multiple environmental axes (Soberón et al., 2018). Distance between contemporary and historic hypervolume centroids is a measure of Euclidean distance between niche core conditions. Distance between niche centroid and Mahalanobis distance may not be optimal metrics for all species (Santini et al., 2019). Alternative approaches, such as using metrics of distance to niche margins rather than distance to niche centroids (Santini et al., 2019), may be better measures for some species (Osorio-Olvera et al., 2020). Distance between hypervolume centroids may prove misleading in some instances, for example if the hypervolumes are irregularly shaped or hollow, the niche centroids may be close, but the niche space would have changed shape radically

(Blonder, 2016). Despite this, distance to niche centroid has been tied to key demographic variables of interest such as species abundance (Martinez-Meyer et al., 2012), density and population trends (Manthey et al., 2015; Osorio-Olvera et al., 2020).

2.1.4 Limitations of the method

This study does have limitations. Although demographic variables may correlate with niche centrality, a species may have a skewed niche space or response curve (Guo et al., 2005; See Chapter 7 for discussion). As such, distance from the niche centroid may not be a consistently good metric of poor performance across all species (Santini et al, 2017; although see Olson et al, 2020). The analysis can conclude whether species are pushed to the climatic, topographic and niche extremes but cannot conclude whether these extremes are sub-optimal without further species-specific study.

Furthermore, the analysis can only assess ecological marginalisation at a macroecological level. Many species may experience ecological marginalisation at a population level such that they have large variation in the performance between populations. For example, the Cape Mountain zebra (*Equus zebra zebra)* have been ecologically marginalised, becoming protected in sub-optimal conditions where they perform poorly across their contemporary range (Lea et al., 2016). However, the species also occupies a few high-quality protected areas where performance is comparatively high. Species-level range polygons for mountain zebra (*Equus zebra*) cover a large proportion of the historic species range and, as such, do not show ecological marginalisation at a macroecological level.

Finally, species may not have occupied all the available historic habitat equally. The methods applied here attempt to account for this by only assessing the biomes and elevation ranges which species are known to occupy. However, there is a possibility that species occupy specific habitats in very low density or occupy these biomes for small periods of time when dispersal is possible. Nonetheless, although the analysis cannot make inferences of where animals preferred habitats were in historic time periods, it can examine shifts in available niche conditions between time periods.

2.2 Chapter 4: Endocrinological analysis of faecal glucocorticoid Cape mountain zebra (*Equus zebra zebra*)

2.2.1 Rationale

Lea et al. (2017) have previously used a corticosterone enzyme immunoassay (EIA) (Munro, CJM006) to relate faecal glucocorticoid hormone concentrations to habitat quality, demography and population performance in the Cape mountain zebra. From their results, they argued that male-biased sex ratio and habitat quality were important factors relating to the hypothalamic-pituitary axis (HPA) activity of the Cape mountain zebra. They recommended that management focus on translocating individuals into grassier areas and removing males to balance sex ratios (Lea et al., 2017). During my thesis, it came to my attention that multiple studies have shown there was no corticosterone found in the faeces of equids. Hence, it was unlikely that the corticosterone enzyme immunoassay used had accurately assessed HPA activity (Palme & Möstl, 1997; Palme et al., 2005). It also became apparent that the corticosterone assay used in Lea et al. 2017 was unvalidated and therefore had not been proven to be accurately measuring HPA activity. A recent validation study demonstrated that a closely related corticosterone EIA was not sensitive to acute stress responses in horses (*Equus caballus ferus;* Hinchcliffe et al., 2021). A recent review highlighted that 37% of faecal GC papers do not demonstrate validation (Palme 2019). As such, Chapter 4 stemmed from the requirement to biologically validate GC assays in Cape mountain zebra in order to reassess the drivers of HPA activity across a species range and re-evaluate the conservation suggestions of Lea et al. 2017 to ensure they were reliable.

2.2.2 Glucocorticoid levels and interpreting what they mean

Glucocorticoids (GCs) are produced from the hypothalamic-pituitary axis as a response to acute or chronic stress. Stress is defined here as a state of real or perceived threat to homeostasis (Smith and Vale, 2006). GC levels can change due to perturbations in environmental conditions and many other threats such as food limitation or exposure to predators (McEwen and Wingfield, 2003; Busch and Hayward, 2009). An increase in GC level is adaptive in the short term as it physiologically primes the organism to respond to the threat (Busch, 2010). However, long term repeated exposure, known as chronic stress or distress, can lead to negative consequences for animal fitness (Linklater and Gedir, 2011). These negative consequences are thought to include suppression of the immune system and growth, or inhibition of reproductive behaviour and ultimately performance (Busch and Hayward, 2009). Chronic stress can be measured with long term monitoring of GC concentrations as levels have been demonstrated to be largely repeatable (Schoenemann and Bonier, 2018).

The relationships between GCs and fitness are not straightforward (Moberg 2000). The relationship between GC levels, GC reaction potential and individual fitness (Breuner et al. 2008, Bonier et al. 2009) can be environmentally dependent or health dependent, such that during good habitat or in good health status high GC responsiveness can be associated with poor vital rates, whereas, in poor conditions, the opposite may be true (Blas et al. 2007). The concept of chronic stress has also been challenged, with some suggesting “chronic” GC responses are always adaptive (Boonstra, 2013). Others have demonstrated that there is no consistent profile to identify chronic stress across species (Dickens and Romero 2013). Although elevated GC levels are not analogous to “stress” levels in the usual use of the term what they do provide is evidence for a perceived challenge or stressor. It is important to outline these caveats to provide context for the findings in Chapter 4.

2.2.3 Study site choice and limitations of explanatory variables

As chapter 4 assesses the use of validated vs unvalidated assays, the analysis, explanatory variables used, and samples analysed were identical to those used in Lea et al. 2017. However, there are limitations to the inferences on HPA axis activity that can be drawn from these variables and analysis.

Lea et al*.* (2017) collected faecal samples for fGCM measurement from seven CMZ populations in separate reserves between 2010 and 2015. The seven populations included Bakkrans Nature Reserve, Welgevonden Game Farm, De Hoop Nature Reserve, Camdeboo National Park, Mount Camdeboo Private Game Reserve, Swartberg Private Game Reserve and Gamkaberg Nature Reserve (see Chapter 1, Figure 1.7 for geography).

Resource differences are likely greatest between summer and winter, due to variations in rainfall. However, these samples were collected seasonally (spring/summer/autumn), and therefore, the current sampling design may not compare the most limiting period across the range. Two reserves experienced summer rainfall, two winter rainfall and three aseasonal rainfall. Summer rainfall is associated with greater grass abundance and winter rainfall is associated with greater shrub abundance (du Toit & O'Connor, 2020). The north-eastern portions of the species historic range receive summer rainfall promoting grasslands, while the southwest portion experiences winter-rainfall resulting in fynbos and succulent Karoo vegetation communities with low grass cover (Boshoff et al., 2009). Hence, rainfall seasonality is crucial for grass availability, but the current categorisation may not capture the amount of rainfall experienced during the sampling period. Rainfall quantity can be heavily dependent upon the year of sampling. Variations in interannual rainfall can influence resource availability, diet quality (Chapter 5) and therefore may influence HPA activity (Joly et al., 2015).

Lea et al. (2017) assessed habitat quality for each reserve by an index of grass abundance (see Lea et al., 2016). Briefly, the vegetation index was compiled by defining vegetation type by a list of highly abundant and dominate taxa. Each grass species was ranked by its palatability to Cape mountain zebra (1 = low, 2 = medium, 3 = high) using available data-sources. This vegetation index provides a systematic and repeatable assessment of grass dominance and richness that can be readily estimated across populations. However, it does not take into consideration the grass availability in the year of sampling or the diets of the animals within the populations. As such, although vegetation index gives a proxy of overall habitat grassiness, it may not accurately reflect the diet of the animals.

2.2.4 Translocation as a biological validation experiment

A biological validation of HPA axis activity is when the species of interest experiences a known acute stress event. In these relatively controlled events, we can predict what glucocorticoid concentrations should do and hence we can assess whether a variety of enzyme immunoassay can accurately assess the response in the material of interest. The gold standard of biological validation is an Adrenocorticotrophic hormone (ACTH) and dexamethasone injections. ACTH directly stimulates the HPA axis while dexamethasone directly suppresses the HPA axis activity (Touma & Palme, 2005). If an EIA does not detect a significant increase or decrease respectively, then the assay is not appropriate for use. However, these gold standard procedures require invasive procedures which can be impossible to perform on wild animals. Hence, translocation, the movement of animals from one area to another is frequently used as a proxy. Transportation is a well-established scenario for biological validation of methods to measure faecal glucocorticoid metabolite responses to an acute stressor. It increases HPA axis activity across species (Dickens et al., 2010; Palme, 2019) allowing us to compare responses in multiple metabolite assays to select the best assay (e.g. Bashaw et al., 2016; Lavin et al., 2019). In Chapter 4, individuals were darted from a helicopter, loaded onto vehicles for transportation, driven to the adjacent property and released upon waking. Following translocation, all individuals quickly ran to the fenceline that separates the two reserves and tracked the fenceline boundary for the subsequent 8 days.

2.2.5 Sample size and number of sampling points per individual

Our biological validation used the translocation of five CMZ (1 male and 4 females) between two neighbouring properties. Although the sample size is relatively small, it is not unusual for biological validation studies (Wasser et al., 2010; Montiglio et al., 2012; Bashaw et al., 2016). Sample collection was relatively sparse. We collected faecal samples from ~12 days before translocation to establish a baseline and ~24-72 hours post translocation. Preferably, sample collection would have taken place daily from the date of translocation until at least day 5-6. If sampling was better the GC peak could have been monitored more accurately. However, with the level of increase seen in Chapter 4 and the difference between validated and unvalidated EIAs, it is highly unlikely that assay selection would have differed with a greater resolution of sampling.

2.2.6 Extraction and assay selection for fGCM analysis and data analysis

As Chapter 4 is partly methodological, a critical evaluation of the methods and data analysis used is discussed there. Additionally, in Chapter 4, I formally test the extraction and EIA methods and provide justification for why I did.

2.3 Chapter 5: Macrophysiology and marginality in the Cape mountain zebra (*Equus zebra zebra*)

2.3.1 Rationale

CMZ provide an ideal model species to understand the mechanisms of population limitation. The availability of C_3 and C_4 grasses, CMZ's preferred food source, varies widely across populations and across the historic species range. If possible, CMZ select grasslands throughout the year, utilising only large-tufted, leafy perennial species: predominately Red grass (*Themeda triandra),* Bitter Turpentine Grass (*Cymbopogon pospischilii), Sporobolus fimbriatus, Panicum stapfianum and Tristachya leucothrix* (Smith *et al.*, 2008). For example, the annual diet of the CMZ in Baviaannaskloof Wilderness area was predominately two grass species: *Thristachya leucothrix* (39%) and *Themeda triandara* (28%) (Weel *et al.*, 2015). Long term population growth rates, density and fecundity vary widely between populations and are associated with grass availability (Lea et al., 2016).

Food availability is a major factor dictating population limitation across animal species (White, 2008). However, the link between diet variation over space and time and physiological consequences are unknown, especially in large mammals. As CMZ exist in fenced environments, migration between populations is not possible without human intervention and each population is an independent “closed system”, such that all individuals are exposed to near identical environmental constraints and resource availabilities (Lea et al., 2016). Although, this is ideal to understand the impacts of food limitation on physiology and dietary ecology, it may not reflect responses in an unfragmented system (See Chapter 5).

2.3.2 Field work

2.3.2.1 Potential causes of variation from study populations and limitation from sample collection

Non-invasive sampling of species in the field has many potential causes of variation outside of the study design. Here I outline potential sources of non-random variation. Although my experimental design captured a large proportion of habitat types in the historic range (Figure 2.1), I could not account for the total variety of habitats historically occupied. Hence, my findings may not apply to all historic habitat types.

Firstly, sampling was conducted by vehicle, using public access and/or management roads and therefore is biased to areas of the reserve which have higher accessibility.
Some populations were supplementarily fed such as Sanbona Wildlife Reserve. Supplementary feed may increase performance levels and population density above what the productive capacity of the landscape would be. Supplementary food location also influences species movement patterns which may affect parasite transmission. This may influence nemabiome composition and faecal egg count measures. Furthermore, although I enquired about whether and when supplementary feed was available to each population, there is a possibility that during drought or food limitation, reserves supplied supplementary feed and did not inform me. If this is the case, it could bolster population numbers by helping animals avoid starvation during lean periods. Although population performance would still be linked to diet, it would not be due to the resource availability that naturally occurs in the environment. Supplementary feed may also have knock-on consequences for microbiome communities. Finally, many surveyed reserves had artificial water sources and may use salt licks or antihelminth treatment. Although I inquired as to the use of these items, it is possible that even single uses may have long term consequences for the nemabiome and microbiome of the animals (Walshe et al., 2019). Within Bushman's Kloof private reserve, Cape mountain zebra had access to mown and planted lawns which likely influenced the amount of grass in their diet.

Gamkaberg Nature Reserve experienced a fire in 2017 which burned ~80% of the reserve. Fires in mountainous fynbos can stimulate grass production. Therefore, the proportion of grass currently in the diet in GNR may not reflect the long-term diet as grass availability may change once a climax community is reached.

Population density for some reserves were not officially known and so were calculated from the area of the reserve and the most recent population count. Population monitoring can vary in intensity between reserves and as such population growth rates and demographic changes may be misestimated for some. Hence, I used the number of foals per adult male (foal:mare ratio) as an additional proxy for recruitment and performance. Populations varied in their predation community which could lead to changes in the observed foal:mare ratio. Nonetheless, it is unlikely that this influenced overall results as predator communities were only present in three reserves: two with high grass availability and one with low.

Sample collection did account for interannual variation in climate but could not account for seasonal variation. Seasonal variation may result in the formation of “grass banks” where good quality conditions in part of the year provide high resource availability for the coming limiting season. If this occurs, climatic conditions at the time of sampling would not drive grass availability. Hence animals may display high proportion of Poaceae (grasses) in their diets despite harsh environmental conditions due to a lag effect. Our sampling design controlled for time of year to ensure changes in grass availability and physiological consequences were not due to seasonality. However, seasonality could play an important role in the dietary ecology and performance of CMZ populations (See Chapter 5).

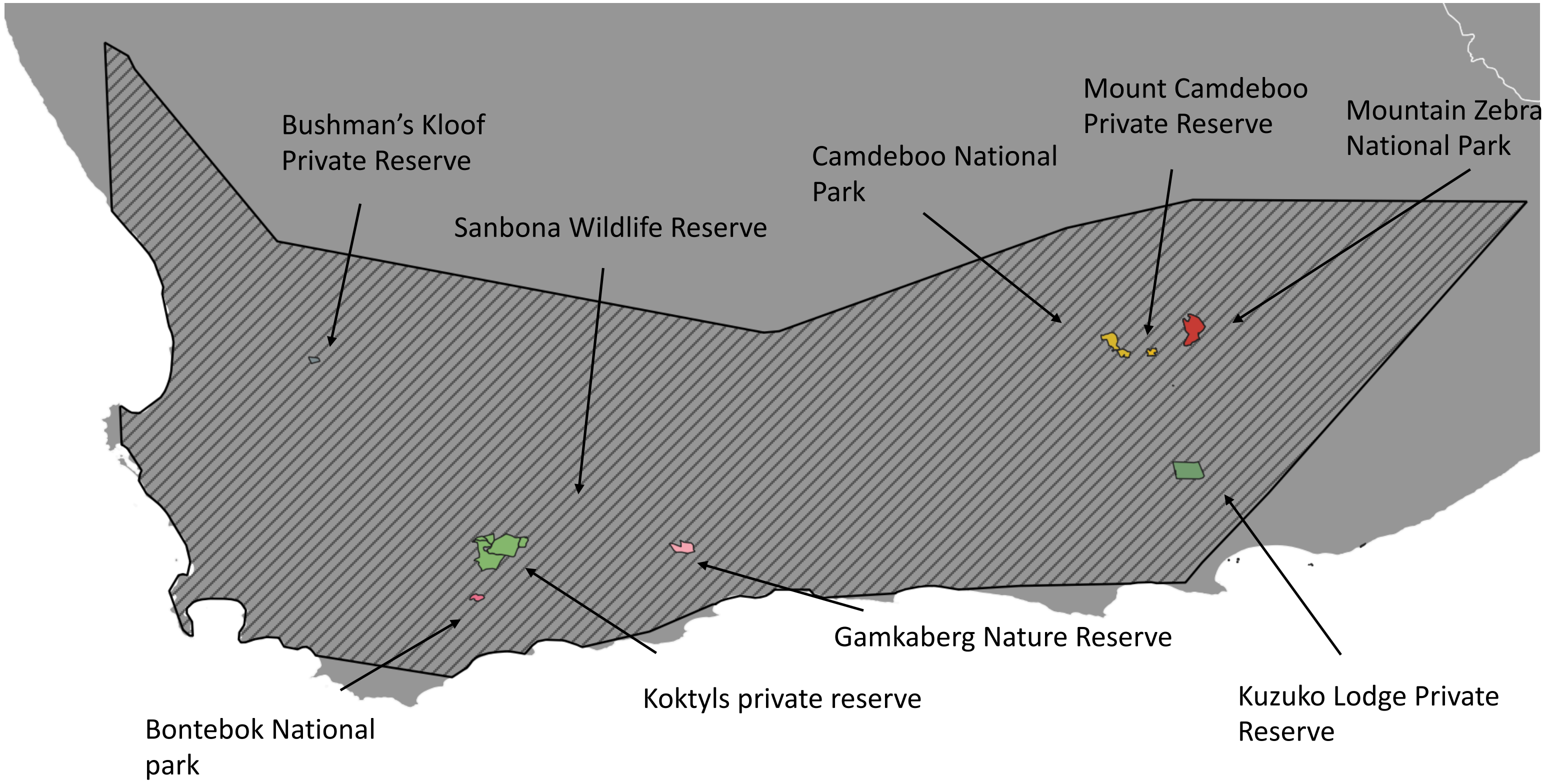


Figure 2.1) Sampling sites in relation to the estimate potential/historic range of Cape mountain zebra. Reserve are coloured polygons. Dashed outline represents the estimated historic/potential distribution of Cape mountain zebra. Historic/potential distribution was estimated as a minimum convex hull (alpha = 0.5) from historic points collated in Boshoff et al, 2016 and contemporary sampling points from my thesis, the thesis of Dr.Jessica Lea and occurrence points from the global biodiversity information facility (GBIF). Koktyls Private reserve is not depicted on the map as a shapefile for the reserve is not available

2.3.2.3 Demographic data collection and Hotspotter

During zebra sighting, I collected GPS location and band membership (demography including age and sex of all individuals). Due to the unique stripe patterns of mountain zebra, each zebra was photographed and identified *via* stripe identification software Hotspotter (Crall et al. 2013; Figure 2.2). Individuals were identified and catalogued to avoid pseudoreplication within years and to identify repeat individuals between years. However, zebra have different stripe patterns on their right and left flanks which could lead to some of the same individuals being treated as different animals.

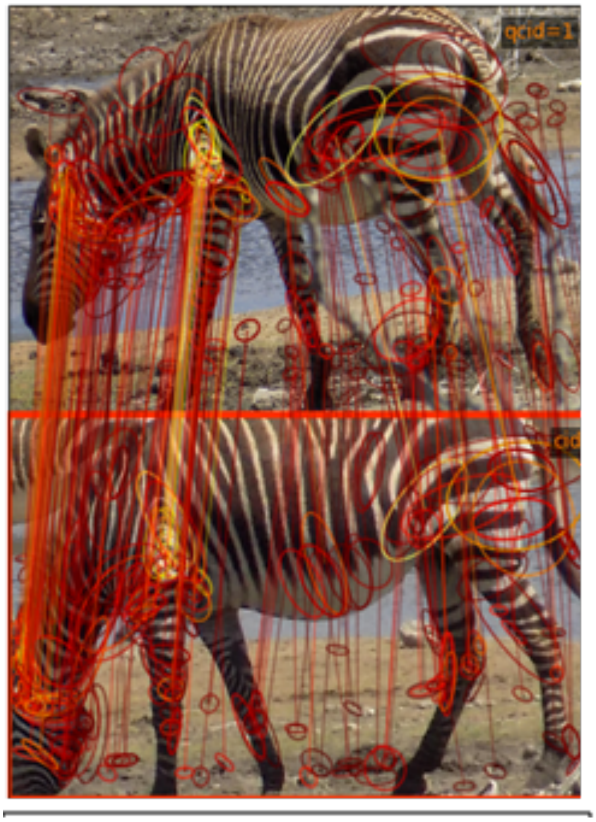


Figure 2.2) Example of stripe recognition software Hotspotter for individual identification. The stripe recognition software identifies hotspots in pattern similarity and displays those areas for manual user verification. The pattern similarity are display by yellow and red circles and areas of interest are linked by red and yellow lines. Red depicts high similarity hotspots and yellow relatively low similarity hotspots.

2.3.2.4 Vegetation transects

For a proxy of forage availability and grass availability, I conducted 50m vegetation transects on foot at every site of sample collection. An average of 24 transects were conducted in each reserve, in each sampling season, with ~12 in zebra occupied areas and 12 in randomly assigned “non-zebra” areas. Zebra occupied areas were identified by rangers, management and by population surveys. Transects were selected relatively close to the road due to ease of access which biases vegetation surveys to accessible areas of the reserves. Transect direction was randomly selected. I only recorded broad categories of vegetation: grasses, forbs, shrubs (including woody shrubs, trees and succulents) and bare ground using a point-transect method (Barbour et al. 1980). Plant height was not recorded, but can influence zebra feeding behaviour and habitat selection. To reduce inconsistent measurements between sites and years, a single observer conducted all vegetation sampling. Non-occupied transects were selected by a random sampling technique of dividing established non-occupied areas of landscape into equal sized sites, assigning a number to each site and using a random number generator to assign location of transect. Non-occupied areas were identified through surveying and through discussion with management and rangers. Again, this procedure was limited by access to areas due to roads. Furthermore, there is a possibility that “non-occupied” areas are used infrequently. Grass availability and forage availability per group were assessed at a scale of ~2.3km^2. I merged both non-occupied and occupied vegetation transects within a circle of ~2.3km^2 around site of sample collection. Sampling at this scale accounts for daily movements of animals before dietary items pass through the digestive system. ~2.3km^2 is the average daily movement pattern of the closely related Grevy’s zebra (Kartzinel et al., 2015). There is a possibility that CMZ movement varies across reserves or limited resources cause the formation of home ranges (Penzhorn, 1984). Whether home ranges exist could not be established due to limited time on reserves.

2.3.2.5 Faecal egg counts

Faecal egg counts (FECs) are a method of estimating gastrointestinal helminth burden, primarily of *Strongylidae* (Coulson et al., 2018). This approach has been widely used to understand the causes and consequences of parasite burdens between individuals (Coltman, Pilkington et al. 2001, Snaith, Chapman et al. 2008). This technique is widely used for monitoring burden in domestic and wild animals (Uhlinger, 1990; Ezenwa, 2004). The method is highly repeatable within individuals and does not vary with time of day (Rinaldi et al., 2009; Scheuerle et al., 2016). There are strong positive associations between FECs and the parasite burden calculated from autopsies in domestic livestock (Rieu et al., 2007). Faecal egg counts are negatively correlated with body condition and health in feral horses (Debeffe et al., 2016). Although widely used for monitoring parasite burden for domestic and wild animals, it does not provide a fine resolution picture of actual parasite infection or the impact on individual condition. Egg shedding rates are highly variable, such that low FEC may not necessarily be associated with low parasite burden. It can be difficult to identify helminths at species level from egg morphology alone. Furthermore, FECs can have a high false negative result which can limit their ability to assess specific groups or species of helminths. Hence, I did not perform species identification during FECs. Although different helminths have different consequences for the host (McKay, 2009), I also weighted each faecal egg equally, irrelevant of the group of helminth it originated from.

Three grams of fresh faeces were suspended in 42ml of saturated sodium chloride solution and analysed using a McMaster FEC protocol for parasite egg abundance. Eggs per gram of faeces were calculated using the following equation:

$$\text{(1)} \quad \text{Eggs per gram (EPG)} = (\text{Egg count in grid 1} + \text{Egg count in grid 2}) * 50$$

Parasite eggs were counted as inside the grid when more than half of the egg was within the grid lines. While saturated salt solution is not an optimal solution to use as it floats fewer eggs than other solutions, it is readily available, cheap and therefore convenient for use in the field (Cringoli et al., 2004). Faecal egg counts were performed fresh as storing in formalin or ethanol can lead to reduction in overall egg count after time (Crawley et al., 2016). All analyses were conducted within 8-12 hours of collection and faeces was kept cold in coolers while in the field to minimise egg denaturation or maturation.

2.3.2.6 Rainfall

Both cumulative rainfall (3 months leading up to sampling date) and annual precipitation were calculated for each population using the Climate Hazards Group Infrared Precipitation combined with station observations (CHIRPS) dataset at a resolution of 0.05° (Funk et al, 2015). CHIRPS uses a combination of weather station and satellite data to give daily, ten-day and monthly estimates of precipitation levels from 1981-present day. Interpolation is used when adequate coverage from local weather stations is not available. CHIRPS is accurate for measuring rainfall across eastern and southern Africa (Dinku et al, 2018). Rainfall was calculated for the pixel under each sample, which is roughly equivalent to 5.5km^2.

2.3.3 DNA metabarcoding

Methods for the DNA metabarcoding are outlined thoroughly in Chapter 5 and 6. Here I outline a short rationale and potential limitations of each step.

2.3.3.1 Rationale

DNA metabarcoding allows automated identification of multiple taxa within environmental samples such as faeces. Increasingly it is used to understand communities of commensal groups with species of conservation concern and the positive and negative health benefits of these interactions. There are growing calls for greater use of DNA metabarcoding in ecology, evolution and conservation science (Trevelline et al., 2019).

The DNA metabarcoding protocols used for Cape mountain zebra and Grevy's Zebra are outlined in Chapter 5 and 6 respectively. The bacterial universal (16s) primer is used extensively in microbiome research. Best practice guidelines are well established (Pollock et al., 2018). For diet, conventional approaches to establish diet are time consuming and error prone such as feeding observations or microscopic analyses of diet content and quality from faecal matter (Holechek et al., 1982). Dietary DNA metabarcoding uses trnL-P6 to evaluate the composition of the species' dietary items with reduced error and can include identification of specific plant species, a resolution not possible with other methods

(Kartzinel & Pringle, 2020). The reference library used has been ground-truthed in East African species and has a large degree of overlap with dominant species found throughout Africa (Gill et al, 2019). However, there may be rare or endemic plants in South Africa that were not available in the reference library. To counter this, unidentified sequences with high read number were BLAST searched and the reference library was updated for greater specificity. Nemabiome has been investigated previously (Avramenko et al., 2015), using ITS-2 primer. Due to the limitations of FEC, I used DNA metabarcoding to accurately assess helminth community composition.

2.3.3.2 Sample storage

We collected samples from at least two complete boluses per dung pile, from several areas of each bolus. Boluses were collected, placed in sealed plastic bags, had excess air removed and were homogenised to ensure a representative sample. We stored samples in 8ml of 100% ethanol during the field season. Storage in ethanol occurred within 8 hours of collection. This method has been shown to be effective for long-term storage for protocols involving DNA metabarcoding (Choo et al., 2015)

2.3.3.3 Microbial functional analysis

Microbiome functional analysis was conducted using FAPROTAX (Louca et al., 2016). FAPROTAX uses a reference library of known bacteria and their functions to assess compositional and functional roles of microbial species. FAPROTAX has been used to evaluate the functional changes in gut microbiota of both rats and rabbits (Xiang et al., 2019; Xing et al., 2019).

2.3.4.4 Limitations of DNA metabarcoding

DNA metabarcoding does have drawbacks and methodological limitations. Firstly, errors during amplification or sequencing can lead to incorrect taxonomic assignment (Taberlet et al., 2012). To minimise this risk and the risk of degradation, samples were stored in ethanol and kept frozen within 8 hours of collection. Samples were kept cool in a cooler (~4^{o}C) prior to freezing while in the field. Erroneous reads are usually caused by sequencing chemistry and can influence diversity estimates (Coissac et al., 2012). To prevent this, I also performed quality control using the DADA2 analysis pipeline which can detect and correct amplicon errors (Callahan et al., 2016). We also removed any assigned sequence variants (ASVs) which were not assigned to any taxonomic levels. We also filtered out ASVs which were found in the blank runs of microbiome, nemabiome and diet. Taxonomic assignment utilised a naïve Bayesian classier algorithm designed for microbiome analysis in DADA2 to prevent biasing assignment (Callahan et al., 2016). Biasing towards common or rare species could occur during quality control however this is unlikely to affect results as we are only assessing overall composition change.

2.4. Chapter 6: Macrophysiology and marginality in the Grevy's zebra (*Equus grevyi*)

2.4.1 Rationale

For this study, I wanted to assess whether ecological marginalisation impacted species within less fragmented landscapes where dispersal opportunities were not as severely limited as in South Africa. Furthermore, I needed to assess whether intra-annual (seasonal) variation in abiotic conditions across ecological gradients also leads to food limitation and similar physiological consequences. Due to the limited fencing between Laikipia, Samburu and Meru counties, it provided an ideal opportunity to test ecological marginalisation in a less fragmented landscape. As animals could move relatively freely, I needed to form relatively distinct groups for comparison. I chose to separated categories by geographic area which led to three different "populations": 1) Lewa wildlife conservancy, 2) Westgate community conservancy, and 3) Mpala, Karisia and OlJogi (Mpala-Oljogi). Information on individual populations is available in Chapter 6.

2.4.2 Demographic data and sample collection

Surveys were conducted by vehicle using available roads which would cause sampling to be biased to areas which have higher accessibility. If the sex of the defecating individual could not be confirmed, then it was labelled as “unknown” sex. The possibility of pseudoreplication was minimized due to the openness of the landscapes, but as hotspotter was not used, the possibility cannot be excluded.

2.4.3 Environmental variables

2.4.3.1 Rainfall

Proxies for rainfall were estimated using the same methods as above and have the same limitations.

2.4.3.2 Normalised difference vegetation index

As vegetation transects were not conducted on foot in Kenya, I needed a proxy of grass availability/productivity across the ecological gradient. To do this I used remote sensing of 8-day aggregated normalised difference vegetation index (NDVI). NDVI measures habitat greenness. It has been ground-truthed and shown to correlate with forage biomass in Eastern Africa (Meshesha et al., 2020). Vegetation greenness has been used as a proxy of forage palatability and quality for Grevy’s zebra (Sundaresan et al., 2008). However, during green-up following the rains, browse also becomes greener (Adole et al., 2018) which may lead to a mis-estimation of grass availability in browse dominated areas. As gut retention time for Grevy’s zebra is approximately 24-48 hours – diet composition assessed with DNA metabarcoding estimates the diet ~24 hours prior to collection. As Grevy’s zebra can move ~ 2.3km per day (Kartzinel et al., 2015) all productivity estimates were conducted at a resolution of 2.3km^2 around the sample collection point.

2.4.4 DNA metabarcoding

Rationale, primer choice and reference libraries as well as the limitations of DNA metabarcoding are identical to those used in Chapter 5 and have been described above.

2.5 References

Adole, T., Dash, J., & Atkinson, P. M. (2018). Large-scale prerain vegetation green-up across Africa. *Global Change Biology*, *24*(9), 4054–4068.

Araújo, M. B., & Pearson, R. G. (2005). Equilibrium of species' distributions with climate. *Ecography*, *28*(5), 693–695.

Barbour, M.G . Burk, J.H. & Pitts, W.D. (1980) Terrestrial Plant Ecology, 1st edn._Benjamin/Cummings, Menlo Park

Bashaw, M. J., Sicks, F., Palme, R., Schwarzenberger, F., Tordiffe, A. S. W., & Ganswindt, A. (2016). Non-invasive assessment of adrenocortical activity as a measure of stress in giraffe (*Giraffa camelopardalis*). *BMC Veterinary Research*, *12*(1), 1–13.

Berti, E., & Svenning, J. C. (2020). Megafauna extinctions have reduced biotic connectivity worldwide. *Global Ecology and Biogeography*, *29*(12), 2131–2142.

Blonder, B. (2016). Do hypervolumes have holes? *American Naturalist*, *187*(4), E93–E105.

Boonstra, R. (2013). Reality as the leading cause of stress: rethinking the impact of chronic stress in nature. *Functional Ecology*, *27*(1), 11–23.

Boshoff, A. F., Kerley, G. I. H., & Cowling, R. M. (2009). Estimated spatial requirements of the medium- to large-sized mammals, according to broad habitat units, in the Cape Floristic Region, South Africa. *African Journal of Range & Forage Sciences*, *19*(1), 29–44.

Boshoff, A., Landman, M., & Kerley, G. (2016). Filling the gaps on the maps: historical distribution patterns of some larger mammals in part of southern Africa. *Transactions of the Royal Society of South Africa*, *71*(1), 23-87.

Braunisch, V., Bollmann, K., Graf, R. F., & Hirzel, A. H. (2008). Living on the edge—Modelling habitat suitability for species at the edge of their fundamental niche. *Ecological Modelling*, *214*(2–4), 153–167.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583.

Choo, J. M., Leong, L. E. X. E., & Rogers, G. B. (2015). Sample storage conditions significantly influence faecal microbiome profiles. *Scientific Reports*, *5*(1), 16350.

Coissac, E., Riaz, T., & Puillandre, N. (2012). Bioinformatic challenges for DNA metabarcoding of plants and animals. *Molecular Ecology*, *21*(8), 1834–1847.

Coulson, G., Cripps, J. K., Garnick, S., Bristow, V., & Beveridge, I. (2018). Parasite insight: assessing fitness costs, infection risks and foraging benefits relating to gastrointestinal nematodes in wild mammalian herbivores. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*(1751).

Crall, J. P., Stewart, C. v., Berger-Wolf, T. Y., Rubenstein, D. I., & Sundaresan, S. R. (2013). HotSpotter-Patterned species instance recognition. *Proceedings of IEEE Workshop on Applications of Computer Vision*, 230–237.

Crawley, J. A. H., Chapman, S. N., Lummaa, V., & Lynsdale, C. L. (2016). Testing storage methods of faecal samples for subsequent measurement of helminth egg numbers in the domestic horse. *Veterinary Parasitology*, *221*, 130–133.

Cringoli, G., Rinaldi, L., Veneziano, V., Capelli, G., & Scala, A. (2004). The influence of flotation solution, sample dilution and the choice of McMaster slide area (volume) on the reliability of the McMaster technique in estimating the faecal egg counts of gastrointestinal strongyles and *Dicrocoelium dendriticum* in sheep. *Veterinary Parasitology*, *123*(1–2), 121–131.

Cromsigt, J. P. G. M., Kerley, G. I. H., & Kowalczyk, R. (2012). The difficulty of using species distribution modelling for the conservation of refugee species - the example of European bison. *Diversity and Distributions*, *18*(12), 1253–1257.

Davis, M., Faurby, S., & Svenning, J. C. (2018). Mammal diversity will take millions of years to recover from the current biodiversity crisis. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(44), 11262–11267.

Debeffe, L., McLoughlin, P. D., Medill, S. A., Stewart, K., Andres, D., Shury, T., Wagner, B., Jenkins, E., Gilleard, J. S., & Poissant, J. (2016). Negative covariance between parasite load and body condition in a population of feral horses. *Parasitology*, *143*(8), 983–997.

Dickens, M. J., Delehanty, D. J., & Michael Romero, L. (2010). Stress: An inevitable component of animal translocation. *Biological Conservation*, *143*(6), 1329–1341.

Di Marco, M., Pacifici, M., Maiorano, L. and Rondinini, C. (2021). Drivers of change in the realised climatic niche of terrestrial mammals. *Ecography*, 44. 1180-1190.

Dinerstein, E., Olson, D., Joshi, A., Vynne, C., Burgess, N. D., Wikramanayake, E., Hahn, N., Palminteri, S., Hedao, P., Noss, R., Hansen, M., Locke, H., Ellis, E. C., Jones, B., Barber, C. V., Hayes, R., Kormos, C., Martin, V., Crist, E., … Saleem, M. (2017). An Ecoregion-Based Approach to Protecting Half the Terrestrial Realm. *BioScience*, *67*(6), 534–545.

du Toit, J. C. O., & O’Connor, T. G. (2020). Long-term influence of season of grazing and rainfall on vegetation in the eastern Karoo, South Africa. *African Journal of Range & Forage Sciences 37*(2), 159–171.

Ellis, E. C., Gauthier, N., Goldewijk, K. K., Bird, R. B., Boivin, N., Díaz, S., Fuller, D. Q., Gill, J. L., Kaplan, J. O., Kingston, N., Locke, H., McMichael, C. N. H., Ranco, D., Rick, T. C., Rebecca Shaw, M., Stephens, L., Svenning, J. C., & Watson, J. E. M. (2021). People have shaped most of terrestrial nature for at least 12,000 years. *Proceedings of the National Academy of Sciences of the United States of America*, *118*(17),

Ellis, E. C., Goldewijk, K. K., Siebert, S., Lightman, D., & Ramankutty, N. (2010). Anthropogenic transformation of the biomes, 1700 to 2000. *Global Ecology and Biogeography*, *19*(5), 589–606.

Ezenwa, V. O. (2004). Interactions among host diet, nutritional status and gastrointestinal parasite infection in wild bovids. *International Journal for Parasitology*, *34*(4), 535–542.

Faurby, S., Davis, M., Pedersen, R., Schowanek, S. D., Antonelli, A., & Svenning, J. C. (2018). PHYLACINE 1.2: The Phylogenetic Atlas of Mammal Macroecology. *Ecology*, *99*(11), 2626.

Fick, S. E., & Hijmans, R. J. (2017). WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*, *37*(12), 4302–4315.

Guo, Q., Taper, M., Schoenberger, M., & Brandle, J. (2005). Spatial-temporal population dynamics across species range: from centre to margin. *Oikos*, *108*(1), 47–57.

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, *25*(15), 1965–1978.

Hirzel, A. H., & Lay, G. le. (2008). Habitat suitability modelling and niche theory. *Journal of Applied Ecology*, *45*(5), 1372–1381.

Holechek, J. L., Vavra, M., & Pieper, R. D. (1982). Botanical Composition Determination of Range Herbivore Diets: A Review. *Journal of Range Management*, *35*(3), 309.

Joly, K., Wasser, S. K., & Booth, R. (2015). Non-Invasive Assessment of the Interrelationships of Diet, Pregnancy Rate, Group Composition, and Physiological and Nutritional Stress of Barren-Ground Caribou in Late Winter. *PloS One*, *10*(6).

Kaczensky, P., Burnik Šturm, M., Sablin, M. v., Voigt, C. C., Smith, S., Ganbaatar, O., Balint, B., Walzer, C., & Spasskaya, N. N. (2017). Stable isotopes reveal diet shift from pre-extinction to reintroduced Przewalski's horses. *Scientific Reports*, *7*(1), 1–9.

Kartzinel, T. R., Chen, P. A., Coverdale, T. C., Erickson, D. L., Kress, W. J., Kuzmina, M. L., Rubenstein, D. I., Wang, W., & Pringle, R. M. (2015). DNA metabarcoding illuminates dietary niche partitioning by African large herbivores. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(26), 8019–8024.

Kartzinel, T. R., & Pringle, R. M. (2020). Multiple dimensions of dietary diversity in large mammalian herbivores. *Journal of Animal Ecology*, 1365-2656.13206.

Kerley, G. I. H., Kowalczyk, R., & Cromsigt, J. P. G. M. (2012). Conservation implications of the refugee species concept and the European bison: King of the forest or refugee in a marginal habitat? *Ecography*, *35*(6), 519–529.

Knutti, R., Masson, D., & Gettelman, A. (2013). Climate model genealogy: Generation CMIP5 and how we got there. *Geophysical Research Letters*, *40*(6), 1194–1199.

Lavin, S. R., Woodruff, M. C., Atencia, R., Cox, D., Woodruff, G. T., Setchell, J. M., & Wheaton, C. J. (2019). Biochemical and biological validations of a faecal glucocorticoid metabolite assay in mandrills (*Mandrillus sphinx*). *Conservation Physiology*, *7*(1).

Lea, J. M. D. M., Walker, S. L., Kerley, G. I. H. I., Jackson, J., Matevich, S. C., & Shultz, S. (2018). Non-invasive physiological markers demonstrate link between habitat quality, adult sex ratio and poor population growth rate in a vulnerable species, the Cape mountain zebra. *Functional Ecology*, *32*(2), 300–312.

Lea, J. M., Kerley, G. I., Hrabar, H., Barry, T. J., & Shultz, S. (2016). Recognition and management of ecological refugees: A case study of the Cape mountain zebra. *Biological Conservation*, *203*, 207–215.

Louca, S., Parfrey, L. W., & Doebeli, M. (2016). Decoupling function and taxonomy in the global ocean microbiome. *Science*, *353*(6305), 1272–1277.

Manthey, J. D., Campbell, L. P., Saupe, E. E., Soberón, J., Hensz, C. M., Myers, C. E., Owens, H. L., Ingenloff, K., Townsend Peterson, A., Barve, N., Lira-Noriega, A., & Barve, V. (2015). A test of niche centrality as a determinant of population trends and conservation status in threatened and endangered North American birds. *Endangered Species Research*, *26*(3), 201–208.

Martin, L. J., Blossey, B., & Ellis, E. (2012). Mapping where ecologists work: Biases in the global distribution of terrestrial ecological observations. *Frontiers in Ecology and the Environment* 10(4) 195–201.

Martinez-Meyer, E., Diaz-Porras, D., Peterson, A. T., & Yanez-Arenas, C. (2012). Ecological niche structure and rangewide abundance patterns of species. *Biology Letters*, *9*(1), 20120637–20120637.

McKay, D. M. (2009). The therapeutic helminth? *Trends in Parasitology*, *25*(3), 109–114.

Merow, C., Smith, M. J., & Silander, J. A. (2013). A practical guide to MaxEnt for modeling species' distributions: what it does, and why inputs and settings matter. *Ecography*, *36*(10), 1058–1069.

Meshesha, D. T., Ahmed, M. M., Abdi, D. Y., & Haregeweyn, N. (2020). Prediction of grass biomass from satellite imagery in Somali regional state, eastern Ethiopia. *Heliyon*, *6*(10), e05272.

Monsarrat, S., Boshoff, A. F., & Kerley, G. I. H. (2019). Accessibility maps as a tool to predict sampling bias in historical biodiversity occurrence records. *Ecography*, *42*(1), 125–136.

Monsarrat, S., & Kerley, G. I. H. (2018). Charismatic species of the past: Biases in reporting of large mammals in historical written sources. *Biological Conservation*, *223*, 68–75.

Monsarrat, S., & Svenning, J. C. (2021). Using recent baselines as benchmarks for megafauna restoration places an unfair burden on the Global South. *Ecography*.

Montiglio, P. O., Pelletier, F., Palme, R., Garant, D., Réale, D., & Boonstra, R. (2012). Noninvasive monitoring of fecal cortisol metabolites in the eastern chipmunk (Tamias striatus): Validation and comparison of two Enzyme Immunoassays. *Physiological and Biochemical Zoology*, *85*(2), 183–193.

Osorio-Olvera, L., Yañez-Arenas, C., Martínez-Meyer, E., & Peterson, A. T. (2020). Relationships between population densities and niche-centroid distances in North American birds. *Ecology Letters*, *23*(3), 555–564.

Palme, R (1997). Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood. *Inst J Mammal Biol*, *62*, 192–197.

Penzhorn, B. L. (1984). A Long-term Study of Social Organisation and Behaviour of Cape Mountain Zebras *Equus zebra zebra*. *Zeitschrift Für Tierpsychologie*, *64*(2), 97–146.

Pili, A. N., Tingley, R., Sy, E. Y., Diesmos, M. L. L., & Diesmos, A. C. (2020). Niche shifts and environmental non-equilibrium undermine the usefulness of ecological niche models for invasion risk assessments. *Scientific Reports 2020 10:1*, *10*(1), 1–18.

Pironon, S., Villellas, J., Morris, W. F., Doak, D. F., & García, M. B. (2015). Do geographic, climatic or historical ranges differentiate the performance of central versus peripheral populations? *Global Ecology and Biogeography*, *24*(6), 611–620.

Pollock, J., Glendinning, L., Wisedchanwet, T., & Watson, M. (2018). The madness of microbiome: Attempting to find consensus "best practice" for 16S microbiome studies. *Applied and Environmental Microbiology* 84(7). e02627-17.

Ramankutty, N., & Foley, J. A. (1999). Estimating historical changes in global land cover: Croplands from 1700 to 1992. *Global Biogeochemical Cycles*, *13*(4), 997–1027.

Rieu, E., Recca, A., Bénet, J. J., Saana, M., Dorchies, P., & Guillot, J. (2007). Reliability of coprological diagnosis of Paramphistomum sp. infection in cows. *Veterinary Parasitology*, *146*(3–4), 249–253.

Rinaldi, L., Veneziano, V., Morgoglione, M. E., Pennacchio, S., Santaniello, M., Schioppi, M., Musella, V., Fedele, V., & Cringoli, G. (2009). Is gastrointestinal strongyle faecal egg count influenced by hour of sample collection and worm burden in goats? *Veterinary Parasitology*, *163*(1–2), 81–86.

Santini, L., Pironon, S., Maiorano, L., & Thuiller, W. (2019). Addressing common pitfalls does not provide more support to geographical and ecological abundant-centre hypotheses. *Ecography*, *42*(4), 696–705.

Scheuerle, M. C., Stear, M. J., Honeder, A., Becher, A. M., & Pfister, K. (2016). Repeatability of strongyle egg counts in naturally infected horses. *Veterinary Parasitology*, *228*, 103–107.

Soberón, J., Peterson, A. T., & Osorio-Olvera, L. (2018). A comment on "Species are not most abundant in the centre of their geographic range or climatic niche." *Rethinking Ecology* *3*, 13–18.

Sundaresan, S. R., Fischhoff, I. R., Hartung, H. M., Akilong, P., & Rubenstein, D. I. (2008). Habitat choice of Grevy's zebras (*Equus grevyi*) in Laikipia, Kenya. *African Journal of Ecology*, *46*(3), 359–364.

Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., & Willerslev, E. (2012). Towards next-generation biodiversity assessment using DNA metabarcoding. *Molecular Ecology*, *21*(8), 2045–2050.

Trevelline, B. K., Fontaine, S. S., Hartup, B. K., & Kohl, K. D. (2019). Conservation biology needs a microbial renaissance: A call for the consideration of host-associated microbiota in wildlife management practices. *Proceedings of the Royal Society B: Biological Sciences* 286(1895), 20182448.

Uhlinger, C. (1990). Effects of three anthelmintic schedules on the incidence of colic in horses. *Equine Veterinary Journal*, *22*(4), 251–254.

Walshe, N., Duggan, V., Cabrera-Rubio, R., Crispie, F., Cotter, P., Feehan, O., & Mulcahy, G. (2019). Removal of adult cyathostomins alters faecal microbiota and promotes an inflammatory phenotype in horses. *International Journal for Parasitology*, *49*(6), 489–500.

Wasser, S. K., Azkarate, J. C., Booth, R. K., Hayward, L., Hunt, K., Ayres, K., Vynne, C., Gobush, K., Canales-Espinosa, D., & Rodríguez-Luna, E. (2010). Non-invasive measurement of thyroid hormone in feces of a diverse array of avian and mammalian species. *General and Comparative Endocrinology*, *168*(1), 1–7.

White, T. C. R. (2008). The role of food, weather and climate in limiting the abundance of animals. *Biological Reviews*, *83*(3), 227–248.

Wiens, J.J. & Graham, C.H., 2005. Niche conservatism: integrating evolution, ecology, and conservation biology. *Annual Review of Ecology, Evolution and Systematics*, *36*, 519-539.

Xiang, Z., Tong, W., Guo, Z., Xu, Y., Guo, J., Ruan, Y., & Zhao P. (2019). Rat H1 parvovirus infection leads to alterations in gut microbiota. *Pathogens and Disease*, *77*(5), 58.

Xing, Y., Liu, J., Lu, F., Wang, L., Li, Y., & Ouyang, C. (2019). Dynamic distribution of gallbladder microbiota in rabbit at different ages and health states. *PLoS ONE*, *14*(2), e0211828.

Yackulic, C. B., Sanderson, E. W., & Uriarte, M. (2011). Anthropogenic and environmental drivers of modern range loss in large mammals. *Proceedings of the National Academy of Sciences*, *108*(10), 4024–4029.

Chapter 3) Conservation in the margins: ecological marginalisation increases extinction risk in mammals

Submitted as Britnell J.A[1,2], Zhu Y[1], Kerley G.I.H.[3], Shultz S[1] Conservation in the margins: ecological marginalisation increases extinction risk in mammals to PNAS

3.1 Significance

Habitat loss has led to widespread range contraction and population declines across taxa. The impact of range contraction on niche occupancy, is rarely, if ever, assessed or incorporated into conservation assessments. We evaluate the impact of range loss on niche parameters of 4785 terrestrial mammals. Geographic range contraction results in more homogenous ranges and reduced niche sizes. Some species become restricted to ecologically extreme habitats at the periphery of their historic niche. This ecological marginalisation increases extinction risk, independent of geographic range loss. Marginalisation can result in a “double jeopardy” for vulnerable taxa, such as large-bodied and small-ranged species, where poor performance in marginalised relict populations exacerbates population declines. We suggest this phenomenon is an underappreciated global conservation threat that may partially explain the failure of protected areas to buffer species from further decline.

3.2 Abstract

Human land-use results in widespread range contractions across taxa. Anthropogenic pressures can result in species’ realized niches expanding, shifting or contracting. Marginalisation occurs when contraction constrains species to the geographic or ecological extremes of their historic niche. Using 4785 terrestrial mammal species, we show that range contraction results in niche space and habitat diversity loss. Additionally, ecological marginalisation is a common consequence of range contraction caused by human land use change. Remnant populations become located in the climatic and topographic extremes of their historic niche that are more likely to be at the periphery of their historic niche at greater distances from historic niche centroids. This ecological marginalisation is associated with poor performance and increased extinction risk independent of geographic range loss. Range loss and marginalisation may create a ‘double jeopardy’ in vulnerable groups such as large-bodied species and species with small geographical range size. Our results reveal a hitherto unrecognised conservation threat that is vital to incorporate into conservation planning.

3.3 Introduction

Human impacts, such as land-use changes (1), persecution and community change (2) have globally altered the size, habitat diversity and connectivity of species' ranges. Efforts to address pervasive habitat loss and conversion include sustainable harvesting mandates, regulations limiting land clearing and habitat fragmentation, and the creation and expansion of protected areas (PAs) (3). Despite increases in established PAs to ~250,000 terrestrial PAs (22 million km^2) and ~20,000 marine PAs (28 million km^2) (4), species loss continues. Continuing declines may be the result of extinction debt, or a lag between habitat loss and population extirpation (5). However, the "Protected Area Paradox" (6) whereby declines continue within protected areas may be caused by protected areas being located in marginal (7), less productive (8) "pristine" lands under little pressure for human conversion (9,10).

Protected area bias towards marginal habitats results from a global mosaic of human agriculture and settlement concentrated in highly productive lands (2, 11) that have shallow slope, fertile soils and close proximity to surface water (12). Temperate and tropical grasslands and tropical dry, Mediterranean and temperate broadleaf forests are preferentially converted and fragmented for high-intensity agriculture (13; Figure S3.1). As most protected areas have been created after agricultural intensification, they are likely to occur in areas that are unattractive for agriculture (8,9) such as steep slopes or far from water rather than areas with the most biodiversity. Moreover, biomes such as mountain, desert and cold tundra that only account for 10% of global net primary productivity are over-represented in protected areas (6,14).

3.3.1 Range dynamics under anthropogenic pressures

Species' ranges encompass abiotic and biotic gradients ranging in quality. In high quality habitats, individual vital rates (survival and reproduction) and population performance (abundance, density and growth) are strong, whereas in low quality, marginal habitats, individual and population metrics are comparatively poor (15). High quality habitats can support higher population density and produce more emigrants that disperse from 'sources' to non-self-sustaining marginal ‘sinks’ (16). Such productive habitats are crucial to maintain meta-population processes and trophic diversity (22). In response to threats, environmental change and persecution, organisms can: 1) retreat into refugia (17), 2) contract into high quality “core” environments that buffer the threat (18), or 3) move into or co-opt a novel habitat (7).

Range change can lead to ecological niche or geographic range shifts, expansion or collapse (Figure 3.1; Figure S3.2). *Random* habitat loss will result in a smaller range but will not necessarily impact habitat diversity or the niche centre. Habitat loss focused on ecological or geographic margins will lead to niche contraction toward the geographic or ecological “core”. Such contraction to high quality habitats with high vital rates can lead to increased resilience of the remaining metapopulation (18). In contrast, loss of habitat in the range core can lead to contraction towards ecological extremes at the periphery of the historically occupied niche (Figure 3.2). Localised human pressures may extirpate or shift individual populations into marginal conditions (e.g. partial refugee species; 19), whereas marginalisation at a macroecological scale can restrict entire species to marginal conditions (7). Given that human activities and land conversion are concentrated in productive accessible lands, range contraction and habitat loss are likely to be associated with shifts towards niche margins. However, the relationship between range contraction and niche shift in mammals has not been evaluated.

Here, we evaluate the intrinsic and extrinsic factors associated with range collapse using a combination of phylogenetic and niche modelling (polyhedral convex hull and n-dimensional hypervolumes). Our estimates of a species niche follow Hutchinson, defined as a multidimensional volume (hyperspace) where axes correspond to conditions and resources (20). In our case, we conceived of the species native and contemporary ranges as physical spaces of available environmental conditions with each point (or cell) expressed as a set of local topographic and climatic factors, similar to Hutchinson’s definition of biotope. We define each species’ native and contemporary geographic range as the set of habitats and elevation ranges known to be occupied by a species from IUCN assessments. We use the three principal components of the occupied topographic and climatic variables as axes for an estimate of each species historic and contemporary abiotic niche space. We then evaluate how range contraction impacts niche shift and extinction risk in 4785 mammal species. We predict that human pressures in ecologically productive landscapes will exclude species from historic niche centres and lead to ecological marginalisation. We also predict that ecological marginalisation will increase extinction risk where niche centroids shift from core, high quality habitats to more marginal areas (21, 22).

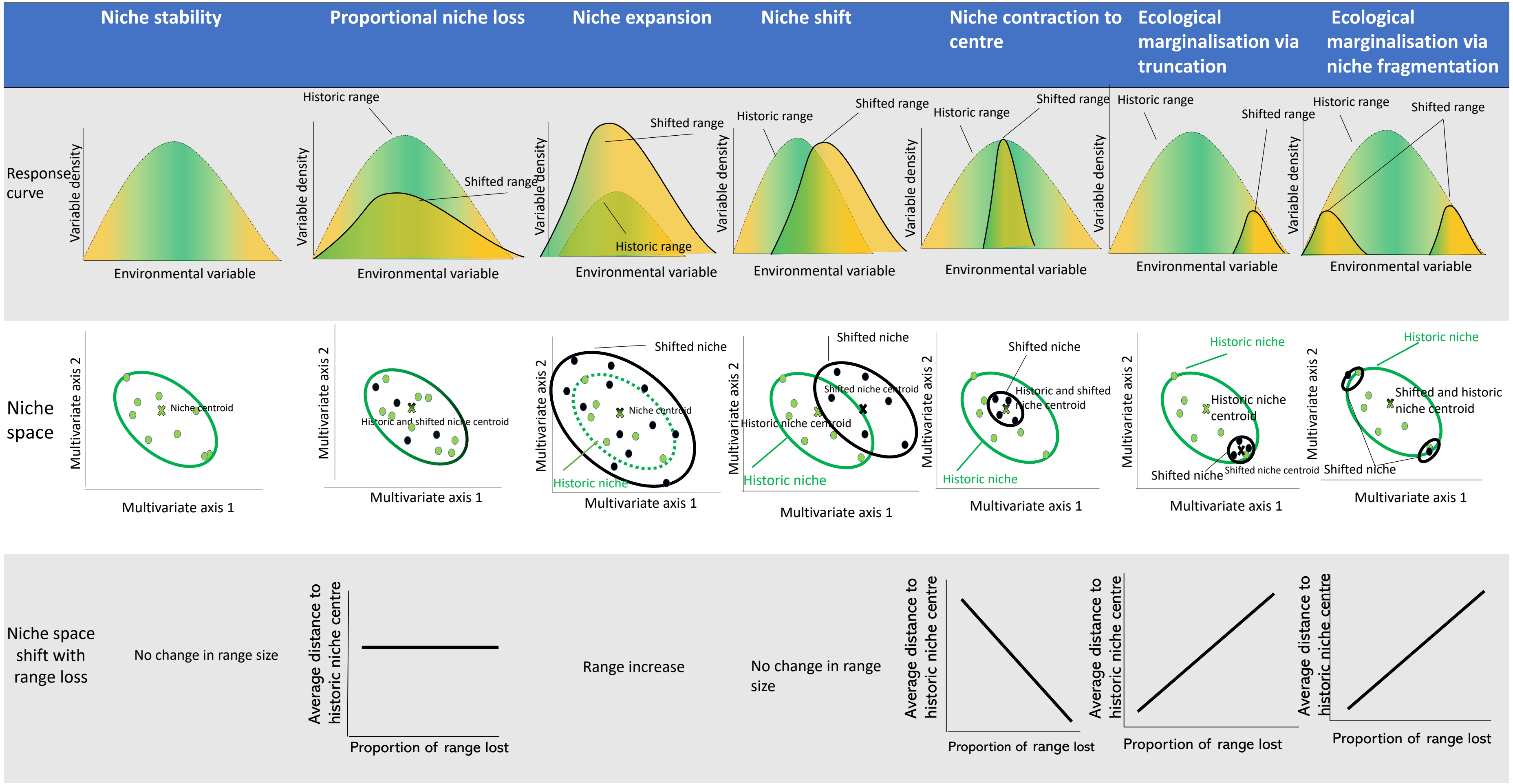


Figure 3.1) **Dynamics of species response curves and niche spaces following shifts from anthropogenic pressures**. Top panel: possible outcomes of niche shifts where green to yellow gradients represent high quality to low quality conditions respectively. Centre panel: changes in niche space in relation to these shifts where crosses represent niche centroids, black-contemporary and green-historic. Ellipses depict niche space, black- contemporary, green–historic . Bottom panel: predicted changes in average distance to historic niche centre with increasing range contraction.

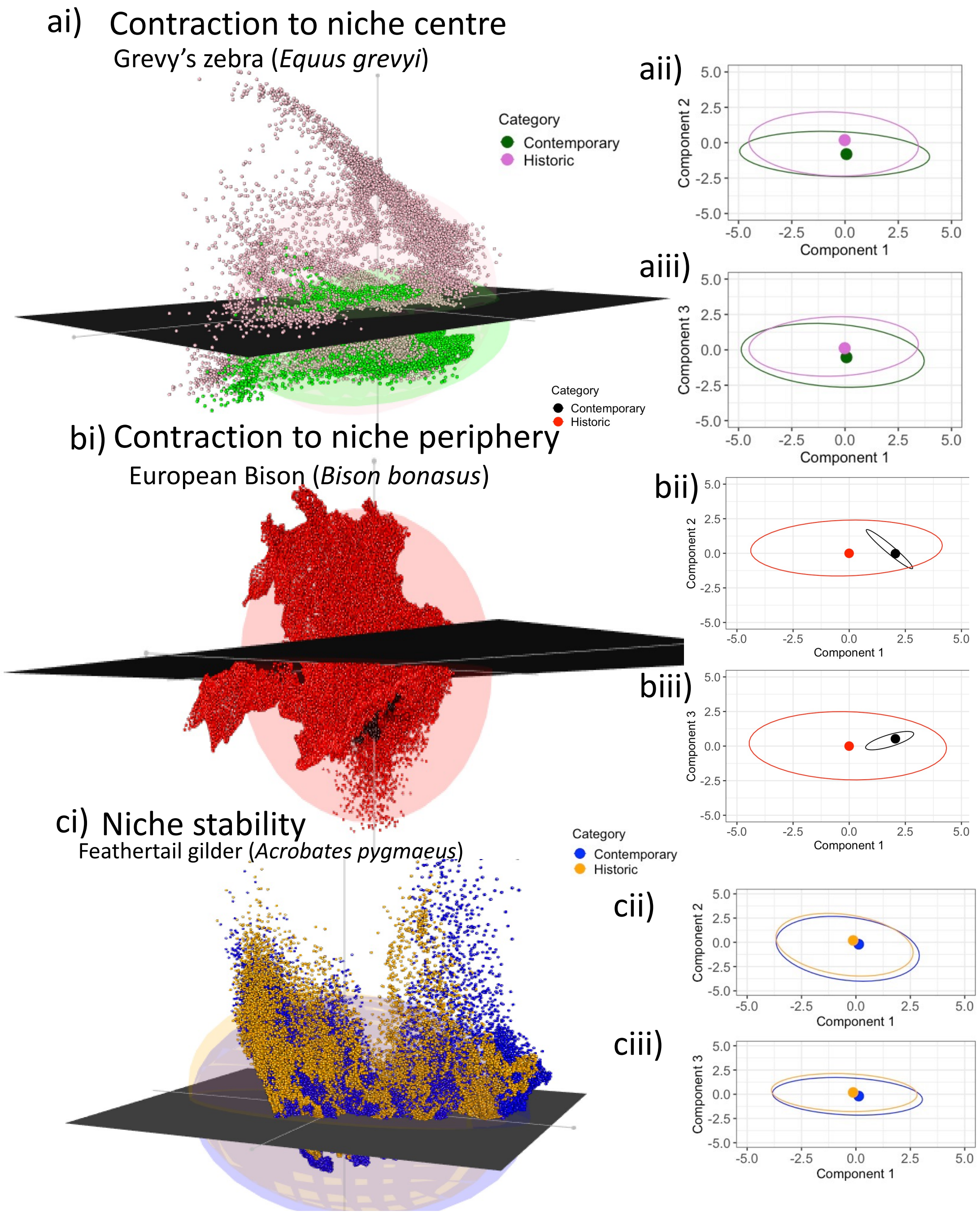


Figure 3.2) **Examples of species demonstrating contraction to niche centroid, ecological marginalisation and stable niches under range contraction in three-dimensional environmental space and simplified to two-dimensional multivariate space.** Top) Grevy's zebra (*Equus grevyi*) as an example of contraction to niche centroid under very high range contraction (~90%). ai) Three-dimensional niche spaces constructed from the first three principal components of contemporary and historic topographic and climatic variables of Grevy's zebra. Pink dots represent PCA coordinate points of niche conditions in the estimated species range without anthropogenic pressure *i.e.* "historic" niche points (modelled with Mid-Holocene period climate, ~6000 years ago). The Pink ellipse represents species' niche space without anthropogenic pressure *i.e.* "historic" niche space. Green dots are contemporary niche points (modelled with 1970-2000 climate) and green ellipse is

contemporary niche space. aii) Principal component 1 and 2 of Grevy's zebra contemporary (green) and historic (pink) niche space displaying lack of shift in niche centroids or overall niche space following widespread range contraction aiii) Principal component 1 and 3 of Grevy's zebra contemporary (green) and historic (pink) niche space Middle) European bison (*Bison bonasus*) *as an example of ecological marginalisation*. bi) Three-dimensional niche space constructed from the first three principal components of contemporary and historic topographic and climatic variables of the European Bison. Red dots represent PCA coordinate points of the species' "historic" niche with red ellipse representing "historic" niche space. Black dots are contemporary niche points and black ellipse is contemporary niche space. bii) Principal component 1 and 2 of European Bison contemporary (Black) and historic (Red) niche space displaying a significant shift in niche centroids and overall niche space following widespread range contraction. biii) Prinicipal component 1 and 3 of European Bison contemporary (Black) and historic (Red) niche space. Bottom) Feathertail gilder (*Acrobates pygmaeus*) as an example of a stable niche under very low levels of range contraction. ci) Three-dimensional niche space constructed from the first three principal components of contemporary and historic topographic and climatic variables separately. Orange dots are PCA coordinate points of the species' "historic" niche and orange ellipse is the "historic" niche space. Blue dots are contemporary niche points and blue ellipse is contemporary niche space. cii) Principal component 1 and 2 of Feathertail gilder contemporary (Blue) and historic (Orange) niche space displaying a significant shift in niche centroids and overall niche space following widespread range contraction. ciii) Principal component 1 and 3 of Feathertail gilder contemporary (Blue) and historic (Orange) niche space

3.4 Results

Thirteen percent, or 627, of 4785 mammal species have undergone geographic range contraction but the intensity of range loss varied (Table S3.1). Across all species, whether geographic range contraction occurred was explained by the following factors: small geographic range size, large body mass and the proportion of the historic range transformed for human landuse (Table S3.2). The extent of range contraction was a function of human transformation of the historic range, large body mass and small geographic range sizes (Table 3.1).

Table 3.1) **Species with more human transformation in their historic range and smaller geographic range sizes are most vulnerable to geographic range loss. ANOVA table for geographic range contraction in range contracted species.** *ANOVA table of best model fit from dredge function of global model of phy.model.mammal.range.loss <-pgls(logit(geographic range loss) ~ order + log(body mass) + log(geographic range size) + logit(human transformation) + log(body mass) * log(geographic range size) + log(body mass) *logit(human transformation), data = mammal.phy.glm.range.loss, lambda = "ML"). Delta AIC (***ΔAIC***) was estimated by dropping each variable from the best model following dredge results. .* **Σ model weights** *referred to the Summed model weights i.e variable importance and inclusion in models with* **ΔAIC** *< 5 from the best fit model. Human transformation denotes the proportion of historic range converted to rangelands, agriculture or dense settlements. Dredge results for global results in Table S3.5. *p<0.05, **p< 0.01, ***p<0.001. Dredge results for global results in Table S3.5.* λ =0.39, delta = 1.00, kappa = 1.00 df = 585, adjusted R^2= 0.37, AICc = 2092.11.

Variable	**df**	**Slope**	**s.e.**	**F**	***P***	**ΔAIC**	**Σ model weights**
Geographic range size	1, 585	-1.68	0.17	333.08	<0.001 ***	257.80	1
Body mass	1, 585	0.03	0.13	8.12	0.005**	40.0	1
Human transformation	1, 585	0.15	0.04	7.88	0.004**	11.08	1
Body mass: Geographic range size	1, 585	0.03	0.02	2.45	0.11	0.41	0.55

As species' geographic ranges contract, their niches shrink and become more homogeneous (higher proportion of habitat diversity loss) (Figure 3.3, Table 3.2). Large body size, large geographic ranges and anthropogenic transformation all predict the extent of habitat diversity loss (Table S3.1; Table 3.2). Mammals with greater geographic range loss lose more habitat diversity with smaller changes in body mass.

Table 3.2) **Species with greater geographic range contraction and human transformation of historic range have lost greater amounts of habitat diversity and niche space.** Top) *ANOVA table of best model fit from dredge function of global model of pgls(asin(hypervolume_loss) ~ logit(geographic_range_loss) + order + logbody + logrange + logit(human_transformation) + logbody*logrange + logbody*logit(geographic_range_loss) + logbody* logit(human_transformation)*. λ =0.20, delta = 1.00, kappa = 1.00, df = 560, adjusted R^2= 0.30, AICc = 541.7. Bottom) **ANOVA table for habitat diversity loss in range contracted species.** *ANOVA table of best model fit from dredge function of global model of pgls(habitat diversity loss ~ order + log(body mass) + log(geographic range size) + logit(proportion of agricultural and rangelands in historic range) + logit(geographic range loss) + log(body mass)*log(geographic range size) + log(body mass)**logit(proportion of agricultural and rangelands in historic range) + log(body mass)**logit(geographic range loss), data = mammal.phy.glm.habitat.loss, lambda = "ML").). Delta AIC (**ΔAIC**) was estimated by dropping each variable from the best model following dredge results. Agricultural and Rangelands denotes the proportion of historic range converted to rangelands or agriculture. *p<0.05, **p< 0.01, ***p<0.001.. Delta AIC (**ΔAIC**) was estimated by dropping each variable from the best model following dredge results.* **Σ model weights** *referred to the Summed model weights i.e variable importance and inclusion in models with* **ΔAIC** < *5 from the best fit model.* λ =0.01, delta = 1.00, kappa = 1.00, df = 583, adjusted R^2= 0.58, AICc = -589.7. Dredge tables can be found in Table S3.6, and Table S3.7 respectively. **p<0.05, **p< 0.01, ***p<0.001*

Model	**Variable**	**df**	**Slope**	**se**	**F**	***P***	**ΔAIC**	**Σ model weights**
Hypervolume Loss	Geographic range loss	1, 560	0.15	0.009	245.2	<0.001***	541.7	1
Habitat Diversity Loss	Geographic range loss	1, 584	0.053	0.009	638.3	<0.001***	406.66	1
	Body mass:Geographic range loss	1, 584	0.006	0.0009	38.9	<0.001***	43.82	1
	Body mass	1, 584	0.012	0.003	70.7	<0.001***	43.79	1
	Geographic range size	1, 584	0.051	0.01	33.5	<0.001***	22.88	1
	Agricultural and Rangelands	1, 584	0.0070	0.004	8.9	0.003 **	0.93	0.67

Most range-restricted species were displaced toward more extreme climate or topography (Table S3.4). Range collapse and human transformation shift species to more extreme temperature (Table S3.8), precipitation (Table S3.10), elevation (Table S3.12) and slope ranges (Table S3.14; Figure 3.3). Species with smaller geographic range are more vulnerable to shifts in temperature and precipitation (Figure 3.3, Table S3.8, S3.10).

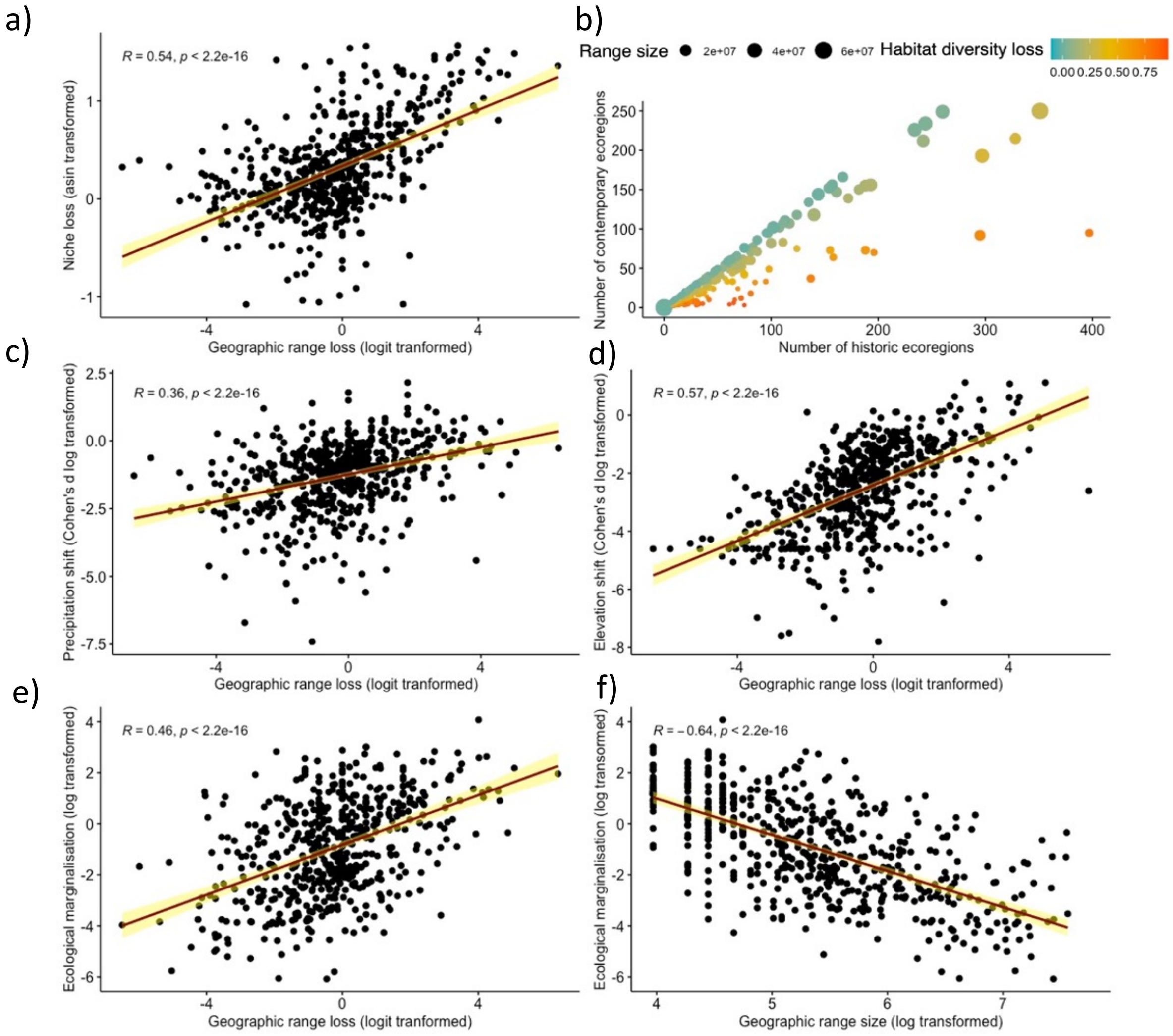


Figure 3.3) **Geographic range loss has severe consequences for species niches – restricting them to smaller, less diverse, ecologically extreme niche spaces.** *a) Geographic range loss results in* niche shrinkage, *b) Small ranged species are more vulnerable to displacement to less diverse ranges- Range size is in* km^2*, Habitat diversity loss is estimated as proportion of ecoregions lost,* Middle)*: Geographic range contraction restricts species to climatic and topographic extremes ,c) precipitation,d) elevation, Bottom row)* Geographic range loss leads to *ecological marginalisation. e) Effect size of* Mahalanobis *distance to historic niche centre, f) Small ranged species are most vulnerable to ecological marginalisation. R and p values presented were calculated from Pearson's correlation coefficient. Yellow outlines represent 95% confidence interval of Pearson's CC. Species correspond to range restricted species only. Ecological marginalisation against geographic range loss with species names is available Figure S3.4.*

Geographic range loss displaces species to the geographic (Table S3.17; Figure S3.3) and ecological periphery of their historic niche (Figure 3.3, Table S3.25). Larger range loss results in more ecological marginalisation, quantified by a greater Mahalanobis distance between the contemporary niche points and the historic niche centroid and greater distances between historic and contemporary niche (hypervolume) centroids (Figure 3.3, Table S3.19). Small geographic ranges were most vulnerable to ecological marginalisation using both Mahalanobis distance and centroid shifts (Figure 3.3). As such, ecological and geographic marginalisation is a common consequence of geographic range loss in range-restricted mammals (Table S3.24).

Across all species, increased extinction risk was associated with large body size and small geographical range (Table S3.21). For species that have experienced range contraction, greater geographic range loss and ecological marginalisation also increased extinction risk (Table 3.3). Geographic marginalisation did not influence extinction risk (Table 3.3).

Table 3.3) **Geographic range loss and both metrics of ecological marginalisation independently increase extinction risk** *ANOVA table of best model fit from dredge function of global model pgls(IUCN status ~ order + logit(geographic range loss) + geographic marginalisation + log10(ecological* marginalisation*) + log10(distance niche centroids+0.1), data = mammalIUCN.phy, lambda = "ML")). Dredge models are available Table S3.24. Delta AIC (*Δ**AIC***) was estimated by dropping each variable from the best model following dredge results.* $\Lambda = 0.53$*, df= 458, adjusted* $R^2 = 0.19$*.* $^*p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$*. Dredge model available in Table S3.23. .* **Σ model weights** *referred to the Summed model weights i.e variable importance and inclusion in models with* Δ**AIC** < *5 from the best fit model.*

Variable	**df**	**Slope**	**se**	**F**	***P***	**ΔAIC**	**Σ model weights**
Ecological marginalisation	1, 458	0.064	0.044	15.20	0.001***	44.3	1
Geographic range loss	1, 458	0.23	0.032	48.27	<0.001***	42	1
Distance between niche centroids	1, 458	0.18	0.12	51.07	<0.001***	0.2	0.54

3.5 Discussion and Conclusion

Human landuse leads to non-random range and habitat diversity losses in terrestrial mammals. As species' ranges contract, they become increasingly restricted to ecologically marginal habitats and extremes of their historical niche. Ecological, but not geographic, marginalisation increases extinction risk independent of geographic range loss. In addition to large body size and small geographic range predicting extinction risk (23), these same traits render species more vulnerable to habitat diversity loss, niche loss and ecological marginalisation. Our analysis suggests ecological retreat (24) and marginalisation may be a global phenomenon in terrestrial mammals undergoing range contraction.

The core-periphery abundance hypothesis argues that abundance, density and performance decrease from the geographic or ecological (niche centroid) core to the periphery of a species range/niche (25, 26). Historic niche and range limits form where death rates and emigration exceed birth rates and immigration (27) and further dispersal was unsustainable (28). Niche peripheries can reflect species' physiological tolerance limits (29) to climatic extremes, resource limitation, disease, predation or competition (30). Geographic edges are not always associated with reduced population performance (31–33), however, studies have linked geographically peripheral populations to reduced patch occupancy, lower abundance and higher rates of genetic differentiation and inbreeding (22). Ecologically marginal populations likewise have been associated with patchier occurrence (34), lower abundance and population density (21,22), lower genetic diversity (35) and lower growth rates (36, 37). However, the core-periphery hypothesis may not hold for all species across time (20, 30, 31, 34,38). Spatiotemporal variation in abiotic conditions may alter species niche centroid position. As species disperse to occupy optimal conditions or populations recover after a period of marginal conditions, a lag time may influence relationships between observed demographic metrics (abundance, density) and proximity to niche centroid (32, 39). Furthermore, spatial manifestation of a species niche may be complex. Species may have a symmetric or asymmetric niche space (39) such that habitat suitability declines towards range edges in a step or a ramp function respectively (30). Small alterations in abiotic conditions could lead to large variation in habitat suitability and performance in some species but not others. The centre (or average) of all conditions across the range, which forms the ecological niche centroid, may not correspond to optimal conditions for performance across species. Ecological marginalisation occurs when these populations become more common than core populations. Marginalisation worsens as contraction continues and it is vital to address habitat suitability issues in declining populations before pressures accumulate (40). Partial refugee species have some key populations confined to sub-optimal habitat with poor performance (6,19) whereas complete refugees are confined to, and protected in, solely marginal conditions (7). Species' are likely to experience increased extinction risk through progressive declines in fitness and resilience as ranges contract towards niche and ecological boundaries (28).

Large-bodied animals are vulnerable to extinction or extirpation due to their high energetic requirements, long generation time and low population density (23, 41). Large-bodied species are also more likely to be in conflict with humans over areas suitable for rangelands or agricultural purposes, pushing them into poor productivity or barren lands (11). Small range size is associated with ecological marginalisation as land-use change within their core "primary" habitat can leave low density, capped populations in the remaining marginal habitats (42,43). We show intrinsic (geographic range size and body mass) and extrinsic factors (geographic range loss) can interact to influence key metrics associated with extinction and marginalisation. Mammals with greater geographic range loss lose more habitat diversity with smaller changes in body mass. Mammals with small geographic ranges are less vulnerable to the effects of body size on shifts in niche centroids. This latter relationship is either due to the lack of scaling of the metric, as it does not control for niche size, or that small geographic ranges result in smaller niches where there cannot be large niche centroids shifts before extinction. Thus, intrinsic characteristics can exacerbate the impacts of geographic range loss and marginalisation. We suggest large-bodied and small-ranged species can experience a form of "double jeopardy" (44) where extinction risk is a result of both intrinsic vulnerability and confinement to marginal habitats (40).

Our findings have major implications for evaluating the effectiveness of protected areas and identifying conservation priorities for species. Protected areas are a final stronghold for many species (45). Many PAs are established in areas with little pressure for human land-use, because of low economic value and productivity, rather than for biodiversity value (9, 10). Steep, bare and sparsely vegetated habitats (e.g., cold mountains, deserts) are overrepresented in protected areas (8). Hence, the full spectrum of historical niche space is unprotected for the vast majority of species' (93.1% of amphibians, 89.5% of birds and 90.9% of terrestrial mammal species) (46). Current estimates suggest PAs fail to adequately protect half of the terrestrial mammals globally with hundreds of species having no viable protected area (47). PAs may be inefficient and potentially detrimental when they protect species in marginal habitat (7, 19) and may effectively enforce ecological marginalisation. This may explain why populations of vulnerable species continue to decline even within protected areas (e.g., 48, 49) and why increasing protected area coverage has not reversed the growing number of threatened species (6). Ecological marginalisation, however, is not widely recognised in conservation management or planning (50). To our knowledge, the only species' conservation assessment to discuss ecological marginalisation as a conservation threat is that for the European bison (*Bison bonasus*) (51).

Our findings also have significant implications for the establishment of appropriate conservation baselines. We demonstrate that contemporary distributions and niche spaces are shaped through ecological retreat *i.e.,* withdrawal into marginal refugia to escape human pressures (24). Without reference to historical information, studying species following widespread range and niche loss can lead to stereotyped perceptions of a species and conservation complacency (52). Models that describe the ecology of a species based on these stereotypes, so called "shifted models", may result in erroneous conservation recommendations (53*)* and inaccurate estimates of potential distributions, habitat suitability and niche breadth (54) or species' sensitivity to environmental change (55). Ecological marginalisation must be considered a hitherto unrecognised threat that compromises conservation efficacy and exacerbates extinction risk.

3.6 Methods

3.6.1 Species traits and extinction risk

Mammal adult body mass (g) and IUCN status were obtained from the PanTHERIA (56) and COMBINE databases (57). 'Data Deficient', extreme outliers, and assumed input errors were excluded. A total of 4785 species' data were included in the analyses. IUCN status was converted to an ordinal scale from 0 to 5 (least concern = 0, near threatened = 1, vulnerable = 2, endangered = 3, critically endangered = 4, extinct/extinct in wild = 5) as a proxy of extinction risk.

3.6.2 Species distributions, geographic range loss and habitat diversity loss

For species distributions, we used present natural *i.e.,* species ranges without human pressures and contemporary ranges from the PHYLACINE dataset (58). Use of the PHYLACINE distributions as estimates of "historic" and contemporary ranges is well established (59). Geographic range loss was assessed from the proportional loss between historic and contemporary ranges. To assess which intrinsic and extrinsic factors were associated with geographic range loss across all 4785 species, geographic range loss was converted to an ordinal scale: zero range loss = 0, 1st quartile of range contraction = 1, 2nd quartile = 2, 3rd quartile = 3, 4th quartile = 4. Range maps were intersected with the *Resolve2017 map* (60) to assign ecoregions and biomes. Habitat diversity loss was estimated from the proportional loss of ecoregions between historic and contemporary ranges. Freshwater or marine bodies within ranges were excluded.

3.6.3 Anthrome and land-use within ranges

The Anthropogenic Biomes (anthromes) Version 2 dataset (1700-2000) (61) was used to assess contemporary land-use within historic species range. Human transformation was calculated as the proportion of a species' historic range converted to dense settlements (urban, mixed settlements and villages anthromes) and Agricultural and Rangelands (anthromes of croplands and rangelands with substantial/significant human populations).

3.6.4 Assessing geographic marginalisation

For geographic marginalisation, 100,000 sampling points were randomly generated throughout both the historic and contemporary ranges of each species. The minimum

Euclidean distance from a historic range boundary was then generated for each point. This approach is analogous to the niche margin index (62) but applied to geographic instead of ecological space. A Cohen's *d effect size* of the average minimum distance to historic range boundaries for contemporary and historic points was calculated. Positive values depict an increase in distance to the historic range boundary *i.e.,* contraction to geographic centre and negative values depict a decrease in distance *i.e.,* contraction to edges.

3.6.5 Assessing ecological marginalisation

For ecological marginalisation, only range-contracted species that lost geographic area between historic and contemporary time points (0 < x < 100%), were assessed. Species' ranges were trimmed by biome type and elevation limits collated from IUCN assessments. Only biome types and elevation ranges known to be occupied by each species were used to estimate species' niches. Conditions within a species' niche at each time were assessed by sampling all cells for climatic and topographic variables within a species' trimmed historic and contemporary range.

For present climate conditions, we assessed the period 1970–2000 using Worldclim version 2 (63). For past climatic conditions we used the Mid-Holocene period (~6000 years before present) using Worldclim version 1 (64). We averaged three climatic (general circulation) models (CNRM_CM5, IPSL-CM5A-LR and the MPI-ESM-P) as estimates of the Holocene climate variability (65). For topographic variables, we evaluated elevation (https://www.worldclim.org/data/2.5m) and calculated slope as the average difference in elevation across eight adjacent cells using the *terrain* function in the package raster (66). Annual temperature and precipitation, precipitation and temperature seasonality, minimum and maximum temperature (assessed in the coldest and hottest months respectively), minimum and maximum precipitation (assessed in the driest and wettest months respectively), slope and elevation were used to represent ecological niches. These factors constitute all major abiotic factors impacting the distribution, diversity, and performance of terrestrial mammals (67). All climatic and topographic conditions were measured by sampling the centroid of each 2.5 arc-minutes grid cell (approximately 5 × 5 km at the equator) as a compromise between spatial coverage and computational feasibility.

We assessed ecological marginalisation by calculating distance between niche centroids and average Mahalanobis distance of contemporary and historic niche points to the historic niche centroid. Firstly, climatic and topographic conditions of historic and contemporary niches were reduced to three principal dimensions using principal component analysis (PCA). PCA coordinates were separated into corresponding historic and contemporary niches. For each, a three-dimensional hypervolume was modelled using a one-class support vector machine (SVM) algorithm (68) (with default γ value of 0.5) using the "hypervolume" package (69). This one-class SVM estimates optimal features that best classify randomly generated points into "inside" or "outside" of the input dataset. Through this procedure, the SVM produces a "tight" fit shape around the input data where boundaries represent niche edges. A one-class SVM is the most suitable algorithm as presence-only data were used and the extremes of the input variables reflect the strict boundaries of species niche space (70). Niche loss was calculated as the proportional loss of hypervolume volume between historic and contemporary hypervolumes. Distance between centroids was calculated as Euclidean distance between contemporary and historic hypervolume centres.

For Mahalanobis distances, we used convex hull modelling (71) to model ecological niches at each time point as minimum-volume ellipsoids (MVE). A MVE for both historic and contemporary niche spaces was modelled using the first three principal components of environmental variables (as per hypervolumes). We then calculated the centroid of historic MVE using the cov_center function in the ntbox package (72). The Mahalanobis distance of all points to the historic niche centroid was calculated. The difference in average Mahalanobis distance between historic and contemporary MVEs was calculated as a Cohen's d effect size. Negative effect sizes occur when the species contracts to its historic core and positive effect sizes occur when species contracts to its historic periphery. Mahalanobis distances are calculated on the observed distribution only and account for the covariance of multiple environmental axes giving a more accurate representation of the distance in environmental space (73).

3.6.6 Ecological variables shift

To investigate variable shifts to ecological extremes, we also investigated the Cohen's d effect size shift in elevation, slope, annual temperature, and annual precipitation. These variables represent key abiotic characteristics that dictate species' distributions, niche dynamics and habitat selection across geographic space and time (74).

3.6.7 Modelling and data analysis

All data-analysis was performed in R (75). We used phylogenetic generalized least squares (PGLS) models to assess the effect of intrinsic variables and human transformation on the metrics of loss, marginalisation, shift, and extinction risk. PGLS models use phylogenetic relationships to account for the expected covariance between species from close phylogeny (76). PGLS models were conducted using "caper" package (78). We calculated Mahalanobis distances using the *Mahalanobis* function in the package *stats* (75). All GIS operations were conducted using the *raster* package (77). Random points were generated using *sp* package (75). All Cohen's *d* estimates at 95% confidence intervals were conducted using rstatix (75). Model selection maximised AICc of global models using dredge function in MuMIn package in R (75). Explanatory variables were transformed to Gaussian distributions to conform to model assumptions. Contemporary range size, body size, effect size of Mahalanobis distance (ecological marginalisation) and distance between niche centroids were log transformed. Geographic range loss, habitat diversity loss and human transformation were logit transformed. Geographic marginalisation was asin transformed. Absolute effect size of shift of annual precipitation, temperature, slope, and elevation were log transformed. Variable importance for each model was analysed by dropping variables from models one at a time to assess change in AIC and through summed weights in models within <5 delta AIC from best fit model using MuMIn::importance function from MuMIn package in R.

3.7 References

1. E. C. Ellis, *et al.*, People have shaped most of terrestrial nature for at least 12,000 years. *Proceedings of the National Academy of Sciences* **118** (2021)
2. E. C. Ellis, Anthropogenic transformation of the terrestrial biosphere. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* **369**, 1010–1035 (2011).
3. D. Tilman, *et al.*, Future threats to biodiversity and pathways to their prevention. *Nature 2017 546:7656* **546**, 73–81 (2017).
4. UNEP-WCMC and IUCN Protected Planet Report 2020. UNEP-WCMC and IUCN: Cambridge UK; Gland, Switzerland. (2020)
5. M. Kuussaari, *et al.*, Extinction debt: a challenge for biodiversity conservation. *Trends in Ecology & Evolution* **24**, 564–571 (2009).
6. G. I. H. Kerley, M. Beest, J. P. G. M. Cromsigt, D. Pauly, S. Shultz, The Protected Area Paradox and refugee species: The giant panda and baselines shifted towards conserving species in marginal habitats. *Conservation Science and Practice* **2** (2020).
7. G. I. H. Kerley, R. Kowalczyk, J. P. G. M. Cromsigt, Conservation implications of the refugee species concept and the European bison: King of the forest or refugee in a marginal habitat? *Ecography* **35**, 519–529 (2012).
8. R. Sayre, *et al.*, An assessment of the representation of ecosystems in global protected areas using new maps of World Climate Regions and World Ecosystems. *Global Ecology and Conservation* **21**, e00860 (2020).
9. O. Venter, *et al.*, Bias in protected-area location and its effects on long-term aspirations of biodiversity conventions. *Conservation Biology* **32**, 127–134 (2018).
10. L.N. Joppa, A. Pfaff, High and far: biases in the location of protected areas. *PLoS One*. 2009;**4**:e8273. (2009)
11. M. A. Huston, The three phases of land-use change: Implications for biodiversity. *Ecological Applications* **15**, 1864–1878 (2005).
12. E. C. Ellis, N. Ramankutty, Putting people in the map: Anthropogenic biomes of the world. *Frontiers in Ecology and the Environment* **6**, 439–447 (2008).
13. A. P. Jacobson, J. Riggio, A. M. Tait, J. E. M. Baillie, Global areas of low human impact (‘Low Impact Areas’) and fragmentation of the natural world. *Scientific Reports* **9**, 1–13 (2019).
14. E. C. Ellis, Anthromes. *Encyclopedia of the World’s Biomes* **5–5**, 5–11 (2020).
15. T. J. Kawecki, Adaptation to marginal habitats. *Annual Review of Ecology, Evolution, and Systematics* **39**, 321–342 (2008).

16. H. R. Pulliam, Sources, Sinks, and Population Regulation. *The American Naturalist* **132**, 652–661 (1988).
17. B. C. Scheele, C. N. Foster, S. C. Banks, D. B. Lindenmayer, Niche Contractions in Declining Species: Mechanisms and Consequences. *Trends in Ecology and Evolution* **32**, 346–355 (2017).
18. R. Channell, M. V. Lomolino, Trajectories to extinction: spatial dynamics of the contraction of geographic ranges of endangered species. *Journal of Biogeography* **27**, 169–180 (2000).
19. J. M. Lea, G. I. Kerley, H. Hrabar, T. J. Barry, S. Shultz, Recognition and management of ecological refugees: A case study of the Cape mountain zebra. *Biological Conservation* **203**, 207–215 (2016).
20. R. Cowell, T. Rangel, Hutchinson's duality: The once and future niche *Proceedings of the National Academy of Sciences* **106**, 19651-19658. (2009)
21. L. Osorio-Olvera, C. Yañez-Arenas, E. Martínez-Meyer, A. T. Peterson, Relationships between population densities and niche-centroid distances in North American birds. *Ecology Letters* **23**, 555–564 (2020).
22. S. Pironon, *et al.*, Geographic variation in genetic and demographic performance: new insights from an old biogeographical paradigm. *Biological Reviews* **92**, 1877–1909 (2017).
23. W. J. Ripple, *et al.*, Extinction risk is most acute for the world’s largest and smallest vertebrates. *Proceedings of the National Academy of Sciences of the United States of America* **114**, 10678–10683 (2017).
24. B. E. Crowley, *et al.*, Extinction and ecological retreat in a community of primates. *Proceedings of the Royal Society B: Biological Sciences* **279**, 3597–3605 (2012).
25. J. H. Brown, On the Relationship between Abundance and Distribution of Species. *The American Naturalist*. **124**, 255–279 (1984).
26. E. Martínez-Meyer, D. Díaz-Porras, A. T. Peterson, C. Yáñez-Arenas, Ecological niche structure and rangewide abundance patterns of species. *Biology Letters* **9** (2013).
27. R. D. Holt, *et al.*, Theoretical models of species’ borders: single species approaches. *Oikos* **108**, 18–27 (2005).
28. R. D. Sagarin, S. D. Gaines, B. Gaylord, Moving beyond assumptions to understand abundance distributions across the ranges of species. *Trends in Ecology & Evolution* **21**, 524–530 (2006).
29. J. E. Lee, C. Janion, E. Marais, B. J. van Vuuren, S. L. Chown, Physiological tolerances account for range limits and abundance structure in an invasive slug. *Proceedings of the Royal Society B: Biological Sciences* **276**, 1459 (2009).
30. G. Caughley, D. Grice, R. Barker, B. Brown, The Edge of the Range. *The Journal of Animal Ecology* **57**, 771 (1988).

31. M. Chevalier, O. Broennimann, A. Guisan, Using a robust multi-settings inference framework on published datasets still reveals limited support for the abundant centre hypothesis: More testing needed on other datasets. *Global Ecology and Biogeography* **30**, 2211–2228 (2021).

32. L. Osorio-Olvera, J. Soberón, M. Falconi, On population abundance and niche structure. *Ecography* **42**, 1415–1425 (2019).

33. L. Santini, S. Pironon, L. Maiorano, W. Thuiller, Addressing common pitfalls does not provide more support to geographical and ecological abundant-centre hypotheses. *Ecography* **42**, 696–705 (2019)

34. R. C. Burner, A. R. Styring, M. A. Rahman, F. H. Sheldon, Occupancy patterns and upper range limits of lowland Bornean birds along an elevational gradient. *Journal of Biogeography* **46**, 2583–2596 (2019).

35. A. Lira-Noriega, J. D. Manthey, Relationship of genetic diversity and niche centrality: a survey and analysis. *Evolution* **68**, 1082–1093 (2014).

36. J. D. Manthey, *et al.*, A test of niche centrality as a determinant of population trends and conservation status in threatened and endangered North American birds. *Endangered Species Research* **26**, 201–208 (2015).

37. P. G. Martínez-Gutiérrez, E. Martínez-Meyer, F. Palomares, N. Fernández, Niche centrality and human influence predict rangewide variation in population abundance of a widespread mammal: The collared peccary (*Pecari tajacu*). *Diversity and Distributions* **24**, 103–115 (2018).

38. T. Dallas R.R. Decker, A. Hastings, Species are not most abundant in the centre of their geographic range or climatic niche. *Ecology Letters*, **20** 1526-1533 (2017)

39. R.D. Holt, Reflections on niches and numbers. *Ecography*, **43**, 387-390 (2020)

40. G. Caughley, Directions in Conservation Biology. *The Journal of Animal Ecology* **63**, 215 (1994).

41. A. Purvis, J. L. Gittleman, G. Cowlishaw, G. M. Mace, Predicting extinction risk in declining species. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **267**, 1947–1952 (2000).

42. A. Benítez-López, *et al.*, The impact of hunting on tropical mammal and bird populations. *Science* **356**, 180–183 (2017).

43. D. O. Fisher, Trajectories from extinction: Where are missing mammals rediscovered? *Global Ecology and Biogeography* **20**, 415–425 (2011).

44. T. P. Hughes, D. R. Bellwood, S. R. Connolly, H. v. Cornell, R. H. Karlson, Double jeopardy and global extinction risk in corals and reef fishes. *Current biology* **24**, 2946–2951 (2014).

45. M. Pacifici, M. di Marco, J. E. M. Watson, Protected areas are now the last strongholds for many imperiled mammal species. *Conservation Letters* **13**, e12748 (2020).

46. J. O. Hanson, *et al.*, Global conservation of species' niches. *Nature* **580**, 232–234 (2020).

47. D. Williams, C Rondinini, D Tilman, Global protected areas seem insufficient to safeguard half of the world's mammals from human-induced extinction *Proceedings of the National Academy of Sciences* **119,** e2200118119 (2022)

48. A. Robson, *et al.*, Over 80% of Africa's savannah conservation land is failing or deteriorating according to lions as an indicator species. *Conservation Letters*, e12844 (2021).

49. S. Rada, *et al.*, Protected areas do not mitigate biodiversity declines: A case study on butterflies. *Diversity and Distributions* **25**, 217–224 (2019).

50. C. R. Margules, R. L. Pressey, Systematic conservation planning. *Nature* **405**, 243–253 (2000).

51. Plumb, G., Kowalczyk, R. & Hernandez-Blanco, J.A. *Bison bonasus*. *The IUCN Red List of Threatened Species* 2020: e.T2814A45156279 (2020)

52. J. A. Britnell, *et al.*, Species stereotypes as a result of unconscious research biases compromise conservation efficacy. *Biological Conservation* **261**, 109275 (2021).

53. G. I. H. Kerley & S. Monsarrat, Shifted models cannot be used for predicting responses of biodiversity to global change: the African elephant as an example. *African Zoology,* **57** 70-73 (2022)

54. S. Monsarrat, P.A. Novellie, I. Rushworth, G.I.H. Kerley, Shifted distribution baselines: neglecting long-term biodiversity records risks overlooking potentially suitable habitat for conservation management. *Philosophical Transactions of the Royal Society B* **374** (2019).

55. L. P. Sales, *et al.*, The effect of past defaunation on ranges, niches, and future biodiversity forecasts. *Global Change Biology* (2022).

56. K. E. Jones, *et al.*, PanTHERIA: a species-level database of life history, ecology, and geography of extant and recently extinct mammals. *Ecology* **90**, 2648–2648 (2009).

57. C. D. Soria, *et al.*, COMBINE: a coalesced mammal database of intrinsic and extrinsic traits. *Ecology* **102**, e03344 (2021).

58. S. Faurby, *et al.*, PHYLACINE 1.2: The Phylogenetic Atlas of Mammal Macroecology. *Ecology* **99**, 2626 (2018).

59. M. di Marco, M. Pacifici, L. Maiorano, C. Rondinini, Drivers of change in the realised climatic niche of terrestrial mammals. *Ecography* **44** 1180-1190 (2021)

60. E. Dinerstein, *et al.*, An Ecoregion-Based Approach to Protecting Half the Terrestrial Realm. *BioScience* **67**, 534–545 (2017).

61. E. C. Ellis, K. K. Goldewijk, S. Siebert, D. Lightman, N. Ramankutty, Anthropogenic transformation of the biomes, 1700 to 2000. *Global Ecology and Biogeography* **19**, 589–606 (2010).

62. O. Broennimann, *et al.*, Distance to native climatic niche margins explains establishment success of alien mammals. *Nature Communications 2021 12:1* **12**, 1–8 (2021).

63. S. E. Fick, R. J. Hijmans, WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology* **37**, 4302–4315 (2017).

64. R. J. Hijmans, S. E. Cameron, J. L. Parra, P. G. Jones, A. Jarvis, Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* **25**, 1965–1978 (2005).

65. R. Knutti, D. Masson, A. Gettelman, Climate model genealogy: Generation CMIP5 and how we got there. *Geophysical Research Letters* **40**, 1194–1199 (2013).

66. R.J. Hijmans, J van Etten, raster: Geographic data analysis and modeling. The R Foundation for Statistical Computing, Vienna, R package version 2.2-31. (2014). https://cran.r-project.org/

67. P. Visconti, *et al.*, Projecting Global Biodiversity Indicators under Future Development Scenarios. *Conservation Letters* **9**, 5–13 (2016).

68. J. M. Drake, C. Randin, A. Guisan, Modelling ecological niches with support vector machines. *Journal of Applied Ecology* **43**, 424–432 (2006).

69. B. Blonder, C. Lamanna, C. Violle, B. J. Enquist, The n-dimensional hypervolume. *Global Ecology and Biogeography* **23**, 595–609 (2014).

70. B. Blonder, *et al.*, New approaches for delineating n-dimensional hypervolumes. *Methods in Ecology and Evolution* **9**, 305–319 (2018).

71. H. Qiao, *et al.*, NicheA: creating virtual species and ecological niches in multivariate environmental scenarios. *Ecography* **39**, 805–813 (2016).

72. L. Osorio-Olvera, *et al.*, ntbox: An r package with graphical user interface for modelling and evaluating multidimensional ecological niches. *Methods in Ecology and Evolution* **11**, 1199–1206 (2020).

73. J. Soberón, A. T. Peterson, L. Osorio-Olvera, A comment on "Species are not most abundant in the centre of their geographic range or climatic niche." *Rethinking Ecology 3: 13-18* **3**, 13–18 (2018).

74. M. W. Tingley, W. B. Monahan, S. R. Beissinger, C. Moritz, Birds track their Grinnellian niche through a century of climate change. *Proceedings of the National Academy of Sciences* **106**, 19637–19643 (2009).

75. R Core Team R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. (2021). https://www.R-project.org/.

76. M. R., Symonds, & S. P. Blomberg. A primer on phylogenetic generalised least squares. In *Modern phylogenetic comparative methods and their application in evolutionary biology*, 105-130. Springer, Berlin, Heidelberg. (2014).

77. E. Paradis, J. Claude & K. Strimmer, APE: analyses of phylogenetics and evolution in R language. *Bioinformatic*s **20**, 289–29 (2004)

78. D. Orme, R. Freckleton, G. Thomas, T. Petzoldt, S. Fritz, N. Issac & W. Pearse caper: comparative analysis of phylogenetics and evolution in R. R package v. 0.5.2 (2013) http://CRAN.R-project.org/package=caper.

3.8 Supplementary information

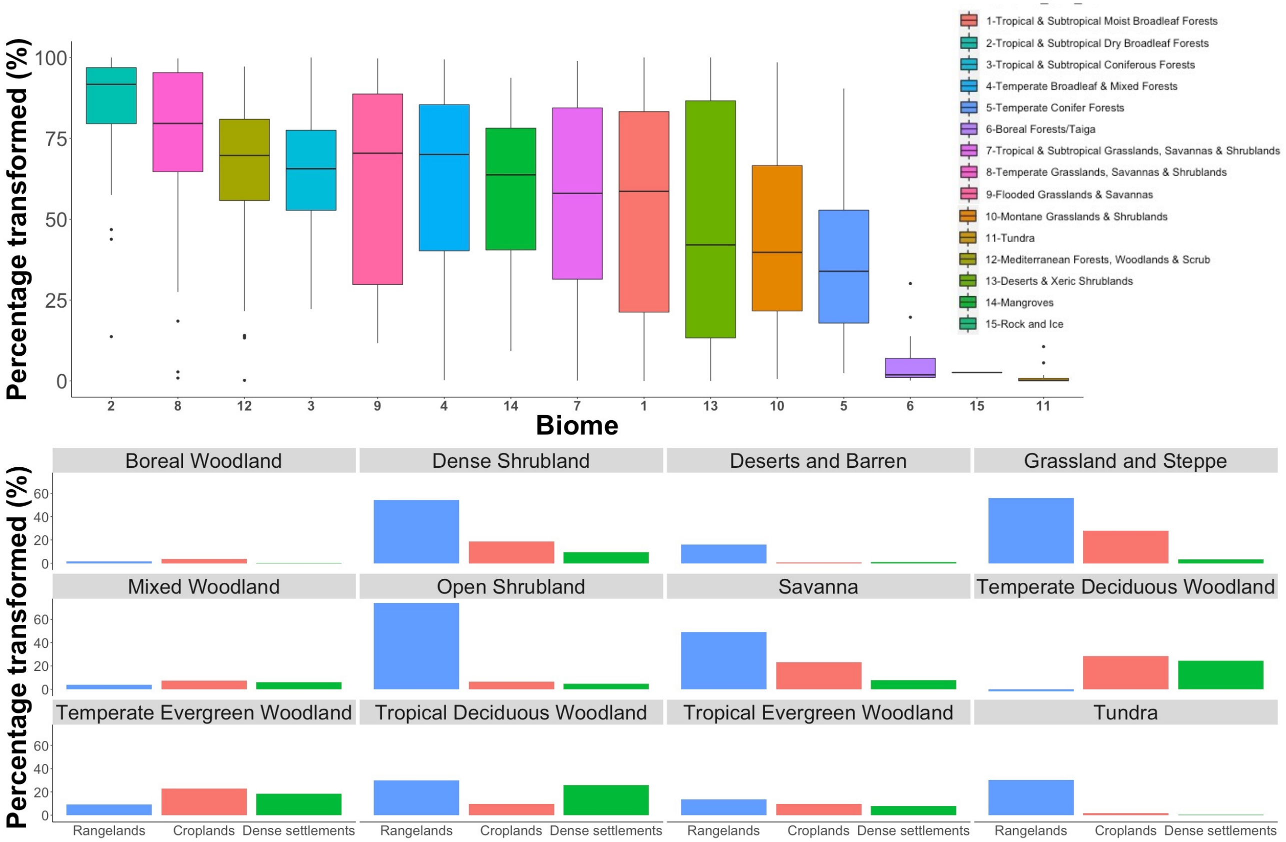


Figure S3.1) Human land-use disproportionately impacts specific biomes. **Top -** Variation in percentage transformation of Olsen's biome types (Adapted from Jacobson *et al*, 2019). **Bottom -** variation in biome type habitat conversion each human landuses category (dense settlements (urban villages and rural settlements) and Croplands and Rangelands (substantial/significant human populations). (Adapted from Ellis *et al*, 2011).

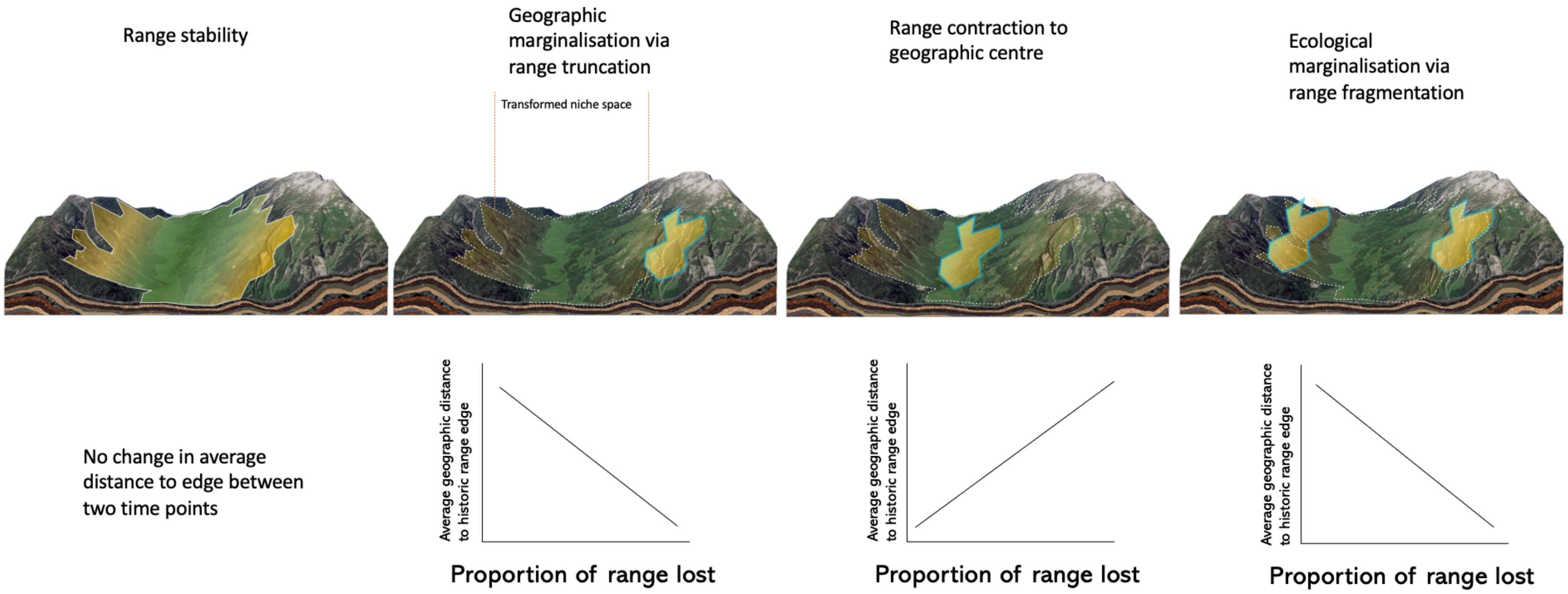


Figure S3.2) Visual conceptualisation of geographic marginalisation and the average minimum geographic distances to an historic range edge predictions. Depicted here is a conceptualized range centred on a valley basin and bounded by upper elevations of adjacent uplands, with potential outcomes of range contraction within this system. Top) possible range dynamics under geographic range contraction, green to yellow gradient represents geographic core to periphery. Blue outlines depict contemporary ranges following range contraction. Bottom) predictions of average minimum geographic distances to an historic range as range contraction continues.

Table S3.1) Extent of geographic range loss and habitat diversity loss across mammals. Proportion of area and ecoregions lost between present_natural (pre-anthropogenic) and contemporary Phylacine ranges. The accuracy of range loss may be biased by historical information available for the species and extent of study. Degree of range contraction and habitat diversity loss may be underestimated for species with little historical information or those that have been poorly studied. This may explain the higher percentage of species displaying no range loss.

Geographic range loss			
	Degree of contraction	Number of species	Percentage of species (%)
	Occupy entire historic range (0% loss)	4139	86.5
	Mild levels of geographic range loss (<25% historic range lost)	167	3.5
	Moderate levels of geographic range loss (<25-50% historic range lost)	176	3.7
	High levels of geographic range loss (<50-75% historic range lost)	140	2.9
	Very high levels of geographic range loss (<75-100% historic range lost)	144	3
	Range expansion	19	0.4
Habitat diversity			
	Degree of loss	Number of species	Percentage of species (%)
	Occupy entire historic range (0% loss)	4357	91.0
	Mild levels of habitat diversity loss (<25% ecoregions lost)	195	4.1
	Moderate levels of geographic range loss (<25-50% ecoregions lost)	108	2.3
	High levels of geographic range loss (<50-75% ecoregions lost)	57	1.2
	Very high levels of geographic range loss (<75-100% ecoregions lost)	52	1.1
	Increase in habitat diversity	16	0.3

Table S3.2) Large body size, small geographic range and large proportion of historic range transformed for human landuse are associated with geographic range loss. *ANOVA table of best model fit from dredge function of pgls(formula = range_quartile ~ order + log(body_weight) + log(geographic_range_size)+ logit(human_transformation), data = mammal.phy.glm.range.loss, lambda = "ML".* λ =0.67, df: 4131, adjusted R^2 = 0.09, AICc = 9502.7 **p<0.05, **p< 0.01, ***p<0.001*

Variable	**df**	**Slope**	**se**	**F**	***P***	**ΔAIC**	**Σ model weights**
Body mass	1, 4156	0.11	0.014	71.5	<0.001***	68.04	1
Geographic range size	1, 4156	-0.25	0.014	350.8	<0.001***	334.97	1
Human transformation	1, 4156	0.40	0.14	9.6	0.004**	5.93	1
Residuals	4156						

Table S3.3) Dredge table for ordinal range contraction across all terrestrial mammals - *global model: pgls(formula = range_quartile ~ order + log(body_weight) + log(geographic_range_size)+ logit(human_transformation), data = mammal.phy.glm.range.loss, lambda = "ML". *denotes variable is not included in the model, models are ranked by AICc, only models within AIC < 5 from the best fit model are displayed**denotes variable is included in the model, models are ranked by AICc, only models within AIC < 5 from the best for model are displayed. Df = 4131.

	Variable									
Model	(Int) (1)	Body size (2)	Human transformation (3)	Range size (4)	Order (5)	Df	Log lik	AICc	Delta	Weight
1234	2.58	0.11	0.39	-0.25	*	4	-4747.3	9502.7	0	0.951

Table S3.4) Summary of significant (Cohen's d >0.2) displacement to climatic and topographic extremes

Annual temperature

	Number of species with absolute Cohen's *d > 0.2*	Number of species assessed	Percentage displaying significant displacement to extremes (%)	Hotter extreme of historic niche	Colder extreme of historic niche
	483	587	75	403	35

Annual precipitation

	Number of species with absolute Cohen's *d > 0.2*	Number of species assessed	Percentage displaying significant displacement to extremes (%)	Drier extreme of historic niche	Wetter extreme of historic niche
	391	587	66.6	225	166

Elevation

	Number of species with absolute Cohen's *d > 0.2*	Number of species assessed	Percentage displaying significant displacement to extremes (%)	High elevation extremes of historic niche	Low elevation extremes of historic niche
	183	587	31.2	87	96

Slope

	Number of species with absolute Cohen's *d > 0.2*	Number of species assessed	Percentage displaying significant displacement to extremes (%)	High slope of historic niche	Low slope of historic niche
	130	587	22.1	80	50

Table S3.5) Dredge table for geographic range contraction in range contracted species - *global model pgls(logit(geographic range loss) ~ order + log(body mass) + log(geographic range size) + logit(human transformation) + log(body mass) * log(geographic range size) + log(body mass) *logit(human transformation), data = mammal.phy.glm.range.loss, lambda = "ML") ". *denotes variable is not included in the model, models are ranked by AICc, only models within AIC < 5 from the best fit model are displayed. DF= 585*

Intercept	Body mass	Human transformation	Range size	Order	Body mass: human transformation	Body mass: geographic range size	Df	Log lik	AICc	Delta	Weight
7.356	0.029	0.149	-1.679	*	*	0.034	5	-1041.01	2092.11	0	0.38
6.030	0.224	0.141	-1.439	*	*	*	4	-1042.23	2092.53	0.41	0.31
7.314	0.041	0.207	-1.686	*	-0.009	0.034	6	-1040.78	2093.71	1.59	0.17
5.996	0.234	0.201	-1.447	*	-0.010	*	5	-1041.99	2094.08	1.96	0.14

Table S3.6) Dredge table for habitat diversity loss *with global model: pgls(logit(geographic range loss) ~ order + log(body mass) + log(geographic range size) + logit(*proportion of agricultural and rangelands in historic range*) + log(body mass) * log(geographic range size) + log(body mass) *logit(*proportion of agricultural and rangelands in historic range*), data = mammal.phy.glm.range.loss, lambda = "ML"). *denotes variable is not included in the model, models are ranked by AICc, only models within AIC < 5 from the best fit model are displayed.* Df = 585

Model	**(Intercept) (1)**	**Body mass (2)**	**Geographic range loss (3)**	**Agricultural and Rangelands (4)**	**Geographic range size (5)**	**Order (6)**	**Body mass* Geographic range loss (7)**	**Body mass* Agricultural and Rangelands (8)**	**Body mass* Geographic range size (9)**	**df**	**logLik**	**AICc**	**delta**	**weight**
123457	-0.118	0.012	0.053	0.007	0.051	*	0.006	*	*	6	298.162	-584.180	0.000	0.224
1234579	-0.267	0.032	0.060	0.006	0.079	*	0.005	*	-0.004	7	299.179	-584.165	0.015	0.222
123579	-0.297	0.034	0.062	*	0.086	*	0.005	*	-0.004	6	297.976	-583.808	0.372	0.186
12357	-0.130	0.011	0.054	*	0.055	*	0.006	*	NA	5	296.679	-583.254	0.925	0.141
1234578	-0.118	0.012	0.053	0.014	0.050	*	0.006	-0.001	NA	7	298.536	-582.880	1.300	0.117

Table S3.7) Dredge for table proportion of niche loss *using global model: pgls(formula = asin(hypervolume_loss) ~ order + log(body_weight) + log(geographic_range_size)+ logit(geographic_range_loss)+ logit(human_transformation), data = mammal.phy.glm.hypervolume.loss, lambda = "ML") *denotes variable is not included in the model, models are ranked by AICc, only models within AIC < 5 from the best fit model are displayed.*Df = 560.

Model	(Intercept) (1)	Body mass (2)	Geographic range loss (3)	Human Transformation (4)	Geographic range size (5)	Order (6)	Body mass:Geographic range loss (7)	Body mass:Human Transformation (8)	Body mass:Geographic range size (9)	df	logLik	AICc	delta	weight
13	0.32	*	0.15	*	*	*	*	*	*	2	-268.83	541.68	0.00	0.14
123	0.21	0.02	0.14	*	*	*	*	*	*	3	-267.92	541.88	0.19	0.13
1234	0.23	0.02	0.14	-0.01	*	*	*	*	*	4	-266.97	542.01	0.32	0.12
1237	0.21	0.02	0.12	*	*	*	0.00	*	*	4	-267.35	542.78	1.10	0.08
12347	0.23	0.02	0.12	-0.01	*	*	0.00	*	*	5	-266.41	542.93	1.24	0.08
135	0.25	*	0.15	*	0.01	*	*	*	*	3	-268.70	543.45	1.76	0.06
134	0.32	*	0.15	-0.01	*	*	*	*	*	3	-268.72	543.48	1.79	0.06
1235	0.19	0.02	0.14	*	0.00	*	*	*	*	4	-267.90	543.88	2.20	0.05
12345	0.20	0.02	0.14	-0.01	0.01	*	*	*	*	5	-266.92	543.96	2.27	0.05
12348	0.24	0.02	0.14	-0.02	*	*	*	0.00	*	5	-266.96	544.02	2.34	0.04
12357	0.21	0.02	0.12	*	0.00	*	0.00	*	*	5	-267.35	544.82	3.13	0.03
123457	0.21	0.02	0.12	-0.01	0.00	*	0.00	*	*	6.00	-266.40	544.95	3.27	0.03
123478	0.23	0.02	0.12	-0.02	*	*	0.00	0.00	*	6.00	-266.41	544.96	3.28	0.03
123459	0.02	0.04	0.15	-0.02	0.04	*	*	*	0.00	6.00	-266.63	545.40	3.72	0.02
12359	0.06	0.04	0.14	*	0.03	*	*	*	0.00	5.00	-267.75	545.60	3.92	0.02
123458	0.20	0.02	0.14	-0.02	0.01	*	*	0.00	*	6.00	-266.91	545.97	4.29	0.02

Table S3.8) Human transformation restricts species to temperature extremes of historic niche. *ANOVA table of best model fit from dredge function of global model* pgls(log(abs(Temperature shift+0.001)) ~ logit(geographic range loss) + order + log(body mass) + log (geographic range size) + logit(human transformation) + log(body mass) *logit(geographic range loss) + log(body mass)* log (geographic range size) + log(body mass)*logit(human_transformation) , data =mammal.phy.glm.BIO1.loss, lambda = "ML"). λ =0.00, df= 578, R^2 = 0.06, AICc = 1787.5. *Delta AIC (***ΔAIC***) was estimated by dropping each variable from the best model following dredge results. Human transformation denotes the proportion of historic range converted to rangelands, agricultural lands or dense settlements. *p<0.05, **p< 0.01, ***p<0.001*

Variable	**df**	**Slope**	**s.e.**	**F**	***P***	**ΔAIC**	**Σ model weights**
Body mass	1, 578	0.031	0.015	0.24	0.62	2.21	0.85
Human transformation	1, 578	0.060	0.030	5.31	0.022*	5.19	0.81
Geographic range size	1, 578	-0.35	0.058	8.9	0.001***	29.24	1

Table S3.9) Dredge table for proportion of absolute effect size change in annual temperature between historic and contemporary ranges using global model: pgls(log(abs(Temperature shift+0.001)) ~ logit(geographic range loss) + order + log(body mass) + log (geographic range size) + logit(human transformation) + log(body mass) *logit(geographic range loss) + log(body mass)* log (geographic range size) + log(body mass)*logit(human_transformation) , data =mammal.phy.glm.BIO1.loss, lambda = "ML").*)* *denotes variable is not included in the model, models are ranked by AICc, only models within AIC < 5 from the best fit model are displayed. Df = 578

(Intercept) (1)	Body mass (2)	Geographic range loss ()	Human Transformation	Geographic range size	Order	Body mass: Geographic range loss	Body mass:Human Transformation	Body mass: Geographic range size	df	logLik	AICc	delta	weight
0.63	0.03	NA	0.06	-0.35	NA	NA	NA	NA	4	-889.72	1787.52	0.00	0.22
0.68	0.03	-0.01	0.06	-0.36	NA	NA	NA	NA	5	-889.69	1789.48	1.97	0.08
0.64	0.03	NA	0.05	-0.35	NA	NA	0.00	NA	5	-889.72	1789.54	2.02	0.08
0.68	0.02	NA	0.06	-0.36	NA	NA	NA	0.00	5	-889.72	1789.55	2.03	0.08
0.74	0.03	NA	NA	-0.35	NA	NA	NA	NA	3	-891.77	1789.58	2.06	0.08
0.60	NA	NA	0.05	-0.30	NA	NA	NA	NA	3	-891.85	1789.73	2.21	0.07
0.70	0.03	-0.06	0.06	-0.37	NA	0.01	NA	NA	6	-889.20	1790.54	3.02	0.05
0.71	NA	NA	NA	-0.31	NA	NA	NA	NA	2	-893.53	1791.09	3.57	0.04
0.68	0.03	-0.01	0.05	-0.36	NA	NA	0.00	NA	6	-889.68	1791.51	4.00	0.03
0.74	0.02	-0.01	0.06	-0.37	NA	NA	NA	0.00	6	-889.69	1791.52	4.00	0.03

Table S3.10) Geographic range contraction and human transformation restricts species to precipitation extremes of historic niche. ANOVA table for Precipitation shift in range contracted species. *ANOVA table of best model fit from dredge function of global model of pgls(log(abs(BIO12_shift))* ~ logit(geographic range loss) + order + log(body mass) + log (geographic range size) + logit(human transformation) + log(body mass) *logit(geographic range loss) + log(body mass)* log (geographic range size) + log(body mass)*logit(human_transformation) , data =mammal.phy.glm.BIO12.loss, lambda = "ML").. *Delta AIC (***ΔAIC***) was estimated by dropping each variable from the best model following dredge results. Human transformation denotes the proportion of historic range converted to rangelands, agricultural lands or dense settlements. *p<0.05, **p< 0.01, ***p<0.001.* Body size, contemporary range size and effect size in annual precipitation has been log transformed λ =0.0, delta = 1.00, kappa = 1.00, df = 578, adjusted R^2= 0.19, AICc = 1807.5

Variable	**df**	**Slope**	**s.e.**	**F**	***P***	**ΔAIC**	**Σ model weight**
Geographic range size	1, 578	-0.42	0.062	45.80	0.001***	35.67	1
Geographic range loss	1, 578	0.14	0.030	92.42	0.001***	19.53	1
Human transformation	1, 578	0.06	0.030	4.89	0.03*	2.46	0.84

Table S3.11) **Dredge table for absolute effect size change in annual precipitation between historic and contemporary ranges** using global model: pgls(log(abs(BIO12_shift))) ~ logit(geographic range loss) + order + log(body mass) + log (geographic range size) + logit(human transformation) + log(body mass) *logit(geographic range loss) + log(body mass)* log (geographic range size) + log(body mass)*logit(human_transformation) , data =mammal.phy.glm.BIO12.loss, lambda = "ML"). . *denotes variable is not included in the model, models are ranked by AICc, only models within AIC < 5 from the best fit model are displayed.DF= 585

(Intercept)	Body mass	Geographic range loss	Human Transformation	Geographic range size	Order	Body mass* Geographic range loss	Body mass* Human Transformation	Body mass* Geographic range size	df	logLik	AICc	delta	weight
0.94	*	0.14	0.06	-0.42	*	*	*	*	4	-899.77	1807.61	0.00	0.36
0.94	0.00	0.14	0.06	-0.42	*	*	*	*	5	-899.77	1809.65	2.03	0.13
1.02	*	0.15	*	-0.42	*	*	*	*	3	-902.02	1810.07	2.46	0.10
0.95	-0.01	0.14	-0.01	-0.41	*	*	0.01	*	6	-899.21	1810.56	2.95	0.08
0.96	0.00	0.09	0.06	-0.43	*	0.01	*	*	6	-899.37	1810.89	3.28	0.07
0.54	0.06	0.14	0.06	-0.35	*	*	*	-0.01	6	-899.60	1811.34	3.73	0.06
0.97	-0.01	0.09	0.00	-0.42	*	0.01	0.01	*	7	-898.87	1811.93	4.32	0.04
1.00	0.00	0.15	*	-0.41	*	*	*	*	4	-901.97	1812.01	4.39	0.04
0.59	0.04	0.14	-0.01	-0.34	*	*	0.01	-0.01	7	-899.06	1812.32	4.71	0.03

Table S3.12) Geographic range contraction and human transformation restricts species to elevational extremes of historic niche. *ANOVA table of best model fit from dredge function of global model pgls(log(abs(Elevation_shift + 0.01)))* ~ logit(geographic range loss) + order + log(body mass) + log (geographic range size) + logit(human transformation) + log(body mass) *logit(geographic range loss) + log(body mass)* log (geographic range size) + log(body mass)*logit(human_transformation) , data =mammal.phy.glm.elevation.loss, lambda = "ML"), *Body size, contemporary range size and effect size in elevation has been log transformed* λ =0.06, delta = 1.00, kappa = 1.00, df = 579, adjusted R^2= 0.32, AICc = 1906.4.

Variable	**df**	**Slope**	**s.e.**	**F**	***P***	**∆AIC**	**Σ model weight**
Geographic range loss	1, 579	0.46	0.029	271.46	0.001***	211.04	1
Human transformation	1, 579	0.10	0.034	7.96	0.004*	5.87	1

Table S3.13) Dredge table for absolute effect size change in elevation between historic and contemporary ranges using global modelpgls(formula = log(abs(elevation_shift + 0.01)) ~ order + logbody + logrange + logit(human_transformation) + logit(geographic_range_loss), data = mammal.phy.glm.elevation.loss, lambda = "ML")*denotes variable is not included in the model, models are ranked by AICc, only models within AIC < 5 from the best fit model are displayed. DF= 579.

Intercept	Body mass	Geographic range loss	Human Transformation	Geographic range size	Order	Body mass* Geographic range loss	Body mass* Human Transformation	Body mass* Geographic range size	df	logLik	AICc	delta	weight
-2.39	*	0.46	0.10	*	*	*	*	*	3	-950.19	1906.42	0.00	0.27
-2.31	-0.03	0.47	0.08	*	*	*	*	*	4	-949.71	1907.48	1.06	0.16
-2.29	*	0.46	0.10	-0.02	*	*	*	*	4	-950.16	1908.38	1.96	0.10
-2.26	-0.04	0.47	0.03	*	*	*	0.01	*	5	-949.46	1909.02	2.60	0.07
-2.51	-0.04	0.48	0.08	0.04	*	*	*	*	5	-949.55	1909.19	2.78	0.07
-2.31	-0.03	0.47	0.08	*	*	0.00	*	*	5	-949.70	1909.51	3.09	0.06
-2.50	-0.05	0.48	0.02	0.05	*	*	0.01	*	6	-949.21	1910.58	4.16	0.03
-2.11	-0.09	0.48	0.09	-0.03	*	*	*	0.01	6	-949.39	1910.93	4.51	0.03
-1.73	-0.13	0.47	0.03	-0.08	*	*	0.01	0.02	7	-948.40	1910.99	4.57	0.03
-2.26	-0.04	0.47	0.03	*	*	0.00	0.01	*	6	-949.45	1911.05	4.64	0.03
-2.51	-0.04	0.48	0.08	0.04	*	0.00	*	*	6	-949.55	1911.24	4.82	0.02
-1.49	-0.15	0.43	0.09	-0.14	*	0.01	*	0.02	7	-948.54	1911.28	4.86	0.02

Table S3.14) Geographic range contraction and human transformation restricts species to topographically extreme portions of historic niche. *ANOVA table of best model fit from dredge function of global model:* ~ logit(geographic range loss) + order + log(body mass) + log (geographic range size) + logit(human transformation) + log(body mass) *logit(geographic range loss) + log(body mass)* log (geographic range size) + log(body mass)*logit(human_transformation) , data =mammal.phy.glm.slope.loss, lambda = "ML"). λ =0, delta = 1.00, kappa = 1.00, df = 579, adjusted R^2= 0.29, AICc = 1951.1

Variable	**df**	**Slope**	**s.e.**	**F**	***P***	**ΔAIC**	**Σ Model weights**
Body mass	1, 579	-0.036	0.016	1.38	0.23	3.12	0.80
Geographic range loss	1, 579	0.46	0.030	238.56	<0.001***	192.76	1

Table S3.15) Dredge table proportion of absolute effect size of slope using global model: *pgls(formula = log(abs(slope_shift + 0.01~* logit(geographic range loss) + order + log(body mass) + log (geographic range size) + logit(human transformation) + log(body mass) *logit(geographic range loss) + log(body mass)* log (geographic range size) + log(body mass)*logit(human_transformation) , data =mammal.phy.glm.slope.loss, lambda = "ML"). . *denotes variable is included in the model, models are ranked by AICc, only models within AIC < 5 from the best fit model are displayed. DF= 579.

(Intercept)	**Body mass**	**Geographic range loss**	**Human Transformation**	**Geographic range size**	**Order**	**Body mass* Geographic range loss**	**Body mass* Human Transformation**	**Body mass* Geographic range size**	**df**	**logLik**	**AICc**	**delta**	**weight**
-2.45	-0.04	0.46	*	*	*	*	*	*	3	-972.51	1951.07	0.00	0.15
-2.02	-0.03	0.43	*	-0.09	*	*	*	*	4	-971.82	1951.72	0.65	0.11
-1.90	*	0.42	*	-0.15	*	*	*	*	3	-972.85	1951.74	0.68	0.11
-2.42	-0.04	0.46	-0.02	*	*	*	*	*	4	-972.28	1952.62	1.56	0.07
-2.52	-0.02	0.46	0.08	*	*	*	-0.02	*	5	-971.36	1952.83	1.76	0.06
-2.02	-0.01	0.43	0.10	-0.11	*	*	-0.02	*	6	-970.41	1952.96	1.90	0.06
-2.45	-0.04	0.46	*	*	*	0.00	*	*	4	-972.51	1953.09	2.03	0.06
-2.00	-0.03	0.44	-0.02	-0.09	*	*	*	*	5	-971.63	1953.36	2.30	0.05
-2.32	0.02	0.43	*	-0.04	*	*	*	-0.01	5	-971.74	1953.59	2.53	0.04
-1.88	*	0.42	-0.01	-0.15	*	*	*	*	4	-972.76	1953.60	2.53	0.04
-2.02	-0.03	0.42	*	-0.10	*	0.00	*	*	5	-971.81	1953.73	2.66	0.04
-2.69	*	0.45	*	*	*	*	*	*	2	-975.08	1954.18	3.12	0.03
-2.42	-0.04	0.46	-0.02	*	*	0.00	*	*	5	-972.28	1954.66	3.59	0.03
-2.46	0.05	0.44	0.10	-0.03	*	*	-0.02	-0.01	7	-970.24	1954.67	3.60	0.03
-2.52	-0.02	0.46	0.08	*	*	0.00	-0.02	*	6	-971.36	1954.86	3.80	0.02
-2.01	-0.01	0.42	0.10	-0.11	*	0.00	-0.02	*	7	-970.38	1954.95	3.88	0.02
-2.38	0.03	0.44	-0.02	-0.02	*	*	*	-0.01	6	-971.50	1955.15	4.09	0.02
-2.00	-0.03	0.43	-0.02	-0.09	*	0.00	*	*	6	-971.62	1955.38	4.32	0.02
-2.34	0.02	0.44	*	-0.03	*	0.00	*	-0.01	6	-971.74	1955.63	4.57	0.02
-2.68	*	0.45	-0.01	*	*	*	*	*	3	-975.01	1956.05	4.99	0.01

Table S3.16) Dredge table of ecological marginalisation (effect size of Mahalanobis distance) using global model: *pgls(ecological_marginalisation ~ logit(geographic_range_loss) + order + logbody + logrange + logit(human_transformation) + logbody*logit(geographic_range_loss) + logbody*logrange + logbody*logit(human_transformation) , data =mammal.phy.glm.geographic_marginalisation.loss, lambda = "ML"* *denotes variable is included in the model, models are ranked by AICc, only models within AIC < 5 from the best fit model are displayed. Df = 559.

(Intercept)	**Body mass**	**Geographic range loss**	**Human Transformation**	**Geographic range size**	**Order**	**Body mass* Geographic range loss**	**Body mass* Human Transformation**	**Body mass* Geographic range size**	**df**	**logLik**	**AICc**	**delta**	**weight**
5.50	*	0.21	*	-1.19	*	*	*	*	3	-992.59	1991.22	0.00	0.31
5.54	*	0.21	-0.04	-1.19	*	*	*	*	4	-992.16	1992.39	1.17	0.17
5.45	-0.01	0.21	*	-1.17	*	*	*	*	4	-992.48	1993.03	1.81	0.12
5.48	-0.01	0.22	-0.04	-1.17	*	*	*	*	5	-991.98	1994.06	2.85	0.07
5.50	-0.03	0.22	-0.15	-1.15	*	*	0.02	*	6	-991.06	1994.27	3.05	0.07
5.48	-0.01	0.17	*	-1.18	*	0.01	*	*	5	-992.29	1994.68	3.47	0.05
5.73	-0.05	0.21	*	-1.22	*	*	*	0.01	5	-992.42	1994.95	3.74	0.05
5.50	-0.01	0.18	-0.04	-1.17	*	0.01	*	*	6	-991.80	1995.74	4.52	0.03
5.52	-0.03	0.19	-0.15	-1.15	*	0.00	0.02	*	7	-990.93	1996.06	4.85	0.03
5.64	-0.04	0.22	-0.04	-1.20	*	*	*	0.00	6	-991.96	1996.07	4.85	0.03
6.18	-0.10	0.14	*	-1.31	*	0.01	*	0.02	6	-992.02	1996.20	4.98	0.03

Table S3.17) Geographic range contraction is more likely to push species to their geographic periphery. *ANOVA table of best model fit from dredge function of global model pgls(geographic_marginalisation* ~ logit(geographic range loss) + order + log(body mass) + log (geographic range size) + logit(human transformation) + log(body mass) *logit(geographic range loss) + log(body mass)* log (geographic range size) + log(body mass)*logit(human_transformation) , data =mammal.phy.glm.geographic.marginalisation.loss, lambda = "ML"). λ =0.10, delta = 1.00, kappa = 1.00, df = 585, adjusted R^2= 0.03, AICc = 61.0. *Delta AIC (***ΔAIC***) was estimated by dropping each variable from the best model following dredge results. Human transformation denotes the proportion of historic range converted to rangelands, agricultural lands or dense settlements. *p<0.05, **p< 0.01, ***p<0.001*

Variable	**df**	**Slope**	**s.e.**	**F**	***P***	**ΔAIC**	**Σ model weight**
Body mass	1, 585	-0.053	0.021	1.38	0.13	4.89	0.95
Geographic range loss	1, 585	-0.017	0.0072	238.56	0.001***	3.76	0.92
Geographic range size	1, 585	-0.051	0.032	2.34	0.13	28.18	1
Body mass: Geographic range size	1, 585	0.010	0.0038	7.56	0.006**	5.53	0.95

Table S3.18) Dredge table for Geographic marginalisation using global model: *pgls(geographic_marginalisation* ~ ~ logit(geographic range loss) + order + log(body mass) + log (geographic range size) + logit(human transformation) + log(body mass) *logit(geographic range loss) + log(body mass)* log (geographic range size) + log(body mass)*logit(human_transformation) , data =mammal.phy.glm.geographic.marginalisation.loss, lambda = "ML". *denotes variable is not included in the model, models are ranked by AICc, only models within AIC < 5 from the best fit model are displayed. DF= 587

(Intercept)	Body mass	Geographic range loss	Human Transformation	Geographic range size	Order	Body mass* Geographic range loss	Body mass* Human Transformation	Body mass* Geographic range size	df	logLik	AICc	delta	weight
0.22	-0.05	-0.02	*	-0.05	*	*	*	0.01	5	-25.43	60.96	0.00	0.24
0.35	-0.07	-0.04	*	-0.08	*	0.00	*	0.01	6	-24.57	61.29	0.33	0.20
0.21	-0.05	-0.02	-0.01	-0.05	*	*	*	0.01	6	-25.04	62.22	1.27	0.13
0.34	-0.07	-0.04	-0.01	-0.07	*	0.00	*	0.01	7	-24.23	62.64	1.69	0.10
0.21	-0.05	-0.02	-0.01	-0.05	*	*	0.00	0.01	7	-25.03	64.26	3.30	0.05
0.34	-0.07	-0.04	-0.01	-0.07	*	0.00	0.00	0.01	8	-24.22	64.69	3.73	0.04
0.10	-0.05	*	*	-0.02	*	*	*	0.01	4	-28.32	64.72	3.76	0.04
0.09	-0.05	*	-0.01	-0.02	*	*	*	0.01	5	-27.56	65.22	4.27	0.03
-0.20	*	-0.01	*	0.03	*	*	*	*	3	-29.84	65.72	4.76	0.02
-0.19	*	-0.01	-0.01	0.03	*	*	*	*	4	-28.92	65.92	4.96	0.02

Table S3.19). Geographic range loss increases distance between historic and contemporary niche centroids ANOVA table for Distance between niche centroids in range contracted species. *ANOVA table of best model fit from dredge function of global model of pgls(*Distance between niche centroids**)** ~ logit(geographic range loss) + order + log(body mass) + log (geographic range size) + logit(human transformation) + log(body mass) *logit(geographic range loss) + log(body mass)* log (geographic range size) + log(body mass)*logit(human_transformation) , data =mammal.phy.glm.distancenichecentroids.loss, lambda = "ML"). *Delta AIC (*Δ**AIC***) was estimated by dropping each variable from the best model following dredge results. Human transformation denotes the proportion of historic range converted to rangelands, agricultural lands or dense settlements. *p<0.05, **p< 0.01, ***p<0.001.* . λ =0.24, delta = 1.00, kappa = 1.00, df = 561, adjusted R^2= 0.21, AICc = 1067.8*).*

Variable	**df**	**Slope**	**s.e.**	**F**	***P***	**ΔAIC**	**Σ model weights**
Body Mass	1, 561	-0.15	0.066	0.047	0.82	3.17	0.91
Geographic range loss	1, 561	0.0028	0.042	94.07	<0.001***	14.06	1
Geographic range size	1, 561	-0.550	0.095	55.43	<0.001***		1
Body Mass:Geographic range loss	1, 561	0.0075	0.0048	0.055	0.81	0.39	0.46
Body Mass: Geographic range size	1, 561	0.031	0.012	6.89	0.0089**	4.88	0.84

Table S3.20) Dredge table for Distance between niche centroids in range contracted species using global model: *pgls(geographic_marginalisation* **)** ~ logit(geographic range loss) + order + log(body mass) + log (geographic range size) + logit(human transformation) + log(body mass) *logit(geographic range loss) + log(body mass)* log (geographic range size) + log(body mass)*logit(human_transformation) , data =mammal.phy.glm.distancenichecentroids.loss, lambda = "ML"). **denotes variable is not included in the model, models are ranked by AICc, only models within AIC < 5 from the best fit model are displayed. DF= 561*

(Intercept)	Body mass	Geographic range loss	Human Transformation	Geographic range size	Order	Body mass* Geographic range loss	Body mass* Human Transformation	Body mass* Geographic range size	df	logLik	AICc	delta	weight
3.27	-0.15	0.00	*	-0.55	*	0.01	*	0.03	6	-527.85	1067.85	0.00	0.25
2.87	-0.10	0.06	*	-0.47	*	*	*	0.02	5	-529.07	1068.24	0.39	0.20
3.23	-0.15	0.01	-0.01	-0.54	*	0.01	*	0.03	7	-527.66	1069.53	1.68	0.11
2.83	-0.10	0.06	-0.01	-0.46	*	*	*	0.02	6	-528.82	1069.80	1.95	0.09
2.09	*	0.07	*	-0.31	*	*	*	*	3	-532.10	1070.25	2.40	0.08
2.07	0.02	0.06	*	-0.33	*	*	*	*	4	-531.34	1070.75	2.90	0.06
2.08	*	0.07	-0.02	-0.31	*	*	*	*	4	-531.51	1071.09	3.24	0.05
3.23	-0.15	0.01	-0.01	-0.54	*	0.01	0.00	0.03	8	-527.66	1071.59	3.74	0.04
2.07	0.02	0.07	-0.02	-0.32	*	*	*	*	5	-530.86	1071.83	3.98	0.03
2.83	-0.10	0.06	-0.01	-0.46	*	*	0.00	0.02	7	-528.82	1071.85	4.00	0.03
2.08	0.02	0.06	*	-0.33	*	0.00	*	*	5	-531.31	1072.73	4.88	0.02

Table S3.21) Body size and geographic range size impact extinction risk *ANOVA table of best model fit from dredge function of* pgls(formula = IUCN_status ~ order + logbody + logrange + logit(human_transformation), data = mammal.phy.glm.IUCN, lambda = "ML"). λ = 0.58, df = 4158, adjusted R^2 = 0.37

Variable	**df**	**Slope**	**se**	**F**	***P***	**ΔAIC**	**Σ model weights**
Body size	1,4158	0.16	0.014	97.9	<0.001	115.10	1
Geographic range size	1, 4158	-0.89	0.018	2332.9	<0.001	1850.98	1
Residuals	4158						

Table S3.22) Dredge table for extinction risk across all species using global model: pgls(formula = IUCN_status ~ order + logbody + logrange + logit(human_transformation), data = mammal.phy.glm.IUCN, lambda = "ML")---*denotes variable is not included in the model, models are ranked by AICc, only models within AIC < 5 from the best fit model are displayed. DF =4158.

	Variable								
Model	Int (1)	Body size (2)	Human transformation (3)	Range size (4)	Order (5)	df	Log lik	AICc	Delta
124	6.1	0.16	*	-0.89	*	3	-5262.0	10530	0.00
1235	6.4	0.16	0.08	-0.89	*	4	-5261.9	10532	1.75

Table S3.23) Dredge table extinction risk with marginality metrics using Global model: pgls(formula = IUCN_status ~ order + logit(geographic_range_loss) + geographic_marginalisation + log(ecological_marginalisation) + log(distance_between_niche_centroids + 0.1), data = mammal.phy.glm.IUCN, lambda = "ML, *denotes variable is included in the model, models are ranked by AICc, only models within AIC < 5 from the best fit model are displayed. DF = 458

	Variable									
Model	int	Geographic marginality	Distance between centroids	Ecological marginality	Geographic range loss	order	df	logLik	AICc	delta
1345	2.97	*	0.18	0.06	0.23	*	4.00	-705.26	1418.60	0.00
145	3.10	*	*	0.11	0.23	*	3.00	-706.37	1418.79	0.19
1234	2.97	0.16	0.18	0.07	0.23	*	5.00	-704.92	1419.97	1.37
134	3.10	0.17	*	0.11	0.23	*	4.00	-705.98	1420.05	1.45

Table S3.24) Summary of species geographically and ecologically marginalised. Significant effect defined as Cohen's *d* effect size > 0.2.

Geographic marginalisation					
	Number of species with Cohen's *d > 0.2*	Number of species assessed	% significant displacement to extremes	# species collapse towards historic geographic centre	# species collapse towards historic geographic edge
	163	587	27.8	259 (87)	248 (76)
Ecological marginalisation					
	Number of species with Cohen's *d > 0.2*	Number of species assessed	% significant displacement to extremes	# species collapse towards historic niche centre	# species collapse towards historic niche edge
	346	587	59	20 (0 significant)	567 (346 significant)

Table S3.25) **Geographic range loss leads to ecological marginalisation as measured by Mahalanobis distance.** *ANOVA table of best model fit from dredge function of global model of pgls(Ecological_marginalisation)* ~ logit(geographic range loss) + order + log(body mass) + log (geographic range size) + logit(human transformation) + log(body mass) *logit(geographic range loss) + log(body mass)* log (geographic range size) + log(body mass)*logit(human_transformation) , data =mammal.phy.glm.ecological.marginalisation.loss, lambda = "ML"). λ =0.00, delta = 1.00, kappa = 1.00, df = 559, adjusted R^2= 0.44, AICc = 1991.2*)*Contemporary range size and effect size of Mahalanobois distance to historic niche centroid has been log transformed. *Delta AIC (***ΔAIC***) was estimated by dropping each variable from the best model following dredge results. .* **Σ model weights** *referred to the Summed model weights i.e variable importance and inclusion in models with* **ΔAIC** < *5 from the best fit model.* Dredge tables can be found in Table S16.. _*p<0.05, **p< 0.01, ***p<0.001. Dredge model available in Table S3.16._

Variable	df	Slope	se	F	*P*	**ΔAIC**
Geographic range size	1, 559	-1.19	0.080	221.53	<0.001***	129.07
Geographic range loss	1, 559	0.21	0.038	218.33	<0.001***	27.55

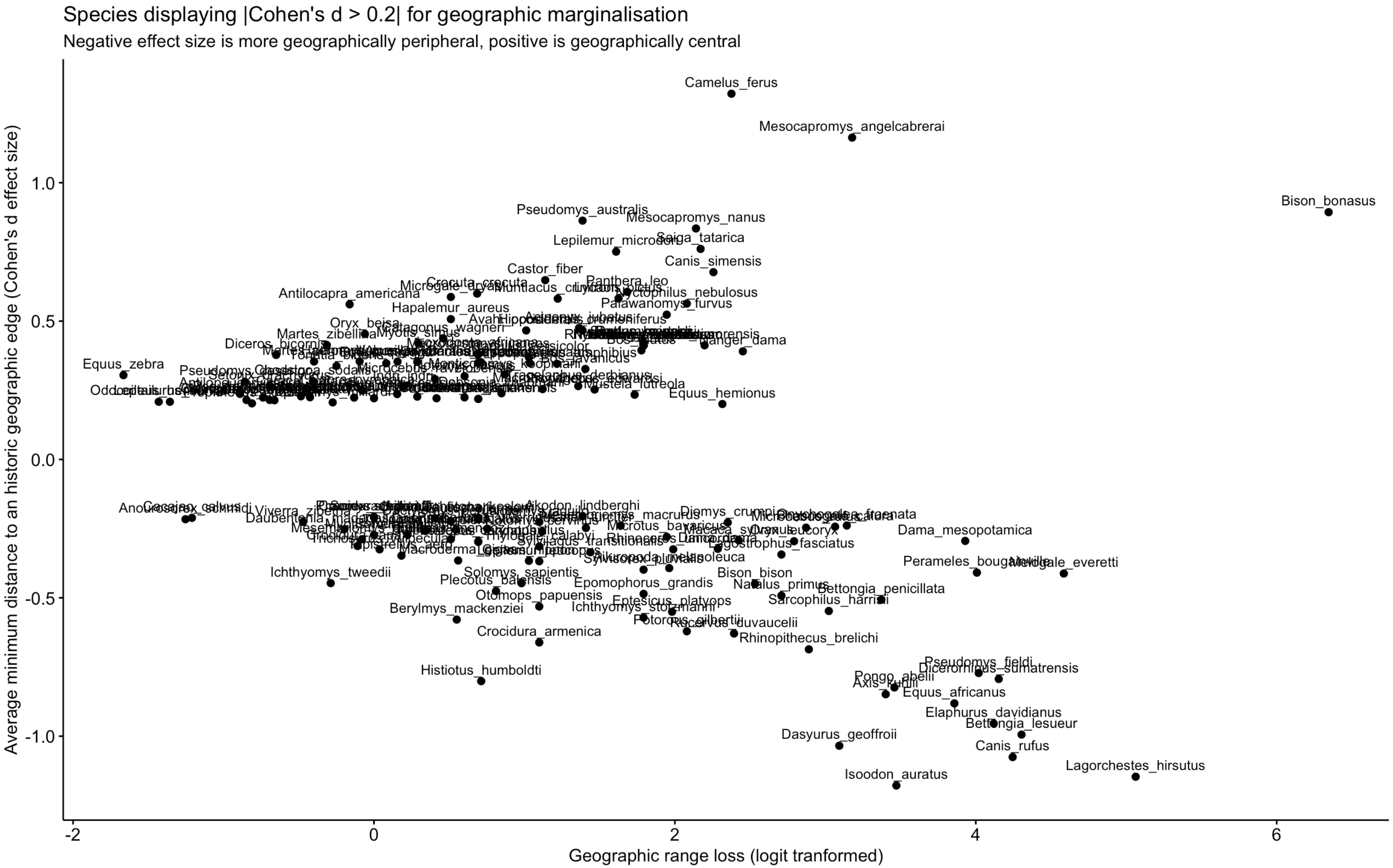


Figure S3.3) Species which have experience significant (Cohen’s d > 0.2) geographic shift towards geographically central or peripheral parts of their niche. Negative denotes shift towards geographic edge, positive denotes shift towards geographic centre. Geographic range loss is logit transformed. Outliers represent species which have experience disproportionate contraction to geographic periphery or centre

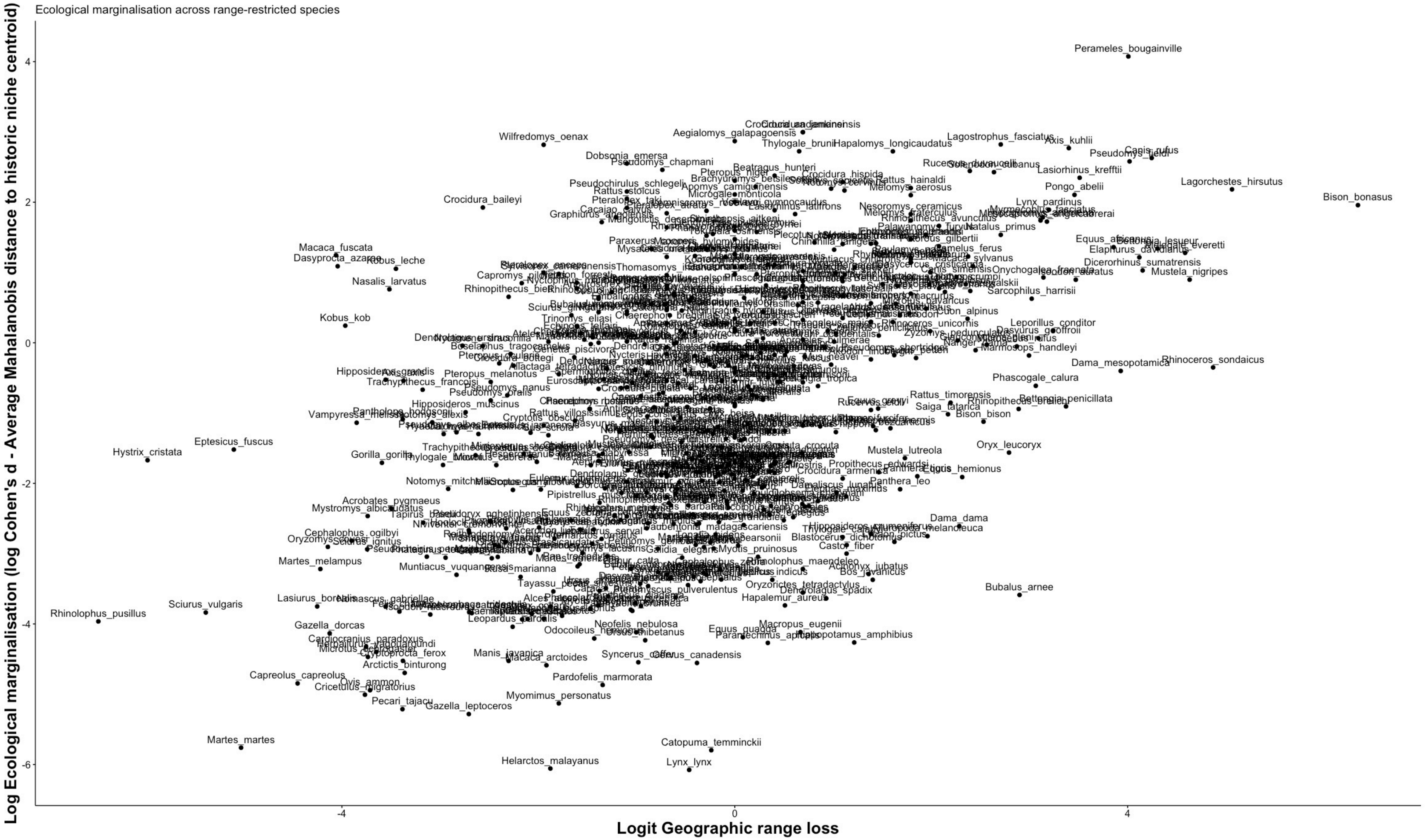


Figure S3.4) Ecological marginalisation is a common consequence of range contraction. Graph displaying ecological marginalisation (log effect size difference in average Mahalanobis distance to historic niche centroid) of all range contracted species against proportional loss of geographic range (logit transformed). Outliers represent species which have experience disproportionate contraction to niche periphery or centre.

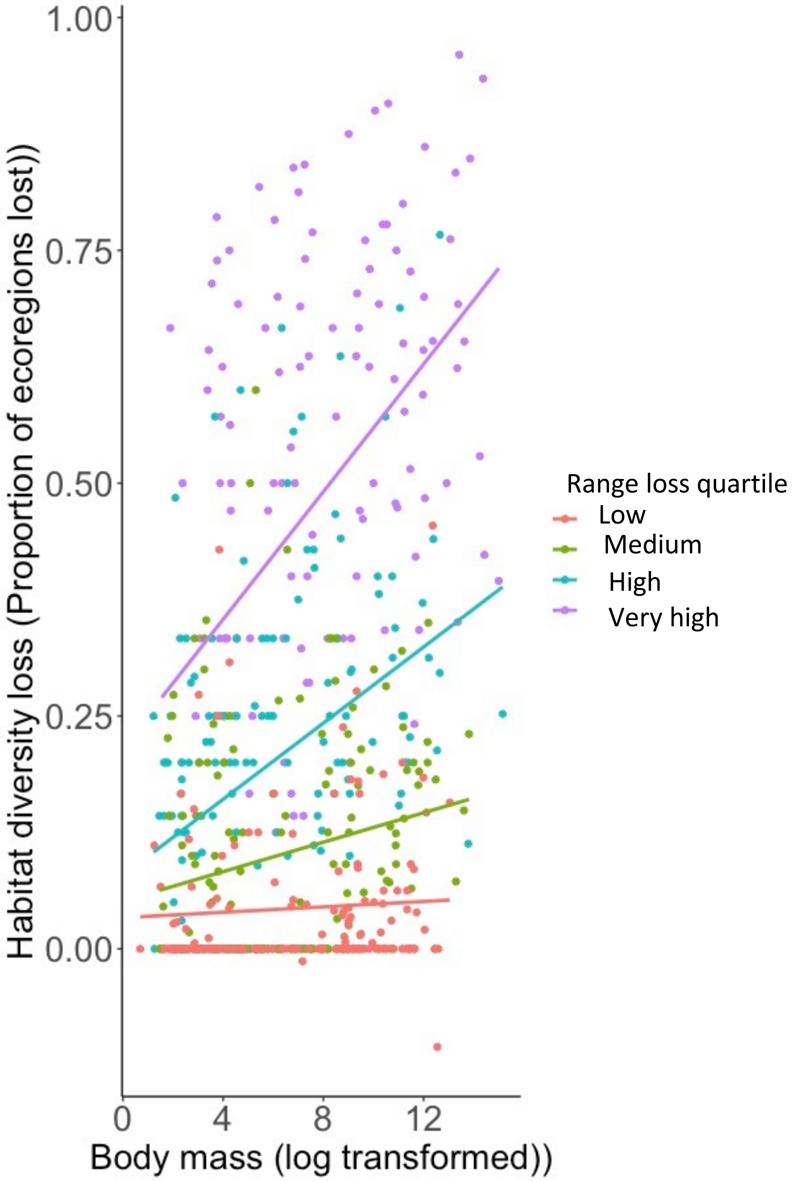


Figure S3.5) Interaction plot between body mass and geographic range loss for habitat diversity loss. Animals with greater geographic range loss lose more habitat diversity with smaller changes in body mass. Range loss quartile bins geographic range loss of range restricted species into 0-25%, 25%x<50%, 50%<x<75%, >75%.

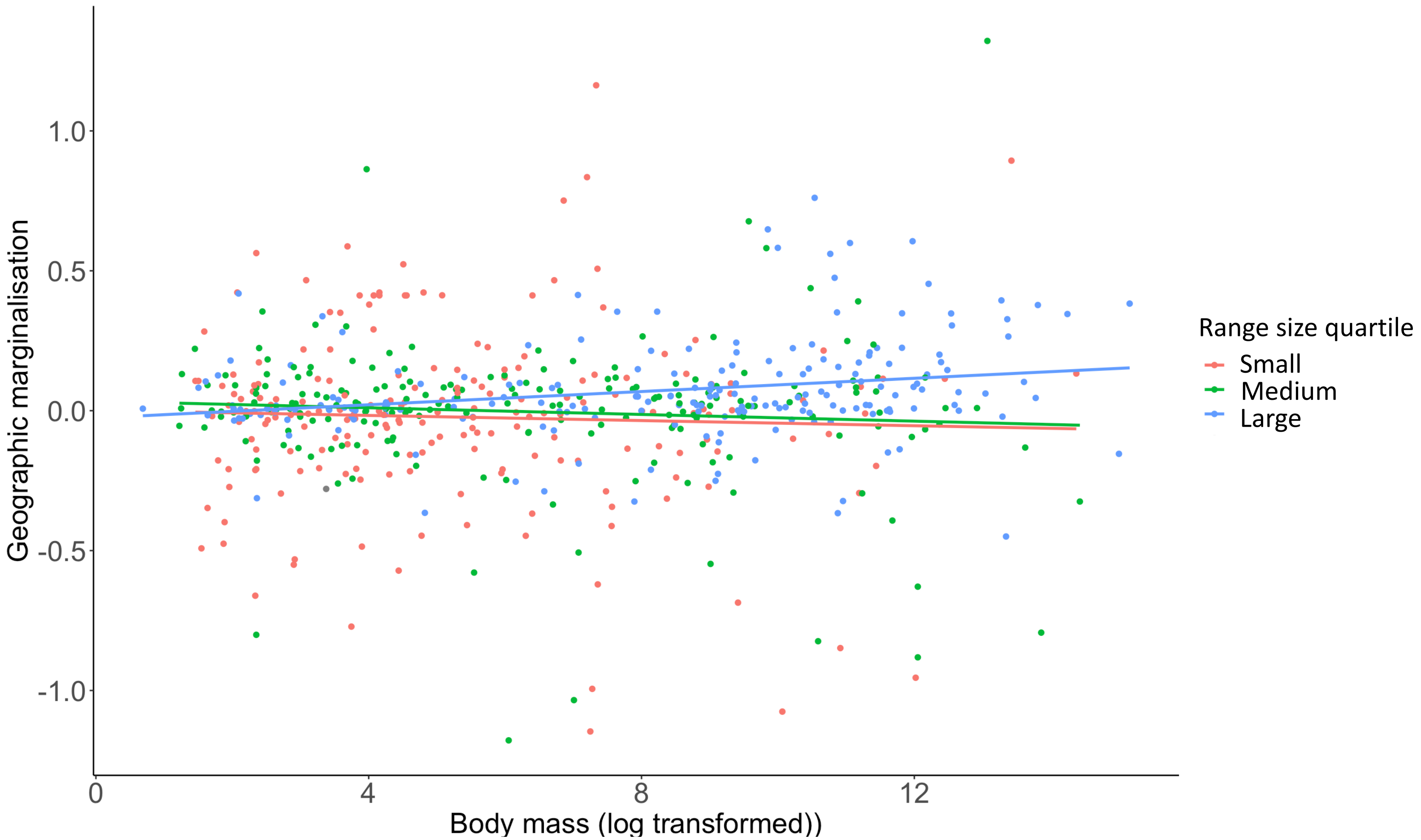


Figure S3.6) Interaction plot between body mass and geographic range size for geographic marginalisation. The interaction between body mass:geographic range size demonstrates large bodied mammals with large geographic ranges are more likely to be pushed to their geographic centre whereas large body with small geographic ranges are more likely to be pushed to geographic peripheries. Range size quartiles were estimated as range restricted organisms with range sizes within the first 33%,66% and 99% of range sizes. Range size was log transformed for this metric.

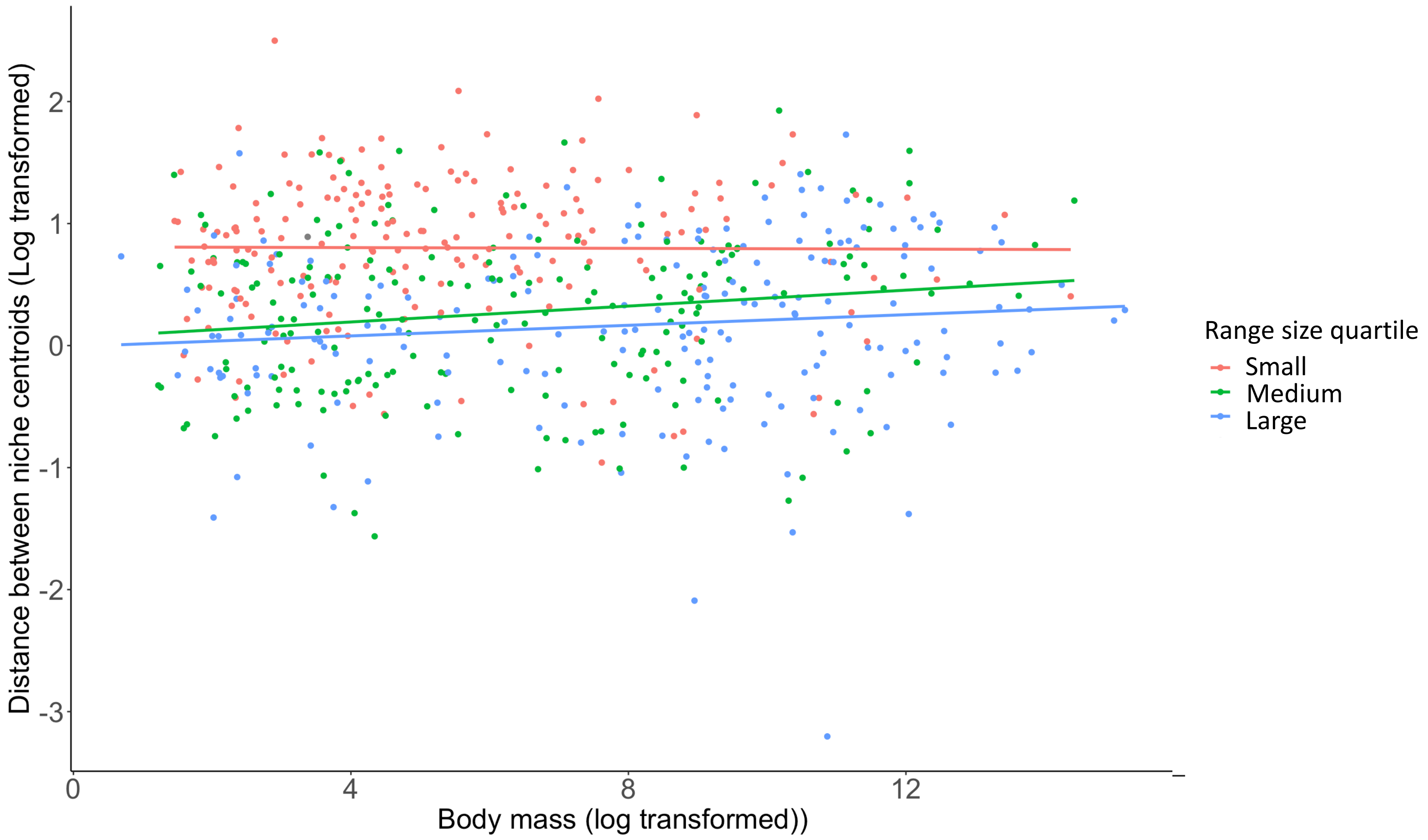


Figure 3.7) Interaction plot between body mass and geographic range size for distance between niche centroid. The interaction between Body Mass: Geographic range demonstrates that small bodied animals with small geographic ranges are less vulnerable to the effects of body mass on increased distances between niche centroids. This is either due to the lack of scaling of the metric (not controlling for niche size) or that small bodied animals have small niches and therefore cannot afford to shift large niche centroids before extinction of their entire range. Range size quartiles were estimated as range restricted organisms with range sizes within the first 33%,66% and 99% of range sizes. Range size was log transformed for this metric.

Chapter 4) The importance of validation: using a validated faecal glucocorticoid metabolite assay links Cape mountain zebra (*Equus zebra zebra*) HPA activity to population performance and sex-specific effects, but not habitat quality or adult-sex ratio

Submitted to Functional Ecology as Jake A. Britnell , Rupert Palme, Jessica M.D Lea, Graham I.H Kerley, John Jackson, Susanne Shultz The importance of validation: using a validated faecal glucocorticoid metabolite assay links Cape mountain zebra (*Equus zebra zebra*) HPA activity to population performance and sex-specific effects, but not habitat quality or adult-sex ratio

4.1 Summary

1. Many studies use steroid hormones, especially glucocorticoids (GCs), to assess physiological responses to pressures. Prior to excretion steroid hormones are heavily metabolised. Thus, it is critical to assess the validity and sensitivity of enzyme immunoassays (EIAs) for measuring faecal glucocorticoid metabolites (fGCMs) in excreta. Although the importance of assay validation has been raised repeatedly, the use of unvalidated assays is widespread. Few studies assess how inappropriate assays can impact biological insights on pressures on animals.

2. Here, we revisit Lea et al. (2017) by validating fGCM assays for Cape mountain zebra (*Equus zebra zebra*; CMZ) and then compare findings between a validated fGCM assay and an unvalidated corticosterone EIA.

3. fGCM concentrations significantly increased following an acute stressor (translocation) using two 11-oxoaetiocholanolone (lab codes: 72T and 72a) assays and an 11ß-hydroxyaetiocholanolone (69a) EIA, but did not with two corticosterone EIAs. As the latter two EIAs did not detect an acute stress response, they do not measure hypothalamic-pituitary-adrenal axis activity in CMZ faeces. This raises concerns to the validity of the results produced by the previously unvalidated corticosterone EIA (Munro, CJM006) used in Lea et al. (2017).

4. Re-analysing samples collected by Lea et al. (2017) using a validated 72T EIA showed that adult sex ratio and habitat quality (measured by grassiness) did not influence fGCMs levels. However, the validated assay did reveal sex-specific effects of rainfall and season, and lower fGCMs concentrations associated with higher numbers of foals per female.

5. We suggest that correlations between the unvalidated Munro corticosterone EIA data and ecological factors likely arose from cross-reactivity and non-specific binding to various unidentified steroid hormone metabolites from multiple physiological processes. We introduce the terminology of an "invalid" assay for a tested assay that failed validation to differentiate this from unvalidated (*i.e.* not tested) and validated assays. Our results

demonstrate how inappropriate assay selection can lead to incorrect conclusions and possibly counterproductive conservation recommendations.

4.2 Introduction

Organisms experience a vast array of stressors and challenges in contemporary landscapes. Landscapes and habitat types vary in quality and threats from predation and disease risk, resource availability and anthropogenic disturbance (Scheele et al., 2017). Organisms can buffer challenges through behavioural or physiological responses, maintaining homeostasis and reducing allostatic load (McEwen, 1998). However, when intense or concurrent challenges occur, allostatic load builds and organisms experience negative fitness consequences (McEwen & Wingfield, 2003). The functional marginality hypothesis states that populations in poor quality conditions should display increased biomarkers associated with negative physiological status and poor fitness (Shultz et al., 2021). Physiological measures and biomarkers can provide mechanistic insights into how organisms adapt, or fail to adapt, to a dynamic landscape of stressors (Chown & Gaston, 2008).

The hypothalamic-pituitary-adrenal (HPA) axis has received much interest as a key pathway for how organisms respond to stressors (Palme, 2019). Following exposure to a stressor, the adrenal glands release glucocorticoids (GCs): cortisol (4-pregnene-11ß,17α,21-triol-3,20-dione) or corticosterone (4-pregnene-11ß,21-diol-3,20-dione), depending upon the species. A short-term glucocorticoid response can assist the animal in coping with stressors by mobilising energy for vital processes and altering behaviour (Sapolsky et al., 2000). However, persistent elevation or depletion of GC concentrations may indicate deleterious effects on individual fitness (Linklater & Gedir, 2011). High parasite load (O’Dwyer et al., 2020), social and anthropogenic stress (van Meter et al., 2009) and presence of invasive species (Santicchia et al., 2018) all may elicit GC responses. While GC levels are not a simple reflection of an organism’s “stress” levels (MacDougall-Shackleton et al., 2019), they do consistently show physiological responses to acute stressors (Shultz et al., 2021).

Glucocorticoids can be measured from various materials, such as blood, feathers, hair, tissues, and excreta (Sheriff et al., 2011). Faeces are commonly used for non-invasive evaluation of HPA axis activity in mammals (Palme, 2019). In addition to non-invasive collection, another advantage of faecal material is that it provides an integrated and smoothed estimate of HPA activity (Palme, 2019). Prior to excretion, steroid hormones are metabolised to various metabolites in the liver and then excreted *via* the bile into the intestinal system (Palme, 2019). While in the intestinal system, microbial enzymatic activities can deconjugate metabolites (Möstl & Palme, 2002), which results in a species-specific pattern of excreted faecal glucocorticoid metabolites (fGCMs; Palme, 2019). Very little or no hormones remain unmetabolized when excreted in the faeces (Palme & Möstl, 1997; Palme et al., 2005).

4.2.1 Native off the shelf vs group-specific Enzyme Immunoassays (EIAs)

Commercially available and ready-to-use enzyme immunoassay (EIA) kits are advertised as “validated” for general use in many biological materials. These kits assess endocrine levels using antibodies for native, *i.e.* unmetabolized steroid hormones, assuming that the native hormone is present in the faeces or that the antibody has sufficient specificity to bind to metabolites from the parent hormone (Montiglio et al., 2013). Group-specific metabolite assays are an alternative approach, where antibodies are developed to measure specific groups of steroid metabolites (e.g. Möstl & Palme, 2002). This approach can more accurately detect fGCMs and give a more robust, biologically meaningful approximation of endocrine levels (and therefore HPA activity) in the species of interest. Despite long-standing calls for accurate validation of EIAs (*e.g.* Touma & Palme 2005), many studies continue to use unvalidated, inappropriate, or sub-optimal assays (see Palme 2019).

Corticosterone radioimmunoassays and EIAs can be used to assess HPA activity from faecal samples in some species (*e.g.* Wasser et al., 2000), but are not appropriate for all species (Palme & Möstl, 1997). Several equid studies use corticosterone EIAs (e.g. York & Schulte, 2014; Yarnell et al., 2016; Merkies et al., 2016; Yarnell & Walker, 2017), but their validity has been recently challenged by Palme (2019). A recent validation study demonstrated that a corticosterone EIA was not sensitive to acute stress responses in horses (*Equus caballus ferus;* Hinchcliffe et al., 2021). Thus, corticosterone EIAs may not give biologically meaningful results of HPA axis activity in other equids (and other species). As a result, inaccurate biological inferences and possibly inappropriate management suggestions may follow (Hinchcliffe et al., 2021).

Group-specific fGCM assays have been analytically and biologically validated for some equids (Merl et al., 2000; Hinchcliffe et al., 2021). Although modern equids are closely related and diverged relatively recently, patterns of faecal metabolites may vary between species. For example, an 11-oxoaetiocholanolone assay (lab code: 72a) has been validated for domestic (*E. caballus*) (Möstl et al., 1999) and feral horses (*E. c. ferus* (Hinchcliffe et al., 2021) and onagers (*E. hemionus onager*) (Vick et al., 2012). Meanwhile, an 11-oxoaetiocholanolone assay (72T) has been validated for Grevy's zebra (*E. grevyi;* Franceschini et al., 2008) and an 11β-hydroxyaetiocholanolone assay (69a) for plains zebra (*E. quagga;* Seeber et al., 2018). Details of the assays and metabolites measured are available in Table 1. Thus, close phylogeny does not guarantee optimal fGCMs assays will be identical and there is a need to physiologically or biologically validate assays for fGCM measurement even in closely related species.

Lea and colleagues (2017) previously used a corticosterone EIA (Munro, CJM006) to relate glucocorticoid concentrations to habitat quality, demography and population performance in the Cape mountain zebra (*Equus zebra zebra,* CMZ), a sub-species of mountain zebra endemic to southern South Africa (Lea et al., 2017). However, the Munro corticosterone EIA Lea et al. (2017) used to assess GC concentrations is unvalidated (untested) for CMZ. Hinchcliffe and colleagues recently demonstrated native corticosterone EIAs are not sensitive to acute stressors and may cross-react with reproductive hormone metabolites (Hinchcliffe et al., 2021). Thus, the ecological factors that Lea et al. (2017) associated with high GC concentrations in CMZ may not reflect population-level differences in stress responses from the HPA pathway.

At the time of the Lea et al. (2017) study, CMZ were listed as Vulnerable (now downgraded to Least Concern) due to a population crash in the 20th century and small isolated populations with variable population growth rates (Hrabar et al. 2019). Across the species' range fecundity, density, population growth rate and foal:mare ratio vary due to grass-availability (Lea et al., 2016). Based on population differences of metabolite concentrations measured with the Munro corticosterone EIA, Lea et al. (2017) argued that male-biased sex ratio and habitat quality were the most important factors associated with elevated HPA activity in CMZ populations. They advocated management interventions that focus on translocating individuals into grassier areas and removing males to balance sex ratios (Lea et al., 2017).

We compare the sensitivity of five enzyme immunoassays (EIAs) to an acute stress event: the translocation of five individuals. We used three group-specific (72T, 72a, 69a) and two "native" corticosterone EIAs (Arbor assay DetectX® Corticosterone EIA and a corticosterone EIA measuring fGCMs with an 11β,21-diol-20-one structure). The two corticosterone EIAs are similar to the Munro corticosterone EIA (CJM006) used by Lea *et al.* (2017) (for details of the Munro assay see Watson et al. 2013, Table 1). The corticosterone EIA measuring fGCMs with an 11β,21-diol-20-one structure (Palme & Möstl, 1997) uses the same immunogen as the Munro CJM006 corticosterone EIA (corticosterone-3-CMO-BSA) and should, therefore, detect the same structural group of metabolites. We use the most responsive EIA to re-analyse the samples used in Lea et al. (2017) and assess whether assay choice impacts the ecological and demographic factors associated with fGCM concentration and subsequent management recommendations.

4.3 Materials and methods

4.3.1 Translocation (biological validation experiment)

Five CMZ (1 male and 4 females) were translocated between two neighbouring properties (see details below). Human handling and transportation of animals are well-established for biological validation of assays used to measure fGCM concentrations in response to an acute stressor, as it dramatically increases HPA axis activity across species (Dickens et al., 2010; Palme, 2019). We predicted that if the assay is biologically reliable, fGCM concentrations should increase between 24-72 hours post-translocation due to the approximate gut retention time (24-36 hours) of CMZ (Palme et al., 2005; Steuer et al., 2011)

Sanbona Wildlife Reserve (SWR) and Koktyls Private Reserve (KOK) (33.8663°S, 20.5284°E) are privately-owned reserves within the Western Cape of South Africa, dominated by two biomes: fynbos and succulent Karoo. KOK and SWR are adjacent properties separated by game fencing, hence are ecologically similar in terms of climate, vegetation communities, elevation and absence of predator communities. On 9th September 2018, five individuals were moved from KOK to the predator-free southern section of SWR. Individuals were darted from a helicopter, loaded onto vehicles for transportation, driven to the adjacent property and released upon waking. Following translocation, all individuals quickly ran to the fence line that separates the two reserves and tracked the boundary for the subsequent eight days.

Faecal samples were collected approximately twelve days prior to translocation to provide baseline GC levels of undisturbed CMZ. All defecating animals were photographed for identification and the stripe/pattern recognition software ‘hotspotter’ was used to confirm individual ID (Crall et al., 2013). Faecal samples from the translocated individuals were collected 24-72 hours post translocation. Faecal samples were collected non-invasively by watching the animal defecate and collecting the samples from the ground within ~30 minutes of deposition. Each sample was thoroughly homogenized in a plastic bag. Faecal samples were stored in a cooler at ~4 °C in the field. Steroid extraction and storage (drying) were performed within 8 hours of collection.

4.3.2 Predictors of fGCMs in the Cape mountain zebra metapopulation – sampling by Lea et al. (2017)

Lea et al. (2017) collected faecal samples for fGCM measurement from seven CMZ populations in separate reserves between January–May and September–December 2015. The populations occurred at Bakkrans Nature Reserve, Welgevonden Game Farm, De Hoop Nature Reserve, Camdeboo National Park, Mount Camdeboo Private Game Reserve, Swartberg Private Game Reserve and Gamkaberg Nature Reserve. Two reserves experienced summer rainfall, two winter rainfall and three aseasonal rainfall. Lea et al. (2017) assessed habitat quality for each reserve by an index of grass abundance (see Lea et al., 2016). Sampling was seasonal (spring/summer/autumn). Adult sex ratio was estimated during two–six surveys of each population. Sampling was conducted in summer and autumn in year one and spring and summer in year two. Individuals were observed defecating, and faecal samples collected within an hour of defecation. Each sample was thoroughly homogenized. A photographic identity database was used to ensure independent samples within seasons. Each reserve was sampled twice, except De Hoop Nature Reserve, which was only sampled during spring following a winter rainfall period. Lea et al. (2017) collected 365 faecal samples from 163 adult CMZ across all their study populations, including 89 stallions for analysis with a testosterone EIA (polyclonal testosterone R156/7).

4.3.3 Extraction and assay selection for fGCM analysis

For the biological validation samples, GC metabolites were extracted from faecal samples by adding 5 ml of 80% methanol to 0.5 g of faeces (Palme et al., 2013), the mixture was shaken by hand for 5 minutes, left to settle for 30 minutes (following Shutt et al., 2012) and the supernatant decanted into Eppendorf tubes. All extracts were completely evaporated at 50^{o}C using a waterbath and were stored dry at -20^{o}C in the field and -80^{o}C in the laboratory.

Lea et al. *(*2017) extracted steroids in the field using a modified extraction technique (Edwards et al., 2014) using HyperSep™ octyl bonded silica (C8) cartridges (Thermo Fisher Scientific, UK). After extraction, samples were exported to the UK on cartridges, extracted and stored as liquid extracts at -20^{o}C until EIA analysis. We assessed the effect of extraction method and storage (HyperSep™ octyl bonded silica (C8) cartridges vs methanol extraction and drying) to ensure cartridge extraction method did not influence trends found in Lea et al. (2017). We found a strong positive correlation between extraction and storage methods (t = 10.6, df = 38, cor = 0.86 $p < 0.001$) suggesting extraction method did not impact trends found in Lea et al. (2017). However, concentrations were significantly lower (52.3% loss) using the HyperSep™ octyl bonded silica (C8) cartridges method (Figure S4.2).

For the current study, sample extracts from Lea et al. (2017) were stored for an additional ~2.5-3 years at -20^{o}C before reanalysis using group-specific glucocorticoid metabolite assays. Storing liquid extracts in frozen, dark conditions is well established for long-term storage, although slight changes at or post 50 weeks of storage may occur due to evaporation (Kalbitzer & Heistermann, 2013). However, evaporation is unlikely to impact our findings as samples were dried and resuspended in the same volume of assay buffer as the original sample before analysis. Validation samples were stored dry for ~6 months at -80^{o}C.

Faecal samples were measured for immunoreactive fGCM concentrations using several EIAs (Table 1). Dried samples were redissolved in EIA buffer and stored at −20°C until analysis. Analyses were performed within 2 days of resuspension. For fGCM assays, we also compared results from non-concentrated versus concentrated samples using diethylether and 5% sodium bicarbonate (Merl et al., 2000). This step was performed as metabolite traces may be under the detection limit of the enzyme immunoassay. DetectX$^{®}$ Corticosterone EIA and Munro Corticosterone EIA used in Lea et al. (2017) are highly correlated across several species (unpub data). DetectX$^{®}$ corticosterone EIAs showed parallelism from pooled faecal extracts (Figure S4.1).

Table 4.1) Details of the enzyme immunoassays (EIAs) used. Comparison of group-specific EIAs is available in Ganswindt et al. (2003).

Enzyme immunoassay	***"Native" vs group-specific EIAs***	**Metabolites assessed**	**Reference**
DetectX$^{®}$ Corticosterone	Native	unknown	
Corticosterone (Palme and Möstl, 1997)	Native	fGCMs with an 11β,21-diol-20-one structure	Palme & Möstl (1997)
Corticosterone (Munro, CJM006)	Native	fGCMs with an 11β,21-diol-20-one structure	Watson et al. (2013)
11-oxoaetiocholanolone (lab code 72a)	Group-specific	11,17-dioxoandrostanes (11,17-DOA)	Palme& Möstl (1997)
11-oxoaetiocholanolone (lab code 72T)	Group-specific	fGCMs with a 5β-3α-ol-11-one structure (3α,11-oxo-CM)	Möstl et al. (2002)
11β-hydroxyaetiocholanolone **(**lab code 69a)	Group-specific	fGCMs with a 5β-3 α,11β-diol structure (3α,11β-diol-CM)	Frigerio et al. (2004)

4.3.4 Data analysis

We assessed whether there were significant increases in fGCMs levels pre- and post-translocation using one-tailed paired t-tests. We also calculated the average times increase (x-times) from pre-translocation levels and Z score (increase measured by the average number of standard deviations away from the pre-translocation mean across all individuals) (Bashaw et al., 2016).

We evaluated the ecological and demographic association with each assay using linear mixed effect models using the *lme4* package in R (R Development Core Team, 2021). We replicated the analysis from Lea et al. (2017) using concentrations from 11-oxoaetiocholanolone (72T) EIA to compare to results from the corticosterone (Munro, CJM006) EIA . First, following Lea et al. (2017), we assessed whether fGCM concentrations from the 72T EIA varied between populations, using reserve as a fixed effect while including zebra ID and sampling trips as intercept-only random effects. We then built a global linear mixed model for the 72T EIA identical to that of Lea et al. (2017) to investigate impact of assay choice on the relative impacts of demographic and ecological factors. This linear mixed model included grass abundance, season (spring/summer/autumn), rainfall type (seasonal/non-seasonal), log transformed group size (number of individuals) and population sex ratio. We built three separate models (as conducted by Lea et al., 2017), one including all individuals and then separated by sex (mares and stallions). We evaluated the relative predictive performance of each by calculating the Akaike information criterion (AIC) (Akaike, 1973) and the change in AIC after dropping each variable sequentially (ΔAIC).

We also assessed the impact of male social position (herd stallion vs bachelor) and female's maternal state (foal at foot vs no foal) on metabolite concentrations for both 72T and Munro EIAs using linear mixed effect models with individual ID included as a fixed factor.

4.3.5 Correlation between endocrine parameters and performance metrics

To assess the relationship between endocrine parameters and both fecundity and population growth rate, we first replicated the analysis of Lea et al. 2017. We compared average fGCM levels from the 72T assay from each reserve across the two seasons with foal:mare ratio (averaged across both visits) and population growth rate using one-tailed Spearman's rank correlation coefficient. We compared results to those in Lea et al. (2017).

After repeating analyses from Lea et al. (2017), we also compared results from linear regression analysis to compare the goodness-of-fit for both average metabolite concentrations (72T and Munro EIAs) and foal:mare ratio (averaged across both visits) and population growth rate. Female fecundity and population growth rate were log-transformed for linear regression analysis in an attempt to transform data to satisfy an approximate Gaussian distribution.

4.4 Results

4.4.1 Biological validation

The three group-specific fGCMs displayed large fold-increases following the acute stressor, but native corticosterone EIAs did not. Faecal GCMs measured by the 72T EIA showed the greatest average increase and highest Z score between pre- and post-translocation values (Figure 4.1). Diethylether extraction did not improve 72T EIA sensitivity. 72a and 69a were also suitable EIAs. However, fGCMs assessed by the 72a assay required an additional concentration step using diethylether. The DetectX corticosterone EIA did not show a significant increase post-translocation (Figure 4.1). The corticosterone EIA measuring fGCMs with a 11β,21-diol-20-one structure did not yield concentrations above the EIA detection limit even after diethylether extraction. This is likely due to there being very little or no fGCMs with an 11β,21-diol-20-one structure in CMZ faeces due to metabolic breakdown. Thus, concentrations of fGCMs with an 11β,21-diol-20-one structure were too low to accurately measure HPA activity and therefore also did not show a significant response post-translocation. The fGCM concentrations assessed with three group-specific EIAs (72T, 72a, 69a) were highly correlated with each other, whereas none were significantly correlated with the DetectX corticosterone EIA (Figure S4.3).

As both corticosterone EIAs did not show any response to the acute stressor, they cannot measure HPA axis activity in CMZ faeces. We found the positive correlation between metabolites assessed by the Munro corticosterone EIA and fGCMs assessed by the validated 72T EIA ($t = 6.015$, $df = 363$, $r = 0.30$, 95% C.I. 0.20 to 0.39, $p < 0.001$) was weaker than the correlation between fGCMs (72T) and metabolite concentrations assessed by a testosterone EIA, steroid hormones, which are unrelated to HPA axis activity ($t = 6.04$, $df = 160$, $r = 0.43$, 95% C.I. 0.30 to 0.55, $p < 0.001$; Text S4.2; Figure S4.3). As we do not know which steroid hormones or metabolites are binding to the Munro (CJM006) corticosterone EIA, we refer to concentrations from this EIA as unspecified/unidentified “faecal steroid” concentrations from this point onwards. Concentrations from validated assays are referred to as fGCMs as they were sensitive to HPA axis activity and bind to specific faecal glucocorticoid metabolites.

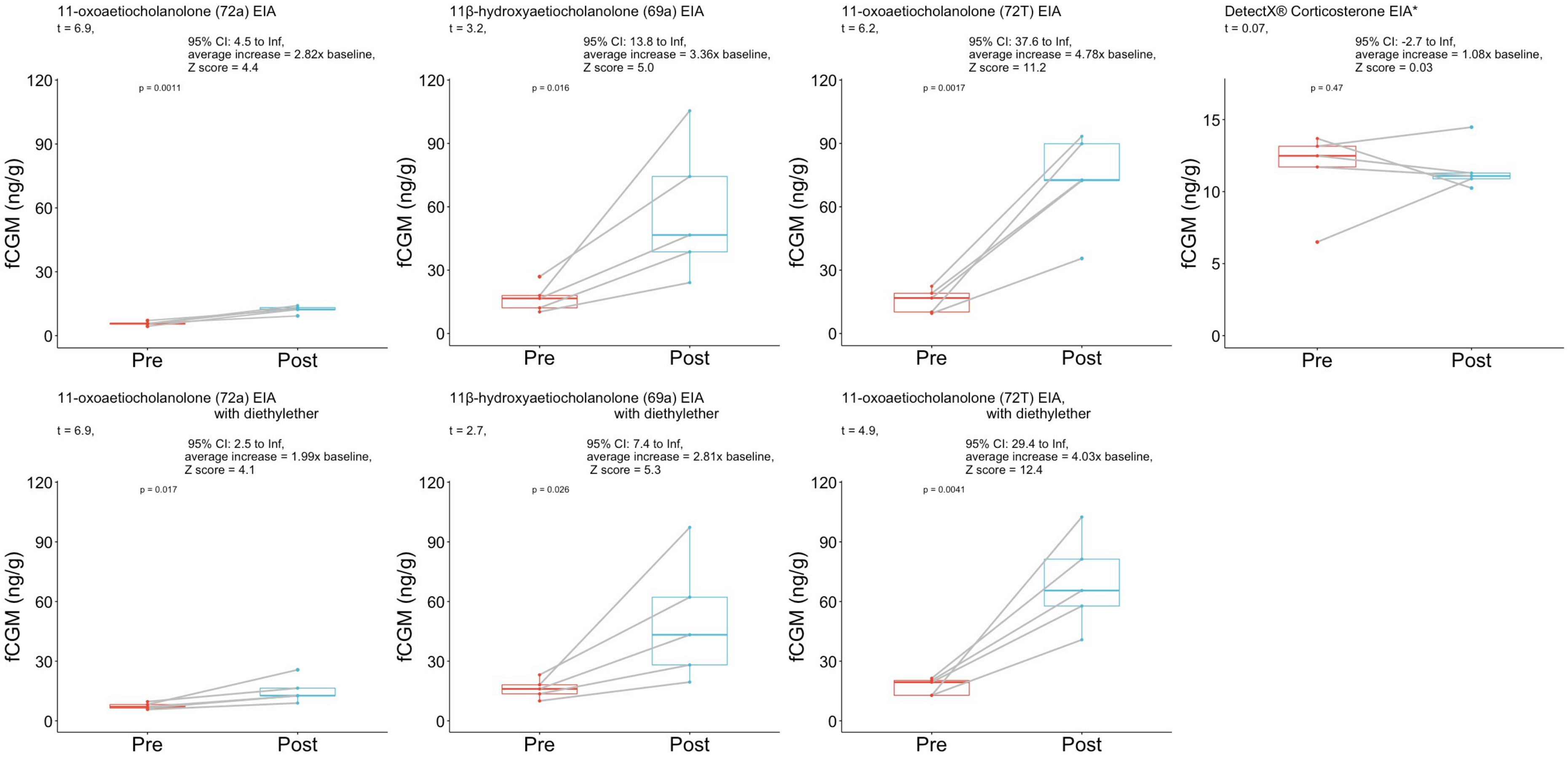


Figure 4.1) Biological validation of fGCM assays. Comparison of fGCM concentrations in Cape mountain zebra per assay in response to an acute stressor (translocation) for the purpose of biological validation. 'Pre' represents fGCM levels approximately twelve days before translocation. All 'post' samples were collected 24-72 hours post-translocation. 95% CI denotes 95% confidence interval, Z score: increase measured by the number of standard deviations away from the pre-stressor mean. "With diethylether" means that a further extraction step utilizing diethylether was applied prior to analysis with the EIA. Results of the other corticosterone EIA measuring fGCMs with a 11β,21-diol-20-one structure are not included as several samples had values below the detection limit and therefore were not accurately measurable. * notes native EIA.

4.4.2 Reanalysing samples from Lea et al. (2017)

When controlling for individual and sampling season, we found fGCMs measured by the 72T EIA varied between reserves ($F_{6,159} = 13.96$, $p < 0.001$) as did unspecified faecal steroid hormone concentrations assessed using the Munro corticosterone EIA ($F_{6,159} = 10.57$, $p < 0.001$). However, the average concentrations of populations changed relative position between Munro corticosterone and 72T EIAs (Figure S4.4).

When comparing the results of the unvalidated Munro corticosterone EIA and the validated 72T metabolite assay, some factors were consistently strong predictors (ΔAIC > 2) across most models, such as season. However, the direction of change was different (Table 2). Habitat grassiness was a strong predictor for unspecified faecal steroid concentrations (Munro corticosterone EIA) across all individuals (Table 2), but was not a strong predictor (ΔAIC < 2, $p > 0.05$) for fGCMs (72T assay) across all samples or when assessing females and males separately (Table 2). Sex ratio was an important predictor for unspecified faecal steroid concentrations (Munro corticosterone EIA) but was a poor predictor (ΔAIC < 2, $p > 0.05$) for fGCM concentrations (72T) in all models (Table 2).

4.4.2.1 Males

Ecological and demographic drivers of unspecified steroid concentrations (Munro corticosterone EIA) and fGCMs (72T) were not the same in males (Table 2). In males, unspecified faecal steroid concentrations (Munro EIA) were high in low grassiness habitats (Lea et al., 2017). This effect was not found in fGCMs (72T; Table 2). Although including group size improved model fit (ΔAICc > 2), group sizes did not significantly impact fGCMs (72T EIA) in males (Table 2). Group size did not improve model fit and was non-significant for unspecified steroid concentrations (Munro EIA).

Male fGCMs (72T EIA) were lower in sites with summer rainfall (Table 4.2, Figure S4.5), and varied with male social position (bachelor vs herd stallion) ($F_{1,90} = 10.3$, p = 0.002). On average, herd stallion males had higher levels of fGCMs than bachelors when using the 72T

EIA. No effect of male social position was found in unspecified faecal steroid concentrations from the Munro corticosterone EIA *(*$F_{1, 90}$ = 0.006, p = 0.94).

4.4.2.2 Females

Ecological and demographic drivers of unspecified steroid concentrations (Munro EIA) and fGCMs (72T EIA) were also not the same in females (Table 2). In females, unspecified steroid concentrations (Munro EIA) were high in low grassiness habitats. This effect was not found for fGCMs (72T EIA), although grassiness improved model fit (ΔAICc > 2; Table 2). In females, unspecified steroid concentrations were higher in high sex-biased populations, again this effect was not found for fGCMs (72T EIA) (Table 4.2).

Females had elevated fGCM (72T EIA) concentrations compared to males, while sex had no effect on unspecified steroid concentrations (Munro EIA) (Table 4.2). Mare fGCM (72T assay) concentrations were lowest in summer compared to spring and autumn (Table 4,2; Figure S4.5). Female fGCMs (72T assay) and unspecified steroid hormone concentrations (Munro EIA) were not influenced by maternal state (with foal at foot vs no foal) ($F_{1, 73}$ = 0.17, p = 0.68 & $F_{1, 73}$ = 0.07, p = 0.79, respectively).

Table 4.2. Linear mixed effect model coefficients AIC change with sequentially dropping terms and p values for the 72T and Munro corticosterone (Munro) assays. Green shading highlights factors which change model AICc more than 2 (when dropped sequentially). Red shading in coefficient column indicates that either the relative coefficients have changed direction (Sign change) between assays or factors have been added or dropped between assays. Green/red shading in the Model Change column indicates factors that have or have not changed in impact or relative coefficient/sign between assays. Green in the p value column highlights factors that had similar trends and significant p values with both assays, red shading indicates changes and no shading (and N.S) factors that were non-significant with both assays.

	Factor	Categories	72T	Munro	ΔAIC 72T	ΔAIC Munro	Model Change	*p value* 72T	*p value Munro*	*p value change*
All individuals	Season	Spring-Autumn Summer-Autumn	-0.27±0.37 -2.94±0.62	0.33±0.09 -0.411±0.14	21.50	41.17	Sign Change	0.001	<.001	Sign Change
	Grassiness		-1.28±2.12	-1.93±0.42	1.70	18.58	Lost	0.55	<.001	Lost
	Rainfall Seasonality	Aseasonal-Summer Aseasonal-Winter	0.15±1.07 -1.91±0.72	0.69±0.22 -0.43±0.14	6.24	7.21	No change	0.03	<0.001	No change
	Sex Ratio		-1.19± 10.01	-0.20±0.10	0.78	-1.01	N.S	0.137	0.05	Lost
	Group Size		-1.19± 10.01	0.001±0.20	1.24	-3.36	N.S.	0.25	0.99	N.S
	Sex		-0.99±0.36	-0.04±0.07	5.48	-5.11	Gained	0.01	0.52	Gained
Males	Androgens		3.16±0.92	0.66±0.22	45.29	10.06	No change	0.001	0.004	No change
	Season	Spring-Autumn Summer-Autumn	0.66±0.52 -1.34±0.89	0.31±0.10 -0.15±0.17	6.43	6.61	No change	0.06	0.013	Lost
	Grassiness		-0.66±2.73	-1.35±0.55	1.90	4.46	Lost	0.8	0.017	Lost
	Rainfall Seasonality	Aseasonal-Summer Aseasonal-Winter	-1.20±1.36 -3.16±0.92	0.29±0.27 -0.22±0.18	13.77	-4.63	Gained	0.001	0.059	Gained
	Sex Ratio		0.54±0.66	-0.24±0.13	-0.34	-0.89	N.S.	0.42	0.22	N.S
	Group Size		-1.81±1.13	0.29±0.24	2.53	-1.53	Gained	0.11	0.38	N.S
Females	Season	Spring-Autumn Summer-Autumn	-0.75±0.52 -3.93±0.81	0.42±0.13 -0.57±0.20	19.52	33.97	Sign Change	0.001	0.000	Sign change
	Grassiness		-0.09±3.10	-2.14±0.60	2.09	10.69	No change	0.98	0.001	Lost
	Rainfall Seasonality	Aseasonal-Summer Aseasonal-Winter	0.59±1.56 -0.23±1.10	0.97±0.32 -0.33±0.21	0.77	3.64	Lost	0.93	0.011	Lost
	Sex Ratio		0.66±0.79	-0.34±0.15	0.05	1.04	N.S.	0.41	0.028	Lost
	Group Size		0.46±1.95	-0.37±0.38	1.22	-1.14	N.S.	0.82	0.355	N.S

4.4.3 Faecal GCMs (72T EIA) correlate with female fecundity but not population growth rate

Lea and colleagues (2017) found that unspecified faecal steroid concentrations (Munro corticosterone EIA) negatively correlated with female fecundity (foal:mare ratio; ρ: −0.68, $S = 94$, $p = 0.055$) and long-term population growth (ρ: −0.71, $S = 96$, $p = 0.04$, $n = 7$). Using the 72T EIA, fGCMs were negatively correlated with female fecundity (foal:mare ratio; ρ: −0.68, $S = 94$, $p = 0.055$), but not with long-term population growth rate (ρ: -0.39, $S = 78$, $p = 0.20$, n =7) (Figure 4.2).

Using linear regression, average unspecified faecal steroid concentrations (Munro) was related to population performance but not female fecundity ($\beta = -0.18 \pm 0.069$, t = -2.5, adjusted $R^2 = 0.48$, $p = 0.05$, *and* $\beta = -0.17 \pm 0.11$, t = -1.6, adjusted $R^2 = 0.20$, $p = 0.17$ *,* respectively, n = 7). Average fGCM concentration (72T EIA) was associated with foal:mare ratio but not population growth rate ($\beta = -0.01 \pm 0.0029$, t = -3.6, $R^2 = 0.67$ $p = 0.015$ and $\beta = -0.006 \pm 0.003$, t = -1.86, $R^2 = 0.29$, $p = 0.12$, respectively, n =7).

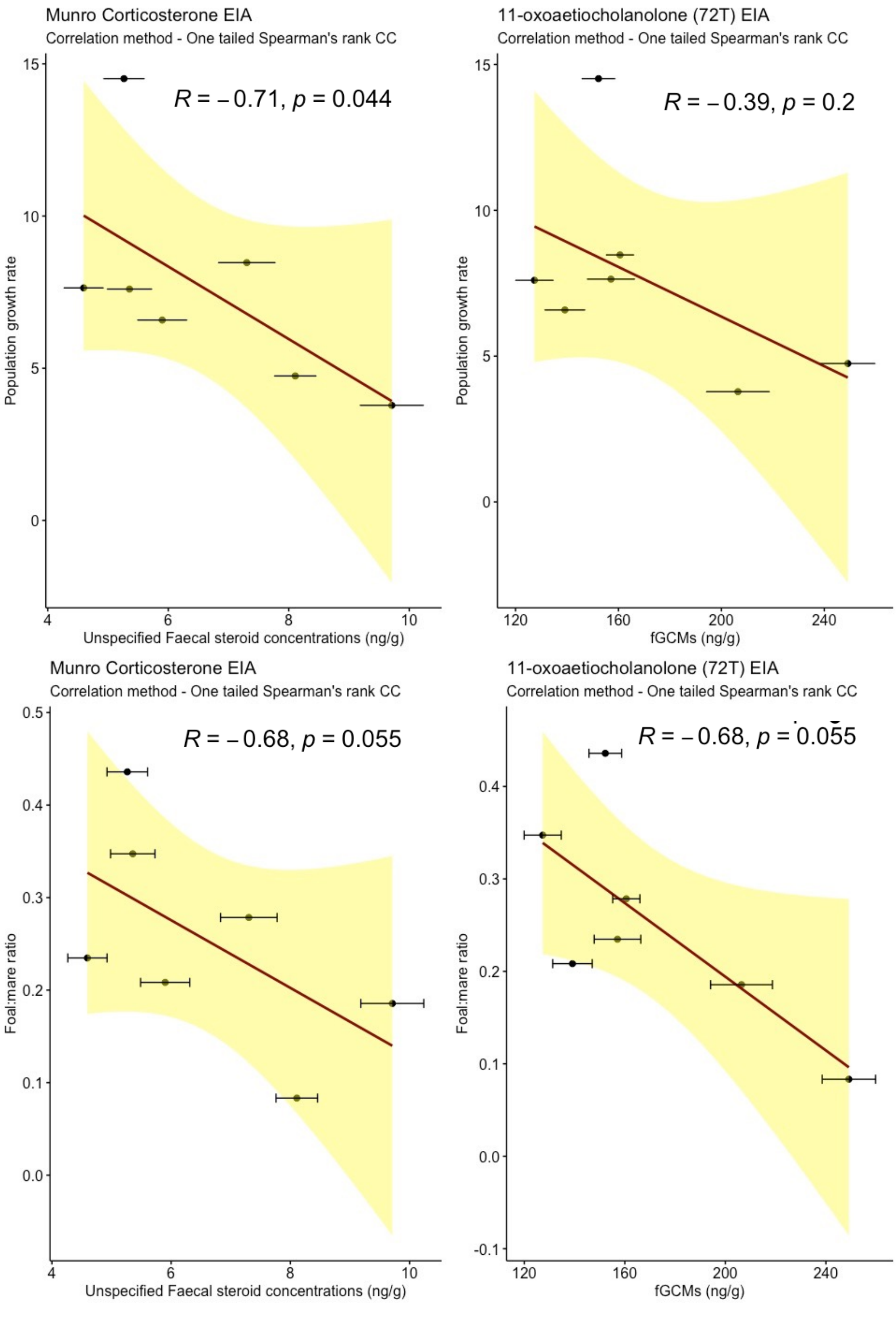


Figure 4.2) Unspecified steroid concentrations from the unvalidated Munro corticosterone EIA and fGCMs assessed with the 72T EIA negatively correlated with Cape mountain zebra foal:mare ratios. However, fGCMs did not correlate with population growth rates. Error bars represent ± se of mean.

4.5 Discussion

This study and Hinchcliffe et al. (2021) are the first, to our knowledge, to compare the impact of assay choice on associations between fGCMs and ecological and demographic factors. Of the five assays tested here, the three group-specific assays (72T, 72a, 69a) measured an acute stress response to translocation. 11-oxoaetiocholanolone (72T) measured the strongest response to the acute stressor. In contrast, both corticosterone EIAs detected no consistent change in fGCM, despite animals being tranquilised, moved, and handled upon release and showing clear behavioural signs of distress by tracking the fence line between the two reserves for the duration of the post-translocation sampling. This raises serious doubts as to the ability of the Munro (CJM006) corticosterone EIA, and corticosterone EIAs in general, to assess HPA activity in the faeces of CMZ. The Munro (CJM006) corticosterone EIA remains unvalidated and highly similar corticosterone EIA are invalid (failed to validate).

4.5.1 Ecological and demographic predictors change with assay choice

Given that two corticosterone EIAs were not able to detect an acute stress response in CMZ, the next question is how assay choice impacts on physiological inferences in free-ranging populations? Re-evaluating the same samples used in Lea et al. (2017) with a validated 11-oxoaetiocholanolone (72T) EIA demonstrated differences with the unvalidated Munro corticosterone assay. Two of the main results of the Lea et al. (2017) study were not repeatable: fGCMs concentrations from the 72T assay did not vary with habitat quality (grassiness) nor with demography (adult sex ratio). Grassiness *via* a vegetation index may not accurately capture habitat quality. The vegetation index likely does not adequately account for changes in seasonal availability in food items or which food items are consumed at the time of sampling. Seasonal effects remained significant but changed direction of effect. One result was consistent between assays: winter rainfall areas, which are marginal habitats for the CMZ (Lea et al., 2016), were associated with higher fGCM (72T EIA) and unspecified faecal steroid (Munro EIA) concentrations. However, as we cannot be sure what the Munro corticosterone EIA is binding to, we can only speculate what drives similar patterns between assays, but it is highly unlikely that the Munro EIA is detecting HPA activity, as by the 72T EIA. Given that native steroid hormones and their metabolites have closely related molecular structure, cross-reactivity between native assays and steroid metabolites may occur across biologically distinct pathways. Hinchcliffe et al. (2021) recently demonstrated invalid “native” corticosterone EIAs may cross react with faecal metabolites from reproductive physiology pathways. Hence, associations between unspecified faecal steroid hormones from Munro EIA may be due to cross-reactivity with metabolites from completely different physiological pathways.

The elevated faecal GCMs levels (72T assay) found in herd stallions may be due to the increased social stress of maintaining, or being part of, a breeding group. Correlations between fGCMs (72T assay) and metabolite levels assessed by Testosterone EIA may suggest causal links with social stress or may simply be due to non-specific binding and cross-reactivity of the testosterone assay (Hinchcliffe et al., 2020). Our results suggest larger group size may decrease HPA activity in males as group size improved model fit when using 72T EIA.

4.5.2 Glucocorticoids as a biomarker of population health and resilience

Lea et al. (2017) found negative relationships between unspecified steroid hormone concentrations (Munro corticosterone EIA), fecundity (foal:mare ratio) and population growth rate. We also found that fGCMs (72T assay) were negatively correlated with foal:mare ratio, but not with population growth rate. At this coarse population level, average population fGCMs may be associated with population level performance metrics but we cannot make inferences on the adaptiveness of individual level response on over fitness. However, a similar relationship in both EIAs does not demonstrate that the two EIAs are measuring the same physiological activities. If the Munro corticosterone EIA is binding non-specifically, the measured concentrations may be a ‘composite’ of a range of physiological functions that *could* inadvertently measure something equivalent to allostatic load. However, without characterising what is actually binding in the Munro corticosterone EIA or the physiological processes it can predictably measure, it cannot be used to make inferences about the physiological state of the animals.

Moreover, the mechanistic relationship between fGCMs, ecological challenges, and population performance are also difficult to disentangle. GCs have complex effects on processes such as metabolism, immune function, reproduction, cognition, development and fluid homeostasis, and GC response may adaptive (Boonstra, 2013). Changes in GCs concentrations have been linked to long-term population dynamics and fitness (Bonier et al., 2009), sociality and behaviour (Packard et al., 2016; Raulo & Dantzer, 2018), macroecological and macrophysiological patterns (Jessop et al., 2013), disease susceptibility and immune regulation (Cain & Cidlowski, 2017), drivers of habitat and space use (Madliger & Love, 2016), adaptive maternal effects (MacLeod et al., 2021) and physiological responses to environmental heterogeneity and marginality such as the formation of range edges, limits and species distributions (Shultz et al. 2021). We need to recognise from these studies that associations between GCs and population performance may indicate a range of underlying physiological processes. Thus, an increase in circulating GC is not necessarily an indication of a compromised physiological state. However, these complex effects also mean that GCs can potentially provide broad-brush insights into physiology, life history and behavioural adaptations to environmental perturbations (Boonstra, 2005; Crespi et al., 2013).

As a solution to the issue of identifying drivers, we recommend using multiple biomarkers to identify mechanistic associations between population performance and environmental challenges (Shultz et al. 2021). Multiple factors such as demography, behaviour, and ecology simultaneously affect physiology and may act independently or in tandem causing additive, synergistic or antagonistic effects (Todgham & Stillman, 2013; Shultz et al., 2021). Adding additional, validated biomarkers can help elucidate both challenges and physiological responses. For example, diet changes can be identified with DNA metabarcoding (e.g. Kartzinel et al. 2015). Thyroid hormone levels can indicate metabolic rates, reproductive hormones can be used to assess cycling and reproductive state of individuals or immune function and inflammatory markers can help identify disease burden (reviewed in Shultz et al. 2021). Thus, rather than over-interpreting correlations from single biomarkers, more insight can be gleaned from a suite of tools that can identify the relative importance of different challenges.

4.5.3 Unvalidated and invalid assays

Our results add to the growing evidence that appropriate validation of assays, especially when using faeces or urine, is essential and should be a prerequisite for all non-invasive studies of the HPA response to stresses (Palme, 2005). In addition to an analytical validation to check sensitivity, accuracy and molecular characterisation, physiological or biological validation is vital to ensure that the assay is able to measure a level of response similar to the conditions present within the study (Palme, 2019). ACTH and dexamethasone challenges, which directly stimulate or suppress the HPA axis activity, have been the gold standard physiological validations (Touma & Palme, 2005). If an EIA does not detect an increase or decrease in measured metabolites following these interventions, it is clearly not appropriate. However, these physiological challenges require invasive experimentation and invoke an intense response and an EIA that shows a mild to moderate increase to an ACTH challenge may not be sensitive enough to detect ecologically relevant stressors. Thus, a biological validation where animals experience a known acute stress event (such as translocation, reintroduction and handling) can provide further evidence that an assay is sensitive enough to pick up ‘real world’ challenges.

The previous study (Lea et al., 2017) is far from the only study to use unvalidated assays. Palme (2019) urged caution in assay selection across species as excreted fGCMs and thus assays and extraction protocols are highly species-specific (Palme et al., 2005). Using an exhaustive list of 1329 studies evaluating GC concentrations, Palme (2019) highlighted that approximately ~37% of the studies lacked adequate physiological or biological validation, which raises the possibility that the methods were unsuitable in the assessed species. Two years following that publication, despite numerous citations and an additional 475 papers collated for the Palme (2019) list (now 1804 papers), the proportion of studies using unvalidated methods has not improved (36.8%) (Palme, personal observation). An important caveat about validation is that an analytical validation demonstrating repeatability, precision and good dilution curves does not mean that it is measuring the compounds or physiological process of interest (Palme, 2019). We demonstrated this with our successful DetectX corticosterone EIA parallelism (Figure S4.1) and the failed biological validation of the same EIA. Currently, the term “unvalidated” encompasses assays that are untested (such as the Munro corticosterone EIA used in this study) and assays which have been tested but fail biological validation (such as the other two corticosterone EIAs in this study). This may be confusing and may not convey the importance of validation. We suggest that the term "*invalid*" assay should be used to identify an assay that has been tested but failed validation. *Unvalidated* would then be used exclusively for untested assays and *validated* assays would remain for tested assays that show predicted responses.

Close phylogenetic relationships do not guarantee similar metabolite specificity, as shown in equids (here and Hinchcliffe et al., 2021), giraffes (Bashaw et al., 2016), primates (Heistermann et al., 2006) and marsupials (Fanson et al., 2017). Although assay selection and validation can be time consuming and logistically challenging, it is essential that best practice be followed as poor assay choice can lead to non-interpretable results or false conclusions. It is important to stress that validation is vital for all assays, not just EIAs assessing HPA activity (see Pribbenow et al. (2016) for the importance of validation for assays assessing male reproductive hormones in faeces).

There is a myriad of potential consequences of using an invalid or unvalidated assay. If an assay cannot detect an HPA response to an acute stressor, its use may underestimate the severity of veterinary procedures or husbandry protocols, such as assessing pain (Merl et al., 2000), inadequate enrichment (Burgener et al., 2008), drivers of stereotypic behaviours (Shepherdson et al., 2013) or inappropriate densities in enclosures and impacts of visitation on captive animals (Scott et al., 2017). The use of invalid or unvalidated assay will likely not help in accurately measuring species responses to a stressful event or their overall physiological status.

Finally, from a conservation perspective, using invalid or unvalidated assays for management recommendations may result in inappropriate recommendations. Glucocorticoids have been linked to major areas of conservation interest such as physiological effects of invasive species (Santicchia et al., 2018), human impacts on animal populations (Rehnus et al., 2014) and the impacts and effectiveness of conservation interventions (Shultz et al., 2021). Some of the recommendations in Lea et al. (2017), namely the expansion of reserves and translocation of individuals into good quality habitat are supported by other empirical data such as demographic data, and common sense. Therefore, increased HPA activity is not required as justification for these recommendations, but it would help to provide physiological understanding of the mechanism. The proximate mechanisms, which drive HPA activity, were not the same when comparing validated and unvalidated assays. Therefore, using a validated assay is essential to determine which environmental and demographic conditions should be considered “high quality”. For example, although grass availability may limit CMZ populations (Lea et al. 2016), our analyses do not provide a link between poor habitat quality (grassiness) and increased HPA activity. Furthermore, Lea et al. (2017) also suggested removing excess males to balance adult sex ratios as a way to reduce social stress. Our validated 72T assay, on the other hand, did not find evidence that sex-biased populations had greater HPA activation (fGCM concentrations). If invalid or unvalidated assays are used and habitat suitability is poorly estimated, conservation complacency can arise from incorrect perception of species ecologies (Britnell et al., 2021).

Lea et al (2017*)* conducted the experiments and assay selection in good faith, based on available recommendations. Their findings and interpretations were also published in good faith. Only as new information became available, has it became clear that a sub-optimal assay had been used and previous results were potentially unreliable. Going forward, all users and developers of assays as biomarkers must ensure adequate biological validation to prevent similar occurrences in the future. Adequate biological validation must be conducted using ACTH and dexamethasone challenges, which directly stimulate or suppress the HPA axis activity when these methods are possible. If invasive procedures are not available or possible (*e.g.* wild free-living species), translocation must be used as an adequate alternative. Species must be uniquely validated as optimal metabolite choice can be different even between closely related species. For example, pptimal assay selection for Plains zebra (*Equus quagga*) and Mountain zebra (*Equus zebra*) is different despite close phylogeny. Ultimately, for assessing stress in Cape mountain zebra, individuals should use 11-oxoaetiocholanolone (72T) metabolite assay as it has proven to be the most sensitive and reliable assay available to date for the species. We would recommend collecting multiple samples from the same individuals overtime to assess the repeatability of GC concentrations in times of non-perturbation. As conservation science has both biological and social aspects, scientists should adapt to new best practice as it is developed. This could include publishing or disclosing all validation results, comparing multiple assays to identify the most appropriate and revisiting findings if more sensitive assays are identified, as we have done here.

4.6 References

Akaike, H. (1973). Maximum likelihood identification of Gaussian autoregressive moving average models. *Biometrika*, *60*(2), 255–265.

Bashaw, M. J., Sicks, F., Palme, R., Schwarzenberger, F., Tordiffe, A. S. W., & Ganswindt, A. (2016). Non-invasive assessment of adrenocortical activity as a measure of stress in giraffe (*Giraffa camelopardalis*). *BMC Veterinary Research*, *12*(1), 1–13.

Bonier, F., Martin, P. R., Moore, I. T., & Wingfield, J. C. (2009). Do baseline glucocorticoids predict fitness? *Trends in Ecology & Evolution*, *24*(11), 634–642.

Boonstra, R. (2005). Equipped for life: the adaptive role of the stress axis in male mammals. *Journal of Mammalogy*, *86*(2), 236–247.

Boonstra, R. (2013). Reality as the leading cause of stress: rethinking the impact of chronic stress in nature. *Functional Ecology*, *27*(1), 11–23.

Britnell, J. A., Lewis, R. N., Elsner-Gearing, F., Harvey, N., Stanbrook, E., & Shultz, S. (2021). Species stereotypes as a result of unconscious research biases compromise conservation efficacy. *Biological Conservation*, *261*, 109275.

Burgener, N., Gusset, M., & Schmid, H. (2008). Frustrated Appetitive Foraging Behavior, Stereotypic Pacing, and Fecal Glucocorticoid Levels in Snow Leopards (*Uncia uncia*) in the Zurich Zoo. *Journal of Applied Animal Welfare Science*, *11*(1), 74–83.

Cain, D. W., & Cidlowski, J. A. (2017). Immune regulation by glucocorticoids. *Nature Reviews Immunology 17*(4), 233–247.

Chown, S. L., & Gaston, K. J. (2008). Macrophysiology for a changing world. *Proceedings of the Royal Society B: Biological Sciences*, *275*(1642), 1469–1478.

Crall, J. P., Stewart, C. v., Berger-Wolf, T. Y., Rubenstein, D. I., & Sundaresan, S. R. (2013). HotSpotter-Patterned species instance recognition. *Proceedings of IEEE Workshop on Applications of Computer Vision*, 230–237.

Crespi, E. J., Williams, T. D., Jessop, T. S., & Delehanty, B. (2013). Life history and the ecology of stress: How do glucocorticoid hormones influence life-history variation in animals? *Functional Ecology*, *27*(1), 93–106.

Dickens, M. J., Delehanty, D. J., & Michael Romero, L. (2010). Stress: An inevitable component of animal translocation. *Biological Conservation*, *143*(6), 1329–1341.

Edwards, K. L., McArthur, H. M., Liddicoat, T., & Walker, S. L. (2014). A practical field extraction method for non-invasive monitoring of hormone activity in the black rhinoceros. *Conservation Physiology*, *2*(1) cot037.

Fanson, K. v., Best, E. C., Bunce, A., Fanson, B. G., Hogan, L. A., Keeley, T., Narayan, E. J., Palme, R., Parrott, M. L., Sharp, T. M., Skogvold, K., Tuthill, L., Webster, K. N., & Bashaw, M. (2017). One size does not fit all: Monitoring faecal glucocorticoid metabolites in marsupials. *General and Comparative Endocrinology*, *244*, 146–156.

Frigerio, D., Dittami, J., Möstl, E., & Kotrschal, K. (2004). Excreted corticosterone metabolites co-vary with ambient temperature and air pressure in male Greylag geese (*Anser anser*). *General and Comparative Endocrinology*, *137*(1), 29–36.

Ganswindt, A., Palme, R., Heistermann, M., Borragan, S., & Hodges, J. K. (2003). Non-invasive assessment of adrenocortical function in the male African elephant (*Loxodonta africana*) and its relation to musth. *General and Comparative Endocrinology*, *134*(2), 156–166.

Gobush, K. S., Booth, R. K., & Wasser, S. K. (2014). Validation and application of noninvasive glucocorticoid and thyroid hormone measures in free-ranging Hawaiian monk seals. *General and Comparative Endocrinology*, *195*, 174–182.

Heistermann, M., Palme, R., & Ganswindt, A. (2006). Comparison of different enzyme immunoassays for assessment of adrenocortical activity in primates based on fecal analysis. *American Journal of Primatology*, *68*(3), 257–273.

Hrabar, H., Birss, C., Peinke, D., Novellie, P. & Kerley, G.I.H. (2019) *Equus zebra* ssp. *zebra*. *The IUCN Red List of Threatened Species* 2019: e.T7959A45171853.

Jessop, T. S., Woodford, R., & Symonds, M. R. E. (2013). Macrostress: do large-scale ecological patterns exist in the glucocorticoid stress response of vertebrates? *Functional Ecology*, *27*(1), 120–130.

Kalbitzer, U., & Heistermann, M. (2013). Long-term storage effects in steroid metabolite extracts from baboon (*Papio sp.)* faeces – a comparison of three commonly applied storage methods. *Methods in Ecology and Evolution*, *4*(5), 493–500.

Kartzinel, T. R., Chen, P. A., Coverdale, T. C., Erickson, D. L., Kress, W. J., Kuzmina, M. L., Rubenstein, D. I., Wang, W., & Pringle, R. M. (2015). DNA metabarcoding illuminates dietary niche partitioning by African large herbivores. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(26), 8019–8024.

Kartzinel, T. R., & Pringle, R. M. (2020). Multiple dimensions of dietary diversity in large mammalian herbivores. *Journal of Animal Ecology*, 1365-2656.13206.

Lea, J. M., Kerley, G. I.H., Hrabar, H., Barry, T. J., & Shultz, S. (2016). Recognition and management of ecological refugees: A case study of the Cape mountain zebra. *Biological Conservation*, *203*, 207–215.

Lea, J. M., Walker, S. L., Kerley, G. I.H., Jackson, J., Matevich, S. C., & Shultz, S. (2017). Non-invasive physiological markers demonstrate link between habitat quality, adult sex ratio and poor population growth rate in a vulnerable species, the Cape mountain zebra. *Functional Ecology*, 32, 300-312

Linklater, W. L., & Gedir, J. V. (2011). Distress unites animal conservation and welfare: towards synthesis and collaboration. *Animal Conservation*, *14*(1), 25–27.

MacDougall-Shackleton, S. A., Bonier, F., Romero, L. M., & Moore, I. T. (2019). Glucocorticoids and “Stress” Are Not Synonymous. *Integrative Organismal Biology*, *1*(1) obz017.

MacLeod, K. J., While, G. M., & Uller, T. (2021). Viviparous mothers impose stronger glucocorticoid-mediated maternal stress effects on their offspring than oviparous mothers. *Ecology and Evolution*, *11*(23), 17238–17259.

Madliger, C. L., & Love, O. P. (2016). Do baseline glucocorticoids simultaneously represent fitness and environmental quality in a declining aerial insectivore? *Oikos*, *125*(12), 1824–1837.

McEwen, B. S., & Wingfield, J. C. (2003). The concept of allostasis in biology and biomedicine. *Hormones and Behavior*, *43*(1), 2–15.

Merkies, K., DuBois, C., Marshall, K., Parois, S., Graham, L., & Haley, D. (2016). A two-stage method to approach weaning stress in horses using a physical barrier to prevent nursing. *Applied Animal Behaviour Science*, *183*, 68–76.

Montiglio, P.-O., Pelletier, F., Palme, R., Garant, D., Réale, D., & Boonstra, R. (2012). Noninvasive Monitoring of Fecal Cortisol Metabolites in the Eastern Chipmunk (*Tamias striatus*): Validation and Comparison of Two Enzyme Immunoassays. *Physiological and Biochemical Zoology: Ecological and Evolutionary Approaches*, *85*(2), 183–193.

Möstl, E., Maggs, J. L., Schrötter, G., Besenfelder, U., & Palme, R. (2002). Measurement of Cortisol Metabolites in Faeces of Ruminants. *Veterinary Research Communications*, *26*(2), 127–139.

Möstl, E., Messmann, S., Bagu, E., Robia, C., & Palme, R. (1999). Measurement of glucocorticoid metabolite concentrations in faeces of domestic livestock. *Zentralblatt Fur Veterinarmedizin. Reihe A*, *46*(10), 621–631.

Möstl, E., & Palme, R. (2002). Hormones as indicators of stress. *Domestic Animal Endocrinology*, *23*(1–2), 67–74.

Nuñez, C. M. V., Adelman, J. S., Smith, J., Gesquiere, L. R., & Rubenstein, D. I. (2014). Linking social environment and stress physiology in feral mares (*Equus caballus*): Group transfers elevate fecal cortisol levels. *General and Comparative Endocrinology*, *196*, 26–33

O’Dwyer, K., Dargent, F., Forbes, M. R., & Koprivnikar, J. (2020). Parasite infection leads to widespread glucocorticoid hormone increases in vertebrate hosts: A meta-analysis. *Journal of Animal Ecology*, *89*(2), 519–529.

Packard, A. E. B., Egan, A. E., & Ulrich-Lai, Y. M. (2016). HPA Axis Interactions with Behavioral Systems. *Comprehensive Physiology*, *6*(4), 1897–1934.

Palme, R & Möstl, E. (1997). Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood. *Inst J Mammal Biol*, *62*, 192–197.

Palme, R. (2005): Measuring fecal steroids: Guidelines for practical application. *Ann. N. Y. Acad. Sci.* 1046, 75-80.

Palme, R. (2019). Non-invasive measurement of glucocorticoids: Advances and problems. *Physiology & Behavior*, *199*, 229–243.

Palme, R., Touma, C., Arias, N., Dominchin, M. F., & Lepschy, M. (2013). Steroid extraction: get the best out of faecal samples. *Wiener Tierärztliche Monatsschrift*, *100*(9/10), 238–246.

Pribbenow, S., Shrivastav, T. G., & Dehnhard, M. (2016). Measuring fecal testosterone metabolites in spotted hyenas: Choosing the wrong assay may lead to erroneous results. *Journal of immunoassay & immunochemistry*, *38*(3), 308–321.

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Raulo, A., & Dantzer, B. (2018). Associations between glucocorticoids and sociality across a continuum of vertebrate social behavior. *Ecology and Evolution*, *8*(15), 7697–7716.

Rehnus, M., Wehrle, M., & Palme, R. (2014). Mountain hares Lepus timidus and tourism: stress events and reactions. *Journal of Applied Ecology*, *51*(1), 6-12.

Santicchia, F., Dantzer, B., van Kesteren, F., Palme, R., Martinoli, A., Ferrari, N., & Wauters, L. A. (2018). Stress in biological invasions: Introduced invasive grey squirrels increase physiological stress in native Eurasian red squirrels. *Journal of Animal Ecology*, *87*(5), 1342–1352.

Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How Do Glucocorticoids Influence Stress Responses? Integrating Permissive, Suppressive, Stimulatory, and Preparative Actions. *Endocrine Reviews*, *21*(1), 55–89.

Scott, K., Heistermann, M., Cant, M. A., & Vitikainen, E. I. K. (2017). Group size and visitor numbers predict faecal glucocorticoid concentrations in zoo meerkats. *Royal Society Open Science*, *4*(4) 161017

Seeber, P. A., Franz, M., Dehnhard, M., Ganswindt, A., Greenwood, A. D., & East, M. L. (2018). Plains zebra (*Equus quagga*) adrenocortical activity increases during times of large aggregations in the Serengeti ecosystem. *Hormones and Behavior*, *102*, 1–9.

Seydack, A., Vermeulen, C., & Huisamen, H. (2000). Habitat quality and the decline of an African elephant population: implications for conservation. *South African Journal of Wildlife Research, 30*, 34–42.

Shepherdson, D., Lewis, K. D., Carlstead, K., Bauman, J., & Perrin, N. (2013). Individual and environmental factors associated with stereotypic behavior and fecal glucocorticoid metabolite levels in zoo housed polar bears. *Applied Animal Behaviour Science*, *147*(3–4), 268–277.

Sheriff, M. J., Dantzer, B., Delehanty, B., Palme, R., & Boonstra, R. (2011). Measuring stress in wildlife: techniques for quantifying glucocorticoids. *Oecologia, 166*(4), 869–887.

Shultz, S., Britnell, J. A., & Harvey, N. (2021). Untapped potential of physiology, behaviour and immune markers to predict range dynamics and marginality. *Ecology and Evolution*, *11*(23), 16446–16461.

Shutt, K., Setchell, J. M., & Heistermann, M. (2012). Non-invasive monitoring of physiological stress in the western lowland gorilla (*Gorilla gorilla gorilla*): Validation of a fecal glucocorticoid assay and methods for practical application in the field. *General and Comparative Endocrinology*, *179*(2), 167–177.

Steuer, P., Südekum, K. H., Müller, D. W. H., Franz, R., Kaandorp, J., Clauss, M., & Hummel, J. (2011). Is there an influence of body mass on digesta mean retention time in herbivores? A comparative study on ungulates. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *160*(3), 355–364.

Touma, C., & Palme, R. (2005). Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of validation. *Annals of the New York Academy of Sciences*, *1046*, 54–74.

van Meter, P. E., French, J. A., Dloniak, S. M., Watts, H. E., Kolowski, J. M., & Holekamp, K. E. (2009). Fecal glucocorticoids reflect socio-ecological and anthropogenic stressors in the lives of wild spotted hyenas. *Hormones and Behavior*, *55*(2), 329–337.

Vick, MM., Wildt, DE., Turner, JB., Palme, R., Wolfe, BA., Pukazhenthi, B. (2012). Glucocorticoid response to changes in enclosure size and human proximity in the Persian onager (*Equus hemionus onager*). *Stress 15*, 52-61.

Watson, R., Munro, C., Edwards, K. L., Norton, V., Brown, J. L., & Walker, S. L. (2013). Development of a versatile enzyme immunoassay for non-invasive assessment of glucocorticoid metabolites in a diversity of taxonomic species. *General and Comparative Endocrinology*, *186*, 16–24.

Wen, B. M. (1998). Stress, Adaptation, and Disease: Allostasis and Allostatic Load. *Annals of the New York Academy of Sciences*, *840*(1), 33–44.

Yarnell, K., Purcell, R. S., & Walker, S. L. (2016). Fecal Glucocorticoid Analysis: Non-invasive Adrenal Monitoring in Equids. *Journal of Visualized Experiments : JoVE*, *2016*(110).

Yarnell, K., & Walker, S. L. (2017). Environmental impact on faecal glucocorticoid metabolite concentrations in Grevy's zebra (*Equus grevyi*). *African Journal of Wildlife Research*, *47*(2), 138-143.

York, C. A., & Schulte, B. A. (2014). The relationship of dominance, reproductive state and stress in female horses (Equus caballus). *Behavioural Processes*, *107*, 15–21.

4.7 Supplementary information

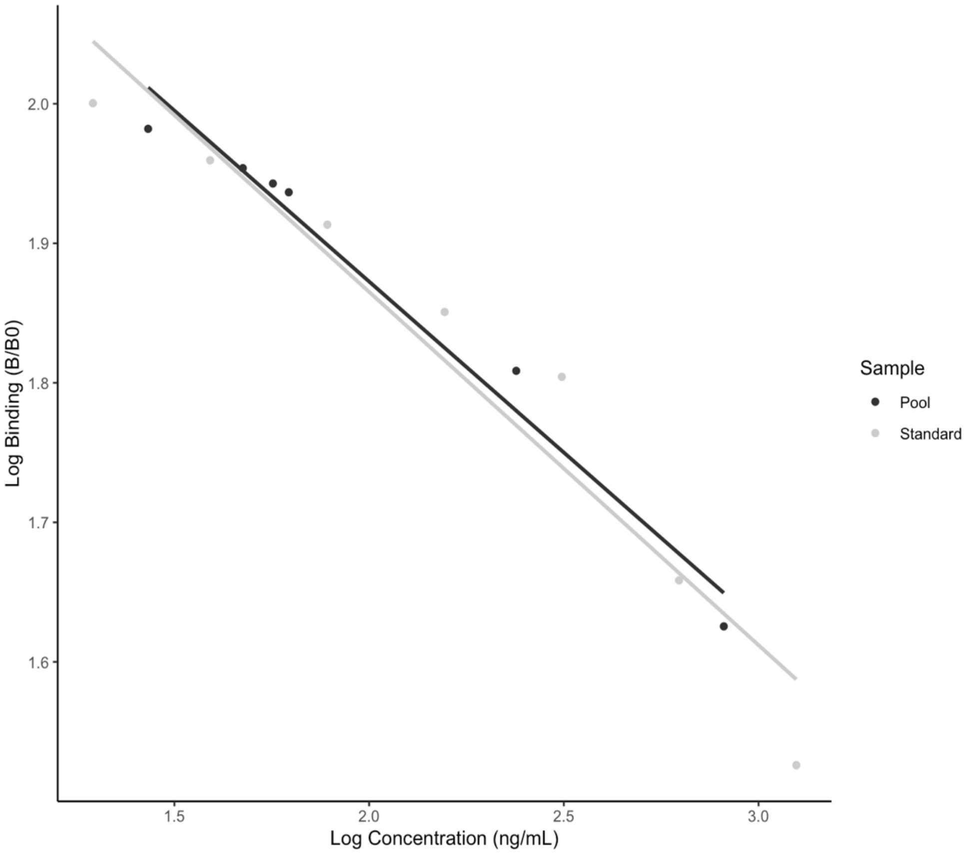


Figure S4.1) Parallelism for DetectX corticosterone EIA

Table S4.1) ANOVA of log_concentration ~ Sample * log_binding demonstrating there is no interaction between sample and binding.

Variable	Df	F value	*P value*
Sample	1,9	5.7	0.04
Binding	1,9	163.2	<0.001
Sample*Binding	**1,9**	**0.2**	**0.7**

4.7.1. Text S1 - Faecal steroid extracts stored on HyperSep C18 cartridges positively correlate with dried extracts.

4.7.1.1. Storage experimentation - Quantification of error using cartridges

It came to our attention that storing hormone metabolites on Hypersep C18 cartridge may not be optimal. To ensure that the prior use of C18 cartridges for sample storage did not influence results in Lea *et* al, 2017, we analysed storage on C18 cartridges against storing samples dried. We combined 0.5 g of faecal material with 80% methanol (5 ml), vortexed and centrifuged the sample for 15 minutes at 2,500 g. We then took 1 ml of extract and we then evaporated the sample under air. For the cartridges, we took the remaining 4 ml of extract processed it through a HyperSep C18 cartridge using the following protocol (Edwards *et al.*, 2014). Once processed, the cartridges were stored upright, overnight. Extract was then eluted using 4ml of 100% methanol. 1 ml of this methanolic extract was used for EIA, analogous to the other (non-cartridge stored samples).

4.7.1.2 Impacts on concentration and correlations

Lea *et al.* (2017) stored samples on HyperSep C18 cartridges rather than drying down extracts. We therefore used a sub-sample to compare concentrations measured with different extraction protocols. We found a strong positive correlation between cartridge and lab extracted sample methods (t = 10.6, df = 38, cor = 0.86 $p < 0.001$). Therefore, HyperSep C18 cartridges and drying method display similar trends when used. *Lea et al 2017* data only used HyperSep C18 cartridges and were not compared to any dried samples hence the results do not influence those found in the manuscript. Instead, they suggest Lea *et al*, 2017 would have found similar associations between GC and ecological and demographic factors if they used either technique (and a validated assay).

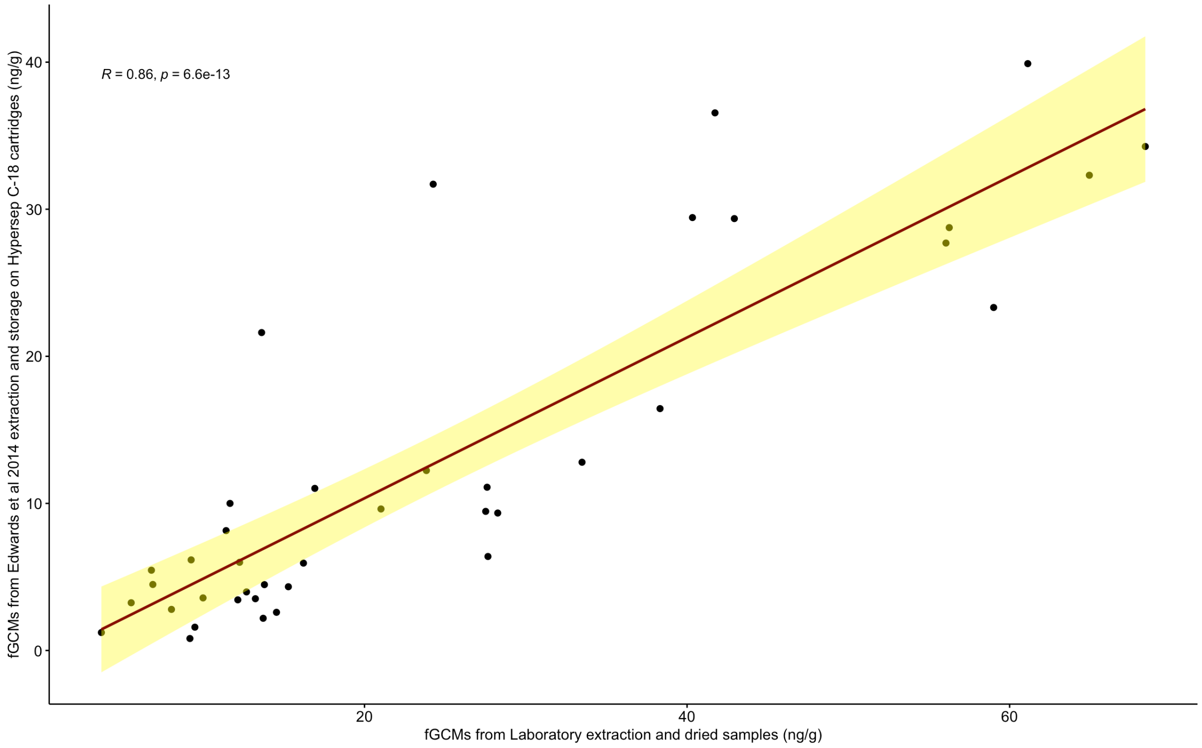
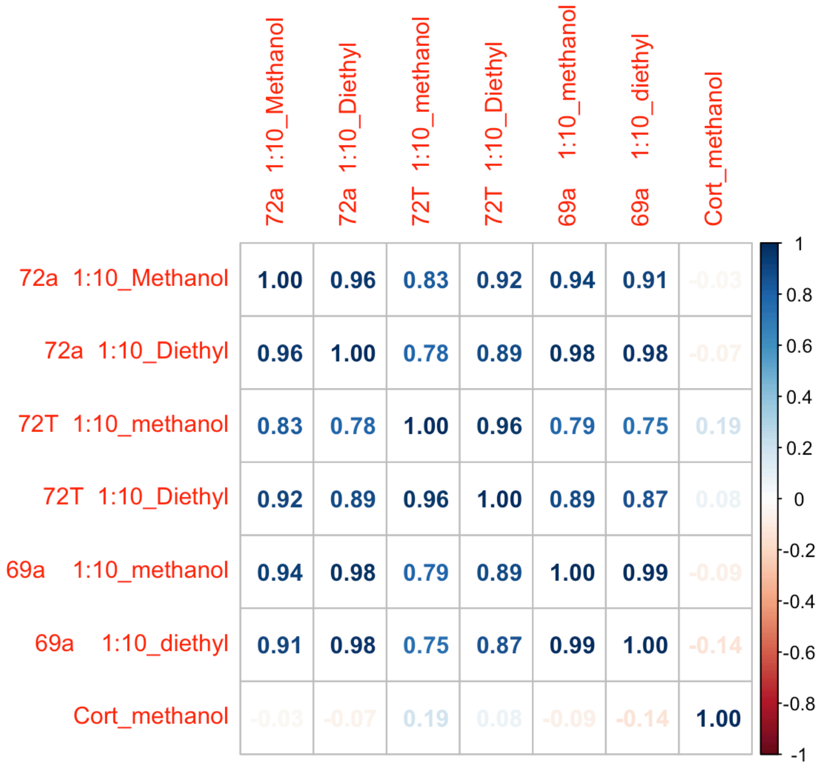


Figure S4.2) Correlation between lab extraction method and cartridge extraction method for the 72T EIA.

4.7.2. Text S2 - Correlation between metabolites in the validation experiment

We investigated correlations between all fGCMs metabolites using Pearson's product-moment correlations during the translocation. Concentrations of fGCMs measured with the two 11-oxoaetiocholanolone (72T; 72a) and the 11β-hydroxyaetiocholanolone (69a) EIAs displayed strong positive correlation between samples independent of the extraction method (Supplementary materials). None of the fGCM levels measured with the three group-specific (72a, 72T, 69a) EIAs significantly correlated with those of the corticosterone EIA during validation



	72a 1:10_Methanol	72a 1:10_Diethyl	72T 1:10_methanol	72T 1:10_Diethyl	69a 1:10_methanol	69a 1:10_diethyl	Cort_methanol
72a 1:10_Methanol	1.00	0.96	0.83	0.92	0.94	0.91	-0.03
72a 1:10_Diethyl	0.96	1.00	0.78	0.89	0.98	0.98	-0.07
72T 1:10_methanol	0.83	0.78	1.00	0.96	0.79	0.75	0.19
72T 1:10_Diethyl	0.92	0.89	0.96	1.00	0.89	0.87	0.08
69a 1:10_methanol	0.94	0.98	0.79	0.89	1.00	0.99	-0.09
69a 1:10_diethyl	0.91	0.98	0.75	0.87	0.99	1.00	-0.14
Cort_methanol	-0.03	-0.07	0.19	0.08	-0.09	-0.14	1.00

Figure S4.3) Correlation matrix of metabolites within validation samples

4.7.2. Text S3 – Covariation between the validated 11-oxoaetiocholanolone (72T) and the unvalidated (Munro, CJM006) corticosterone EIA

Across Lea *et al*, 2017 samples, we found a moderate positive correlation between unspecified faecal steroid concentrations assessed by the Munro corticosterone EIA and fGCMs assessed by the validated 72T EIA (t = 6.015, df = 363, r = 0.30, 95% C.I. 0.20 to 0.39, $p < 0.001$), which may result from cross-reactivity between EIAs or covarying physiological processes (Figure 3). However, the correlation coefficient was much lower than we would expect for two assays that are meant to measure the same biological response. For example, the correlation was weaker than that between fGCMs assessed by 72T EIA and metabolite concentrations assessed by a testosterone EIA, steroid hormones which are unrelated to HPA axis activity (t = 6.04, df = 160, r = 0.43, 95% C.I. 0.30 to 0.55, $p < 0.001$) (Figure 3).

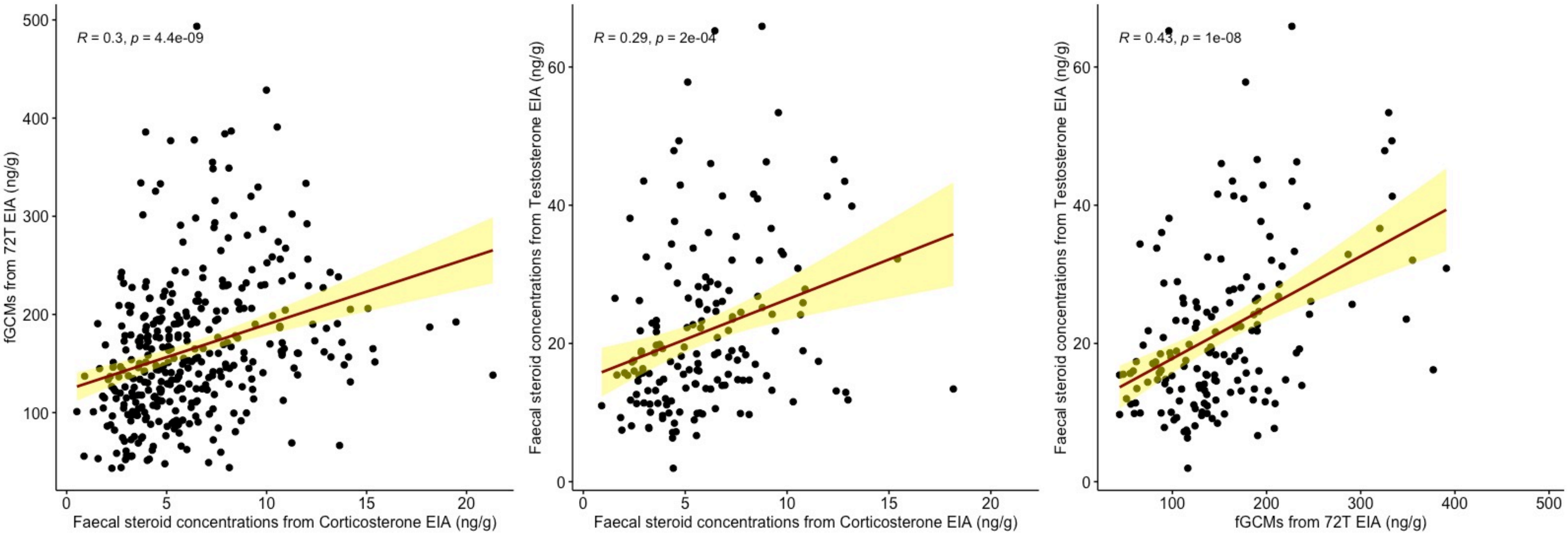


Figure S4.4) Correlation between fGCMs measured by the 11-oxoaetiocholanolone (72T), and unspecified steroid hormone metabolite concentrations from the corticosterone (Munro, CJM006) and testosterone (polyclonal testosterone R156/7) EIAs. R statistic and p value calculated using Pearson's correlation coefficient.

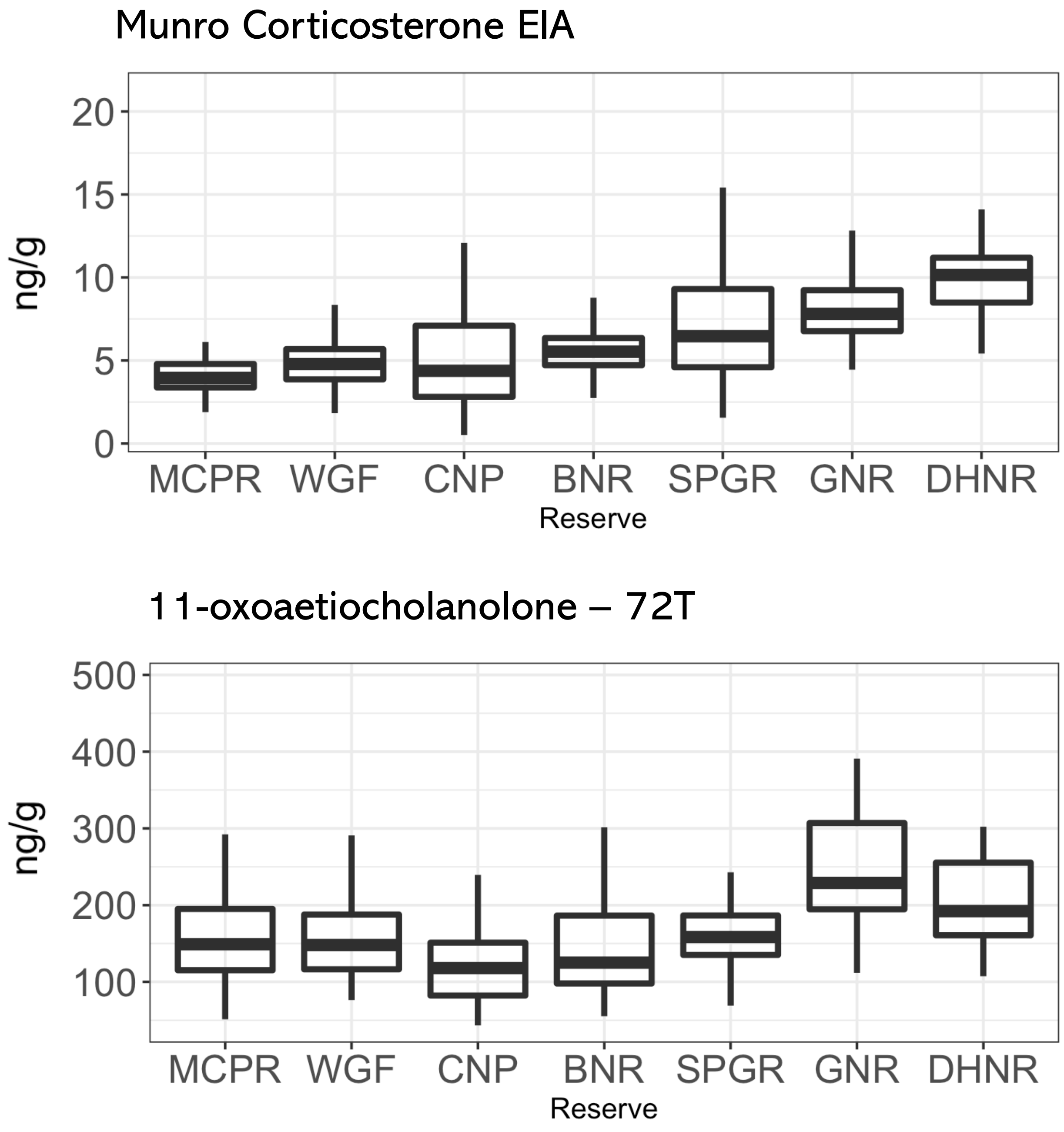


Figure S4.5) Boxplot graphs of concentrations of unspecified faecal steroid metabolites from the Munro corticosterone EIA and fGCM assessed by 11-oxoaetiocholanolone(72T) across reserves (oxoaetiocholanolone – 72T). Average concentration order varied – reserves ordered by the corticosterone concentrations.

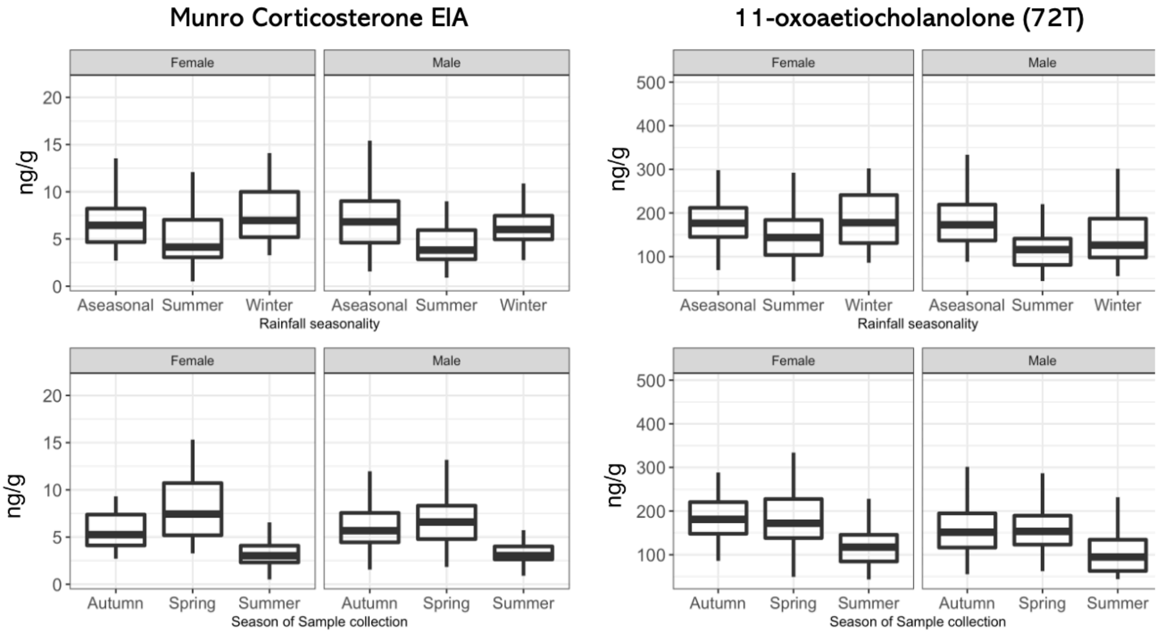


Figure S4.6) Cape mountain zebra unspecified steroid hormone concentrations from Munro Corticosterone EIA (Left) and fGCM concentrations (Right) from 11-oxoaetiocholanolone(72T) show seasonal variation and differ depending on the timing of peak rainfall. However, 11-oxoaetiocholanolone (72T) assay reveals sex-specific effects of season, which differ from that of unspecified steroid hormone concentrations.

Chapter 5) Macrophysiology and marginality in the Cape mountain zebra (*Equus zebra zebra)*

In preparation for submission to the Journal of Animal Ecology as Britnell J.A, Kerley G.I.H., Antwis R, Giandhari J, de Oliveria T, Lee C, Shultz S. Cascading physiological impacts of diet limitation drive performance across a species range in Cape mountain zebra (*Equus zebra zebra*)

5.1 Abstract

1. Food availability and quality are significant factors limiting animal populations. In food rich areas, survival, reproductive rates and/or density are higher than in ecologically marginal areas, where populations are less resilient. Ultimately, range limits result when food availability is inadequate to sustain populations. Despite the importance of food in regulating and limiting large mammal abundance and distributions, dietary shifts (except migration) and their macrophysiological responses have received limited attention.

2. We investigate how diet and macrophysiology link to performance across populations in a large grazer, Cape Mountain zebra (*Equus zebra zebra, CMZ*) using DNA metabarcoding of the diet, nemabiome, and microbiome (*n = 267*).

3. First, we find CMZ dietary niche breadth follows a quadratic relationship with the proportion of Poaceae (Grasses) in the diet. Individual dietary breadth increases as grass items become scarce because they supplement their diet with secondary plant families such as Asteraceae (Asters), Fabaceae (Legumes) or Malvaceae (Mallows). As grass availability decreases, individuals transition to a reliance on these secondary plant families.

4. Diet has cascading consequences for microbiome composition and function and nemabiome composition. Individuals with more dissimilar diets had more dissimilar microbiomes and nemabiomes. The proportion of grass in the diet is associated with functional microbiome shifts to a greater proportion of microbial groups involved in fermentation and xylanolysis. The proportion of grass in the diet was negatively associated with parasite burden, lower burdens were associated with high grass diets.

5. Finally, we find populations with more dissimilar diets had greater population performance differences. We show low proportion of grasses in the diet and increased parasite burden are associated with lower numbers of foals per mare and

lower population densities across the species range. We find that grass consumption in the diet explains performance metrics better than grass availability.

6. *Synthesis:* Our study reveals that dietary switching is common across a species range. Our results demonstrate that diet structures microbiome composition and function and nemabiome composition across a species range. We find links between diet, nemabiome and population performance. Our results suggest food limitation is a significant factor limiting CMZ density and reproductive output across protected populations.

5.2 Introduction

A fundamental question in ecology is what limits species distributions and abundances (Sutherland et al., 2013). This question becomes more crucial as human pressures cause widespread declines in population sizes and species ranges (Channell & Lomolino, 2000; WWF, 2020). Species distributions and population sizes are dictated by ecological gradients formed through variation in biotic and abiotic conditions (Holt, 2009). In resource rich areas, energy availability is greater, such that the potential total biomass of animals is increased (Huston, 2005). These high-quality habitats may promote greater survival or reproductive rates (Pulliam, 1988; Pulliam & Danielson, 1991) or, if dispersal is balanced *i.e.* no net flow of individuals across habitats, a higher carrying capacity (McPeek & Holt, 1992) than ecologically marginal areas. Range limits form where conditions become too extreme or resource availability too poor such that populations are extirpated faster than they are colonized (Gaston, 2009).

Resource availability is a major factor influencing large mammalian herbivore demographics. Large mammalian herbivore population size and growth is more often limited by food abundance and quality (Mduma et al., 1999; Sinclair et al., 1985; White, 2008) than predation, disease or infanticide (Krebs, 1978). Food shortages lead to population fluctuations due to starvation (Mduma et al., 1999) or compromised reproductive rates (Nagy & Holmes, 2005). Despite the importance of diet in regulating and limiting herbivore populations (reviewed in White 2008), the dietary strategies and macrophysiological basis of herbivore population dynamics is poorly understood.

Food availability and herbivore consumption vary across landscapes due to climate (McNaughton et al., 1989). In areas of high resource abundance, animals maximize their energy intake by selecting "optimal" diets (Schoener, 1971). During food limitation, animals can alter their diet according to two contrasting models: optimal foraging theory (OFT) (MacArthur & Pianka, 1966) or niche variation hypothesis (NVH) (van Valen, 1965) Optimal foraging theory predicts conspecifics select, or compete for, the highest quality items available (MacArthur & Pianka, 1966; Stephens & Krebs, 1986). Once these items become scarce, individuals increase dietary breadth by consuming poorer quality items (Krebs et al., 1977; Roughgarden, 1974). Dietary breadth may decline when individuals become heavily dependent on secondary items (Figure 5.1). Alternatively, the niche variation hypothesis predicts, during food limitation, individuals minimize intraspecific competition by specialising in subsets of dietary items (Svanbäck & Bolnick, 2007; van Valen, 1965). NVH posits that food limitation leads to a linear increase in dietary breadth (Augustine & McNaughton, 1998; Sargeant, 2007) through dietary differences between individuals (Figure 5.1; Svanbäck & Bolnick, 2005).

Ultimately, dietary changes may regulate populations through physiological changes within the individuals making up the populations (Chown & Gaston, 2016; Shultz et al., 2021). An essential aspect of an animal's physiology impacted by diet is its gastrointestinal physiology (Karasov et al., 2011). Diet influences the structure of animal gut microbiomes and nemabiomes, the communities of bacteria and helminths in the gastrointestinal system respectively (Kartzinel et al., 2019; Schneider-Crease et al., 2020). Mammalian enzymes cannot digest many plant polysaccharides and mammals therefore depend on their gut microbiome to digest and assimilate nutrients and vitamins from food items (Hanning & Diaz-Sanchez, 2015) and detoxify plant defence chemicals (Kohl et al., 2014). The gut microbiome is crucial in modifying immune responses to maintain health status, with perturbations potentially leading to disease (Duvallet et al., 2017).

Diet can also affect population trends through gastrointestinal parasitic infection (Ezenwa, 2004) and nemabiome composition (Schneider-Crease et al., 2020). Gastrointestinal parasite infections are associated with reduced fitness and population fluctuations (Hudson et al., 1998; Hillegass et al., 2010). Immunocompetence is compromised by resource limitation (Fair & Whitaker, 2008; Budischak et al., 2015) and bolstered by high quality diets (Navarro-Gonzalez et al., 2011). Immunocompromised individuals are at greater risk of gastrointestinal parasitic infection from helminths. Diet is therefore crucial in determining gastrointestinal parasite burdens (Ezenwa, 2004; Navarro-Gonzalez et al., 2011) and their effects on the host (Budischak et al., 2015).

We predict individuals following the predictions of optimal foraging theory or the niche variation hypothesis to have different physiological responses. If microbiome responses to dietary change are deterministic, animals following optimal foraging theory should display directional changes in microbiome composition. Shifts in microbiome and nemabiome dissimilarity - β diversity - should be associated with the food items that animals switch to. Population level diet, microbiome and nemabiome β dispersion (distance from individual composition to a population average) should remain constant irrespective of diet as conspecifics within a population utilise similar items. Furthermore, overlap between conspecifics' diets within populations should remain constant (Figure 5.1). In contrast, animals following the predictions of niche variation hypothesis would display increasing population level diet, microbiome and nemabiome β dispersion with decreasing primary food sources. Overlap between conspecific diets should increase with increasing resource availability (Figure 5.1). In both, we would predict a shift to increased microbial functions associated with digestive breakdown of the ingested dietary items. For the niche variation hypothesis model, microbial functional shifts should diversify with increasing individual specialisation. We predict individuals would experience greater parasite burdens with declining primary food items.

Here we test whether diets limit population performance *via* changes in gastrointestinal macrophysiology in Cape Mountain zebra (*Equus zebra zebra*) across its' species range. We hypothesise:

1. Dietary composition, especially proportion of *Poaceae* (Grasses), will be driven by rainfall, forage availability (proportion of bareground) and grass availability.
2. Individuals will utilise resources according to the predictions of optimal foraging theory. Increases in dietary niche breadth will be due to increases in individual dietary diversity as individuals add poorer quality items to their diet.
3. Individuals will show dietary switching, by increasing consumption of "fallback" or less preferred items, when they cannot saturate their diet with grass.
4. We predict dietary breath will follow a negative quadratic with proportion of grass in the diet. We predict dietary breadth will increase as animals supplement their diet with fallback foods and decreasing as animal transition to greater dependence on fallback foods.
5. Diet will be associated with altered microbiome and nemabiome composition and microbiome function such that animals with more similar diets will have more similar microbiomes and nemabiomes. Diet will also lead to functional changes in the microbiome. Increasing proportion of grasses in the diet will be associated increases in relative abundance of primary cellulolytic degraders (*Ruminococcaceae*, *Lachnospiraceae*, *Fibrobacteraceae*, *Spirochaetes*) and secondary fermentative bacterial groups (*Prevotellaceae* and *Bacteroidales*) (Flint et al., 2008; White et al., 2014). Grass-rich diets will be associated with increases in proportion of bacteria associated with cellulolytic/fermentation activities or activities which break down specific plant polysaccharides.
6. Reduced proportion of grasses in the diet will increase helminth burdens and lead to nemabiome compositions of more pathogenic species such as Strongylus *spp.*
7. Diet and parasite burden will limit CMZ population density and fecundity. Populations with increased average proportion of grasses in the diet will have a higher ratio of foals to females and will have greater population densities. Animals with more dissimilar microbiomes and nemabiomes will have more dissimilar performance and population densities.

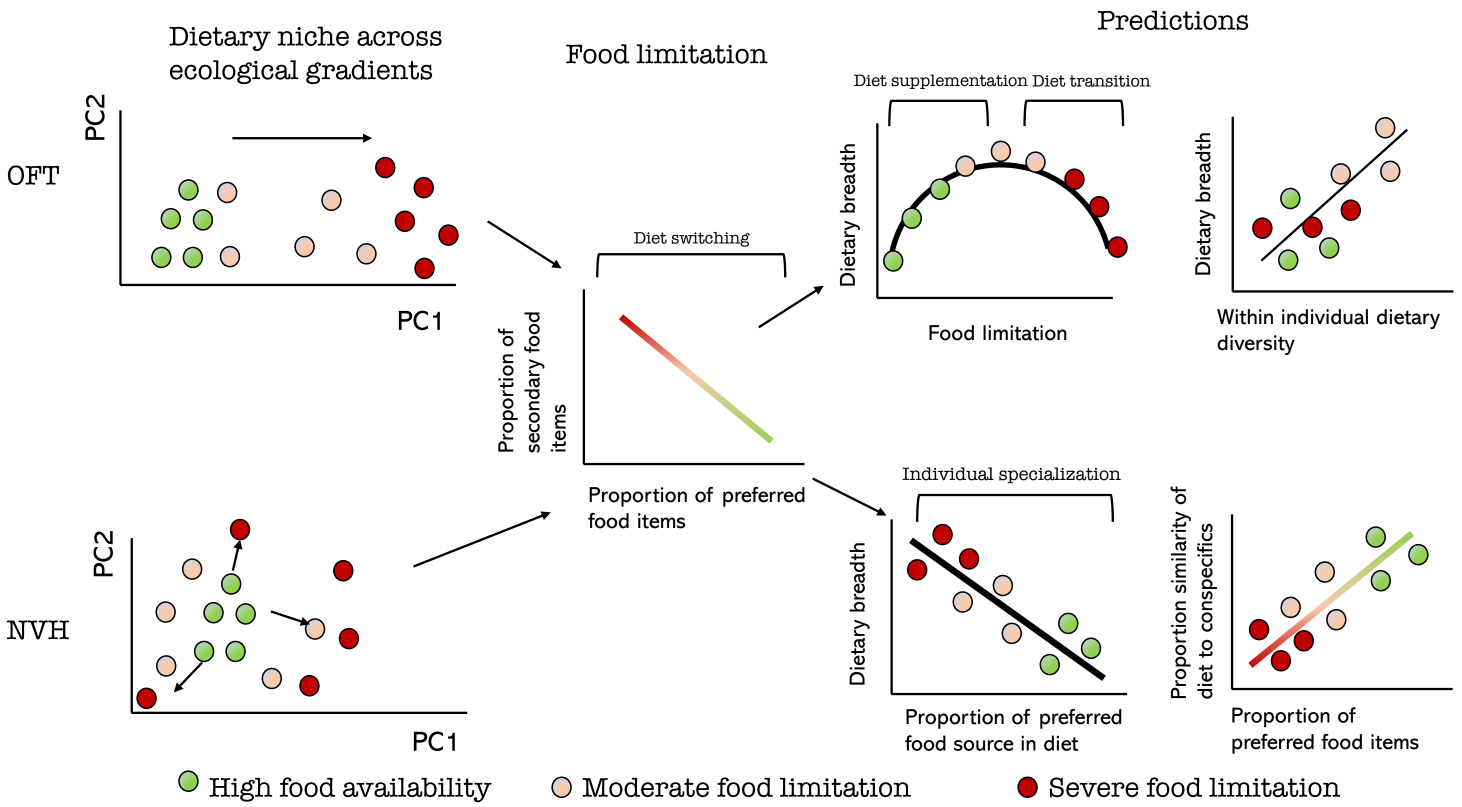


Figure 5.1) Predictions of dietary niche dynamics under optimal foraging theory (OFT) and niche variation hypothesis (NVH). Left) Predictions of OFT and NVH in multivariate dietary niche space. During OFT, dietary niche space is stable, spreads as animals include additional items into their diet and then shifts completely to the dietary space associated with the secondary food item. Occupied NVH dietary niche space increases as individuals eat different items to minimize intraspecific competition. Centre) Diet switching can be evidenced through negative correlations between primary food items and secondary food items. Top right) Diet supplementation indicates animals supplementing their diet with additional food items. Diet transition occurs when animals transition to heavy dependence on secondary food items. According to OFT, increases in dietary breadth should be driven by within individual dietary diversity. Bottom right) According to NVH, dietary breadth decreases with decreasing food limitation. The overlap between conspecific diets should increase with decreasing food limitation.

5.3 Methods

5.3.1. Study sites, species and sampling

Mountain zebra are a vulnerable equid species (IUCN, 2019) divided into two subspecies Cape Mountain zebra (*Equus zebra zebra, CMZ*) found below orange river in South Africa and Hartman's zebra (*Equus zebra hartmannae)* in Namibia. Historically, Cape Mountain zebra had a limited historical distribution across five biomes – Fynbos, Succulent Karoo, Desert, Nama-Karoo and Grassland within the Eastern, Western and Northern Cape provinces of South Africa (Boshoff et al., 2016). CMZ provide an ideal model species to understand the mechanisms of population limitation. The availability of C_3 and C_4 grasses, CMZ's preferred food source, varies widely across populations and the historic species range (*SI Text S1)*. Long term population growth rates, density and fecundity vary widely between populations and are associated with grass availability (Lea et al., 2016). As CMZ exist in fenced environments, migration between populations is impossible without human intervention. Each population is an independent "closed system" such that all individuals experience nearly identical environmental constraints and resource availabilities (Lea et al., 2016).

For this study, we collected 267 faecal samples from 223 individuals across nine populations: Kuzuko Lodge Private Reserve (KZL), Mountain Zebra National Park (MZNP), Camdeboo National Park (CNP), Mount Camdeboo Private Reserve (MCPR), Gamkaberg Nature Reserve (GNR), Bontebok National Park (BNP), Sanbona Wildlife Reserve (SWR), Koktyls Private Reserve (KOK) and Bushman's Kloof private reserve (BMK) (For geography see Figure S7). These nine populations span most of the species historic niche and vary in their climatic conditions and vegetation communities (*SI Text S1*). All faecal samples were collected between Dec-March in the years of 2019-2021. Year 1, samples were collected from KZL, CNP, KOK, SWR and BNP. Year 2, samples were collected from all reserves. Individuals were witnessed defecating, identified by stripe-pattern and faecal samples were collected and stored in 8ml of 100% ethanol until extraction (*SI Text S2*).

5.3.2 DNA metabarcoding

We extracted DNA and undertook amplicon sequencing for microbiome, nemabiome and diet from 267 faecal samples (Text S4). For bacterial identification, we amplified the 16S rRNA (v4 region) gene region (Antwis et al., 2019; Kozich et al., 2013). For nematodes, we amplified the rDNA Internal transcription spacer-2 (ITS-2) region (Avramenko et al., 2015). For plants, we amplified the P6 loop of the chloroplast *trn*L(UAA) region (Kartzinel et al., 2015; Kartzinel & Pringle, 2020). All sequence processing was conducted using Cutadapt (Martin, 2011) , DADA2 (v1.18.0) (Callahan et al., 2016) and analysis was conducted using Rstudio (v1.3.1093) (R Core Team 2020). We rarefied sample count to 2000 reads for diet and nemabiome and 5000 reads for microbiome. Following sample processing and rarefication, 260 samples remained for microbiome, 263 samples for diet and 202 samples for nemabiome. Total rarefied reads for each metabarcoding run were converted into relative read abundance (RRA) to evaluate composition (*Text S4*). RRA is widely used for diet as a proxy of the proportional quantity of foods eaten. RRA is well validated as a reliable proxy of grass:browse consumption ratios for large herbivorous mammals (Kartzinel et al. 2015).

5.3.3 Data analysis

5.3.3.1 Hypothesis 1: Proportion of grasses (Poaceae) will be driven by rainfall, forage and grass availability

Forage and grass availability were determined by 50m vegetation transects using a point transect method (Barbour et al. 1980). Presence of grasses, forbs, shrubs (including woody shrubs, trees and succulents) and bare ground (unvegetated) were recorded at each 1m interval. Line transects were located in areas in active use by CMZ as identified by managers, rangers, sightings or presence of faeces. Gut retention time for Cape mountain zebra is approximate 24-36 hours. To account for movement pattens of CMZ, we estimated grass availability for each sample by pooling transects within ~3km^2 of each other (approximately 2-day movement circumference of closely related Grevy’s zebra (*Equus greyvi*) and lower estimate of the range size of breeding CMZ groups (Penzhorn, 1982) (*SI Text S3*). Rainfall was assessed *via* the CHIRPS dataset at 0.05° resolution. As a proxy of rainfall, we assessed cumulative rainfall for three months prior to sampling and annual rainfall for each reserve to allow for the delay in rainfall and grass growth and to assess overall precipitation limits across years respectively (*SI Text S3*).

Relative read abundances of dietary items were calculated as their relative abundance within each sample using microeco (Liu et al, 2021).

5.3.3.2 Hypothesis 2: Individuals will utilise resources along predictions of OFT.

5.3.3.2.1 Dietary switching

Dietary switching should result in negative correlations between primary and secondary food items (Marshall et al., 2009). We assessed relationships between two most abundant food items (usually RRA of Poaceae, Asterceae and Fabaecae) and secondary food items using Pearson's product-moment correlation.

5.3.3.2.2 Diet diversification - Dietary breadth and compositional similarity between conspecifics

We investigated diet breadth changes by calculating Total dietary niche width (TNW) within-individual diversity (WID), between-individual diversity (BID) and a proportional-similarity index (PS_i). Total niche width can be used as a proxy of dietary breadth (Roughgarden, 1974). Total niche width was calculated using a Shannon Diversity Index of diet composition (Pansu et al., 2019). Total niche width is made up of within-individual diet variation and between-individual variation level (Bolnick et al., 2002; Sargeant, 2007). Under OFT, increases in Total Niche Width will be due to increases in individual diet diversity (Jesmer et al., 2020) (Figure 5.1). Compositional similarity between individuals was analysed using PS_i values generated using the *RInSp* package (Zaccarelli et al., 2013). Proportional similarity index (PS_i) provides a metric of individual specialisation by comparing compositional overlap between plant operational taxanomic units of each individual sample and a population-wide average diet (Bison et al., 2015). We calculated the average PS_i and converted it to a metric of between individual diversity for each reserve at each time period using the equation *V*= (1- average PS_i) (Araújo et al., 2011; Bolnick et al., 2002; Pansu et al., 2019).

5.3.3.2.3 Beta dispersion

We investigated beta dispersion as distance from the reserve centroid (of each time point). Samples were first transformed *via* centre-logged ratio (CLR) using *microbiome* package (Lahti *et al*, 2017). We then produced a Euclidean distance matrix using *distance* function from *phyloseq* (McMurdie & Holmes, 2013). We evaluated the relationship between beta dispersion and proportion of grass in the diet (Poaceae) using Pearson's correlation coefficient.

5.3.3.3 Hypothesis 3) Diet will deterministically change microbiome and nemabiome structure and microbiome function

5.3.3.3.0 Taxonomic level of analysis

In our analyses, we used different taxonomic resolutions to evaluate our hypotheses of interest. For diet, taxa were agglomerated to family level for compositional analysis. Family level analysis represents the most biologically meaningful and intuitive resolution. With this resolution, dietary groups of grasses, legumes, mallows and asters could easily be distinguished. For microbiome and nemabiome, taxa were agglomerated to species level for compositional analysis. Species level analysis was performed for microbiome as bacterial genus can have varying functions depending on the species. For nemabiome, although genus level analysis would have identified key groups, species level analysis can identify specific detrimental species such as *Strongylus equinus*. For both microbiome and nemabiome taxa were agglomerated at species level as we wanted to account for different assigned sequence variants (ASV) 213ccurring for the same species. For diet, diet diversification was evaluated at assigned sequence variant (ASV) level. Dissimilarity analysis was also evaluated at ASV level for diet, nemabiome and microbiome analysis. ASV level for these analyses as we needed to account for all possible ASVs irrelevant of the level of taxonomic assignment.

5.3.3.3.1 Diet, microbiome and nemabiome composition, beta diversity and pairwise associations

We analysed compositional change between reserves and years using permutational analysis of variance (perMANOVA) in the *vegan* package (using 10,000 permutations) (Dixon, 2003). For beta diversity of diet and nemabiome, we calculated weighted unifrac index of compositional dissimilarity between each pair of faecal samples (i.e., individual samples). Weighted Unifrac measures between-sample dissimilarity while controlling for phylogenetic distances between items (Lozupone & Knight, 2005). The weighted UniFrac calculated community distance as a weighted sum of branch lengths in a phylogenetic tree. Phylogenetic trees for each dataset were generated by first aligning sequences using DECIPHER package in R (Wright, 2020). A maximum likelihood tree was generated in phangorn package (Schliep, 2011), and FastTREE (Price et al., 2009) for microbiome phylogenetic relationships due to the size of the alignment. Phylogenetic trees were rooted at their midpoint using for small tree mid.point function in phytools (Revell, 2012) and root_at_midpoint function in caster package due to the size of the tree (Louca & Doebeli, 2018).

We evaluated the effect of environmental variables on between-sample community composition using principal component analysis. Firstly, to account for differences in sequencing depth between samples, the counts for each data set were normalized using the centred-log-ratio (CLR) method (using a small pseudo count of minimum relative abundance/2 for zero counts) using MicroViz package (Barnett et al., 2021). Secondly, we conducted a Principal Component Analysis (PCA) to visually represent between-sample differences according to the predictors. This approach has been recommended for proportional and compositional datasets such as metabarcoding data (Gloor et al., 2017). Using PCA on CLR-transformed data allows for the projection of variable loadings of ASVs (or groups) onto each PC. Principal components can then be extracted and correlated against environmental variables. For diet, taxa were agglomerated to family level and family level dietary groups were loaded onto PCA. For microbiome and nemabiome, taxa were agglomerated to species level and variable loadings of species were evaluated. Thirdly, relationships between environmental variables on principal components were established using linear regression.

To test for associations between beta diversity of microbiome, nemabiome and diet, we compared the weighted Unifrac dissimilarity of each to each other. We assessed the relationship between pairwise distances in each dataset using Mantel tests using 999 permutations and Spearman's multilevel correlations. In other words, we tested whether individuals with more phylogenetically dissimilar diets harbour more phylogenetically dissimilar microbiome and nemabiome communities. Additionally, we also tested whether individuals with more phylogenetically dissimilar microbiome communities had more dissimilar nemabiome assemblages.

5.3.3.3.2 Microbiome functional analysis

Microbiome functional analysis was conducted using FAPROTAX (Louca et al., 2016). The FAPROTAX functional reference database has been used to evaluate the functional changes in gut microbiota of both rats (*Rattus norvegicus*) and rabbits (*Oryctolagus cuniculus*) (Xiang et al., 2019; Xing et al., 2019). We assessed the relationship between functional traits and environmental variables using Spearman's rank correlation coefficient.

5.3.3.3.3 Parasite burden

Intestinal parasite burdens can be non-invasively monitored using faecal egg counts (Ezenwa, 2004; Uhlinger, 1990). Three grams of fresh faeces were suspended in 42ml of saturated salt solution and analysed using a McMaster FEC protocol for parasite egg abundance. Eggs per gram of faeces were calculated using the following equation:

$$\text{(5)} \quad \text{Eggs per gram (EPG)} = (\text{Egg count in grid 1} + \text{Egg count in grid 2}) * 50$$

Parasite eggs were counted as inside the grid when more than half of the egg was within the grid lines. Faecal egg counts are negatively correlated with body condition and health in feral horses (Debeffe et al., 2016). Faecal egg counts were performed fresh as storing in formalin or ethanol can lead to reduction in overall egg count after time (Crawley et al., 2016). All analyses were conducted within 8-12 hours of collection.

5.3.3.4 Hypothesis 4) Cape mountain zebra population growth and size will be limited by diet and associated with macrophysiological biomarkers.

We investigated the relationship between dietary composition, macrophysiological indicators and population performance metrics such as fecundity and population density. We compared average RRA of Poaceae and parasite burden for each reserve with foal:mare ratio and population density using linear regression analysis and Spearman's rank correlation coefficient.

We evaluated the effect of diet, microbiome and nemabiome composition on performance variables by comparing the mean weighted Unifrac dissimilarity to absolute change in foal:mare ratio across each reserve. In other words, we tested whether diet, microbiome and nemabiome dissimilarity were related to dissimilar performance rates. We used linear regression analysis to assess significance.

5.4 Results

5.4.1 Diet composition is driven by rainfall, forage and grass availability

A PERMANOVA test on weighted Unifrac distances (phylogenetically controlled distances between samples) indicated diet composition varied across reserves and years (ADONIS Reserve: $F_{261,8} = 50.3$, df = 8, $R^2 = 0.57$, $p < 0.001$; Year: $F_{261,1} = 20.1$, df = 1, $R^2 = 0.03$, $p < 0.001$, Table S5.1). Across populations, the majority of CMZ diets were made up of four plant families: Poaceae (Grasses, 63%), Asterceae (Asters, 15%), Fabaceae (Legumes, 7%) and Malvaceae (Mallows, 3.5%) (Figure S5.1). Major grass genera included highly palatable species such as *Digitaria* (average diet included 11.5%), *Ehrharta* (8.9%), *Themeda* (6.5%), *Cenchrus* (3.2%), as well as the semi-palatable genus *Heteropogon* (2.6%) and low palatability grasses such as *Diplachne* (1.5%) and *Aristida* (2.8%). Legumes were disproportionately made up of *Medicago spp* (4.2%) and *Aspalathus* (Rooibos shrub) (1.6%). Mallows were disproportionately made up of *Hermannia* (2.6%) and *Grewia* (0.7%).

Proportion of grasses in diet varied across reserves ($F_{8,234} = 52.8$, $p < 0.001$, Figure S5.2 and Table S5.3). Grass availability and cumulative rainfall explained 12% and 3% of dietary composition respectively (ADONIS Weighted Unifrac: Grass availability $F_{261,1} = 36.1$, $R^2 = 0.12$, $p < 0.001$; Proportion unvegetated; $F_{261,1} = 1.2$, $R^2 = 0.04$, $p = 0.25$; Rainfall = $F_{261,1} = 0.03$, $p < 0.001$, $n = 261$; Table S5.2).

Across populations, the proportion of grass in the diet was a function of increasing grass availability and rainfall ($\beta = 0.36$, s.e = 0.05, $F = 60.7$, $p < 0.001$, $n = 234$ and $\beta = 0.005$, s.e = 0.002, $F = 11.2$, $p < 0.001$, $n = 234$, respectively). Although not significant, the proportion of bare ground improved model fit (delta AIC > 2) (SI Table S5.3).

The first principal component of diet β diversity, which explained 15.2% of variation, was negatively correlated with rainfall (β= -0.004, s.e = 0.0007, $t = -5.3$, df = 260, $R^2 = 0.1$, $p < 0.001$) and grass availability (β= -2.72, s.e = 0.17, $t = -16.3$, df = 260, $R^2 = 0.50$, $p < 0.001$). PC1 was positively correlated with proportion of unvegetated ground (β= 2.79, s.e = 0.24, $t = 11.8$, df = 260, $R^2 = 0.35$, $p < 0.001$). The family that loaded negatively (loading scores <-1.0) on PC1 (i.e., correlated with higher rainfall and grass availability) were Poaceae (Grasses) of genera *Digitaria, Themeda, Cenchrus, Enneapogon, Aristida* (Table S5.6). The families associated with low rainfall and greater proportion of unvegetated ground also included Poaceae of genera *Ehrharta spp* and *Diplachne spp,* Ebenaceae *(*evergreen trees and scrubs of the ebony family) and Crassulaceae (succulents), Asteraceae (asters) and Fabaceae (legumes) (Figure 5.2; Table S5.7).

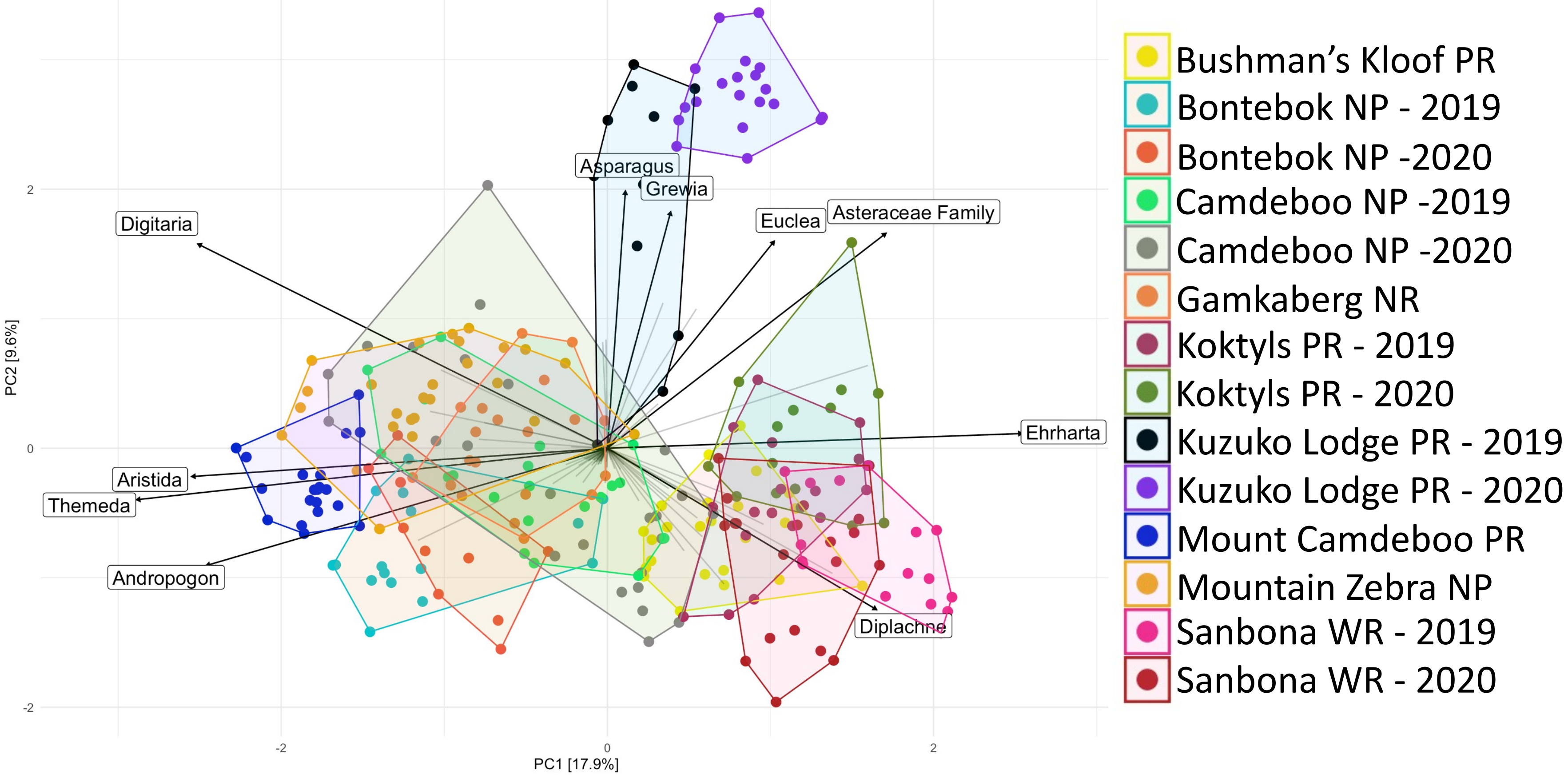


Figure 5.2) Environmental variables drive differences in Cape mountain zebra dietary composition. PCA ordination of dietary beta diversity CLR-transformed and agglomerated at genus level. Arrows indicate loadings. PC1 positively correlated with proportion of bare ground and negatively correlated with cumulative rainfall and grass availability. PC2 weakly positively correlated with proportion of bare ground and negatively weakly correlated with grass availability. *Themeda, Digitaria, Aristida, Ehrharta, Andropogon* and *Diplache* are grasses. *Grewia* are mallows. Asteraceae family includes Asters which could not be assigned to genus level. PR denotes private reserve, NP = National Park, NR = Nature Reserve and WR = Wildlife Reserve/ Some genera are not displayed as a compromise between clarity and information. Loading scores for genera are available in Tables S5.6 and S5.7.

5.4.2 CMZ display dietary switching and utilise items according to the predictions of optimal foraging theory

Individuals displayed changes to a single secondary plant family when they could not saturate their diet with grasses (Figure 5.3). Animals included Fabaceae (legumes), Asteraceae (asters) or Malvaceae (mallows) depending on the reserve. The plant family that was included did not depend on year of sampling (Figure 5.3). As grass availability increased across reserves animals consumed a greater proportion of grasses (β = 0.44, s.e = 0.07, t = 6.5, $p < 0.001$, R^2= 0.28*, df = 108*), fewer asters (β = -0.23, s.e = 0.07, t = -3.58, $p < 0.001$, R^2= 0.12*,* df = 88) and fewer mallows (β = -0.90, s.e = 0.36, t = -2.51, p = 0.02 , R^2= 0.22*, df = 18*) (Figure 5.4).

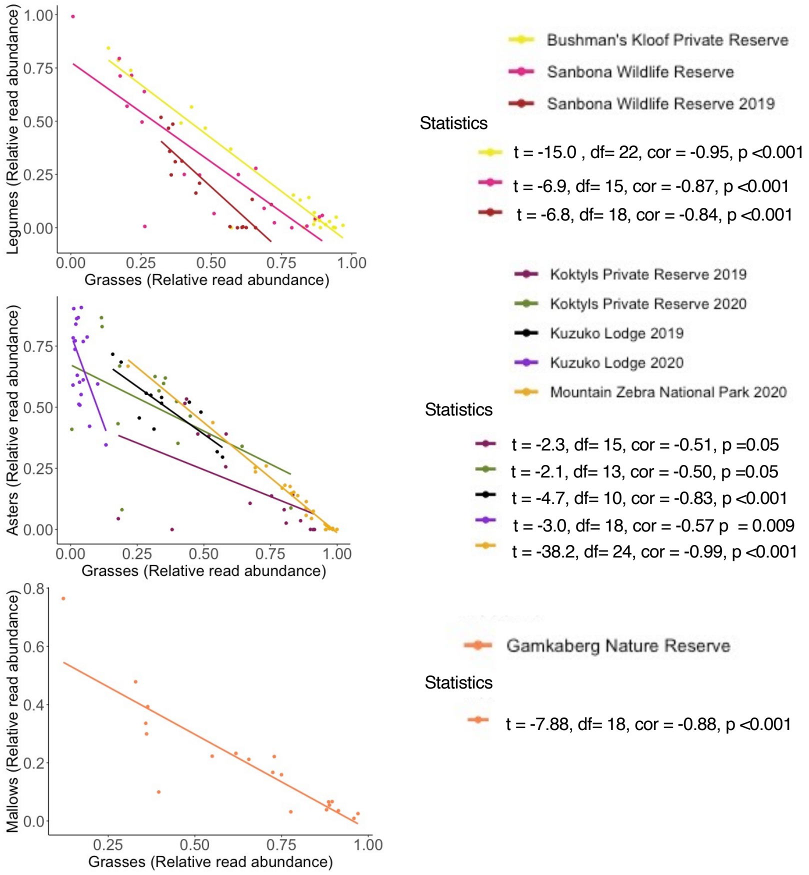


Figure 5.3) Alternative plant families incorporated into zebra diets when they cannot saturate their diet with grass. Top) Reserves where individuals supplement their diet with Fabaceae (Legumes), Middle) Asteraceae (Asters), Bottom) the reserves where individuals supplement their diet with Malvaceae. Results of correlations also available in Table S5.5)

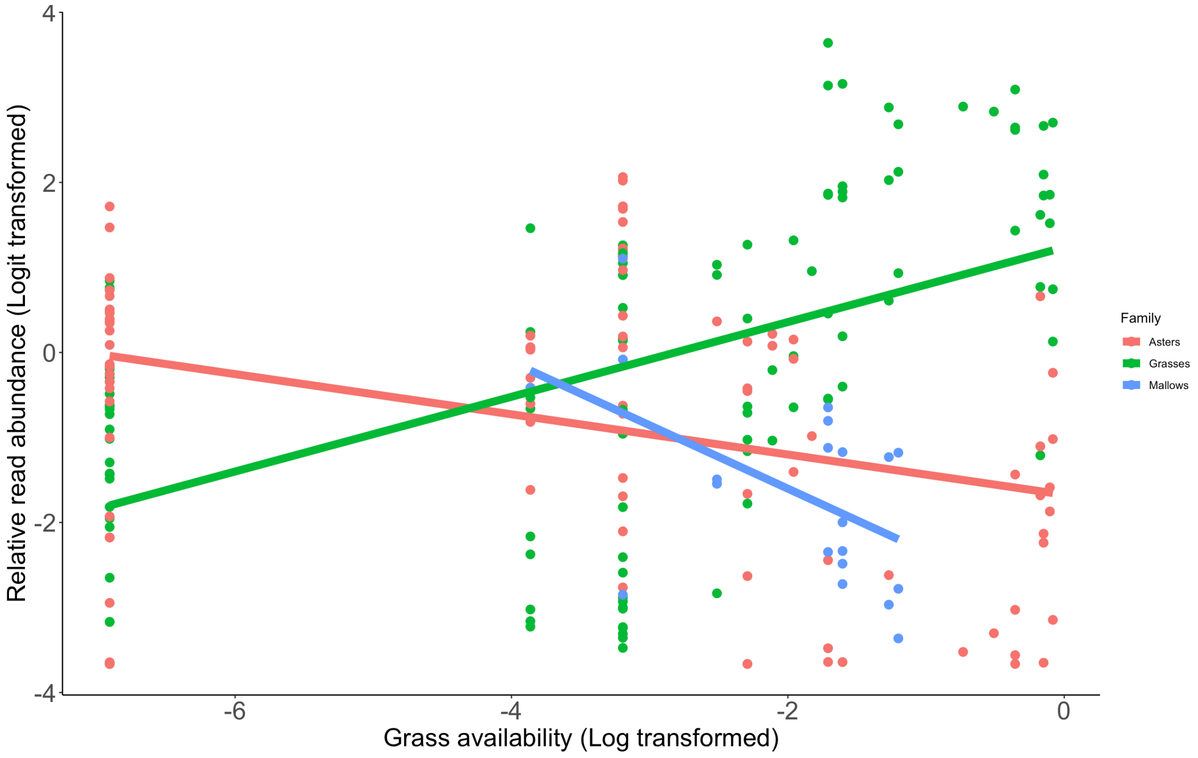


Figure 5.4) Proportion of grass consumed increases with increasing grass availability across reserves. Grass consumption increases with increasing availability of grass whereas aster and mallow consumption decrease. Legumes did not decrease with increasing grass availability and are not displayed on this graph. This is likely because the presence of legume-rich supplementary feed such as *Medicago sativa* aka alfalfa leads to inaccurate interactions between native grass availability and legume consumption.

Dietary niche breadth increased with increasing grass availability across sites (β = 0.25, s.e = 0.11, t = 2.16, p = 0.03 , R^2= 0.02*, df = 223,* Figure S5.7*).* Dietary niche breadth follows a negative quadratic relationship with proportion of grass in the diet (logit(RRA_Poaceae)^2*,* β = -2.80, s.e = 0.56, t = -5.0, p < 0.001, R^2= 0.09, F = 13.9*, df = 259*, Figure 5.5; SI Table S5.4). Populations display no evidence of individual specialisation (SI Figure S5.3). Increases in total niche width were disproportionally due to increases in the within-individual diversity (β = 0.9424, s.e = 0.15, t = 6.2, p < 0.001, R^2= 0.77, n = 14) (Figure 5.5). Dietary beta dispersion followed a negative quadratic with proportion of Poaceae in the diet (logit(RRA_Poaceae)^1*,* β = -7.1, s.e = 4.43, t = -1.6, p = 0.11, logit(RRA_Poaceae)^2, β = -21.2, s.e = 4.6, t = -4.7, p < 0.001, R^2= 0.08, F = 13.3*, df = 259*) and conspecific dietary overlap was not influenced by average proportion of Poaceae consumed by the population (β = 0.02, s.e = 0.07, t = 0.31, p = 0.76, adjusted R squared = -0.07) (Figure S5.3). Metrics of dietary individual specialisation did not change with increasing grass availability (Figure S5.6)

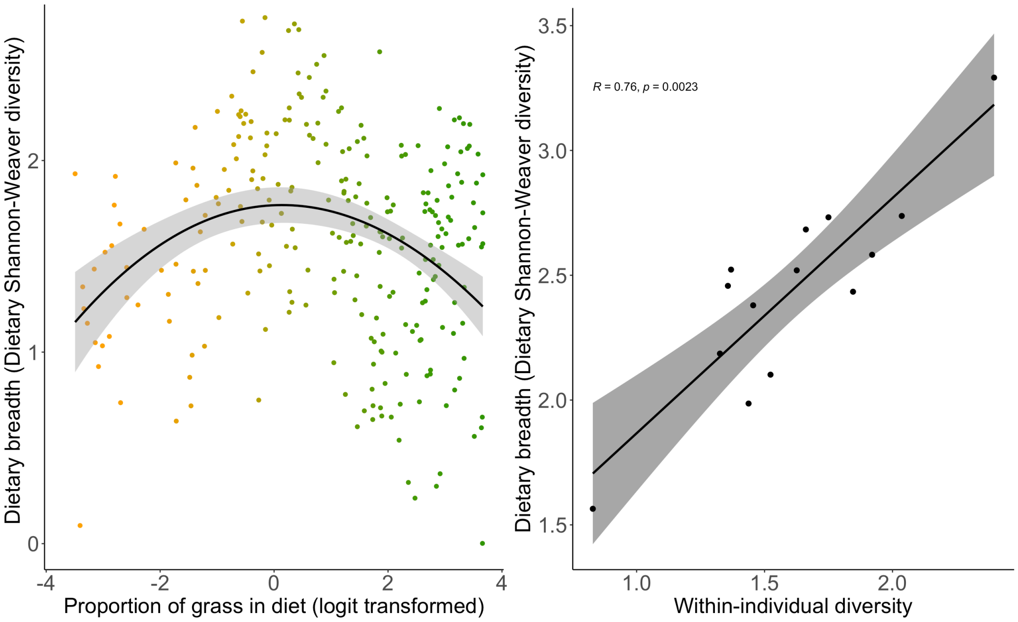


Figure 5.5) Cape mountain zebra dietary breadth (Shannon-Weaver diet diversity) follows a negative quadratic with primary food source (Poaceae, grass). Dietary breadth increases as zebra supplement their diet with additional food items other than Poaceae in a stage we define as “Diet supplementation”. Following that zebra transition their diet to being completely dependent on non-grass items in a process we term dietary transition. Increases in dietary breadth are strongly related to increases in individual dietary diversity following the predictions of OFT. Correlation between dietary breath and within-individual diversity was conducted using Spearman’s rank correlation coefficient. R value and *p* value are displayed. Results of regression analysis are presented above. Points are coloured according to the proportion of grass in the diet – the coloured gradient moves from zero(orange) to 100% (green).

5.4.3 Dietary changes are associated with shifts in microbiome structure and function and nemabiome composition

Individuals with more dissimilar diets had more dissimilar microbiomes (Mantel statistic r: 0. 11, *p < 0.001, n= 255*). Microbiome communities across the species range were made up of four major phyla: Firmicutes (46%), Bacteriodota (22%), Spirochaetota (13.3%)

Verrucomicrobiota (12.3%) (Figure S5.4). Microbial families which are typically found the guts of ruminants and the herbivorous hindgut were also prevalent in the Cape mountain zebra gut. These families include many cellulolytic/fibrolytic (19% *Lachnospiraceae*, 2.3% *Fibrobacteraceae,* 1.1% *Ruminococcaceae*) and fermentative families (5.6% *Rikenellaceae*, 3,2% *Prevotellaceae*, 2.7% *Bacteroidales F082*). The Spirochaetaceae family (13.2%) was mostly composed of *Treponema spp* (13.1%), a genus involved in lignocellulose degradation.

A PERMANOVA test on weighted Unifrac distances indicated microbiome β diversity was associated with proportion of grass, asters and legumes in diet and as well as parasite burden (ADONIS weighted Unifrac: Grass: $F_{254,1} = 12.5$, $R^2 = 0.05$, $p < 0.001$; Legumes: $F_{254,1} = 4.56$, $R^2 = 0.02$, $p < 0.001$; Asters: $F_{254,1} = 3.80$, $R^2 = 0.014$, $p < 0.001$; parasite burden $F_{254,1} = 4.93$, df = 8, $R^2 = 0.018$, $p < 0.001$, Table S5.11). Hence, variation in microbiome composition across reserves was driven partially by dietary differences (Figure 5.6). Beta dispersion of microbiome displayed a weak negative association with increasing grass in diet ($\beta = -1.21$, s.e = 0.50, t = -2.5, p = 0.01, $R^2 = 0.02$, n = 257) but this decline did not occur across all reserves (Table S5.12).

The proportion of grass in the diet was positively correlated with PC1 of microbiome β diversity ($\beta = 2.59$, s.e = 0.23, $t = 11.27$, df = 253, $R^2 = 0.33$, $p < 0.001$) and negatively correlated with PC2 ($\beta = -1.39$, s.e = 0.27, $t = -5.2$, df = 253, $R^2 = 0.09$, $p < 0.001$). PC1 was negatively correlated with proportion of asters in diet ($\beta = -3.39$, s.e = 0.31, $t = -10.85$, df = 253, $R^2 = 0.32$, $p < 0.001$). PC2 was positively correlated with proportion of legumes in diet ($\beta = 1.4$, s.e = 0.54, $t = 2.6$, df = 253, $R^2 = 0.02$, $p < 0.001$) (Figure 5.5, Table S5.8). The bacterial groups that loaded onto PC axes correlated with increased grass consumption included several cellulolytic/fibrolytic taxa from genera such as *Ruminococcus and Lachnospiraceae* (Table S5.9).

Relative abundance of cellulose-degrading bacterial family *Lachnospiraceae* increased with proportion of grass in the diet ($\beta = 0.091$, s.e = 0.016, t = 5.9, $p < 0.001$, F = 34.7, $R^2 = 0.11$, n =253). While groups associated with breaking down non-cellulosic polysaccharides or carbohydrate degrading activities such as *Prevotella* ($\beta = -0.012$, s.e = 0.004, t = -3.5, p <

0.001, F = 12.38, R^2= 0.04, *n* =253 and the order *Bacteroidales* (β = -0.05, s.e = 0.012, t = -4.48, p < 0.001, F = 20.1, R^2= 0.07, *n* =253) decreased with increasing grass in the diet. Dietary composition was associated with functional changes in the microbiome (Figure S5.5). Increased grass consumption was related to increases in microbial communities associated with key digestive functions such as fermentation (β = 0.52, s.e = 0.09, t = 6.0, p < 0.001, F = 36.2, R^2= 0.13, *n* =253, Figure 5.6) and xylanolysis (β = 0.033, s.e = 0.0066, t = 5.1, p < 0.001, R^2= 0.09, F = 25.6, *n* =253; Figure 5.6).

5.4.4 Dietary changes are associated with shifts in nemabiome composition

We found individuals with dissimilar diets had dissimilar nemabiomes (Mantel statistic r = 0.12, *p = 0.*01, n = 162). Nemabiome composition - β diversity - was associated with the proportion of legumes (Fabaceae) in the diet (ADONIS weighted Unifrac: legumes in diet $F_{1,190}$ = 9.4, R^2 = 0.047 p = 0.001). A small amount of variance of nemabiome composition was explained by proportion of grass (Poaceae) in the diet and parasite burden (faecal egg count) but neither were significant (ADONIS weighted Unifrac: grass in diet: $F_{1,190}$ = 2.2, R^2 = 0.009 p = 0.1; parasite burden: $F_{1,190}$ = 2.6, R^2 = 0.013, p = 0.07, Table S5.14). CMZ Nemabiomes were dominated by parasitic helminth families *Strongylidae* (92.6%) and *Trichostrongylidae* (5.4%). *Strongylidae* genera included small strongyles such as *Cylicocyclus* (41.5%) and *Cylicostephanus* (8.8%), cyathostomins such as *Skrjabinodentus spp (9.6%)* as well as large pathogenic strongyles of *Strongylus* (Hookworm, 7.9%), *Triodontophorus* (6%) and *Trichostrongylus* (roundworms, 5.4%) (Figure S5.6).

The proportion of grass (Poaceae) in the diet was negatively correlated with PC1 of nemabiome β diversity (β= -0.78, s.e = 0.21, *t* = -3.6, df = 198, R^2 = 0.06, *p* < 0.001) (Table S5.15). PC1 was positively correlated with proportion of legumes (Fabaceae) in the diet (β= 2.41, s.e = 0.65, *t* = - 3.7, df = 196, R^2 = 0.06, *p* < 0.001). PC2 was not correlated with any dietary items (Figure 5.5). The species that loaded negatively (loading scores > -0.4) on PC1 (*i.e.* correlated with increased grass consumption) were small strongylid species such as *Cylicocyclus adersi and Cylicocyclus auriculatus* as well as the cythanostome *Skrajabinodentus caragandicus* and the common equid parasite *Triodontophorus nipponcis*.

Whereas species associated with increased legume consumption were large pathogenic hookworms such as *Strongylus vulgaris*, *Strongylus edentus*, *Trichostrongylus axei* and small strongylid *Cylicostephanus minutus*. Legume-rich diets were positively correlated with the proportion of *Strongylus spp* in nemabiome ($\beta = 0.64$, s.e = 0.16, $t = 4.0$, $F = 15.8$, $p < 0.001$, $R^2 = 0.08$, $n = 188$). Grass-poor diets were associated with increased parasite burden ($\beta = -1.91$, s.e = 0.25, $t = -7.8$, $F = 46.2$, $p < 0.001$, $n = 233$). Beta dispersion of nemabiome was not a function of grass in diet ($\beta = -0.007$, s.e = 0.29, $t = -0.02$, $p = 0.98$, $R^2 = -0.005$, $n = 198$).

We also found microbiome and nemabiome communities co-vary. Individuals with more dissimilar microbiomes had more dissimilar microbiomes (Mantel statistic $r = 0.24$, $p < 0.01$, $n = 162$).

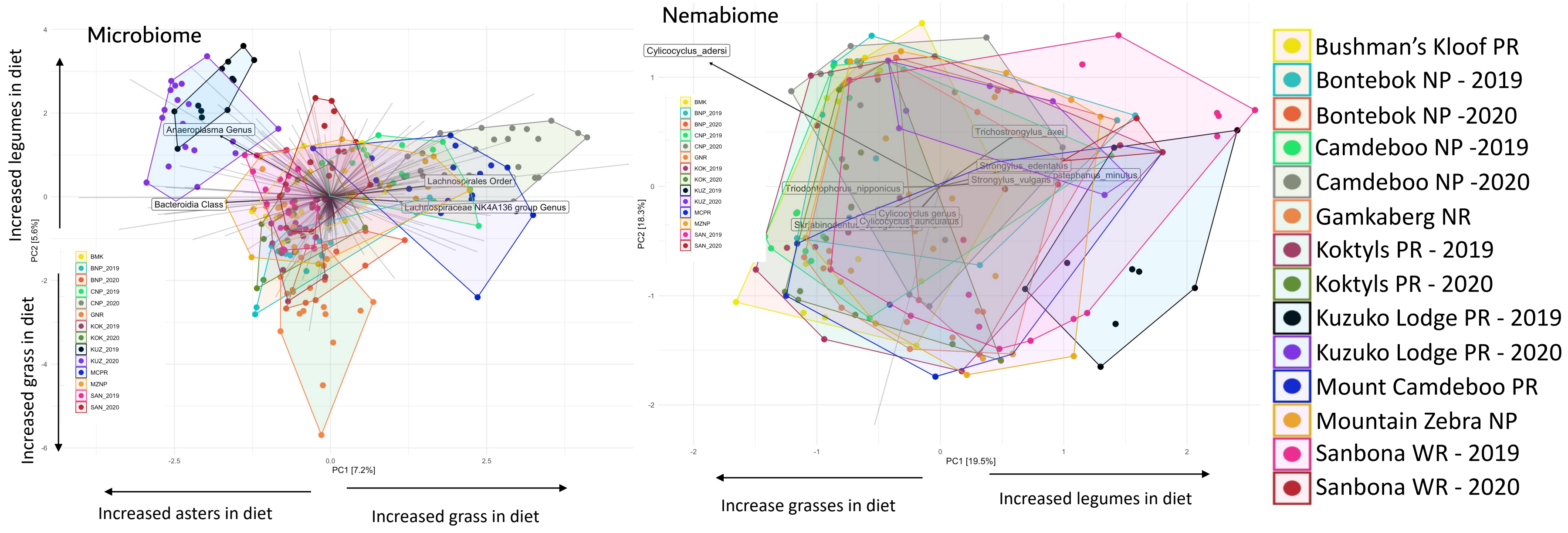


Figure 5.6) Microbiome and nemabiome beta diversity vary across the Cape mountain zebra species range due, in part, to diet. Left) **Microbiome.** PCA ordination of microbiome beta diversity CLR-transformed and agglomerated at species level. PC1 positively correlated with proportion of grass (Poaceae) in the diet and negatively correlated with the proportion of asters in the diet (Asteraceae). PC2 positively correlated with proportion of legumes in the diet (Fabaceae) and negatively correlated with grass in the diet. Right) **Nemabiome.** PCA ordination of nemabiome beta diversity CLR-transformed and agglomerated at species level. PC1 positively correlated with proportion of legumes (Fabaceae) in the diet and negatively correlated with the proportion of grass in the diet (Poaceae). No measured variable could be linked to PC2 for nemabiome composition. PR denotes private reserve, NP = National Park, NR = Nature Reserve and WR = Wildlife Reserve (Helminth species arrow lengths correspond to relative loading scores) Coloured hulls are reserves. Loadings scores and additional groups for microbiome and nemabiome are available in Tables S5.8, S5.9, S5.15.

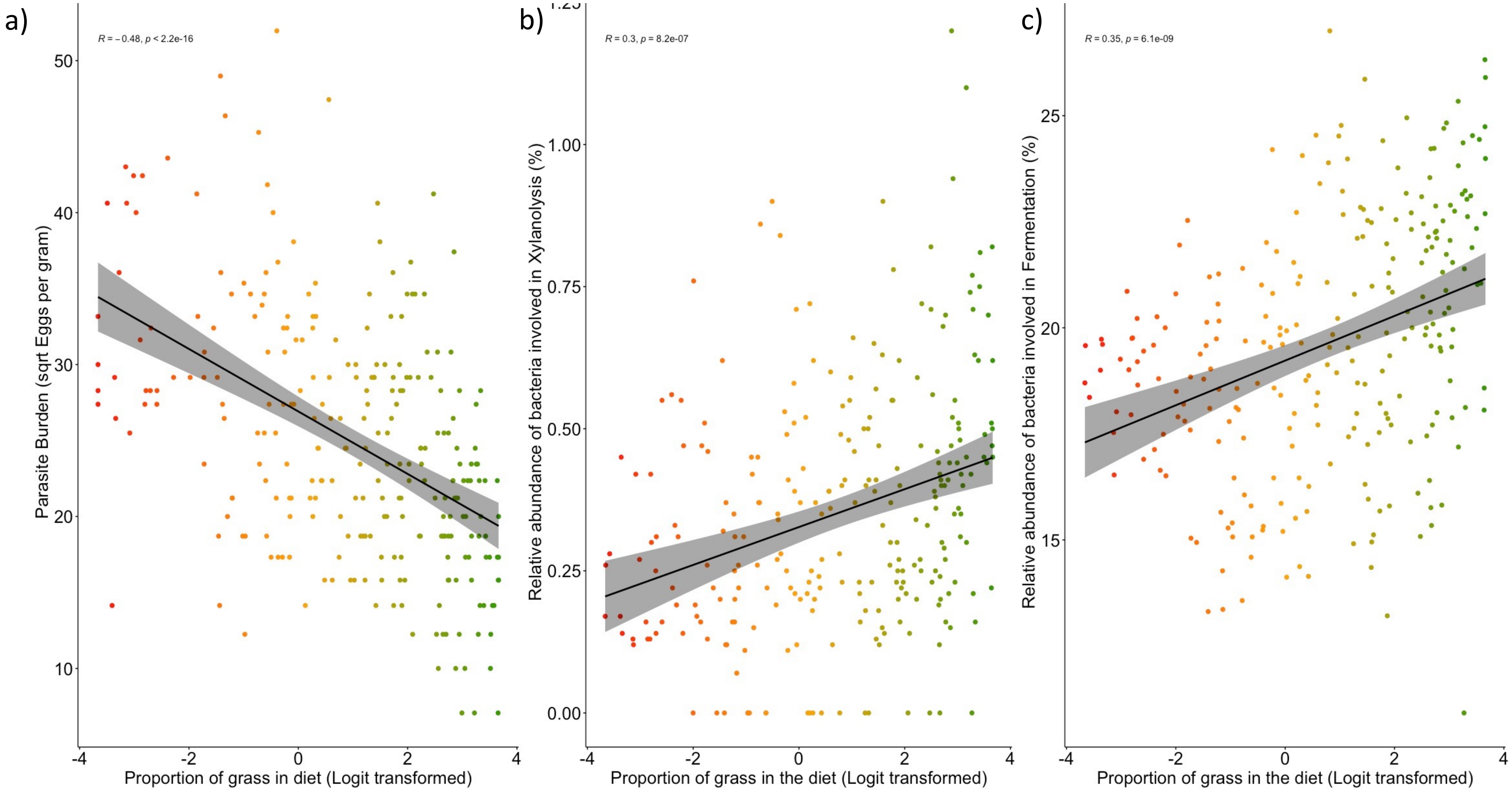


Figure 5.7) Proportion of grass in the diet negatively correlates with parasite burden and influences digestive functions of microbiome. Points are coloured by the proportion of grass in the diet – Green is high proportion of grass in the diet and red low. R value and *p value* were calculated from Pearson's Correlation Coefficient. Xylanolysis and fermentation depict the percentage of microbiome associated with each respective function. Model results for FEC vs proportion of grass in the diet are available in Table S5.16.

5.4.5. Dietary and nemabiome dissimilarity, low grass diets and high parasite burden are associated with performance metrics in CMZ.

Reserves with a greater proportion of grass available displayed an increasing trend towards higher population density (β = 2.62, s.e = 1.28, t = 2.06, p = 0.06, R^2 = 0.23, *n* = 10) and foal:mare ratio (R = 0.44, t= 1.56 p = 0.08, df= 10, one-tailed Spearman's rank correlation, Figure 5.8). Animals with more dissimilar diets had greater absolute differences in foal:mare ratio (β = 0.176, s.e = 0.03, t = 6.18, p < 0.001, R^2 = 0.29, *n* =89). Fecundity, number of foals per mare in the population, increased with increasing proportion of grass in the diet (β = 0.9424, s.e = 0.15, t = 6.2, p < 0.001, R^2 = 0.77, *n* =14) and decreased with increasing faecal egg count (β = -0.0001, s.e = 0.00005, t = -2.2, p = 0.05, R^2= 0.28, *n* =14) (Figure 5.9). Population density was positively correlated with the proportion of grass in the diet (R = 0.54, p = 0.04, one-tailed Spearman's rank correlation) but was not significant when analysed with regression analysis (β = 1.8, s.e = 1.1, t = 1.6, p = 0.14, R^2= 0.17, *n* =14).

Animals with more dissimilar microbiomes did not show greater absolute differences in foal:mare ratio or population density (F:M; β = -3.1, s.e = 9.86, t = -0.31, p <-0.75, R^2 = -0.01, *n* =89; Population density β = 0.19, s.e = 0.35, t = 0.57, p = 0.057, R^2 = -0.008, *n* =89). Greater nemabiome dissimilarity between reserves was associated with reduced foal:mare ratio differences (β = -0.09, s.e = 0.048, t = -1.99, p =0.05, R^2 = 0.03, *n* =89) but were not associated with population density (β = -1.37, s.e = 1.39, t = -0.99, p= 0.327, R^2 = 0, *n* =89).

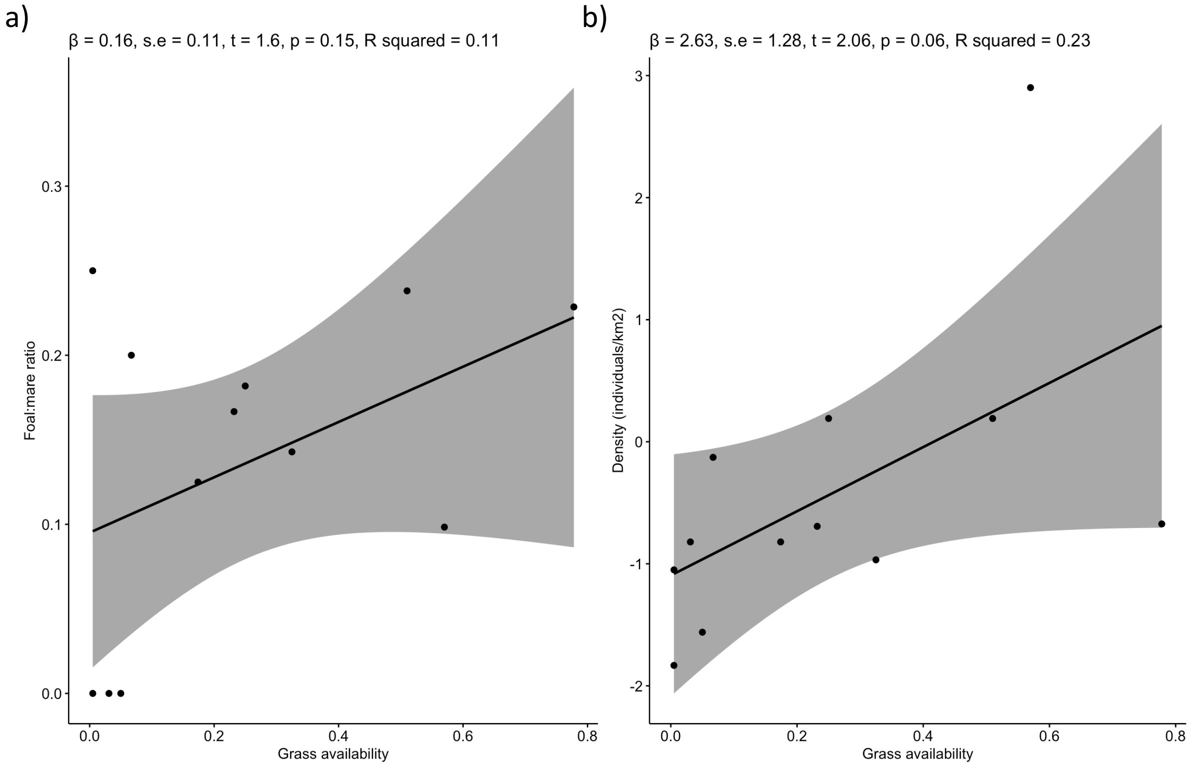


Figure 5.8) Grass availability as a function of performance metrics across Cape mountain zebra species range. a) Foal:mare ratio and b) population density tend to increase with increasing grass availability however neither are statistically significant. Results from linear regression are shown above.

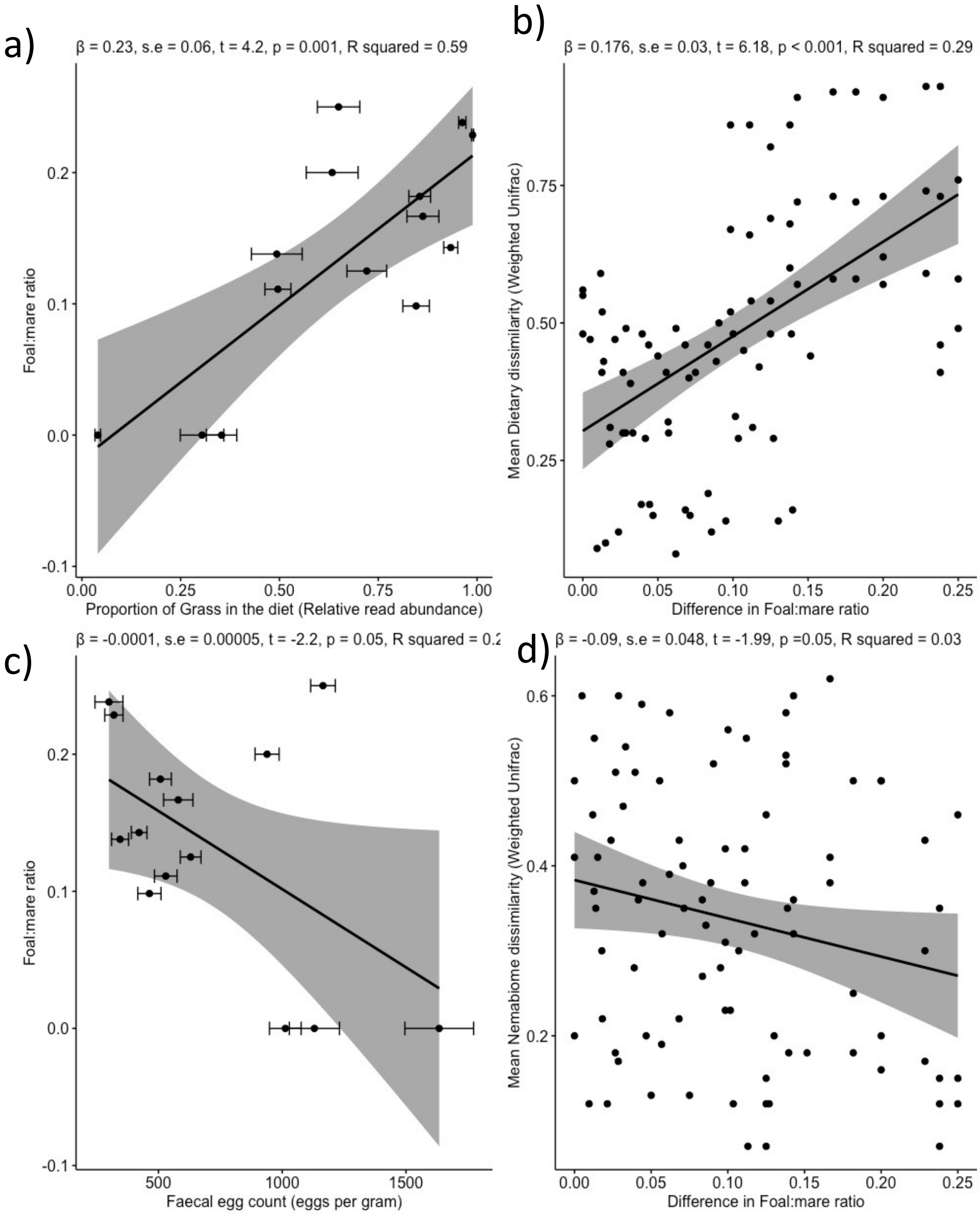


Figure 5.9) Diet composition and the physiological impacts on nemabiome correlate with performance metrics across Cape mountain zebra species range. a) Foal:mare ratio increase with increasing proportion of grass in the diet, b) More dissimilar diets lead to greater differences in foal:mare ratio such that individuals with similar diet compositions will perform similarly, c) Foal:mare ratio decreases with increasing parasite burden (assessed by faecal egg count), d) performance differences are related to individuals having more dissimilar nemabiome suggesting individuals who perform similarly have heterogeneous nemabiomes. Results from linear regression are shown above. Mean weighted Unifrac dissimilarity matrices tables are available Table S16, S17 and S18.

5.5. Discussion

The objective of this study was to uncover the dietary dynamics and macrophysiological basis linking food limitation to population regulation across the species range of a large herbivore. We found diet was strongly associated with microbiome composition and function. We also found diet was strongly associated with helminth burdens and community composition. In addition, we reveal associations between diet, macrophysiological trends and performance. Previous research has demonstrated temporal changes and community niche partitioning in African megafauna (Kartzinel et al., 2015; 2019; Kartzinel & Pringle, 2020; Pansu et al., 2019) but few studies have assessed links between dietary strategies, macrophysiology and population performance in a single species across a heterogeneous range.

Our results reveal Cape mountain zebra are predominately grazers but show dietary flexibility. We found relative abundance of Poaceae (grasses) including *Digitaria spp, Themeda spp, Cenchrus spp* increased with increasing rainfall, grass and forage availability. However, *Ehrharta spp* positively correlated with proportion of unvegetated ground. *Ehrharta spp* were only consumed in Bushman's kloof private reserve which had very patchy grass availability and may be the result of planted lawns. Cape mountain zebra dietary preferences have been studied previously. In Mountain zebra national park, CMZ breeding groups select areas of high rainfall and high palatable grass availability (Penzhorn, 1982) which ensure maximum diet quality across seasons (Novellie et al., 1988). Studies of CMZ in Baviaanskloof Wilderness area have revealed they predominately consume grass (96%) of two species: *Thristachya leucothrix* (39%) and *Themeda triandara* (28%) (Weel et al., 2015). Despite this, animals in Baviaanskloof Wilderness area had poor seasonal nitrogen and phosphorus levels and were concluded to be severely resource limited (Weel et al., 2015). Despite the dominant grazing dietary ecology, our results reveal populations can be heavily reliant on other plant families such as legumes (Fabaceae) or asters (Asteraceae) across years (KUZ, SAN) or at specific time periods (KOK). Increases in legume consumption in Sanbona was likely due to the presence of supplementary feed. Changes to specific fallback foods might have reflected availability or quality but this was not assessed. There is the possibility that CMZ select more varied diets than 100% grass as seen in Plains zebra (*Equus* *quagga*) (Potgieter & Kerley, 2022). However, estimates of grass availability from field transects did not significantly correlate with performance metrics. Field transects may not have accurately assessed habitat use and zebra may have been accessing more resources than were assumed available in field sites due to accessibility of areas. Hence, we can conclude that the proportion of grass in the diet is a more accurate metric of habitat and diet quality than grass availability.

Our results demonstrate that during food limitation Cape mountain zebra employ dietary switching. Dietary switching in response to temporal shifts in resource availability is likely beneficial for African savanna herbivores (Staver & Hempson, 2020). Dietary switching is mostly associated with mixed feeders as they act as grazers in the wet season (Abraham et al., 2019; Codron et al., 2007) and transition to browse as grass availability or quality decline (Murray & Illius, 2000). Dietary switching has been observed in African elephants (*Loxodonta africanus*) based on faecal isotopic analysis and in American "wood" bison (*Bison bison athabascae*) using DNA barcoding (Hecker et al., 2021). However, extended periods of dietary switching may be indicative of an ecologically marginal habitat.

The optimal foraging theory predicts that animals select foods to maximize energy intake and increase total dietary breadth when preferred items become scarce (MacArthur & Pianka, 1966). We reveal dietary breadth had a negative quadratic relationship with Poeceae. As Poeceae became scarce, individuals supplemented their diets with additional secondary food items. This "diet supplementation" increases dietary breadth following predictions of optimal foraging theory. This dietary supplementation has been demonstrated in moose (*Alces alces*) (Jesmer et al., 2020). However, we reveal a second stage of food limitation. When primary food items became depleted, individuals transition to a diet dominated by poor quality secondary food items or "fallback foods" (Marshall et al., 2009) We term this process "dietary transition". We predict that the trend of dietary supplementation and transition should continue with tertiary (or quaternary) items. This pattern should continue until ultimately, food availability or quality is too poor to maintain sufficient nutrition and results in starvation. We found no evidence for CMZ following the predictions of niche variation hypothesis across their range. Increases in dietary breadth were due to increases in within-individual diversity not between-individual diversity, more indicative of Optimal Foraging Theory than Niche Variation Hypothesis (Jesmer et al., 2020). Furthermore, diet β dispersion and conspecific diet similarity did not change with the proportion of grass in the diet. NVH is more likely to occur in ecologically flexible species where highly individual foraging strategies can form between individuals or groups within the population (Araújo et al., 2011; Bolnick et al., 2003).

We found strong evidence that diet composition influences microbiome composition and function. Our results demonstrate diet influences the CMZ microbiome to a similar degree to East African herbivore species (Kartzinel et al., 2019). Grass digestion in herbivores relies on first cellulolytic degraders (*e.g. Ruminococcaceae*, *Lachnospiraceae*, *Fibrobacteraceae*, *Spirochaetes*), which all loaded onto principal components associated with increased grass in the diet. Cellulolytic degraders attach to plant cell walls and break down major structural proteins, such as cellulose, hemicellulose, and xylan into smaller polysaccharides and oligosaccharides (White et al., 2014). These polysaccharides and oligosaccharides are then fermented into more simple sugars which can be easily absorbed through the gut (Flint et al., 2008, 2012).

We found *Lachnospiraceae* positively correlated with increasing grass in the diet. *Lachnospiraceae* is a main cellulolytic taxon in the mammalian gut and, in primate studies, increases in relative abundance when animals consumed a diet rich in leaves and plants (Amato et al., 2015; Springer et al., 2017). In terms of secondary fermentative groups, *Prevotella* which is involved in breaking down polysaccharides not related to cellulose and pectin (Flint et al., 2008, 2012) declined with grass in the diet. In primates *Prevotella* increase in abundance when animals consume high fiber diets (Springer et al., 2017). The increasing relative abundance of *Prevotella* with alternative dietary items to grass may suggest increased dietary fiber through less palatable or woodier plant families. *Bacteroidales* also declined in relative abundance with increasing grass in the diet. Members of *Bacteroidales* are involved in a wide array of carbohydrate degrading activities and breaking down indigestible dietary plant polysaccharides (e.g., amylose, amylopectin, and pullulan) (Hooper et al., 2002). Despite declines in *Bacteroidales and Prevotella, t*he overall proportion of the microbiome associated with fermentation increased with proportion of grass in the diet. Fermentation is key for equids to extract nutrients from vegetation

(Sneddon & Argenzio, 1998). Xylanolysis was also upregulated when grass consumption increased. Xylan is an abundant hemicellulose found in primary and secondary cell walls in monocots (Peña et al., 2016; Scheller & Ulvskov, 2010). The increase in cellulolytic taxa and associated functional changes suggest cape mountain zebra microbiomes shift with grass-rich diets to a community suited to extract nutrients from ingested grasses.

The Functional Marginality hypothesis predicts resource limitation (a persistent stressor) should be associated with increased negative physiological biomarkers (Shultz et al., 2021). From this, we can predict poor quality diets should cause cascading negative physiological effects in nemabiome communities. We found overall diet composition did influence overall nemabiome composition. We found individuals with grass poor diets had increased parasite burdens and a higher prevalence of *Strongylus spp*. *Strongylus spp* are highly pathogenic in equids and have severe health implications (Duncan & Pirie, 1975; McCraw & Slocombe, 1985). The presence of *Strongylus spp* was related to the proportion of legumes (Fabaceae) in the diet. A high proportion of Fabaceae was *Medicago spp* which is used for common forage crop used for supplementary feed. Sanbona Wildlife reserve supplementarily feeds its animals due to poor grass availability. High dung counts which form at supplementary feeding sites may increase the transmission of *Strongylus spp.* As diet quality can affect immunology (French et al., 2009), these parasite burdens may be due to poor diet quality and reduced immunocompetence.

Importantly, our analyses only assess abiotic differences between reserves. Intraspecific effects, such as the composition of sympatric wildlife, could influence diet, microbiome and nemabiome through competition for resources and direct transmission of bacteria and nematodes respectively. Although, all reserves have a similar large mammalian herbivore (LMH) composition, game counts are not available for all reserves and therefore their effects cannot be formally assessed here. Densities of sympatric LMHs do vary between reserves. Therefore the influences of species composition and density cannot be discounted as a potential factor influencing trends.

Our results provide strong evidence that abundance and quality of food items limits Cape mountain zebra population performance across their species range. The food hypothesis states that population performance and size is driven by the abundance and quality of food (Sinclair et al., 1985; White, 2008). Food limitation can result in negative physiological status leading to poor performance (Gobush et al., 2014) while higher quality diets promote better body condition (Parker et al., 2009) and improved pregnancy rates (Wasser et al., 2017). Poor grass availability is associated with reduced population performance, density and abundance across 23 cape mountain zebra populations (Lea et al., 2016). Variation in grass available may therefore influence long term population dynamics. For example, CMZ in Kuzuko Lodge consumed a maximum of 30% grass during our study period, in contrast, Koktyls private reserve shifted between an average of 70% grass in 2019 to 30% in 2020. In 2019, Kuzuko Lodge had an annual rainfall of 360mm and an average 4% grass cover, rainfall and grass declined in 2020 (245mm and an average 3% grass cover). Koktyls private reserve had annual rainfall of 244mm and 5% grass cover in 2019 and 368mm and 1.5% grass cover in 2020. Both populations have poor foal:mare ratio. Our results suggest a constant low availability of grass or resource heterogeneity between years (“boom and bust” availability) could lead to poor long-term performance (Illius & O’Connor, 2000) in CMZ populations.

Our results have important implications for the conservation biology of CMZ and its sympatric communities. All our sampling sites were protected areas. Cape mountain zebra are recognised as a partial refugee species *i.e.* protected in marginal habitats within part of its range (Lea et al., 2016). This confinement to resource poor ecologically marginal areas may be due to protected area placement. For example, in the Cape floristic region of South Africa, where many of the protected areas exist for CMZ, many of the publicly owned parks are in marginal areas which are unsuitable for agriculture and unlikely to bolster large herbivore populations (Boshoff et al., 2009). Relict marginalised populations can remain at small population sizes for many years and experience decreasing genetic diversity (Kotzé et al., 2019). Historically, Cape mountain zebra populations display greater dependence on grassland than contemporary populations and likely altitudinally migrated (Faith, 2012). Irrespective of protection, Cape mountain zebra now occupy many ecologically marginal areas where they experience poor performance rates through food limitation. Confinement to grass poor areas likely limits the population size and performance of sympatric grazers. With increasing levels of droughts across South Africa, the ability of large herbivore species to reach energetic demands and feed on preferred food resources may worsen in the future (Meza et al., 2021).

5.6 References

Abraham, J. O., Hempson, G. P., & Staver, A. C. (2019). Drought-response strategies of savanna herbivores. *Ecology and Evolution*, *9*(12), 7047–7056.

Amato, K. R., Leigh, S. R., Kent, A., Mackie, R. I., Yeoman, C. J., Stumpf, R. M., Wilson, B. A., Nelson, K. E., White, B. A., & Garber, P. A. (2015). The Gut Microbiota Appears to Compensate for Seasonal Diet Variation in the Wild Black Howler Monkey (Alouatta pigra). *Microbial Ecology*, *69*(2), 434–443.

Antwis, R. E., Edwards, K. L., Unwin, B., Walker, S. L., & Shultz, S. (2019). Rare gut microbiota associated with breeding success, hormone metabolites and ovarian cycle phase in the critically endangered eastern black rhino. *Microbiome*, *7*(1), 1–12.

Araújo, M. S., Bolnick, D. I., & Layman, C. A. (2011). The ecological causes of individual specialisation. *Ecology Letters 14*(9), 948–958.

Augustine, D. J., & McNaughton, S. J. (1998). Ungulate Effects on the Functional Species Composition of Plant Communities: Herbivore Selectivity and Plant Tolerance. *The Journal of Wildlife Management*, *62*(4), 1165.

Avramenko, R. W., Redman, E. M., Lewis, R., Yazwinski, T. A., Wasmuth, J. D., & Gilleard, J. S. (2015). Exploring the gastrointestinal “nemabiome”: Deep amplicon sequencing to quantify the species composition of parasitic nematode communities. *PLoS ONE*, *10*(12), e0143559.

Barbour, M.G ., Burk, J.H. & Pitts, W.D. (1980) Terrestrial Plant Ecology, 1st edn._Benjamin/Cummings, Menlo Park

Barnett, D., Arts, I., & Penders, J. (2021). microViz: an R package for microbiome data visualization and statistics. *Journal of Open Source Software*, *6*(63), 3201.

Bison, M., Ibanez, S., Redjadj, C., Boyer, F., Coissac, E., Miquel, C., Rioux, D., Said, S., Maillard, D., Taberlet, P., Yoccoz, N. G., & Loison, A. (2015). Upscaling the niche variation hypothesis from the intra- to the inter-specific level. *Oecologia*, *179*(3), 835–842.

Bolnick, D. I., Snowberg, L. K., Hirsch, P. E., Lauber, C. L., Knight, R., Caporaso, J. G., & Svanbäck, R. (2014). Individuals' diet diversity influences gut microbial diversity in two freshwater fish (threespine stickleback and Eurasian perch). *Ecology Letters*, *17*(8), 979–987.

Bolnick, D. I., Svanbäck, R., Fordyce, J. A., Yang, L. H., Davis, J. M., Hulsey, C. D., & Forister, M. L. (2003). The ecology of individuals: Incidence and implications of individual specialization. *American Naturalist*, *161*(1), 1–28.

Bolnick, D. I., Yang, L. H., Fordyce, J. A., Davis, J. M., & Svanback, R. (2002). Measuring Individual-Level Resource Specialization. *Ecology*, *83*(10), 2936.

Boshoff, A. F., Kerley, G. I. H., & Cowling, R. M. (2009). Estimated spatial requirements of the medium- to large-sized mammals, according to broad habitat units, in the Cape Floristic Region, South Africa. *African Journal of Range & Forage Science 19*(1), 29–44.

Boshoff, A., Landman, M., & Kerley, G. (2016). Filling the gaps on the maps: Historical distribution patterns of some larger mammals in part of southern Africa. *Transactions of the Royal Society of South Africa*, *71*(1), 23–87.

Budischak, S. A., Sakamoto, K., Megow, L. C., Cummings, K. R., Urban, J. F., & Ezenwa, V. O. (2015). Resource limitation alters the consequences of co-infection for both hosts and parasites. *International Journal for Parasitology*, *45*(7), 455–463.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583.

Carla Staver, A., & Hempson, G. P. (2020). Seasonal dietary changes increase the abundances of savanna herbivore species. *Science Advances*, *6*(40).

Channell, R., & Lomolino, M. V. (2000). Trajectories to extinction: spatial dynamics of the contraction of geographic ranges of endangered species. *Journal of Biogeography*, *27*, 169–180.

Chown, S. L., & Gaston, K. J. (2016). Macrophysiology – progress and prospects. *Functional Ecology*, *30*(3), 330–344.

Codron, D., Codron, J., Lee-Thorp, J. A., Sponheimer, M., de Ruiter, D., Sealy, J., Grant, R., & Fourie, N. (2007). Diets of savanna ungulates from stable carbon isotope composition of faeces. *Journal of Zoology*, *273*(1), 21–29.

Crall, J. P., Stewart, C. v., Berger-Wolf, T. Y., Rubenstein, D. I., & Sundaresan, S. R. (2013). HotSpotter-Patterned species instance recognition. *Proceedings of IEEE Workshop on Applications of Computer Vision*, 230–237.

Crawley, J. A. H., Chapman, S. N., Lummaa, V., & Lynsdale, C. L. (2016). Testing storage methods of faecal samples for subsequent measurement of helminth egg numbers in the domestic horse. *Veterinary Parasitology*, *221*, 130–133.

Deagle, B. E., Clarke, L. J., Kitchener, J. A., Polanowski, A. M., & Davidson, A. T. (2018). Genetic monitoring of open ocean biodiversity: An evaluation of DNA metabarcoding for processing continuous plankton recorder samples. *Molecular Ecology Resources*, *18*(3), 391–406.

Debeffe, L., McLoughlin, P. D., Medill, S. A., Stewart, K., Andres, D., Shury, T., Wagner, B., Jenkins, E., Gilleard, J. S., & Poissant, J. (2016). Negative covariance between parasite load and body condition in a population of feral horses. *Parasitology*, *143*(8), 983–997.

Dixon, P. (2003). VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science*, *14*(6), 927–930.

du Toit, J. C. O., & O'Connor, T. G. (2020). Long-term influence of season of grazing and rainfall on vegetation in the eastern Karoo, South Africa. *African Journal of Range & Forage Science 37*(2), 159–171.

Duncan, J. L., & Pirie, H. M. (1975). The pathogenesis of single experimental infections with *Strongylus vulgaris* in foals. *Research in Veterinary Science*, *18*(1), 82–93.

Duvallet, C., Gibbons, S. M., Gurry, T., Irizarry, R. A., & Alm, E. J. (2017). Meta-analysis of gut microbiome studies identifies disease-specific and shared responses. *Nature Communications*, *8*(1).

Ezenwa, V. O. (2004). Interactions among host diet, nutritional status and gastrointestinal parasite infection in wild bovids. *International Journal for Parasitology*, *34*(4), 535–542.

Fair, J. M., & Whitaker, S. J. (2008). Avian cell-mediated immune response to drought. *Wilson Journal of Ornithology*, *120*(4), 813–819.

Faith, J. T. (2012). Palaeozoological insights into management options for a threatened mammal: Southern Africa's Cape mountain zebra (*Equus zebra zebra*). *Diversity and Distributions*, *18*(5), 438–447.

Flint, H. J., Bayer, E. A., Rincon, M. T., Lamed, R., & White, B. A. (2008). Polysaccharide utilization by gut bacteria: Potential for new insights from genomic analysis. *Nature Reviews Microbiology 6*(2) 121–131.

Flint, H. J., Scott, K. P., Duncan, S. H., Louis, P., & Forano, E. (2012). Microbial degradation of complex carbohydrates in the gut. *Gut Microbes 3*(4) 289–306.

French, S. S., Moore, M. C., & Demas, G. E. (2009). Ecological immunology: The organism in context. *Integrative and Comparative Biology*, *49*(3), 246–253.

Gaston, K. J. (2009). Geographic range limits of species. *Proceedings of the Royal Society B: Biological Sciences*, *276*(1661), 1391–1393.

Gill, B. A., Musili, P. M., Kurukura, S., Hassan, A. A., Goheen, J. R., Kress, W. J., Kuzmina, M., Pringle, R. M., & Kartzinel, T. R. (2019). Plant DNA-barcode library and community phylogeny for a semi-arid East African savanna. *Molecular Ecology Resources*, *19*(4), 838–846.

Gloor, G. B., Macklaim, J. M., Pawlowsky-Glahn, V., & Egozcue, J. J. (2017). Microbiome datasets are compositional: And this is not optional. *Frontiers in Microbiology*, *8*, 2224.

Gobush, K. S., Booth, R. K., & Wasser, S. K. (2014). Validation and application of noninvasive glucocorticoid and thyroid hormone measures in free-ranging Hawaiian monk seals. *General and Comparative Endocrinology*, *195*, 174–182.

Gosling, L.M., Muntifering, J., Kolberg, H., Uiseb, K. & King, S.R.B. 2019. *Equus zebra* (amended version of 2019 assessment). *The IUCN Red List of Threatened Species* 2019: e.T7960A160755590.

Hanning, I., & Diaz-Sanchez, S. (2015). The functionality of the gastrointestinal microbiome in non-human animals. *Microbiome 3*(1) 51.

Hecker, L. J., Edwards, M. A., & Nielsen, S. E. (2021). Assessing the nutritional consequences of switching foraging behavior in wood bison. *Ecology and Evolution*, *11*(22), 16165–16176.

Hillegass, M. A., Waterman, J. M., & Roth, J. D. (2010). Parasite removal increases reproductive success in a social African ground squirrel. *Behavioral Ecology*, *21*(4), 696–700.

Holt, R. D. (2009). Bringing the Hutchinsonian niche into the 21st century: Ecological and evolutionary perspectives. *Proceedings of the National Academy of Sciences*, *106*(2), 19659–19665.

Hooper, L. V., Midwedt, T., & Gordon, J. I. (2002). How host-microbial interactions shape the nutrient environment of the mammalian intestine. *Annual Review of Nutrition 22*, 283–307).

Hrabar, H., and G. I. H. Kerley. "Cape mountain zebra 2014/15 status report." (2015). *Port Elizabeth: Centre for African Conservation Ecology, Nelson Mandela Metropolitan University Report* 63

Hudson, P., Dobson, A., & Newborn, D. (1998). Prevention of population cycles by parasites removal. *Science*, *282*, 2256–2258.

Huston, M. A. (2005). The three phases of land-use change: Implications for biodiversity. *Ecological Applications 15*(6),1864–1878.

Illius, A. W., & O'Connor, T. G. (2000). Resource heterogeneity and ungulate population dynamics. *Oikos*, *89*(2), 283–294.

Jesmer, B. R., Kauffman, M. J., Murphy, M. A., & Goheen, J. R. (2020). A test of the Niche Variation Hypothesis in a ruminant herbivore. *Journal of Animal Ecology*, *89*(12), 2825–2839.

Karasov, W. H., Martínez Del Rio, C., & Caviedes-Vidal, E. (2011). Ecological physiology of diet and digestive systems. *Annual Review of Physiology*, *73*, 69–93.

Kartzinel, T. R., Chen, P. A., Coverdale, T. C., Erickson, D. L., Kress, W. J., Kuzmina, M. L., Rubenstein, D. I., Wang, W., & Pringle, R. M. (2015). DNA metabarcoding illuminates dietary niche partitioning by African large herbivores. *Proceedings of the National Academy of Sciences*, *112*(26), 8019–8024.

Kartzinel, T. R., Hsing, J. C., Musili, P. M., Brown, B. R. P., & Pringle, R. M. (2019). Covariation of diet and gut microbiome in African megafauna. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(47), 23588–23593.

Kartzinel, T. R., & Pringle, R. M. (2020). Multiple dimensions of dietary diversity in large mammalian herbivores. *Journal of Animal Ecology*, *89*(6), 1482–1496.

Kohl, K. D., Weiss, R. B., Cox, J., Dale, C., & Dearing, M. D. (2014). Gut microbes of mammalian herbivores facilitate intake of plant toxins. *Ecology Letters*, *17*(10), 1238–1246.

Kotzé, A., Smith, R. M., Moodley, Y., Luikart, G., Birss, C., van Wyk, A. M., Grobler, J. P., & Dalton, D. L. (2019). Lessons for conservation management: Monitoring temporal changes in genetic diversity of Cape mountain zebra (Equus zebra zebra). *PLOS ONE*, *14*(7), e0220331.

Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., & Schloss, P. D. (2013). Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the miseq illumina sequencing platform. *Applied and Environmental Microbiology*, *79*(17), 5112–5120.

Kraaij, T. (2011). The flora of the Bontebok National Park in regional perspective. *South African Journal of Botany*, *77*(2), 455-473.

Krebs, J. R., Erichsen, J. T., Webber, M. I., & Charnov, E. L. (1977). Optimal prey selection in the great tit (*Parus major*). *Animal Behaviour*, *25*(1), 30–38.

Lea, J. M., Kerley, G. I., Hrabar, H., Barry, T. J., & Shultz, S. (2016). Recognition and management of ecological refugees: A case study of the Cape mountain zebra. *Biological Conservation*, *203*, 207–215.

Li, H., Li, T., Beasley, D. A. E., Heděnec, P., Xiao, Z., Zhang, S., Li, J., Lin, Q., & Li, X. (2016). Diet diversity is associated with beta but not alpha diversity of pika gut microbiota. *Frontiers in Microbiology*, *7*, 1169.

Liu, C., Cui, Y., Li, X., & Yao, M. (2021). Microeco: An R package for data mining in microbial community ecology. *FEMS Microbiology Ecology*, *97*(2).

Louca, S., & Doebeli, M. (2018). Efficient comparative phylogenetics on large trees. *Bioinformatics*, *34*(6), 1053–1055.

Louca, S., Parfrey, L. W., & Doebeli, M. (2016). Decoupling function and taxonomy in the global ocean microbiome. *Science*, *353*(6305), 1272–1277.

Love, S., Murphy, D., & Mellor, D. (1999). Pathogenicity of cyathostome infection. *Veterinary Parasitology*, *85*(2–3), 113–122.

Lozupone, C., & Knight, R. (2005). UniFrac: a New Phylogenetic Method for Comparing Microbial Communities. *Applied and Environmental Microbiology*, *71*(12), 8228.

MacArthur, R. H., & Pianka, E. R. (1966). On Optimal Use of a Patchy Environment. *The American Naturalist*, *100*(916), 603–609.

Marshall, A. J., Boyko, C. M., Feilen, K. L., Boyko, R. H., & Leighton, M. (2009). Defining fallback foods and assessing their importance in primate ecology and evolution. *American Journal of Physical Anthropology*, *140*(4), 603–614.

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal*, *17*(1), 10–12.

McCraw, B. M., & Slocombe, J. O. D. (1985). *Strongylus equinus*: development and pathological effects in the equine host. *Canadian Journal of Comparative Medicine*, *49*(4), 372.

McMurdie, P. J., & Holmes, S. (2013a). Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE*, *8*(4).

McNaughton, S. J., Oesterheld, M., Frank, D. A., & Williams, K. J. (1989). Ecosystem-level patterns of primary productivity and herbivory in terrestrial habitats. *Nature* *341*(6238), 142–144.

McPeek, M. A., & Holt, R. D. (1992). The evolution of dispersal in spatially and temporally varying environments. *American Naturalist*, *140*(6), 1010–1027.

Mduma, S. A. R., Sinclair, A. R. E., & Hilborn, R. (1999). Food regulates the Serengeti wildebeest: A 40-year record. *Journal of Animal Ecology*, *68*(6), 1101–1122.

Meza, I., Eyshi Rezaei, E., Siebert, S., Ghazaryan, G., Nouri, H., Dubovyk, O., Gerdener, H., Herbert, C., Kusche, J., Popat, E., Rhyner, J., Jordaan, A., Walz, Y., & Hagenlocher, M. (2021). Drought risk for agricultural systems in South Africa: Drivers, spatial patterns, and implications for drought risk management. *Science of The Total Environment*, *799*, 149505.

Millar, J. C. G. Census of Cape mountain zebras: Part II. (1970) *African Wildlife 24*(2) 104-114.

Murray, M. G., & Illius, A. W. (2000). Vegetation modification and resource competition in grazing ungulates. *Oikos*, *89*(3), 501–508.

Nagy, L. R., & Holmes, R. T. (2005). Food limits annual fecundity of a migratory songbird: An experimental study. *Ecology*, *86*(3), 675–681.

Navarro-Gonzalez, N., Verheyden, H., Hoste, H., Cargnelutti, B., Lourtet, B., Merlet, J., Daufresne, T., Lavín, S., Hewison, A. J. M., Morand, S., & Serrano, E. (2011). Diet quality and immunocompetence influence parasite load of roe deer in a fragmented landscape. *European Journal of Wildlife Research*, *57*(3), 639–645.

Novellie, P. A., Fourie, L. J., Kok, O. B., & Westhuizen, M. C. van der. (1988). Factors affecting the seasonal movements of Cape mountain zebras in the Mountain Zebra National Park. *African Zoology*, *23*(1), 13–19.

Pansu, J., Guyton, J. A., Potter, A. B., Atkins, J. L., Daskin, J. H., Wursten, B., Kartzinel, T. R., & Pringle, R. M. (2019). Trophic ecology of large herbivores in a reassembling African ecosystem. *Journal of Ecology*, *107*(3), 1355–1376.

Parker, K. L., Barboza, P. S., & Gillingham, M. P. (2009). Nutrition integrates environmental responses of ungulates. *Functional Ecology*, *23*(1), 57–69.

Peña, M. J., Kulkarni, A. R., Backe, J., Boyd, M., O'Neill, M. A., & York, W. S. (2016). Structural diversity of xylans in the cell walls of monocots. *Planta*, *244*(3), 589–606.

Penzhorn, B. (1982). Habitat selection by Cape mountain zebras in the Mountain Zebra National Park. S*outh African Journal of Wildlife Reserve 12*, 48–54.

Penzhorn, B. L. (1982). Home range sizes of Cape mountain zebras Equus zebra zebra in the Mountain Zebra National Park. *Koedoe*, *25*, 103–108.

Potgieter, T.-L., & Kerley, G. I. H. (2022). The zebra as a grazer: Selectivity for grass consumption differs as grass availability varies. *African Journal of Ecology*.

Price, M. N., Dehal, P. S., & Arkin, A. P. (2009). FastTree: Computing Large Minimum Evolution Trees with Profiles instead of a Distance Matrix. *Molecular Biology and Evolution*, *26*(7), 1641.

Pulliam, H. R. (1988). Sources, Sinks, and Population Regulation. *The American Naturalist*, *132*(5), 652–661.

Pulliam, H. R., & Danielson, B. J. (1991). Sources, Sinks, and Habitat Selection: A Landscape Perspective on Population Dynamics. *The American Naturalist*, *137*, S50–S66.

Revell, L. J. (2012). phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, *3*(2), 217–223.

Roughgarden, J. (1974). Niche Width: Biogeographic Patterns Among Anolis Lizard Populations. *The American Naturalist*, *108*(962), 429–442.

Rutherford, M. C., Mucina, L., & Powrie, L. W. (2006). Biomes and bioregions of southern Africa. *The vegetation of South Africa, Lesotho and Swaziland*, *19*, 30-51.

Sargeant, B. L. (2007). Individual foraging specialization: niche width versus niche overlap. *Oikos*, *116*(9), 1431–1437.

Scheller, H. V., & Ulvskov, P. (2010). Hemicelluloses. *Annual Review of Plant Biology*, *61*, 263–289.

Schliep, K. P. (2011). phangorn: Phylogenetic analysis in R. *Bioinformatics*, *27*(4), 592–593.

Schneider-Crease, I., Beehner, J. C., Bergman, T. J., Gomery, M. A., Koklic, L., Lu, A., & Snyder-Mackler, N. (2020). Ecology eclipses phylogeny as a major driver of nematode parasite community structure in a graminivorous primate. *Functional Ecology*, *34*(9), 1898–1906.

Schoener, T. W. (1971). Theory Of Feeding Strategies*. Annual Review of Ecology and Systematics 2*.369-404

Seydack, A., Vermeulen, C., & Huisamen, H. (2000). Habitat quality and the decline of an African elephant population: implications for conservation. S*outh African Journal of Wildlife Reserve 30*(1) 34–42.

Shearer, C. L., & Ezenwa, V. O. (2020). Rainfall as a driver of seasonality in parasitism. *International Journal for Parasitology: Parasites and Wildlife*, *12*, 8–12.

Shultz, S., Britnell, J. A., & Harvey, N. (2021). Untapped potential of physiology, behaviour and immune markers to predict range dynamics and marginality. *Ecology and Evolution*, *11*(23), 16446–16461.

Sinclair, A. R. E., Dublin, H., & Borner, M. (1985). Population regulation of Serengeti Wildebeeest: a test of the food hypothesis. *Oecologia*, *65*(2), 266–268.

Sneddon, J. C., & Argenzio, R. A. (1998). Feeding strategy and water homeostasis in equids: the role of the hind gut. *Journal of Arid Environments*, *38*(3), 493–509.

Springer, A., Fichtel, C., Al-Ghalith, G. A., Koch, F., Amato, K. R., Clayton, J. B., Knights, D., & Kappeler, P. M. (2017). Patterns of seasonality and group membership characterize the gut microbiota in a longitudinal study of wild Verreaux's sifakas (*Propithecus verreauxi*). *Ecology and Evolution*, *7*(15), 5732–5745.

Stephens, D. W., & Krebs, J. R. (1986). Foraging Theory. *Foraging Theory*. Princeton University Press.

Sutherland, W. J., Freckleton, R. P., Godfray, H. C. J., Beissinger, S. R., Benton, T., Cameron, D. D., Carmel, Y., Coomes, D. A., Coulson, T., Emmerson, M. C., Hails, R. S., Hays, G. C., Hodgson, D. J., Hutchings, M. J., Johnson, D., Jones, J. P. G., Keeling, M. J., Kokko, H., Kunin, W. E., … Wiegand, T. (2013). Identification of 100 fundamental ecological questions. *Journal of Ecology*, *101*(1), 58–67.

Svanbäck, R., & Bolnick, D. I. (2005). Intraspecific competition affects the strength of individual specialization: An optimal diet theory method. *Evolutionary Ecology Research*, *7*(7), 993–1012.

Svanbäck, R., & Bolnick, D. I. (2007). Intraspecific competition drives increased resource use diversity within a natural population. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1611), 839–844.

Uhlinger, C. (1990). Effects of three anthelmintic schedules on the incidence of colic in horses. *Equine Veterinary Journal*, *22*(4), 251–254.

van Valen, L. (1965). Morphological Variation and Width of Ecological Niche. *The American Naturalist*, *99*(908), 377–390.

Walshe, N., Duggan, V., Cabrera-Rubio, R., Crispie, F., Cotter, P., Feehan, O., & Mulcahy, G. (2019). Removal of adult cyathostomins alters faecal microbiota and promotes an inflammatory phenotype in horses. *International Journal for Parasitology*, *49*(6), 489–500.

Wasser, S. K., Lundin, J. I., Ayres, K., Seely, E., Giles, D., Balcomb, K., Hempelmann, J., Parsons, K., & Booth, R. (2017). Population growth is limited by nutritional impacts on pregnancy success in endangered Southern Resident killer whales (*Orcinus orca*). *PLOS ONE*, *12*(6), e0179824.

Weel, S., Watson, L. H., Weel, J., Venter, J. A., & Reeves, B. (2015). Cape mountain zebra in the Baviaanskloof Nature Reserve, South Africa: resource use reveals limitations to zebra performance in a dystrophic mountainous ecosystem. *African Journal of Ecology*, *53*(4), 428–438.

Weinstein, S. B., Martinez-Mota, R., Stapleton, T. E., Klure, D. M., Greenhalgh, R., Orr, T. J., Dale, C., Kohl, K. D., & Denise Dearing, M. (2021). Microbiome stability and structure is governed by host phylogeny over diet and geography in woodrats (Neotoma spp.). *Proceedings of the National Academy of Sciences of the United States of America*, *118*(47).

White, B. A., Lamed, R., Bayer, E. A., & Flint, H. J. (2014). Biomass utilization by gut microbiomes. In *Annual Review of Microbiology. 68*, 279–296.

White, T. C. R. (2008). The role of food, weather and climate in limiting the abundance of animals. *Biological Reviews*, *83*(3), 227–248.

Workentine, M. L., Chen, R., Zhu, S., Gavriliuc, S., Shaw, N., Rijke, J. de, Redman, E. M., Avramenko, R. W., Wit, J., Poissant, J., & Gilleard, J. S. (2020). A database for ITS2 sequences from nematodes. *BMC Genetics*, *21*(1), 1–4.

Wright, E. S. (2020). RNAconTest: comparing tools for noncoding RNA multiple sequence alignment based on structural consistency. *RNA*, *26*(5), 531–540.

Xiang, Z., Tong, W., Guo, Z., Xu, Y., Guo, J., Ruan, Y., & Zhao, P. (2019). Rat H1 parvovirus infection leads to alterations in gut microbiota. *Pathogens and Disease*, *77*(5), 58.

Xing, Y., Liu, J., Lu, F., Wang, L., Li, Y., & Ouyang, C. (2019). Dynamic distribution of gallbladder microbiota in rabbit at different ages and health states. *PLOS ONE*, *14*(2), e0211828.

Zaccarelli, N., Bolnick, D. I., & Mancinelli, G. (2013). RInSp: an r package for the analysis of individual specialization in resource use. *Methods in Ecology and Evolution*, *4*(11), 1018–1023.

Zaneveld, J. R., McMinds, R., & Thurber, R. V. (2017). Stress and stability: Applying the Anna Karenina principle to animal microbiomes. *Nature Microbiology*. *2*(9). 17121.

5.7 Supplementary information

5.7.1 Text S1 - Study populations and species

Mountain zebra are a vulnerable equid species (IUCN) divided into two subspecies Cape Mountain zebra (*Equus zebra zebra, CMZ*) found below orange river in South Africa and Hartman’s zebra (*Equus zebra hartmannae)* in Namibia. Historically, Cape Mountain zebra (*Equus zebra zebra, CMZ*) have a limited historical distribution across five biomes – Fynbos, Succulent Karoo, Desert, Nama-Karoo, Grassland within the Eastern, Western and Northern cape of South Africa (A. Boshoff et al., 2016). By the 1950s, population numbers were fewer than 80 individuals and the species range was restricted to three relict populations, Gamkaberg Nature Reserve (GNR), the Kammanassie Mountains and the Mountain Zebra National Park (MZNP) due to hunting and pressure from agricultural landuse (Millar, 1970). Since then, population numbers are now above 4791 individuals distributed throughout much of its’ historic range (Hrabar and Kerley, 2015). However, the majority of individuals occur in two populations, MZNP and Karoo National Park.

The north-eastern portions of the species historic range receive summer rainfall promoting grasslands, while the southwest portion experiences winter-rainfall resulting in fynbos and succulent Karoo vegetation communities with low grass cover (Boshoff et al., 2009). Summer rainfall is associated with greater grass abundance and winter rainfall seasonality being associated with greater shrub abundance (du Toit & O'Connor, 2020). Furthermore, all populations of CMZ occupy a heavily fragmented landscape due to fencing. As CMZ exist in fenced environments, migration between populations is not possible without human intervention and each population is independent and a "closed system" such that all individuals are exposed to near identical environmental constraints and resource availabilities (Lea et al., 2016).

Sanbona Wildlife Reserve (SWR) (33.8663°S, 20.5284°E) is a 58 000-ha privately-owned reserve within the Western Cape of South Africa. CMZ were reintroduced to the area in 2016 into a predator free southern section of SWR. CMZ in SWR do not experience predation pressure. In SWR, CMZ have access to supplementary feed throughout the year but is naturally dominated by fynbos and succulent karoo biomes. SWR experiences aseasonal rainfall. SWR is estimated to receive an average ~120mm of rain per year. Rainfall is aseasonal.

Koktyls private reserve (KOK) is the adjacent property to SWR. Koktyls does not have access to supplementary feed but is made up of the same biomes as SWR (Fynbos and succulent karoo biomes). Population size and density is unknown. KOK is estimated to receive an average ~120mm of rain per year. Rainfall is aseasonal.

Mountain Zebra National Park (MZNP) (32.1410°S, 25.5096°E) is a 18 000-hectare reserve (Brown & Bezuidenhout 2000) located in the Eastern Cape Province and under the jurisdiction of South African National Parks authority. The CMZ population at MZNP is unmanaged and subject to natural predation from Lion (*Panthera leo*). MZNP encapsulates four biome types: Grassland, Nama Karoo, Thicket and Savannah. Population size is ~1191 CMZ and population density of 0.0558 individuals/ha (Lea et al., 2016). MZNP is estimated to receive an average ~153-651mm of rain per year with an average 382mm between 1982-1998. Rainfall occurs mostly summer and autumn.

Gamkaberg Nature Reserve (GNR) is a 9428 hectare reserve situated in the Western Cape under the jurisdiction of CapeNature, 33 km SW of Oudtshoorn. GNR is predominately within fynbos biome, with limited availability of grass species. However, GNR experienced a fire in 2017 which burned ~80% of the reserve which can stimulate grass production. Rainfall is between 300-500 mm per year. There is no large predator presence however leopard (*Panthera pardus*) do occur in the area.

Kuzuko Lodge Private Reserve (KZL) is a 15,000 hectare private reserve situated within the larger Addo elephant part. The vegetation is “valley bushveld” or succulent thicket within the Albany thick biome. Valley bushveld is characterised by dense, spiny and evergreen shrubland to low forest which is dominated by Spekboom (*Portulacaria afra*). There is predation pressure in Kuzuko Lodge due to the presence of Lions (*Panthera leo*). Rainfall occurs in spring and autumn with ~400mm per year.

Camdeboo National Park (CNP) is a 19405 hectare reserve under the jurisdiction of SANparks located between (32º18’14.83”S and 24º38’31.41”). CNP has into three distinct biomes: Albany Thicket, Grassland and Nama-karoo biomes (Mucina & Rutherford 2006). Rainfall falls predominantly in the summer with an average 336mm per year.
There is no predation pressure in CNP.

Mount Camdeboo Private Reserve (MCPR) is a 14 000-hectare private reserve. Rainfall occurs mainly in the summer months with 380-310mm year (Stewart & Campbell, 2001) although rainfall can be as high as 600-800mm in nearby areas. Montane escarpment grassland makes up 42.1% of the total area dominated by *Merxmuellera disticha* and *Themeda triandra* grass species. Karoo Shrubland and Thicket make up the lowlands within the reserve. There is predation pressure in Mount Camdeboo Private Reserve due to the presence of Lions (*Panthera leo*).

Bontebok National Park (BNP) is a 3435 hectare national park under the jurisdiction of Sanparks. Rainfall is non-seasonal with annual rainfall of ~537 mm. There is no predator presence in BNP. Vegetation communties include the renosterveld and lowland fynbos vegetation (Kraajj et al, 2011).

Bushman’s Kloof private reserve (BMK) is a 7500-hectare private reserve dominated by Fynbos and Succulent Karoo biomes. Fynbos vegetation consists of small shrubs, grass-like ‘restios’ interspersed with small trees whereas Karoo vegetation is dominated by small shrubs (<1m high) and succulents (Rutherford, Mucina, Powrie, 2006) The area receives approximately 274mm of rainfall per annum. There is no predation pressure in BMK.

5.7.2 Text S2 - Sample collection

Each visit entailed a minimum of seven reserve-wide surveys per sampling season. Surveys were conducted both by vehicle, using public access and/or management roads. This sampling design controlled for time of year and ensured changes in grass vegetation and physiological consequences within and between years were due to seasonality. All faecal samples included in the analysis were from individuals observed excreting from the car. This allowed us to identify the sex of the individual and group composition for foal:mare ratio. Due to the unique stripe patterns of mountain zebra, each zebra was photographed and identified *via* a stripe identification software -Hotspotter (Crall et al. 2013)

We collected samples from at least two complete boluses per dung pile, from several areas of each bolus. Boluses were collected, placed in sealed plastic bags, had excess air removed and homogenised to ensure a representative sample. We stored samples in 8ml of 100% ethanol during the field season. Storage in ethanol occurred within 8 hours of collection. This method has been shown to be effective for long term storage for protocols involving DNA extraction

5.7.3. Text S3 - Environmental data

5.7.3.1 Resource (forage and grass) availability

50m vegetation transects were conducted on foot at every site of sample collection. An average 24 transects were conducted each sampling season – 12 in areas where zebra occupied areas and 12 in randomly assigned “non-zebra” areas. A point-transect method (Barbour et al. 1980) was used to measure vegetation cover and vegetation composition. Vegetation cover was used as a proxy of forage availability by assessing proportion of bare ground recorded along 50 m transects in each habitat type. Transects were selected relatively close to farm roads (< 500 m) for ease of access and transect direction was randomly selected. A pin was placed at 1 m intervals along each transect so percentage cover of grasses, forbs, shrubs (including woody shrubs, trees and succulents) and bare ground could be recorded.   If grass, forbs or shrubs occurred at the identical location both growth forms were recorded at an individual sampling point (Coulloudon et al. 1999), e.g., the occurrence of grass under the canopy of a tree. The percentage cover for each vegetation item and bare ground was calculated as the percentage occurrence along all points. If obstructions occurred within the transect, sampling points were taken at least 3 m away from the obstructions and restarted within the direction of the original transect. To reduce inconsistent measurements between sites and years, a single observer conducted all vegetation sampling. The vegetation sampling procedure was also repeated for habitats not utilised by Cape mountain zebra. Non-occupied transects were selected by a random sampling technique of dividing established non-occupied areas of landscape into equal sized sites, assigning a number to each site and using a random number generator to assign location of transect.

5.7.3.2 Rainfall

Rainfall across populations could be important in determining the availability of forage and grass. We assessed amount of rainfall in each population using the Climate Hazards Group Infrared Precipitation combined with station observations (CHIRPS) dataset at a resolution of 0.05°. Rainfall was estimated for the 30 days before sample collection to allow delay between rainfall and vegetation growth. Rainfall was calculated for the pixel under each sample, which is roughly equivalent to 5.5km^2 resolution close to the average home range size of CMZ (3.1-16 km$^{2)}$ (Penzhorn, 1982). As gut retention time for the equids of similar size is approximately 24-48 hours – faecal sampling will represent diet composition approximately 24-48 hours before collection. To account for this, all metrics were assessed at a scale of ~ 2.3km^2 which is the possible distance of daily movements patterns for closely related species Grevy's zebra (*Equus grevyi*) (Kartinzel *et* al*, 2015*). Grass availability and forage availability per group were also used at this scale by merging vegetation transects within 2.3km^2 of each other.

5.7.4 Text S4 - Metabarcoding

QIAamp DNA Stool Mini Kits (Qiagen, UK) were used for DNA extraction. Extraction was conducted following the manufacturer's protocol with an additional incubation at 95°C for 30 minutes. Extract concentrations were checked using a Qubit 4 Fluorometer and Qubit™ dsDNA HS Assay Kit (Invitrogen, CA, USA).

Samples were analysed for bacterial, plant and nematode composition using amplicon sequencing. For bacteria phylogeny and taxonomy, we amplified the 16S rRNA (v4 region) gene region (Antwis et al., 2019; Kozich et al., 2013). For nematodes, we amplified the rDNA Internal transcription spacer-2 (ITS-2) region (Avramenko et al., 2015). For plants, we amplified the P6 loop of the chloroplast *trn*L(UAA) region (Kartzinel et al., 2015a; Kartzinel & Pringle, 2020a). Amplicon sequences were dual-indexed with index primers to allow for sample identification during sequencing from 158 unique index combinations. Each 5' end of each forward amplicon product was tagged with one of 16 8-nt multiplex identification (MIID) tags. Each 5' end of each reverse amplicon product was tagged with one of 8 8-nt MIID tags.

Bacterial DNA amplification and indexing was conducted in a single round of PCR using dual indexed forward and reverse primers (Kozich et al., 2013). PCR thermocycling conditions were 95 °C for 15 min; 25 cycles of 95 °C for 20s, 50 °C for 60s, 72 °C for 60s; and a final extension at 72 °C for 10 min. PCR reactions were made up of 25µl 5x HOT FIREPol® Blend Master Mix (Solis BioDyne, Estonia), 2µM primers and 3µl of sample DNA.

Diet DNA amplification and indexing was conducted in two rounds of PCR. In the first PCR round, DNA was amplified in 25µl PCRs made up of 22µl Platinum Green Hot Start PCR 2X Master Mix (Thermo Fisher, MA, USA) 0.2µM each primer [trnL(UAA)g/trnL(UAA)h], and 2µl of DNA. PCR cycling conditions were 95 °C for 5 min; 35 cycles of 95 °C for 30s, 55 °C for 40s, 72 °C for 60s; and a final extension at 72 °C for 10 min. Prior to the second round of PCR, primers and small bp lengths (<50bp) were removed from samples using HighPrep PCR clean up beads according to the manufacturer's instructions. In the second round, indexes were added to to the amplicon primers. 2nd round PCR reactions were 25µl reactions using 22µl KAPA HiFI Ready mix (Kapa Biosystems, Millipore Sigma, MI, USA), 1µM index primes and 2µl cleaned PCR product. 2nd round thermocycling conditions were 95 °C for 45 s; 7 cycles of 98 °C for 20s, 63 °C for 20s, 72 °C for 2 mins.

Nematode DNA amplification and indexing was conducted in two rounds of PCR. Firstly, DNA was amplified in 25µl PCRs according to (Avramenko et al., 2015). Amplicon primers were a mixture of four forward and four reverse primers with zero, one, two and three random nucleotides included between the locus specific primer sequence and Illumina adapter sequence due to the variability of the ITS-2 region in nematodes of interest. Primers are mixed in equal proportions. The 1st round of PCR reactions were 25µl, 21.5 µl Kapa HiFi Hotstart PCR kit with dNTPs (Kapa Biosystems, MilliporeSigma, MI, USA), 0.5µM of primer mix along with 4µl of DNA. 1st round PCR conditions used were 95 °C for 2 mins; 35 cycles of 98 °C for 20s, 62°C for 15s, 72°C for 15s; and a final extension at 72 °C for 2 minutes. PCR products were cleaned using HighPrep PCR clean up beads to remove any primer and small unspecific binding (<50bp). The second round of PCRs were used to add the indexes equivalent to the diet. 2nd round PCR were 25µl reactions using 22µl KAPA Kapa HiFi Hotstart PCR kit with dNTPs (Kapa Biosystems, Millipore Sigma, MI, USA), 1µM index primers and 2µl cleaned PCR product. 2nd round PCR conditions were 95 °C for 45 s; 7 cycles of 98 °C for 20s, 63 °C for 20s, 72 °C for 2 mins.

The bacteria, diet and nematode sequencing used the identical protocol after PCR amplification. Adjustments were made regarding differing library concentrations, percentage PhiX spikes, and the Miseq kits used for the full sequencing run for optimisation of each run. A titration pool was made by adding 1µl of each product. Average bp length and PCR quality were checked on an Agilent 2200 TapeStation using High Sensitivity D1000 ScreenTape (Agilent Technologies, CA, USA) according to manufacturer's instructions and library concentration quantified on Qubit 4 Fluorometer using a Qubit™ dsDNA BR Assay Kit (Invitrogen, CA, USA). Using average fragment size and concentration, the titration pool was diluted to a 4nM library and a titration sequencing run was conducted using paired-end reads (2 × 150 bp) with a 50-cycle reagent kit (MiSeq Reagent Kit v2 MS-102-2001). Concentration for all three libraries was 4pM, with a 5% spike of PhiX Control v3 (Illumina, FC-110-3001) for the bacteria and 15% spike for diet and nematodes on the Illumina MiSeq platform. The titration and full MiSeq runs were conducted at the University of Salford, UK.

The titration sequencing was used to normalise read abundance across all samples. Titration sampling allowed concentrations of each sample to be calculated to produce the final pool for full sequencing runs. This step minimises sequencing bias.

The average library size of the final pool was determined using an Agilent 2200 TapeStation using High Sensitivity D1000 ScreenTape (Agilent Technologies, CA, USA). Concentration was quantified on Qubit 4 Fluorometer using a Qubit™ dsDNA BR Assay Kit (Invitrogen, CA, USA). A 4nM pool was produced from calculations. Diet sequencing used paired-end reads (2 × 250 bp) with 300-cycle reagent kits (MiSeq Reagent Kit v2 MS-102-2003) with a 15% spike of PhiX Control v3 at a library concentration of 4pM. Nemabiome sequencing used paired-end reads (2 × 250 bp) with 500-cycle reagent kits (MiSeq Reagent Kit v2 MS-102-2003) with a 15% spike of PhiX Control v3 at a library concentration 9.5pMs Bacterial sequencing used paired-end reads (2 × 300 bp) with a 300 cycle reagent kit (MiSeq Reagent Kit v2 MS-102-3003) at a concentration of 12pM with a 5% spike of PhiX Control v3 (Illumina, FC-110-3001).

As the target amplicon for diet was shorter than the paired reads that were sequenced, adapters were present in the resulting sequences. Using Cutadapt 2.1 (Martin, 2011) on Python (v3.7) (Python Software Foundation 2018), the forward and reverse adapters were removed. The Illumina platform identifies primer sequences in the resulting sequences and automatically trims them. This did not happen for nematodes. We removed primer sequences using Cutadapt 2.1 (Martin, 2011). Any sequences where the number of reads fell to zero were removed from the analysis. Data analysis and visualisation was conducted in Rstudio (v1.3.1093) (RStudio Team 2020) for R (v4.0.3) (R Core Team 2020). Raw amplicon sequencing data was processed using DADA2 (v1.18.0) (Callahan et al., 2016) . Sequences were filtered, trimmed and denoised, merged and chimeras are removed. Assigned sequences variants (ASVs) were identified using taxonomic reference libraries if possible. Reference libraries used were: SILVA v138 database (Quast et al. 2013; Yilmaz et al. 2014), (Workentine et al., 2020) for ITS-2 nematode and (Gill et al., 2019) for chloroplast *trn*L (UAA) region for plants from this region of Eastern Africa. Any ASVs found in negative controls were removed. Any ASV which was not assigned at any taxonomic level was removed from the analysis. If an ASV was not assigned but had high read number, a NCBI blast search was used and highly similar sequences were added to the analysis.

A total of 10,658,674 raw sequence reads from 267 samples were generated during sequencing. Modal contig length was 253bp once paired-end reads were merged. Mean reads per sample was 34624 (range 5768 – 736264). We removed sequence variants (SVs) found in the negative controls. 7 samples with fewer than 5000 overall reads were removed from further analyses during rarefaction. SVs with fewer than 100 total reads were also removed from analyses. SVs were identified 72% to family level.

A total of 6,387,000 raw sequence reads from 267 samples were generated during sequencing. Modal contig length was 52bp once paired-end reads were merged. Chimeras were removed. Four samples had fewer than 2000 overall reads and were removed during rarefaction from further analyses, leaving 263 samples with a mean of 22704 reads per sample after quality control (range 2611 - 162676). ASVs were assigned to 75% at family level.

A total of 4,780,465 raw sequence reads from 267 samples were generated during sequencing. Modal contig length was 370bp once paired-end reads were merged. The longest sequenced SV was 425bp, which is within the expected range of 100bp-700bp (Workentine et al. 2020 p. 2). We removed chimeras. 65 samples with fewer than 2000 overall reads were removed during rarefaction from further analyses, leaving 202 samples with an average of 22656 reads per sample (range 2044 - 199609). ASVs were assigned to 96% at family level.

Phyloseq package (McMurdie & Holmes, 2013) was used to produce a phyloseq object of ASV table, taxonomy table, phylogenetic tree and sample metadata.

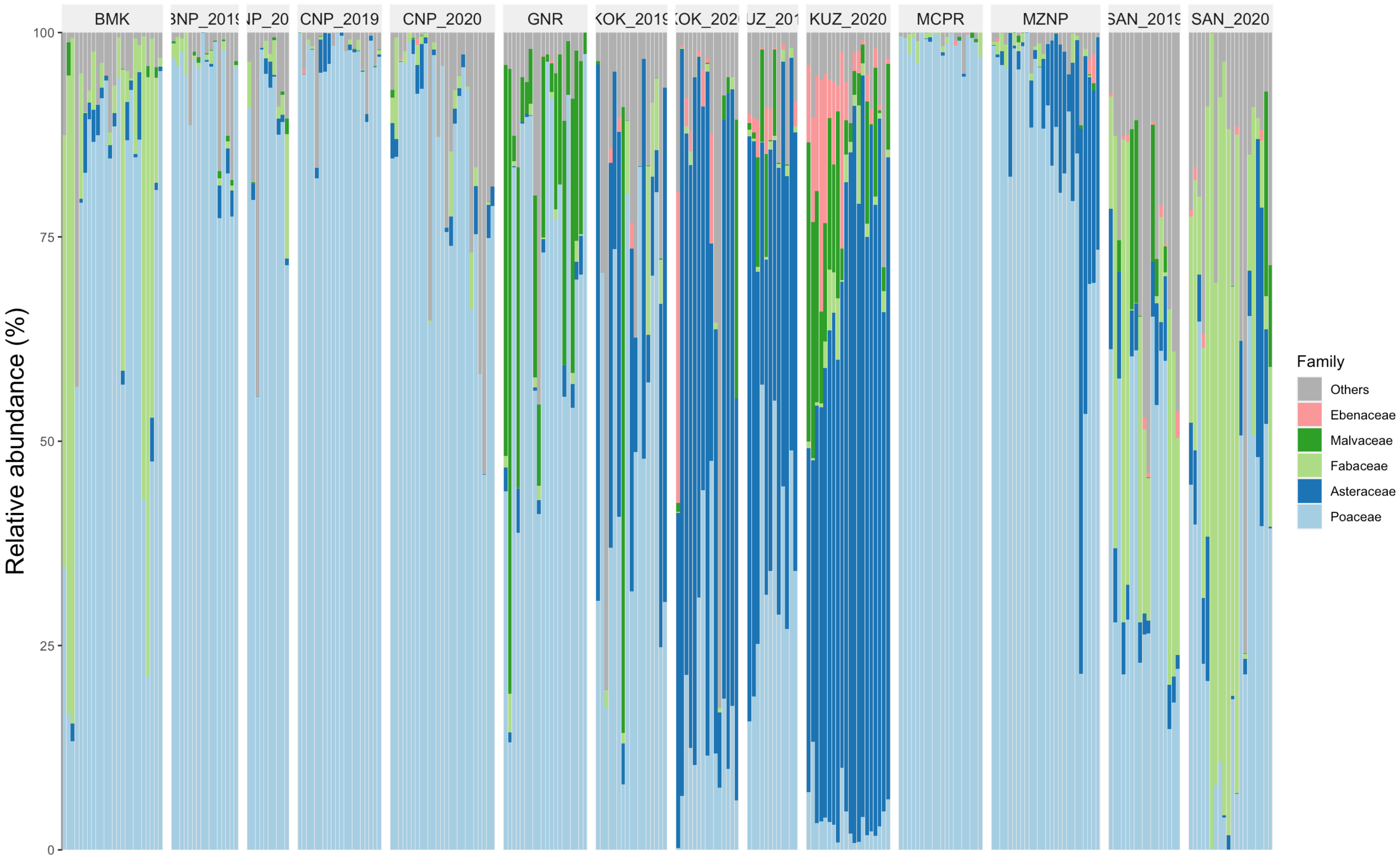


Figure S5.1) Cape Mountain zebra dietary composition across populations per sample at the family level level – Top five most abundant groups. Others will include additional plant families.

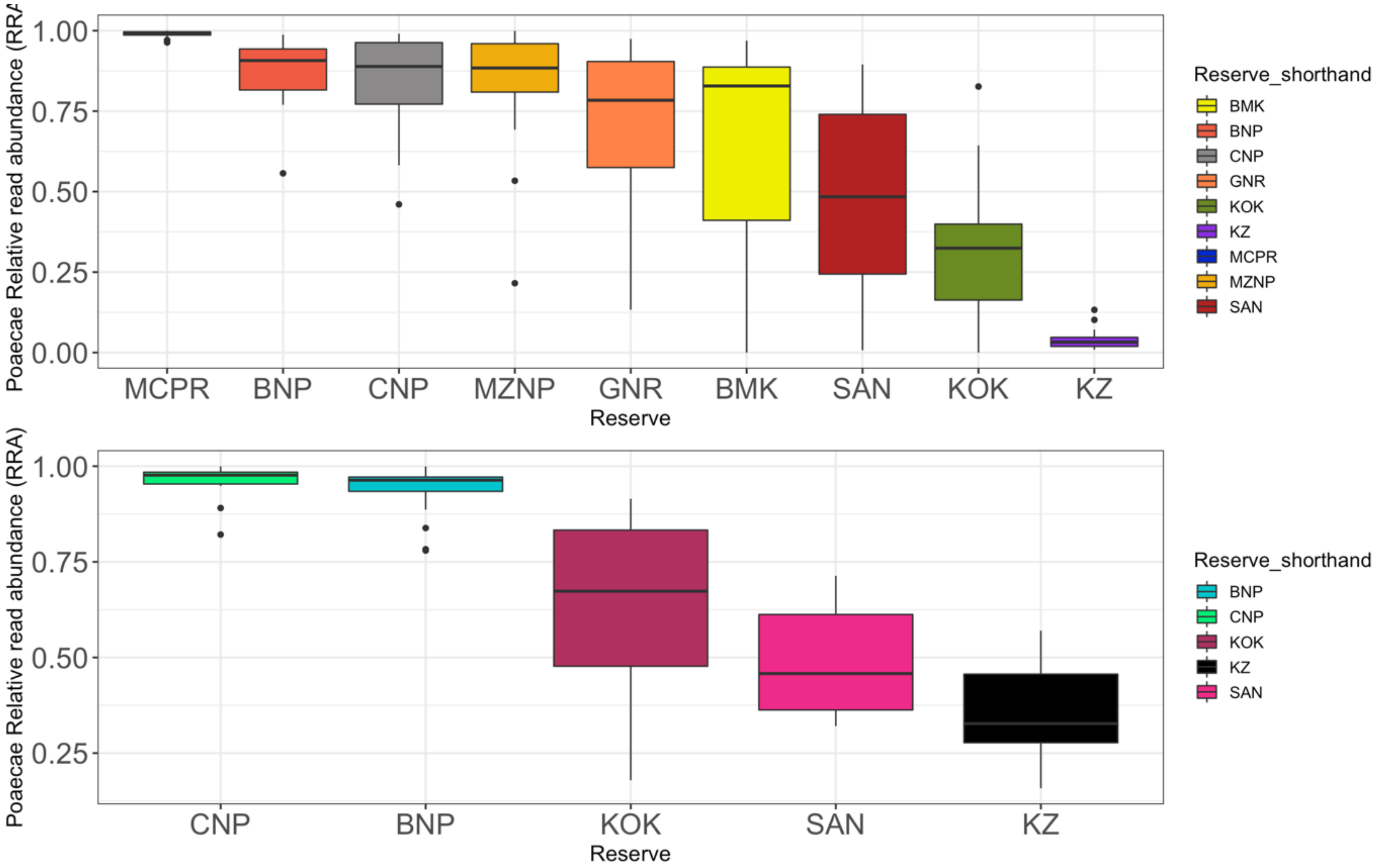


Figure S5.2) Proportion of grass (*Poaceae*) in the diet across nine reserves in 2020 (Top) and across five reserves in 2019 (Bottom)

Table S5.1) The results of PERMANOVA differences between reserve and years in the diet of cape mountain zebra. We used weighted Unifrac distances to assess between-sample dissimilarity. 10000 permutations were carried out. The R-squared values indicate the amount of between-sample variation explained by each variable.

	Df	SumOfSqs	R2	F	Pr(>F)
Reserve_shorthand	8	8.70	0.57	50.3	<0.001
Year	1	0.43	0.03	20.1	<0.001
Reserve_shorthand:Year	4	0.70	0.05	8.03	<0.001
Residual	248	5.37	0.35306853		

Table S5.2) The results of PERMANOVA testing for the effects that significantly structure the diet of cape mountain zebra. We used weighted Unifrac distances to assess between-sample dissimilarity. 10000 permutations were carried out. The R-squared values indicate the amount of between-sample variation explained by each variable.

	Df	SumOfSqs	R2	F	Pr(>F)
Culminative Rainfall	1	0.44	0.029	8.9	<0.001
Grass availability	1	1.801	0.12	36.1	<0.001
Proportion of unvegetated ground	1	0.06	0.004	1.2	0.25
Residual	258	12.9	0.84810148		

Table S5.3: Proportion of grass in the diet is driven by grass availability and rainfall across species range in Cape mountain zebra across years. Global model for logit-transformed proportion of grass in diet, associated with grass availability (log transformed), forage availability (logit transformed) and cumulative rainfall of 3 month period prior to sampling controlling for individual and year. (*Vif < 2)*

Parameter	df	Slope	s.e	t	*F*	*p*	Delta AIC
Intercept	1, 266	1.84	0.48	3.8			
Grass availability	1, 233	0.36	0.05	7.9	60.7	<0.001	48.193
Forage availability	1, 233	-0.14	0.10	-1.4	1.85	0.17	2.77
Rainfall	1, 233	0.005	0.002	3.4	11.2	<0.001	4.05

Table S5.4) Quadratic regression of dietary breadth and proportion of grass in the diet

Parameter	**df**	**Slope**	**s.e**	**t**	***p***
(Intercept)	1, 259	1.58	0.03	49.7	
logit(proportion of grass in the diet)^1	1, 259	-0.51	0.54	-0.95	0.34
logit(proportion of grass in the diet)^2	1, 259	-2.80	0 0.55	-5.0	<0.001

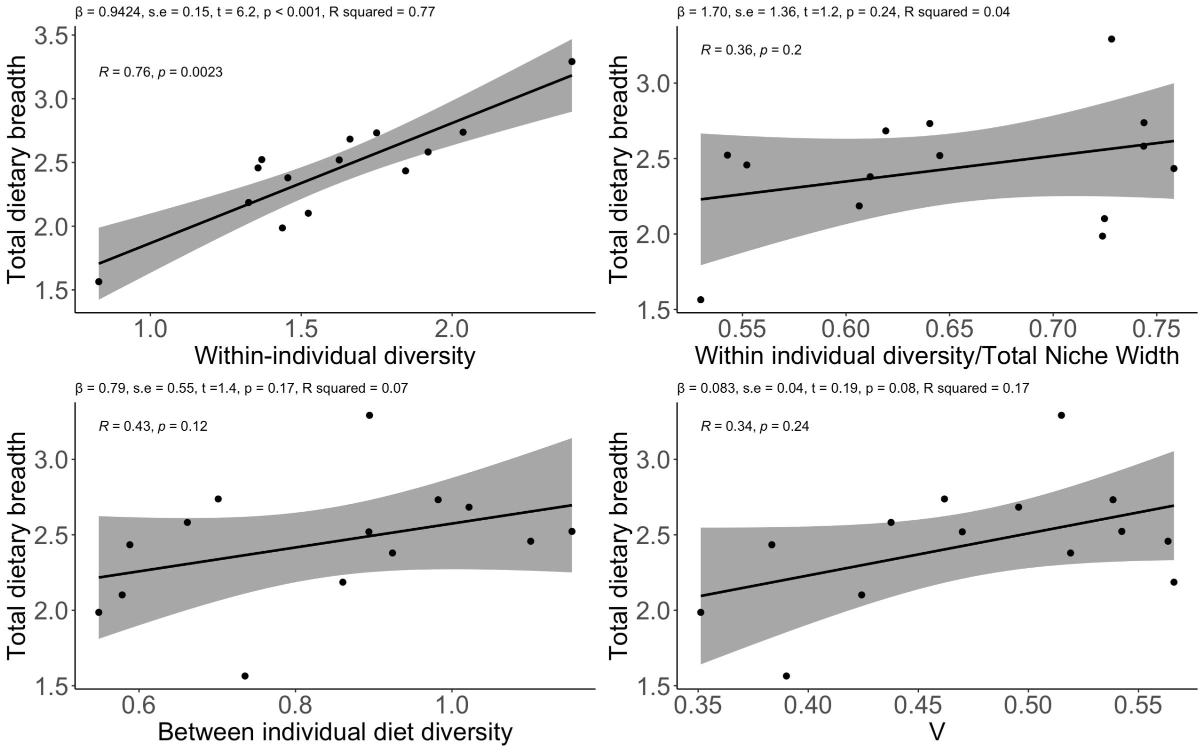


Figure S5.3) Cape mountain zebra do not display individual specialisation with food limitation. The Niche Variation Hypothesis predicts that during food limitation increases in individual diversity should come from between-individual diversity increases (BIC, middle). Instead, and in accordance with OFT, Total niche width (TNW) increases during food limitation stemming primarily from increased within-individual diversity. Furthermore, WIC/TWN is predicted to decrease during individual specialisation whereas our results show a positive correlation. Correlation was analysed using Spearman's rank correlation coefficient. Results of regression analysis are presented above.

Table S5.5) Correlation within each Reserve of primary food item and the 2nd most prevalent plant genus

Correlation test	**Biome type**	**t**	**df**	**cor**	**P value**
Grasses (*Poaecae*) vs legumes (*Fabaceae*)	Bushman's Kloof Private Reserve	-14.976	22	-0.9542883	<0.001
	Sanbona Wildlife Reserve 2018	-6.7678	18	-0.8472802	<0.001
	Sanbona Wildlife Reserve 2020	-6.937	15	-0.8731556	<0.001
Grasses (*Poaecae)* vs asters (*Asteraceae*)	Koktyls Private Reserve 2019	-2.275	15	-0.5064916	0.05
	Koktyls Private Reserve 2020	-2.1	13	-0.50	0.05
	Kuzuko Lodge 2019	-4.6558	10	-0.8272303	<0.001
	Kuzuko Lodge 2020	-2.9523	18	-0.57	0.009
	Mountain Zebra National Park	-38.18	24	-0.9918683	<0.001
Grasses (*Poaecae*) vs mallows (*Malvaceae*)	Gamkaberg Nature Reserve	-7.88	18	-0.88	<0.001

Table S5.6*)* Loading scores of Genus dietary items on the first principal component of diet beta diversity**.** Positive loadings correspond to low rainfall, more barren habitats and negative loadings correspond to high rainfall, high grass availability habitats, Only loading scores >1.0 or <-1.0 are displayed.

Dietary genus	**PC1 loading score**
Themeda	-2.9347911
Aristida	-2.5910762
Digitaria	-2.5525381
Andropogon	-2.5002266
Cenchrus	-1.1057709
Enneapogon	-1.037571
Ehrharta	2.58444351
Asteraceae Family	1.73623286
Diplachne	1.67735689
Crassula	1.62353015
Medicago	1.40286485
Caryophyllales Order	1.32914404
Euclea	1.0399975
Craterocapsa	0.97353394

Table S5.7) Loading scores of Genus dietary items on the second principal component of diet beta diversity. Positive loadings correspond to more barren (unvegetated) habitats and negative loadings did not correspond to environmental variables, Only loading scores >1.0 or <-1.0 are displayed.

Dietary genus	PC2 loading score
Asparagus	2.01832351
Grewia	1.8557297
Asteraceae Family	1.68695077
Euclea	1.62513374
Digitaria	1.60322545
Indigofera	0.3469683
Diplachne	-1.2668685
Cyperus	-1.0660763
Caryophyllales Order	-1.0023701

Table S5.8) Summary of microbiome genera loadings on the first principal component of microbiome beta diversity. Positive loadings correspond to greater proportion of grasses (Poaceae) in the diet. Negative loadings correspond to greater proportion of asters (Asteraceae) in the diet. Only loading scores >0.4 or <-0.4 are displayed. ASV loading scores are not given directly due to the large quantity of ASVs.

Microbial genus and species (if assigned	Loading score
Anaerosporobacter Genus	1.54158595
Monoglobus Genus	1.05264035
Methanobrevibacter Genus	0.9330702
Anaerovibrio Genus	0.82749609
Candidatus Soleaferrea Genus	0.81324487
Coprococcus Genus	0.79434939
Saccharofermentans Genus	0.75925606
Lachnospiraceae NK4A136 group Genus	0.72757674
dgA-11 gut group Genus	0.69955757
Muribaculaceae Family	0.66704348

Desulfovibrio Genus	0.66191703
Lachnospirales Order	0.65486834
Quinella Genus	0.6487955
[Eubacterium] ruminantium group Genus	0.64671185
Marvinbryantia Genus	0.64184814
Phoenicibacter Genus	0.6310406
Anaerovoracaceae Family	0.56842647
Armatimonadota Phylum	0.559717
Family XIII UCG-001 Genus	0.55596304
Blautia Genus	0.5391785
Ligilactobacillus Genus	0.53353782
Eggerthellaceae Family	0.53021308
Anaerofustis Genus	0.52088841
Herbinix Genus	0.51828293
Syntrophomonadaceae Family	0.48939026
Synergistaceae Family	0.48434229
Lachnospiraceae XPB1014 group Genus	0.48394144
XBB1006 Genus	0.48048528
V9D2013 group Genus	0.45971121
Lachnospiraceae NK4B4 group Genus	0.45951836
Moryella Genus	0.45332182
Lactobacillaceae Family	0.45106462
[Eubacterium] hallii group Genus	0.44905138
Defluviitaleaceae UCG-011 Genus	0.42963242
[Eubacterium] coprostanoligenes group Family	0.42259324
Akkermansia Genus	0.41569687
Solobacterium Genus	0.41350442
Paludicola Genus	0.38291611
Ruminococcus Genus	0.37548241
Izemoplasmatales Order	-1.3012851

Erysipelatoclostridiaceae Family	-1.0944359
Marinifilaceae Family	-1.0372495
Candidatus Methanomethylophilus Genus	-0.9685935
Izemoplasmataceae Family	-0.9456369
vadinBE97 Family	-0.8922996
Paludibacteraceae Family	-0.8578326
Clostridia vadinBB60 group Order	-0.8526547
Mycoplasma Genus	-0.7864587
Erysipelotrichales Order	-0.778778
Victivallaceae Family	-0.7587309
Methanomethylophilaceae Family	-0.7536987
Colidextribacter Genus	-0.7344901
UCG-004 Genus	-0.7126726
Bacteroidia Class	-0.6653325
Succinivibrionaceae UCG-002 Genus	-0.6218481
CAP-aah99b04 Family	-0.6056015
Ruminiclostridium Genus	-0.5819484
Anaeroplasma Genus	-0.5700149
T2WK15B57 Genus	-0.5469495
Desulfovibrionaceae Family	-0.5144955
Rhodospirillales Order	-0.5088999
Methanocorpusculum Genus	-0.4971706
Faecalibacterium Genus	-0.4647188
Puniceicoccaceae Family	-0.4511424
EMP-G18 Genus	-0.4326082
Marinilabiliaceae Family	-0.4309614
[Eubacterium] ventriosum group Genus	-0.4186485
Methanimicrococcus Genus	-0.4028832

Table S5.9) Summary of Microbiome genera loadings on the second principal component of microbiome beta diversity**.** Positive loadings greater proportion of legumes (Fabaceae) in the diet. Negative loadings correspond to greater proportion of grass (Poaecae) in the diet. Only loading scores >0.4 displayed. ASV loading scores are not given directly due to the large quantity of ASVs

Microbiome Order	**Loading score**
MVP-15 Class	1.1294873
Colidextribacter Genus	1.0177377
Marinifilaceae Family	0.90644737
Firmicutes Phylum	0.88032021
Monoglobus Genus	0.79412336
Oribacterium Genus	0.7242407
Ruminiclostridium Genus	0.71204711
Oscillospira Genus	0.69215029
Sediminispirochaeta Genus	0.67814477
UCG-009 Genus	0.63160148
UCG-002 Genus	0.60237003
Oscillospirales Order	0.59089934
Synergistaceae Family	0.58419326
Lachnospiraceae UCG-006 Genus	0.55620381
Desulfovibrionaceae Family	0.55481962
Akkermansia Genus	0.5503529
Desulfovibrio Genus	0.52265877
Eggerthellaceae Family	0.52264901
Oscillospiraceae Family	0.51864477
Succinivibrionaceae UCG-002 Genus	0.51504654
Methanocorpusculum Genus	0.51196992
Elusimicrobium Genus	0.51119226
Campylobacter Genus	0.50465344
Bilophila Genus	0.48141396

Anaeroplasma Genus	0.4715477
Marinilabiliaceae Family	0.4667827
SP3-e08 Genus	0.45539239
Prevotellaceae Ga6A1 group Genus	0.44793234
[Eubacterium] siraeum group Genus	0.43721536
Methanobrevibacter Genus	0.4338434
Papillibacter Genus	0.42212555
dgA-11 gut group Genus	0.41883012
Prevotellaceae Family	0.40434385
Rhodospirillales Order	0.40027731
Alphaproteobacteria Class	-1.0357633
Corynebacterium Genus	-0.7761223
Pseudoalteromonas Genus	-0.6485161
Staphylococcus Genus	-0.6387755
Shuttleworthia Genus	-0.6281883
Halomonas Genus	-0.5941058
Streptococcus Genus	-0.5645532
clevelandensis	-0.4847627
[Eubacterium] nodatum group Genus	-0.4469918
Cyanobacteriia Class	-0.4411756
Sphingomonas Genus	-0.4410175
Saccharofermentans Genus	-0.4248333
[Eubacterium] ruminantium group Genus	-0.4136457
EMP-G18 Genus	-0.4082838

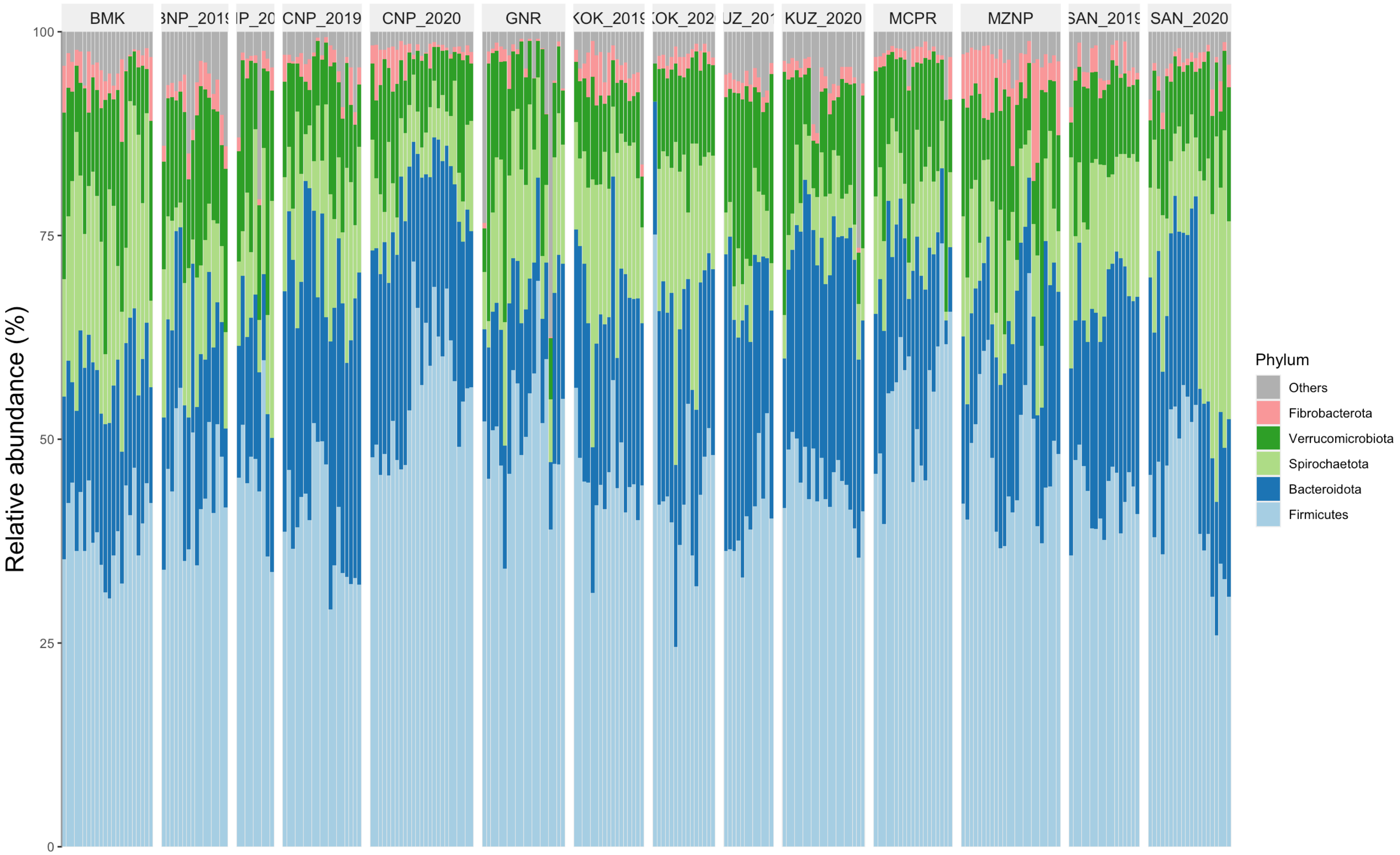


Figure S5.4) Cape Mountain zebra microbiome composition across reserves split per sample at the phyla level – Top five most abundant groups. Others will include bacterial phyla.

Table S5.10) The results of PERMANOVA testing for the variance in the microbiome of cape mountain zebra explained by reserve differences. We used weighted Unifrac distances to assess between-sample dissimilarity. 10000 permutations were carried out. The R-squared values indicate the amount of between-sample variation explained by each variable.

	Df	**SumOfSqs**	**R^2**	**F**	**Pr(>F)**
Reserve	13	3.21	0.37	10.9	<0.001
Residual	241	5.5	0.63		
Total	254	8.7	1		

Table S5.11) The results of PERMANOVA testing for the effects that significantly structure the microbiome of cape mountain zebra. We used weighted Unifrac distances to assess between-sample dissimilarity. 10000 permutations were carried out. The R-squared values indicate the amount of between-sample variation explained by each variable.

	Df	**SumOfSqs**	**R^2**	**F**	**Pr(>F)**
Proportion of grass (Poaecae) in the diet	1	0.39	0.05	12.5	<0.001
Proportion of legumes (Fabaecae) in the diet	1	0.14	0.02	4.6	<0.001
Proportion of asters (Asteraceae) in the diet	1	0.12	0.01	3.8	<0.001
Residual	251	7.85	0.91		
Total	254	8.66	1		

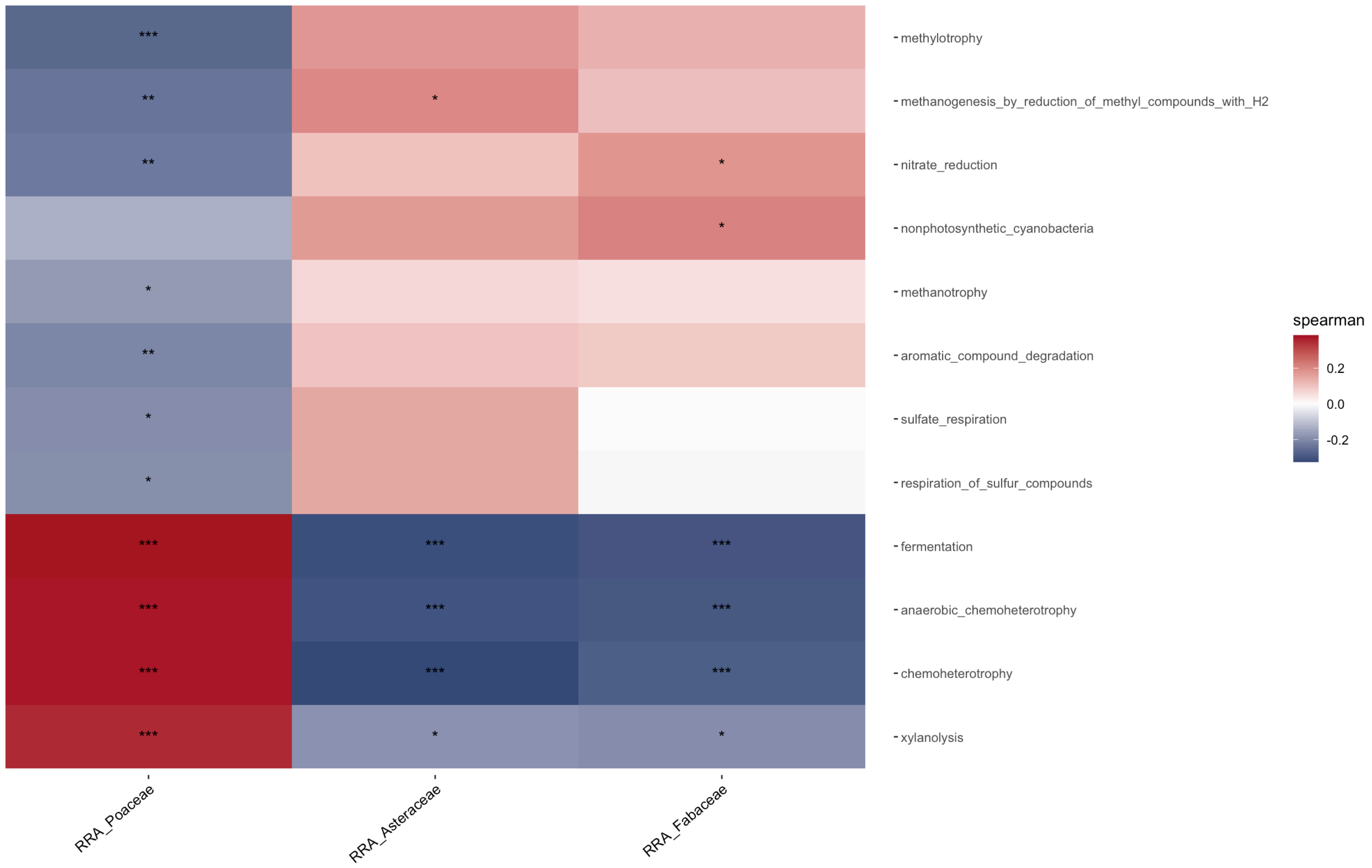


Figure S5.5) Functional analysis of microbiome heatmap from environmental variables including relative abundance of dietary and microbiome groups using using FAPROTAX. Colour of tile relates to the strength of spearman correlation coefficient. Blank represents non significance, * = *p < 0.05, ** p < 0.01, *** p < 0.001.*

Table S5.12) Correlation between microbiome beta dispersion and proportion of grass in the diet by reserves using Pearson's correlation coefficient

Reserve	***t***	**df**	***cor***	***P value***
Bushman's Kloof Private Reserve	0.48	23	0.10	0.63
Sanbona Wildlife Reserve	-2.1	35	--0.34	0.04
Koktyls Private Reserve	2.5	31	-0.41	0.02
Kuzuko Lodge	1.49	30	0.26	0.15
Mountain Zebra National Park	-1.5	22	-0.31	0.14
Gamkaberg Nature reserve	-1.98	18	-0.42	0.06
Camdeboo National Reserve	-3.22	42	-0.44	0.002
Bontebok National Park	1.1	23	0.23	0.28
Mount Camdeboo Private Reserve	0.09	17	0.02	0.92

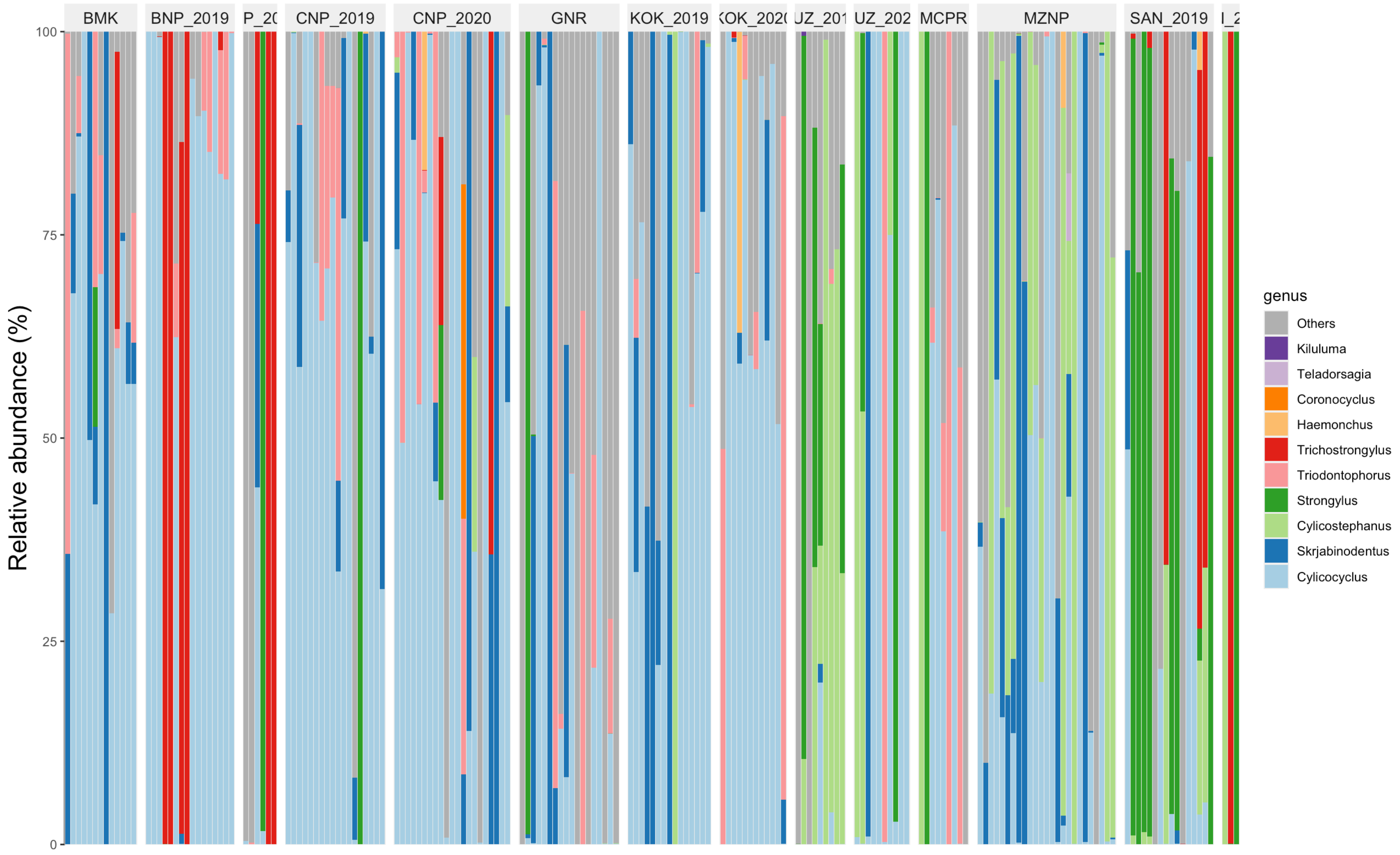


Figure S5.6) Cape Mountain zebra nemabiome composition across reserves per sample at the genera level – Top ten most abundant groups. Others will include nematodes not assigned to genus level and additional nematode groups.

Table S5.13) The results of PERMANOVA testing for the variance in the nemabiome of cape mountain zebra explained by reserve differences. We used weighted Unifrac distances to assess between-sample dissimilarity. 10000 permutations were carried out. The R-squared values indicate the amount of between-sample variation explained by each variable.

	Df	**SumOfSqs**	**R2**	**F**	**Pr(>F)**
Reserve	13	3.75	0.21	3.64	<0.001
Residual	177	14.03	0.79		

Table S5.14) The results of PERMANOVA testing for the effects that significantly structure the nemabiome of cape mountain zebra. We used weighted Unifrac distances to assess between-sample dissimilarity. 10000 permutations were carried out. The R-squared values indicate the amount of between-sample variation explained by each variable.

	Df	**SumOfSqs**	**R2**	**F**	**Pr(>F)**
Grass (Poaecae) in the diet	1	0.20	0.011	2.2	0.09
Legumes (Fabaceae) in the diet	1	0.83	0.047	9.4	0.002
Asters (Asteraceae) in the diet	1	0.02	0.0013	0.26	0.83
Parasite burden (Faecal egg count)	1	0.23	0.013	2.59	0.07
Residual	186	16.50	0.93		
Total	190	17.78	1		

Table S5.15) Summary of Nemabiome species loadings on the first principal component of Nemabiome beta diversity**.** Positive loadings correspond to species associated with higher proportion of grass in the diet and negative loadings corresponds to species associated with greater proportion of legumes (Fabaceae) in the diet. Only loading scores >0.4 or <-0.4 are displayed.

Nemabiome genus and species (if assigned)	**Loading score**
Cylicostephanus_minutus	2.3351607
Strongylus_edentatus	1.3445263
Trichostrongylus_axei	1.2751535
Strongylus_vulgaris	1.1388294
Skrjabinodentus_caragandicus	- 1.345491
Triodontophorus_nipponicus	- 1.5675250
Cylicocyclus_adersi	4.0772507

Table S5.16) Model results of parasite egg count and proportion of grass in the diet .Populations with restricted proportion of grass in the diet were associated with greater parasite burden (FEC) across years

Parameter	df	Slope	s.e	t	*F*	*p*	Delta AIC
Intercept	1, 266	27.5	0.7	39.2			
Proportion of grass in diet	1, 233	-1.91	0.25	-7.8	46.2	<0.001	47.903
Rainfall	1, 233	-0.01	0.006	2.6	2.28	0.13	7.183

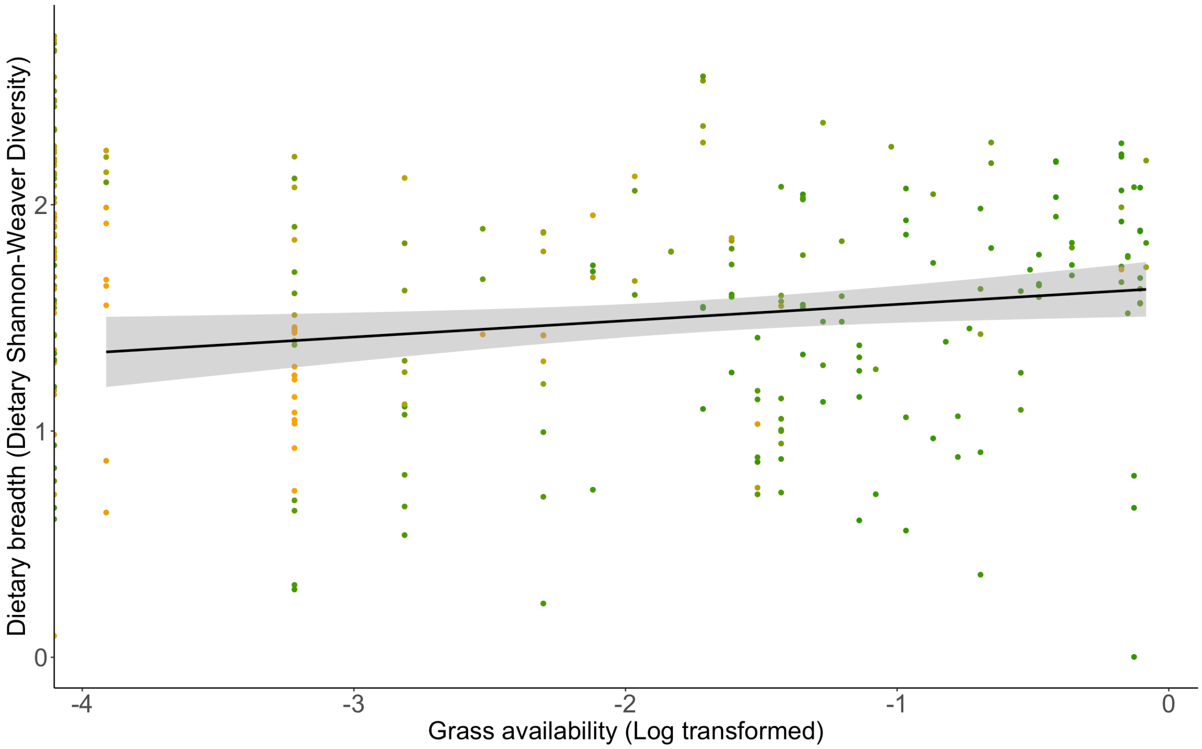


Figure S5.7) Dietary diversity against grass availability (proportion of grass on the ground) from field transects taken at the point of faecal sampling. Dietary breadth shows significant linear increases with increasing grass availability such that animals eat a more varied diet when a greater proportion of palatable food items are available.

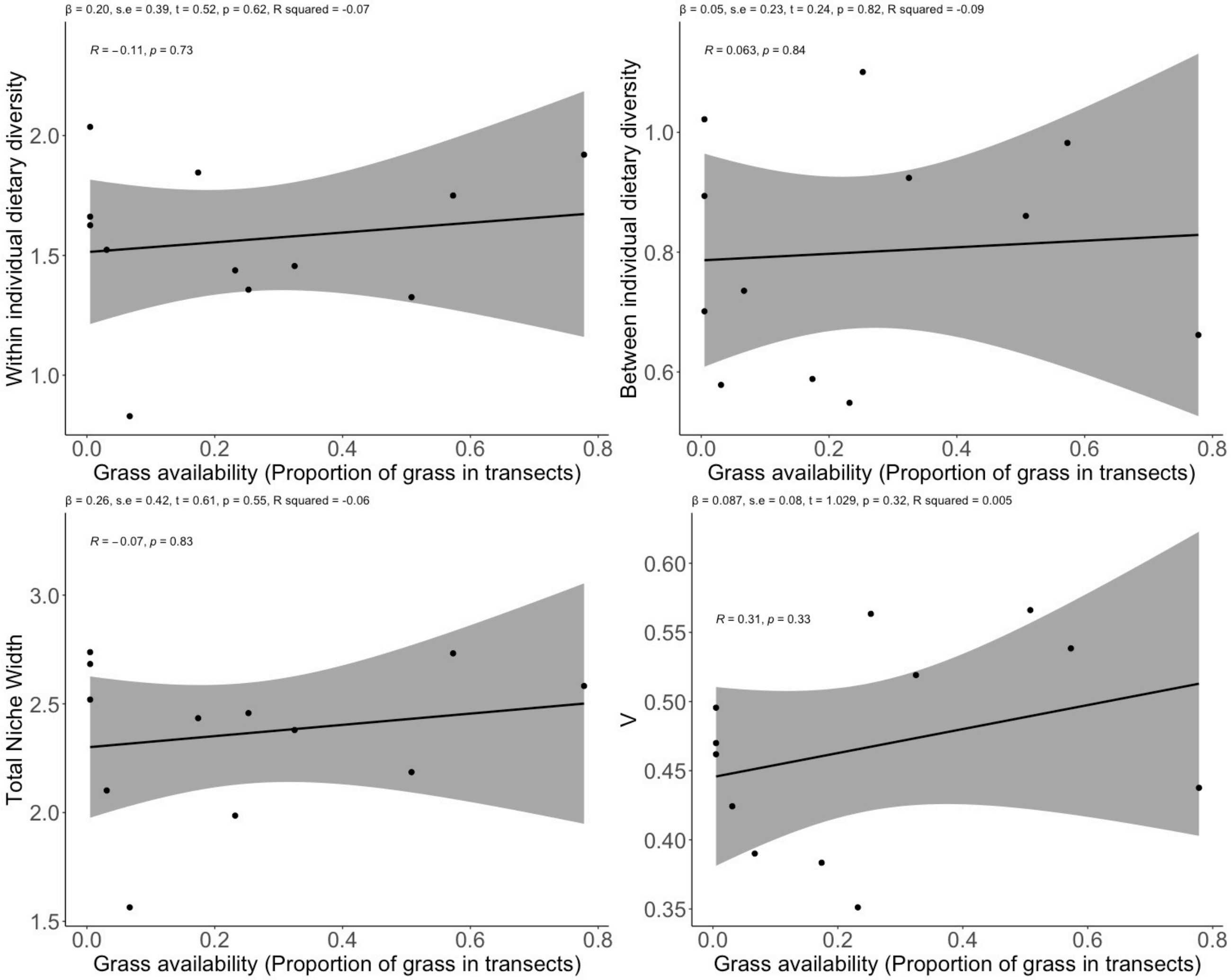


Figure S5.8) Dietary individual specialisation metrics against grass availability across reserves. Sanbona Wildlife reserve across both years has been excluded from this analysis as it is supplementary fed and therefore dietary dynamics should not follow native grass availability.

Table S5.17) Cape mountain zebra interpopulation dietary overlap across populations and across years. Values are weighted means of Unifrac–distance dissimilarities (low values = high overlap). Weighted Unifrac distance account for phylogenetic distance between dietary items. *Indicates supplementary fed population

Diet		**2019**					**2020**								
	Population	BNP	CNP	KOK	KUZ	SAN*	BMK	BNP	CNP	GNR	KOK	KUZ	MCPR	MZNP	SAN*
2019	BNP	N/A													
	CNP	0.14	N/A												
	KOK	0.45	0.46	N/A											
	KUZ	0.57	0.59	0.49	N/A										
	SAN*	0.39	0.41	0.48	0.57	N/A									
2020	BMK	0.30	0.29	0.44	0.62	0.43	N/A								
	BNP	0.12	0.15	0.46	0.58	0.41	0.30	N/A							
	CNP	0.17	0.14	0.46	0.58	0.40	0.31	0.10	N/A						
	GNR	0.28	0.31	0.48	0.54	0.43	0.41	0.29	0.32	N/A					
	KOK	0.72	0.73	0.58	0.48	0.66	0.73	0.73	0.72	0.69	N/A				
	KUZ	0.91	0.93	0.76	0.56	0.86	0.91	0.92	0.92	0.82	0.55	N/A			
	MCPR	0.12	0.09	0.47	0.59	0.42	0.30	0.08	0.15	0.29	0.74	0.93	N/A		
	MZNP	0.17	0.16	0.44	0.52	0.41	0.33	0.16	0.19	0.30	0.67	0.86	0.14	N/A	
	SAN*	0.47	0.48	0.54	0.60	0.41	0.49	0.49	0.46	0.52	0.68	0.86	0.50	0.48	N/A

Table S5.18) Cape mountain zebra interpopulation microbiome overlap across populations and across years. Values are weighted means of Unifrac–distance dissimilarities (low values = high overlap). Weighted Unifrac distance account for phylogenetic distance between dietary items. *Indicates supplementary fed population

Microbiome		**2019**					**2020**								
	Population	BNP	CNP	KOK	KUZ	SAN*	BMK	BNP	CNP	GNR	KOK	KUZ	MCPR	MZNP	SAN*
2019	BNP	N/A													
	CNP	0.26	N/A												
	KOK	0.25	0.24	N/A											
	KUZ	0.27	0.26	0.28	N/A										
	SAN*	0.26	0.25	0.21	0.26	N/A									
2020	BMK	0.25	0.26	0.23	0.28	0.25	N/A								
	BNP	0.24	0.27	0.26	0.29	0.28	0.25	N/A							
	CNP	0.27	0.23	0.25	0.28	0.28	0.27	0.27	N/A						
	GNR	0.27	0.29	0.26	0.32	0.29	0.26	0.25	0.28	N/A					
	KOK	0.25	0.23	0.19	0.28	0.23	0.23	0.25	0.26	0.26	N/A				
	KUZ	0.28	0.28	0.28	0.22	0.30	0.29	0.30	0.28	0.32	0.27	N/A			
	MCPR	0.26	0.27	0.27	0.29	0.29	0.27	0.24	0.23	0.26	0.27	0.29	N/A		
	MZNP	0.25	0.26	0.23	0.28	0.26	0.25	0.25	0.25	0.27	0.25	0.27	0.26	N/A	
	SAN*	0.26	0.25	0.23	0.27	0.23	0.24	0.26	0.26	0.27	0.23	0.28	0.27	0.26	N/A

Table S5.19) Cape mountain zebra interpopulation nemabiome overlap across populations and across years. Values are weighted means of Unifrac–distance dissimilarities (low values = high overlap). Weighted Unifrac distance account for phylogenetic distance between dietary items. *Indicates supplementary fed population

Nemabiome		**2019**					**2020**								
	Population	BNP	CNP	KOK	KUZ	SAN*	BMK	BNP	CNP	GNR	KOK	KUZ	MCPR	MZNP	SAN*
2019	BNP	N/A													
	CNP	0.28	N/A												
	KOK	0.30	0.07	N/A											
	KUZ	0.60	0.46	0.46	N/A										
	SAN	0.47	0.35	0.35	0.50	N/A									
2020	BMK	0.32	0.12	0.13	0.50	0.38	N/A								
	BNP	0.43	0.35	0.36	0.62	0.50	0.54	N/A							
	CNP	0.28	0.18	0.22	0.50	0.40	0.22	0.41	N/A						
	GNR	0.30	0.07	0.07	0.46	0.35	0.13	0.36	0.19	N/A					
	KOK	0.32	0.12	0.12	0.50	0.38	0.16	0.38	0.18	0.12	N/A				
	KUZ	0.36	0.15	0.15	0.41	0.42	0.20	0.41	0.25	0.15	0.20	N/A			
	MCPR	0.33	0.12	0.12	0.43	0.32	0.17	0.39	0.20	0.12	0.17	0.30	N/A		
	MZNP	0.38	0.18	0.18	0.42	0.37	0.23	0.43	0.27	0.18	0.23	0.31	0.20	N/A	
	SAN*	0.60	0.56	0.55	0.53	0.51	0.58	0.60	0.59	0.55	0.58	0.52	0.52	0.51	N/A

Chapter 6) Macrophysiology and marginality in the Grevy's zebra (*Equus grevyi*)

To be submitted as part of a collaborative project investigating dietary strategies and physiological consequences to seasonal fluctuations in a Critically Endangered grazer, Grevy's Zebra (*Equus grevyi*), and a Critically Endangered browser, Black Rhino (*Diceros bicornis michaeli*).

6.1 Abstract

Spatio-temporal changes in populations across heterogeneous landscapes drive metapopulation dynamics. Metapopulations occur across ecological gradients ranging from high quality or “core” habitats to marginal ones. Core habitats promote a higher intrinsic rate of growth and larger population densities at carrying capacity, while supplying potential immigrants to replenish surrounding environments. In core habitats, individuals should have greater access to resources and improved physiological status through time. Understanding the relative importance of cyclic fluctuations in habitat quality, and dietary and physiological responses to these changes, is crucial to understand population dynamics. Here, we evaluate the dietary strategy of the Critically Endangered Grevy’s zebra *(Equus grevyi)* to seasonal fluctuations and their impacts on microbiome structure and function, and nemabiome composition across an ecological gradient. We demonstrate Grevy’s zebra include legumes (Fabaceae, especially *Indigofera spp*) in their diet in poor rainfall areas and periods. Legume consumption decreases with increases in grass availability (assessed by NDVI). Across the rainfall gradient, diets in arid areas were continually grass poor, suggesting ecological marginality. Diet composition altered microbiome structure with low grass diets being associated with functional shifts to increase the breakdown of aromatic compounds, reduced xylanolysis and increased prevalence of pathogenic bacteria. Low grass diets were also associated with less diverse nemabiomes with higher prevalence of pathogenic helminths such as *Strongylus vulgaris, S. edenatus and S. equinus*. Our results suggest the rainfall gradient between Meru, Samburu and Laikipia was a more important driver of dietary composition and macro-physiological trends, through time, than seasonal fluctuations. These results have implications for the environmental and physiological drivers of population dynamics in the Grevy’s zebra metapopulation.

6.2 Introduction

The principles that underlie spatio-temporal dynamics of populations in heterogeneous landscapes are critical for understanding range dynamics through population regulation and dispersal/migration. Metapopulations, spatially separated populations that interact (Levins, 1969), occur across ecological gradients ranging from high quality “core” habitats to marginal ones (Kawecki, 2008). In core habitats conditions should, on average, be favourable to the survival and reproduction of the species. Core habitats will either bolster average fitness levels greater than 1 (increasing population) or be associated with higher population densities than marginal habitats. If quality differences between habitats are severe and marginal populations begin declining, core habitats become source populations by producing excess immigrants, which overflow into marginal sinks (Pulliam, 1988; Pulliam & Danielson, 1991). If differences are mild (fitness > 1 in both habitats), dispersal can become balanced *i.e.* no net flow of individuals between habitats and fitness can equalise (McPeek and Holt, 1992). However, marginal habitats will maintain a lower carrying capacity and population density.

A primary challenge in light of widespread range contraction (*e.g.,* Ripple et al, 2015; Wolf & Ripple, 2016) and population decline (WWF, 2020) is identifying how survival, reproductive and performance rates vary across populations (Holt, 2009). Identifying ecological characteristics that describe marginal habitats is crucial for formation and protection of resilient metapopulations (Hanski, 2012). Ultimately, variation in biotic and abiotic conditions forms ecological gradients influencing animal movement (Kauffman et al., 2021) and demographics (Street et al., 2015). Individual fitness and habitat selection is directly associated with acquiring sufficient food items to support survival, reproduction and buffer periods of scarcity or challenge (Mduma et al. 1999; Kitaysky et al. 2006, 2010). Food availability and quality is a major driver regulating large mammalian herbivore populations (Sinclair et al., 1985; White 2008). Food availability and quality for large herbivores are largely driven by precipitation and soil fertility (Olff et al., 2002). Marginal habitats may be formed where seasonal variability causes periods of food limitation (Jesmer et al., 2020) or where relatively stable climatic patterns lead to a patchy resource distribution. Understanding the relative importance of stable and cyclic patterns of environmental factors and resource availability may be key to understanding the herbivore dietary strategies and population dynamics.

Animals should maximize their energy intake and minimize ingestion of plant chemical defences by choosing the ‘best’ food items available (Freeland & Janzen, 1974). During lean periods animals widen their dietary breadth to include poorer quality but available items (MacArthur & Pianka, 1966). This widening of dietary breadth can result in dietary switching where animals “switch” to using alternative food items (Grueter et al., 2009). For example, browsers can become more dependent on grazing (Kerley et al., 2010), or conversely grazers become more reliant on browse (Bocherens et al., 2015; Hecker et al., 2021; Kaczensky et al., 2017) even though their digestive physiology may be poorly adapted to these diets (Demment & van Soest, 1985). If habitat quality is independent of seasonality, marginal habitats will be heavily dependent on alternative food items across seasons. If habitat qualities are influenced by seasonality, the quality of habitats may converge, diverge or equalise across seasons (Figure 6.1). In this instance, animals will display seasonal dietary switching.

During lean periods, animals are predicted to behave according to two contrasting models. Optimal foraging theory (OFT) predicts that individuals utilize available resources in order of quality, first depleting preferred items and then incorporating lower quality items (MacArthur & Pianka, 1966; Stephens & Krebs, 1986, Figure 6.1). The niche variation hypothesis (NVH) (van Valen, 1965) predicts that during food limitation animals minimize intraspecific competition, by specializing in specific dietary items (van Valen, 1965). Lean periods are, therefore, associated with increased individual specialization (Bolnick et al., 2002; Sargeant, 2007, Figure 6.1). If animals behave according to the predictions of optimal foraging theory, all individuals in marginal environments should incorporate the same secondary food items into their diets during food limitation. When food limitation becomes severe all individuals should become dependent on the same secondary diet item. Alternatively, if animals behave according to the niche variation hypothesis, marginal habitat should display the greatest levels of individual specialization or frequent dietary diversification. (Figure 6.1)

Diet changes can have downstream consequences on animals' physiology, especially on the physiology of the gastrointestinal system (Karasov et al., 2011). Diet changes affect the gastrointestinal microbial community (the 'microbiome'). The microbiome changes with dietary composition (Kartzinel et al., 2019) and fluctuates seasonally with dietary changes (Baniel et al., 2021; Hicks et al., 2018). The microbiome performs critical functional roles in the digestive tract (Hanning & Diaz-Sanchez, 2015; Sommer & Bäckhed, 2013). Microbiomes are essential for modulating immune responses (Duvallet et al., 2017), digesting food items (Hanning & Diaz-Sanchez, 2015), and breaking down plant toxins (Barboza et al., 2010). Microbiome changes may be beneficial for the host, allowing the animal to include food items it otherwise could not digest (Kohl et al., 2014). Alternatively, frequent perturbations may cause microbiomes to stochastically vary (Zaneveld et al., 2017) or completely breakdown (Antwis, 2021). Unstable microbiomes may impact on digestive efficiency, inflammatory responses (Barranco, 2021; Wastyk et al., 2021) or disease state (Couch et al., 2021). Seasonally dependent diet changes may, therefore, impose a physiological or energetic cost on herbivores.

Climatic fluctuations have also been linked to gastrointestinal nematode fluctuations (Cattadori et al., 2005). Diet, microbiome and nemabiome are heavily influenced by each other. Poor diet quality can result in heightened gastrointestinal parasite burden and a greater variety of pathogenic species (Budischak et al., 2015; Ezenwa, 2004). As microbiomes are crucial for modulating the immune response, changes in microbiome composition have been associated with colonisation of pathogenic helminth groups (Lee et al., 2014; Li et al., 2011). Indeed, the microbiota composition can either alleviate or exacerbate the pathology of helminth infection (Peachey et al., 2017). Thus, improved diet quality may promote a microbiome composition which leave hosts less susceptible to pathogenic helminth infections (Glendinning et al., 2014). Although the links are not well understood, microbial community composition, helminth burden and composition, and diet are all associated with fitness (Sinclair et al., 1985; Cattadori et al., 2005; Gould et al., 2018).

We predict seasonal dietary changes will result in different physiological effects dependent on whether animals follow optimal foraging theory or the niche variation hypothesis. If microbiome responses to dietary change are deterministic, animals following optimal foraging theory should display directional changes in microbiome composition. If habitat quality does not fluctuate across seasons, microbiomes and nemabiomes should remain stable across seasons. If habitat quality does fluctuate with season, we would predict microbiome and nemabiome dissimilarity (β diversity) to reduce if habitat quality converges and increase if habitat quality diverges. In contrast, animals following the predictions of niche variation hypothesis would display increasing inter-individual variability of diet, microbiome and nemabiome (β dispersion) during lean periods. If habitat quality does not vary with season, β dispersion in marginal areas should be consistently greater than that in high quality areas. If habitat quality does fluctuate with season, we would predict microbiome and nemabiome β dispersion to reduce if habitat quality increases, and increase if habitat quality decreases.

Here we evaluate the dietary strategy of a Critically Endangered grazer, the Grevy's zebra (*Equus grevyi*), to seasonal fluctuations across an ecological gradient. We also assess the physiological consequences of seasonal dietary change. We assess changes across an ecological gradient, *specifically* rainfall and productivity, in Laikipia, Meru, and Samburu counties, Kenya. Savannas present an excellent opportunity to evaluate seasonal dietary dynamics across ecological gradients. Rainfall is concentrated in specific periods of the year, resulting in wet and dry seasons (Marston et al., 2019). During wet seasons, 'greening up' of herbaceous and deciduous plants means that more plants are available to herbivores than during dry seasons (Adole et al., 2018). Alongside seasonality, there are long term ecological gradients of rainfall and productivity in East Africa, such that some areas receive a greater absolute amount of rainfall (Mumo et al., 2019).

6.3 Hypotheses

1. Consumption of grass will be greatest in wet season and in high rainfall areas along the ecological gradient.
2. Animals will display dietary switching to additional plant groups when they cannot saturate their diet with grasses

3. We predict dietary breadth will increase during the dry season as individuals incorporate additional plant items into their diet. We also predict dietary breadth will decline if animals become completely dependent on secondary food items.
4. We predict Grevy's zebra will follow the predictions of Optimal Foraging Theory instead of Niche Variation Hypothesis. We therefore predict the level of individual specialisation (between-individual variation) will not change with decreasing proportion of grass in the diet. We also predict increases in population-level dietary breadth will be due to all conspecifics eating additional food items (increasing intra-individual dietary breadth), instead of conspecifics having more varied diets between them (inter-individual dietary breadth).
5. Diet will alter microbiome and nemabiome composition. Individuals with more dissimilar diets will have more dissimilar microbiomes and nemabiomes.
6. Low grass diets will be associated increased prevalence of pathogenic helminth groups such as large gastrointestinal parasites e.g. Strongylus *spp.*

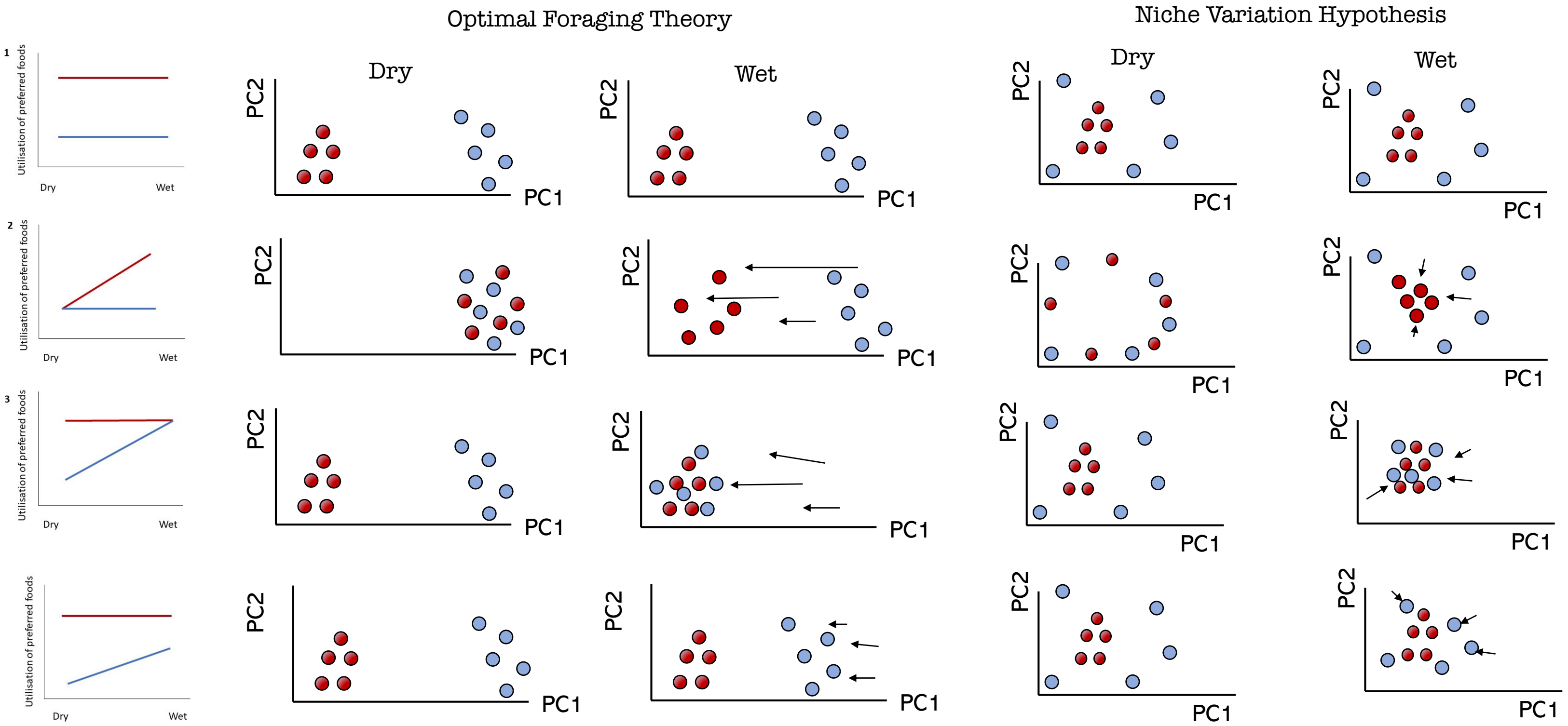


Figure 6.1) Seasonal dietary fluctuations predicted from optimal foraging theory (OFT) and Niche variation hypothesis (NVH) across habitats of varying quality. High quality habitat are coloured red and low quality blue. Scenario 1) Habitat quality is not dependent on seasonal fluctuations. 2) Habitat quality increases in one habitat relative to another due to seasonality, 3) Seasonality equalizes habitat qualities 4) Seasonality improves habitat quality of marginal habitat but they are not equalized. 1st column depicts changing availability of preferred/primary food sources between dry and wet season. 2nd and 3rd demonstrate variation in dietary composition between dry and wet season according to the predictions of optimal foraging theory. 4th and 5th columns demonstrate variation in dietary composition between dry and wet season according to the predictions of Niche variation hypothesis. PC1 and PC2 depict the multivariate position of dietary composition. Arrows indicate movement of individual diets in multivariate space across seasons.

6.4 Methods

6.4.1 Study species

Historically, Grevy's zebra occurred throughout the horn of Africa in Kenya, Ethiopia, Eritrea, Djibouti, South Sudan and Somalia (Bauer et al., 1994). Due to widespread range contraction, Grevy’s zebra now occupy a discontinuous range. In Kenya, they occur from the eastern side of the Rift Valley to the Tana River. In Ethiopia, they occur in a single isolated population in the Alledeghi Plains northeast of Awash National Park (Rubenstein et al., 2016). The largest populations exist in Laikipia, Kenya (Rubenstein et al., 2016).

Grevy’s zebra are predominately grazers (Kartzinel et al., 2015; Kartzinel & Pringle, 2020; Sundaresan et al., 2007) but will include browse (documented at 30%) in their diets during drought (Ginsberg et al,. 1987) or in overgrazed areas (Kartzinel et al., 2015). Grevy’s zebra preferentially occupy habitat with highly palatable grass species such as *Themeda, Cynodon* and *Pennisetum* (Sundaresan et al., 2008). Grevy’s zebra live in arid and semi-arid grass/shrubland with dispersal patterns being largely determined by food and water availability (Klingel, 1974; Rubenstein, 1989; Williams, 1998)

6.4.2 Study populations

Due to the limited fencing between Laikipia, Samburu and Meru counties, most wildlife species can move freely throughout the landscape. Most land in Laikipia, Samburu and Meru counties lacks formal protection. Primary land uses are pastoral or commercial ranchlands which bring livestock rearing and active wildlife conservation into close contact (Low et al., 2009). Due to geographic distance we have split our study area into three different “populations”: Mpala, Karisia and Ol Jogi wildlife conservancy form a single population referred to as Mpala-Oljogi, Lewa wildlife conservancy forms a second, and Westgate community conservancy, the third. Predation communities of lions (*Panthera leo*) and leopards (*Panthera pardus*) are intact across all protected areas.

Mpala research centre is found in central Laikipia (0°17’N, 36°53’E). Mpala receives an average of 500mm rainfall annually. The habitat is a bushed grassland. The woody vegetation is dominated by *Acacia* species while there are abundant grasslands of primarily the genera *Themeda*, *Cynodon* and *Pennisetum*. Vegetation communities in Mpala can be characterised into wooded grasslands, semi-arid savanna and acacia bushland (Franz et al., 2010). Ol Jogi Conservancy is a 235km^2 private reserve sanctuary in Laikipia County (0.32°N, 36.98°E) adjacent to Mpala. Rainfall averages at ~570mm per annum. Vegetation communities include wooded grassland habitat similar to Mpala with mixed species woodland/thicket. Grevy's zebra are able move between the properties freely. Karisia is in eastern Laikipia in an area north of Mount Kenya. Karisia is at the southern extent of the Mpala and Ol Jogi conservancies.

Lewa Wildlife Conservancy is a 250km^2 reserve in Meru County (0.20°N, 37.42°E). The average annual precipitation is ~570mm with distinct wet and dry seasons. Short rains fall between March to May and a longer rainy season falls from October to December. The vegetation communities are predominately wooded grassland but there are small proportions of bushland and mountain forest.

Westgate Community Conservancy is a community led conservancy situated in Samburu County covering 403.5 km^2 in northern Kenya. Average rainfall within Samburu country is between 36mm-279mm per year. Rainfall seasonality follows four key periods: a short dry season between January-February, long wet season between March and May, a long dry season between June and October and finally a short-wet season between November-December. Vegetation communities are characterized as arid acacia bushland.

6.4.3 Environmental data

6.4.3.1 Rainfall and Normalized difference vegetation index (NDVI)

We assessed amount of rainfall across the ecological gradient with Climate Hazards Group Infrared Precipitation combined with station observations (CHIRPS) dataset at a resolution of 0.05°. Rainfall estimates for 30 days prior to sample collection were used to account for the delay between rainfall and vegetation growth. Rainfall was calculated for the pixel under each sample, which is roughly equivalent to 5.5km^2 resolution. 8-day aggregated NDVI maps were used as a proxy for plant productivity. NDVI measures habitat greenness and has been ground-truthed and shown to correlate with forage biomass in Eastern Africa (Meshesha et al., 2020). Vegetation greenness has been used as a proxy of forage palatability and quality for Grevy's zebra (Sundaresan et al., 2008). As gut retention time for Grevy's zebra is approximately 24-48 hours and Grevy's zebra can move ~2.3km per day (Kartinzel et al., 2015), all NDVI estimates were performed at a resolution of ~2.3km^2 of sample collection point.

6.4.4 Sampling

Samples were collected over two field seasons, between June – July 2018 and January – March 2019. These periods were chosen as the former is generally within the wet season in this area of Kenya, and the latter is a dry season. Samples were collected from at least two complete boluses per dung pile. Several areas of each bolus were sampled, avoiding the surface (~1cm depth). Samples were placed in sealed plastic bags and had excess air removed. Samples were stored frozen and were frozen within ~8 hours of collection. 158 Grevy's zebra samples were used for the metabarcoding analysis.

6.4.5 DNA Metabarcoding

6.4.5.1 Extraction and amplicon sequencing

For DNA extraction, we used QIAamp DNA Stool Mini Kit (Qiagen, UK). The manufacturer's protocol was followed with the additional incubation at 95°C for 30 minutes. DNA extractions were performed in pre-PCR rooms to avoid contamination. DNA extraction products were checked for a suitable concentration using a Qubit 4 Fluorometer and Qubit™ dsDNA BR Assay Kit (Invitrogen, CA, USA).

Amplicon sequencing was used for bacterial, plant and nematode composition. Amplified gene loci were 16s rRNA (v4 region) for bacteria (R. E. Antwis et al., 2019; Kozich et al., 2013), ITS-2 region was used according to (Avramenko et al., 2015) for nematodes and the P6 loop of the chloroplast *trn*L(UAA) region was used for plants (Kartzinel et al. 2015;

Kartzinel & Pringle 2020). Dual-indexing with index primers allowed sample identification during the pooled sequencing step. 16 8-nt multiplex identification (MIID) tags were add to the 5’ end of each forward amplicon primer and 8 8-nt MIID tags added to 5’ end of each reverse amplicon primer. Blank samples were run each for each metabarcoding run to account for potential contamination. Any assigned sequence variants (ASVs) found in the blank samples were removed from the analysis.

6.5.4.2 DNA amplification

6.5.4.2.1 Bacteria

A single round of PCR was used for amplification and indexing of the 16S rRNA gene (v4 region). PCR reactions were 30µl using 5x HOT FIREPol® Blend Master Mix (Solis BioDyne, Estonia), 2µM primers and 3µl of DNA. Thermocycling conditions were 95 °C for 15 min; 25 cycles of 95 °C for 20s, 50 °C for 60s, 72 °C for 60s; and a final extension at 72 °C for 10 min.

6.5.4.2.2 Diet

DNA amplification of the *trnL*-P6 region was achieved using Platinum Green Hot Start PCR 2X Master Mix (Thermo Fisher, MA, USA), 0.2µM each primer [trnL(UAA)g/trnL(UAA)h], and 2µl of sample DNA with thermocycling conditions of 95 °C for 5 min; 35 cycles of 95 °C for 30s, 55 °C for 40s, 72 °C for 60s; and a final extension at 72 °C for 10 min. PCR products were cleaned using HighPrep PCR clean up beads to remove DNA sequences (<50bp). A second round of PCRs added indexes. 2nd round PCR used 25µl reactions using KAPA HiFI Ready mix (Kapa Biosystems, Millipore Sigma, MI, USA), 1µM index primers and 2µl cleaned PCR product using thermocycling conditions of 95 °C for 45 s; 7 cycles of 98 °C for 20s, 63 °C for 20s, 72 °C for 2 mins.

6.4.4.2.3 Nematodes

The rDNA ITS-2 region was amplified in 25µl PCRs according to (Avramenko et al., 2015) using Kapa HiFi Hotstart PCR kit with dNTPs (Kapa Biosystems, MilliporeSigma, MI, USA). Following the method set out in Avramenko et al. (2015), four ITS-2 primers of different lengths were mixed in equal proportions. 0.5µM of each primer was used along with 4µl of DNA in PCR reactions. Thermocycling conditions were modified from Avramenko et al. (2015) as conditions showed insufficient DNA quality. Final conditions: 95 °C for 2 mins; 35 cycles of 98 °C for 20s, 62°C for 15s, 72°C for 15s; and final extension of 72 °C for 2 minutes. PCR products were cleaned of small bp products using HighPrep PCR clean up beads. A second round of PCRs added the indexes to the amplicon primers in 25µl reactions using KAPA Kapa HiFi Hotstart PCR kit with dNTPs (Kapa Biosystems, MilliporeSigma, MI, USA), 1µM index primers and 2µl cleaned PCR product using thermocycling conditions of 95 °C for 45 s; 7 cycles of 98 °C for 20s, 63 °C for 20s, 72 °C for 2 mins.

6.4.4.3 Titration sequencing run

We used similar protocols for the three sequencing runs (bacteria, diet and nemabiome), with differing library concentrations, percentage PhiX spikes and the Miseq kits for the bacteria. A titration pool was made up by adding 1µl of each sample. Average library size was determined using an Agilent 2200 TapeStation using High Sensitivity D1000 ScreenTape (Agilent Technologies, CA, USA). Library concentration was quantified on Qubit 4 Fluorometer using a Qubit™ dsDNA BR Assay Kit (Invitrogen, CA, USA), both according to manufacturer's instructions. Average fragment size and concentration were used to dilute to 4nM. A titration sequencing run was conducted using paired-end reads (2 × 150 bp) with a 50-cycle reagent kit (MiSeq Reagent Kit v2 MS-102-2001) at a concentration of 4pM for all three libraries. 5% spike of PhiX Control v3 (Illumina, FC-110-3001) for the bacteria and 15% spike for diet and nematodes on the Illumina MiSeq platform at the International Livestock Research Institute, Nairobi.

Titration sequencing results were used to create the final pool. This minimises sequencing bias between samples. The amount of each sample for the final sequencing run is inversely proportional to the occurrence of tagged sequences in the titration run.

6.4.4.4 Full sequencing

For the full sequencing run, an Agilent 2200 TapeStation using High Sensitivity D1000 ScreenTape (Agilent Technologies, CA, USA) and Qubit 4 Fluorometer using a Qubit™ dsDNA

BR Assay Kit (Invitrogen, CA, USA) were used to calculate average library size and concentration respectively. The final pool was diluted to 4nM. For diet sequencing we used paired-end reads (2 × 250 bp) with 500-cycle reagent kits (MiSeq Reagent Kit v2 MS-102-2003) with a 15% spike of PhiX Control v3 at a library concentration of 4pM. The nematode sequencing run used paired-end reads (2 × 250 bp) with 500-cycle reagent kits (MiSeq Reagent Kit v2 MS-102-2003) with a 15% spike of PhiX Control v3 at a library concentration 9.5pMs. The bacterial sequencing used paired-end reads (2 × 300 bp) with a 600 cycle reagent kit (MiSeq Reagent Kit v3 MS-102-3003) at a concentration of 12pM with a 5% spike of PhiX Control v3 (Illumina, FC-110-3001). All sequencing runs were carried out on the Illumina MiSeq platform at the International Livestock Research Institute, Nairobi.

6.4.4.5 Pre-processing

Cutadapt 2.1 (Martin, 2011) was used to remove any forward and reverse adapters and primers attached to sequences. Any sequences where number of reads fell to zero were removed from the analysis. Data analysis and visualisation was conducted in Rstudio (v1.3.1093) for R (v4.0.3) (R Core Team 2020). Raw amplicon sequencing data was processed (filtered, trimmed and denoised, merged and chimeras removed) using DADA2 (v1.18.0) (Callahan et al., 2016). Assigned sequences variants (ASVs) were identified using taxonomic reference libraries at the highest possible taxonomic level. Reference libraries used were: SILVA v138 database (Quast et al., 2013) for ITS-2 nematode (Workentine et al., 2020) and for chloroplast *trn*L(UAA) region for plants from this region of Eastern Africa (Gill et al., 2019). Phyloseq package (McMurdie & Holmes, 2013) was used to combine the final ASV table, taxonomy table and sample metadata. Samples with less than 2000 overall reads in any of the datasets were removed from further analyses using rarefication. For microbiome, ASVs with fewer than 100 total reads were also removed. Removal of low reads for microbiome was conducted as bacterial sequencing produces many more reads, which can lead to large numbers of ASVs with low read counts. Any ASVs found in negative controls (from blanks) were removed. Total rarefied reads for each metabarcoding run were converted into relative read abundance (RRA) to evaluate composition. RRA is widely used as a proxy of the proportional quantity of foods eaten in dietary ecology studies. RRA has been well validated for large herbivorous mammals in eastern Africa when compared to stable isotope analysis and presence/absence data (Kartzinel et al. 2015)

6.4.4.5.1 Bacteria

A total of 7,573,950 raw sequence reads from 158 samples were generated during sequencing. Modal contig length was 253bp once paired-end reads were merged. We removed sequence variants (SVs) with length < 250 bp and > 260 bp. 2 samples with fewer than 2000 overall reads were removed from further analyses leaving 156 samples with a mean of 23070 reads per samples (range 2846 – 636331). 44% of SVs were identified to genus level, and 75.5% to family level. ASVs which were unassigned to any taxonomic level were removed from the analysis.

6.4.4.5.2 Diet

A total of 795,283 raw sequence reads from 158 samples were generated during sequencing. Modal contig length was 52bp once paired-end reads were merged. Sequence variants were between 50bp to 108bp in length. 8 samples with fewer than 2000 overall reads were removed from further analyses, leaving 150 samples and a mean of 5980.257 reads per sample (range 2121 - 11820). 58% of SVs were identified to genus level, and 88% to family level. ASVs which were unassigned to any taxonomic level were removed from the analysis.

6.4.4.5.3 Nematode

A total of 4,627,476 raw sequence reads from 158 samples were generated during sequencing. Modal contig length was 370bp once paired-end reads were merged. The expected range was 100bp-700bp (Workentine et al., 2020). Sequence variants (SVs) <100bp were removed. The longest sequenced SV was 445bp. 2 samples with fewer than 2000 overall reads were removed from further analyses, leaving 156 samples and an average of 25598 reads per sample (range 3186 - 376328). A relatively low number of SVs were successfully assigned taxonomy. 54% were identified to genus level, and 99% to family level. ASVs which were unassigned to any taxonomic level were removed from the analysis.

6.4.4.6 Analysis

Statistical analysis was conducted in Rstudio (v1.3.1093) (RStudio Team 2020) for R (v4.0.3) (R Core Team 2020).

We used linear mixed effect modelling to evaluate the associations between dietary items, helminth groups and microbial groups using package lme4 (Bates *et al*, 2014). Factors displaying variance inflation factors greater than 2 were reduced to a single variable. Relative abundances were generated using microeco package (Liu et al. 2021).

6.4.4.6.1 Beta diversity metrics and pairwise associations between datasets

For beta diversity, we calculated the Weighted Unifrac (a weighted sum of branch lengths in a maximum likelihood phylogenetic tree of samples) index of dissimilarity between each individual sample. Weighted Unifrac measures between sample dissimilarity while controlling for phylogenetic distances between items (Lozupone & Knight, 2005). Phylogenetic trees for each dataset were generated by first aligning sequences using DECIPHER package in R (Wright, 2020). We then constructed maximum likelihood trees using phangorn package (Schliep, 2011) for diet and microbiome, and FastTREE (Price et al., 2009) for nemabiome phylogenetic relationships, due to the size of the alignment. Phylogenetic trees were rooted at their midpoint using small tree mid.point function in phytools (Revell, 2012). We analysed compositional change across reserves and season using permutational analysis of variance (perMANOVA) in the *vegan* package (Dixon, 2003). We also used perMANOVA to assess the proportion of variance in beta diversity which environmental variables described.

We evaluated the effect of environmental variables on between-sample community composition using principal component analysis. Firstly, to account for differences in sequencing depth between samples, the counts for each dataset were normalized using the centred-log-ratio (CLR) method (using a small pseudo count of minimum relative abundance)/2 for zero counts) in MicroViz package (Barnett et al., 2021). We conducted a Principal Component Analysis (PCA) to visually represent between-samples differences according to the predictors. This approach has been recommended for proportional and compositional datasets such as metabarcoding data (Gloor et al., 2017). Using PCA on CLR-transformed data allows for the projection of variable loadings of ASVs (or groups) onto each PC and principal components can be extracted correlated against environmental variables. For diet, taxa were agglomerated to genus level. Therefore, only taxanomic group at the family level or higher were loaded onto PCA axes. For microbiome and nemabiome, taxa were agglomerated to family level and species level respectively. We agglomerated the datasets at these levels as they provided the most intuitive and biologically meaningful levels to understand composition change. For example, for nemabiome, we agglomerated at species level as the vast majority of helminth differences between reserves were species differences. Impacts of environmental variables on principal components were established using linear regression analysis.

To test for pairwise associations between microbiome, nemabiome and diet, we compared the Weighted Unifrac dissimilarly metrices of each to each other. We assessed the relationship between pairwise distances in each dataset using Mantel tests and spearman's multilevel correlations using the *mantel* function in vegan.

6.4.4.6.2 Community composition

The difference in composition of dietary plant community, microbiome and nemabiome across populations was assessed from the relative abundance of each. Stacked bar plots were used to assess the relative contribution of each taxonomic level to the entire composition using microeco package (Liu et al. 2021).

6.4.4.6.3 Dietary switching

We assessed relationships between relative read abundance of primary and secondary food items (defined as most prevalent and second most prevalent groups) using Pearson's product-moment correlation.

6.4.4.6.4 Assessing Individual specialisation, dietary breadth and beta dispersion

For diet, we investigated individual specialisation by calculating proportional-similarity index (PS_i). Proportional similarity index (PS_i) compares the compositional overlap between operational taxonomic units (OTUs) in each individual sample and a pooled population-wide average diet (Bison et al., 2015). As we do not have discrete populations, we assess composition overlap between all samples within a specific season. Low PS_i values indicate high variability between individuals.

For diet breadth, we calculated Shannon Weaver diversity (Bolnick et al., 2007; Roughgarden, 1974). Shannon Weaver diversity has been used as a proxy of dietary niche width (Total niche width) previously (Pansu et al., 2019) Dietary total niche width (TNW) is composed of a "within-individual component" (*i.e.* intra-individual diet variation, WIC), and "between/among-individual component" (*i.e.* inter-individual variation, BIC) (Bolnick et al., 2002; Sargeant, 2007). If species are conforming to Optimal foraging theory, increases in dietary breadth should stem from increases in within-individual dietary variation (Jesmer et al., 2020). The ratio of WIC to total dietary niche width is a measure of individual specialisation. WIC to TNW ratio is predicted to increase if individuals broaden their diet according to optimal foraging theory *i.e.* increased within-individual dietary breadth, and decrease if they broaden their diet through the Niche Variation Hypothesis (increased between individual diversity). Relationships between WIC, BIC, TNW and average proportion of grass in the diet were investigated using Spearman's rank correlation coefficient. We modelled individual dietary breadth against logit transformed proportion of grass in each individual's diet using quadratic regression to account for potential non-linear relationships.

To assess the impacts of proportion of grass on the diet and microbiome or nemabiome diversity among individuals, we evaluated changes in beta dispersion. We estimated beta dispersion as the Euclidean distance to the population centroid of each geographic area in each time period. Firstly, operational taxonomic unit counts were converted using a centre-log ratio (CLR) transformation (with a small pseudo count for zero counts) using the *transform* function from the microbiome package (Lahti *et al*, 2017). Secondly, a Euclidean distance of all points to the population centroid was calculated using *betadisper* function in vegan package (Dixon, 2003). The relationship between beta dispersion and proportion of grass in diet was assessed quadratic regression to account for potential non-linear relationships.

6.4.4.6.5 Functional analysis

The microbiome functional analysis was performing using FAPROTAX reference library (Louca et al., 2016) and conducted using the package microeco (Liu et al., 2021). FAPROTAX uses a reference database of the functions of bacterial groups/species to estimate the proportion of the microbiome involved in each metabolic function. The FAPROTAX reference database has been used to investigate microbiota changes with age and health status in rabbits (*Oryctolagus cuniculus*) (Xing et al., 2019) and gut microbiota changes with diet in brown rats (*Rattus norvegicus*) (Xiang et al., 2019).

6.4.4.6.6 Taxonomic level of analysis

In our analyses, taxonomic resolutions differed depending on the analysis. For diet, taxa were agglomerated to family level for compositional analysis. Family level analysis represents the most biologically meaningful and intuitive resolution for this analysis. With this resolution, proportions and presence of grasses and legumes in the diet could be analysed over time and space. Whereas, for microbiome and nemabiome, taxa were agglomerated to species level for compositional analysis. Species level analysis was performed for microbiome as bacterial genus can have varying functions depending on the species. For nemabiome, species level analysis is the most useful analysis as we can identify detrimental species. For diet, diet diversification was evaluated at assigned sequence variant (ASV) level. Dissimilarity analysis was also evaluated at ASV level for diet, nemabiome and microbiome analysis. ASV level for these analyses as we needed to account for all possible ASVs irrelevant of the level of taxonomic assignment.

6.6. Results

Diet composition varied across the ecological gradient and across seasons (Weighted Unifrac: Reserve; df = 150, R_2 = 0.63, $F_{2,150}$ = 182.4, p < 0.001; Season; R_2 = 0.06 and $F_{1,150}$ = 37.0, p < 0.001, Table S6.1). Across the ecological gradient, diet composition varied with NDVI and rainfall (Weighted Unifrac: Rainfall; df = 150, R_2 = 0.46, $F_{1,150}$ = 133.2, p < 0.001; NDVI; R_2 = 0.03 and $F_{1,150}$ = 9,5, p < 0.001, Table S6.2). Disproportionately, Grevy's zebra diets were made up of two plant families: Poaecae (grasses) (67% of average diet) and Fabaceae (legumes) (29% of average diet) (Figure S6.3). Amaranthaceae was the third most prevalent taxa in Grevy's zebra diets (1.5%). The *Indigofera* genus was the most prevalent legume consumed (25% of average diet). Grasses were mainly made up of one genus *Cenchrus* (20%) but other genera were also consumed including: *Digitaria* (4%), *Aristida* (3%), *Brachiaria* (2%) and *Themeda* (0.8%).

The first principal component of diet β diversity explained 28.4% of variation and was positively correlated with rainfall (β= 0.0090, s.e = 0.0007, t = 13.15, df = 149, R^2 = 0.53, p < 0.001) and NDVI (β= 0.045, s.e = 0.004, t = 11.24, df = 149, R^2 = 0.46 p < 0.001). PC2 was negatively correlated with NDVI (β= -0.013, s.e = 0.005, t = -2.5, df = 149, R^2 = 0.03, p = 0.01). Poaceae (grasses) of genera *Digitaria, Themeda, Cenchrus, Eriochloa* and *Aristida* loaded positively (loading scores > 1.0) onto PC1 and are therefore consumed more in higher rainfall and NDVI areas (Figure 6.2). *Phyllanthus* and the legume *Chamaecrista* also positively loaded onto PC1. *Cenchrus spp, Digitaria spp, Eriochloa spp* and the Asteraceae and Amaranthaeceae families loaded positively on to PC2 and therefore are consumed more in areas with higher NDVI.

Across populations, proportion of grass (Poaecae) in the diet increased with increasing NDVI (β = 0.04, s.e = 0.01, F = *18.4*, p < 0.001, df = 149) and rainfall (β = 0.014, s.e = 0.002, F = *53.4*, p < 0.001, df = 149) (Table S6.2). Across populations and seasons, animals display evidence of consuming increased legumes (Fabaecae) when they could not saturate their diet with grasses (Figure S6.10). Individuals in Lewa and Mpala-Oljogi maintained the highest level of grass consumption across seasons. Proportion of legumes consumed increased with decreasing NDVI across all reserves (Figure 6.3). Individuals in Westgate did not display a period during our study where the average diet was majority grass (>50%) (Figure 6.3). Disproportionately, Grevy's zebra consumed legumes (Fabaecae) when they could not saturate their diet with grass (Figure S6.10). *Indigofera spp* were the major alternative food type consumed by Grevy's zebra across reserves and seasons ($\beta = 0.998$, s.e = 0.021, t = 47, $p < 0.001$, $R^2 = 0.94$) (Figure S6.6).

Dietary breadth (diet Shannon diversity index) follows a quadratic with increasing grass in the diet (RRA Poaceae^1: $\beta = 1.92576$, s.e = 0.36, t = 5.3, $p < 0.001$, RRA_Poaceae^2*:* $\beta = -3.21$, s.e = 0.36, t = -8.9, $p < 0.001$, $R^2 = 0.41$, n = 148, Figure 6.4). Increases in dietary breadth were driven by increases in both within-individual dietary diversity ($\beta = 1.5$, s.e = 0.07, t = 21.4, $p < 0.001$, $R^2 = 0.99$) and between individual dietary diversity ($\beta = 3$, s.e = 0.29, t = 10.1, $p < 0.001$, $R^2 = 0.96$) (Figure S6.1*).* We found no evidence of increased individual specialisation during food limitation. NDVI did not correlate with between-individual dietary variation, within-individual dietary breadth or the ratio of individual dietary breadth to total dietary diversity (Figure S6.2).

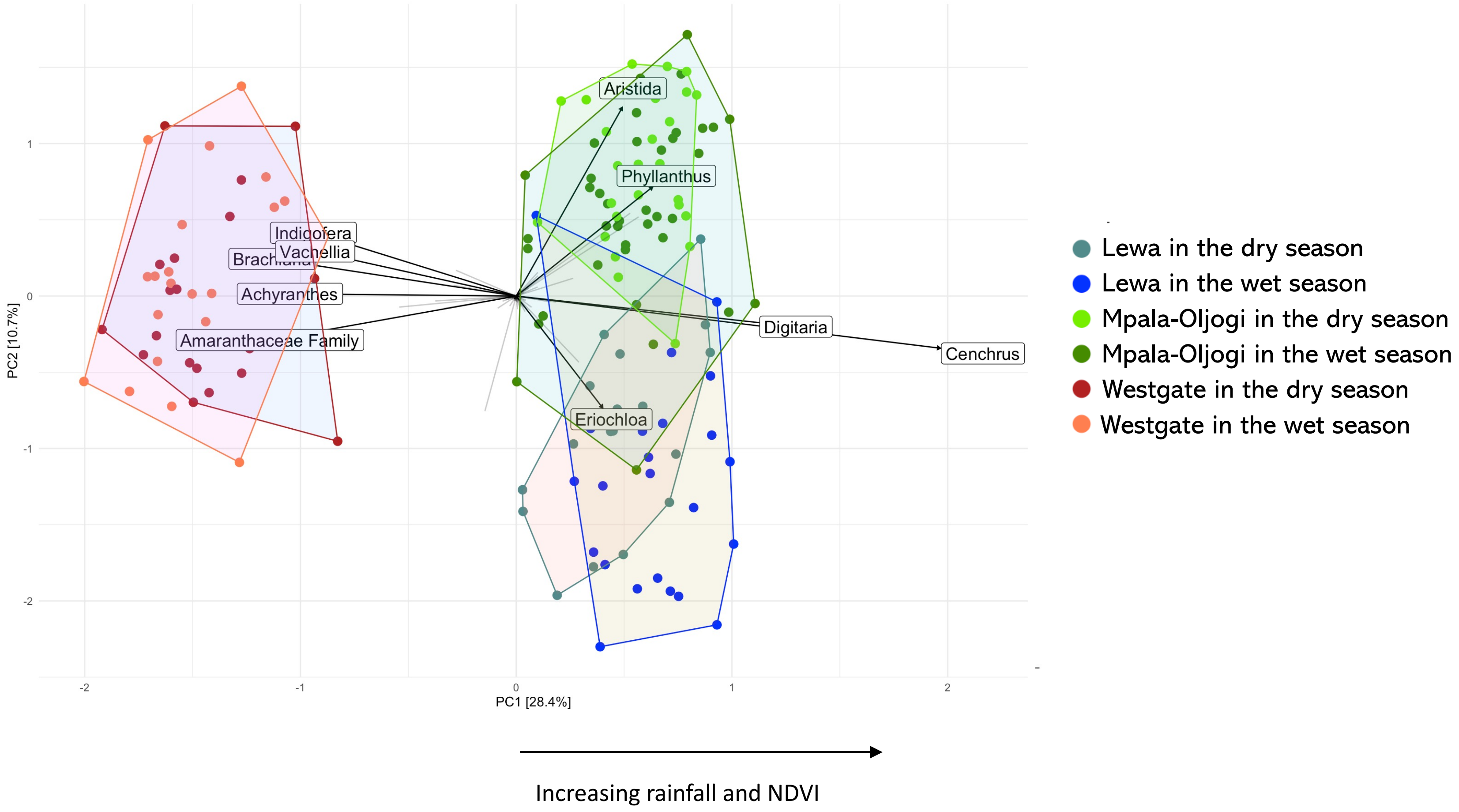


Figure 6.2) Environmental variables drive differences in Grevy's zebra dietary composition across an ecological gradient. PCA ordination of dietary beta diversity CLR-transformed agglomerated at genus level. Arrows indicate loadings of genera with the length of arrow indicating the magnitude of loading score. PC1 positively correlated with rainfall and NDVI. PC2 weakly positively correlated with NDVI. Eriochloa, Digitaria, Aristida and Cenchrus are grasses. Indigofera and Vachellia are legumes. Amaranathaceae family is depicted as ASVs could not be assigned to genus level. Some genera which loaded (>0.4 or <-0.4) are not displayed for clarity. Loading scores for each PCA axis are available in Tables S6.4

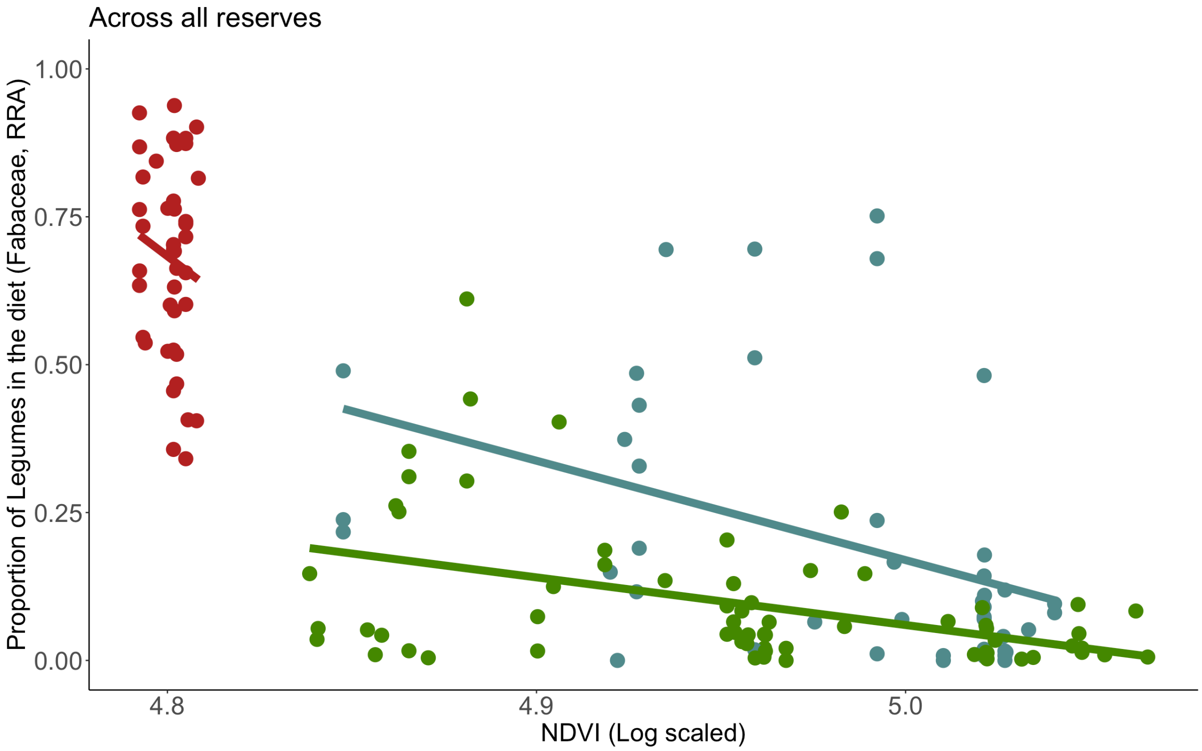


Figure 6.3) Grevy's zebra legume consumption decreases with increasing Normalized difference vegetation index (NDVI) across all reserves and across seasons. Wet and dry seasons are both represented as reserve so that variation in NDVI and legume consumption can be assessed across seasons. NDVI is a measure of habitat greenness which correlates with grass abundance and palatability in non-browse dominated areas. Red points are individuals sampled within Westgate, green points are individuals sampled within Mpala-Ol-Jogi is in green and blue points are individuals sampled within Lewa.

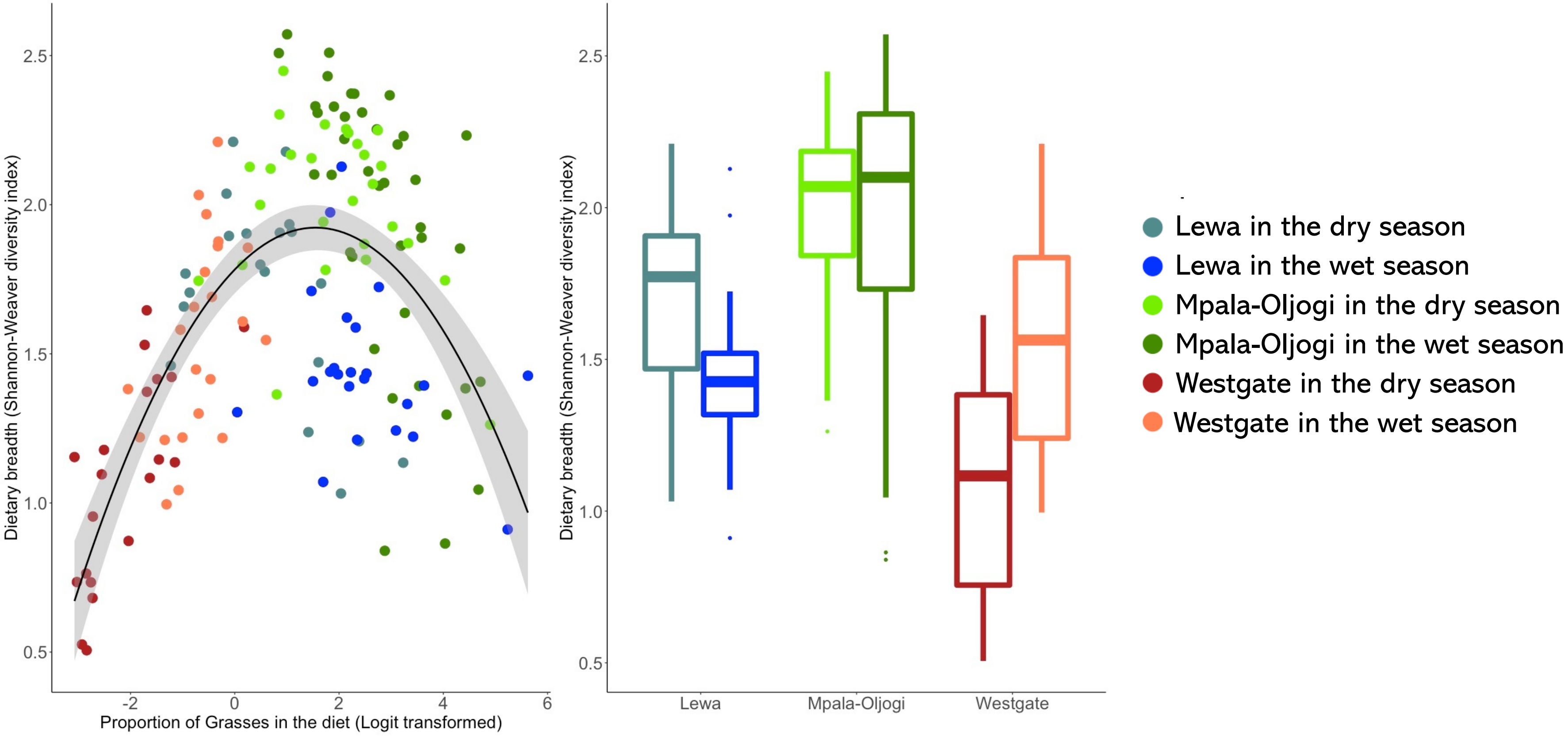


Figure 6.4) Grevy's zebra dietary breadth (Shannon Weaver diversity index) follows a negative quadratic with proportion of grasses (Poaecae) in the diet across an ecological gradient. Dietary breadth is greater in areas where zebra must supplement their diet with additional food items other than Poaceae" and is lowest in areas where animals can saturate their diet with grasses or are completely dependent on non-grass items, Dietary breadth increases as animals include additional secondary items in their diet and declines when their diet either becomes saturated with grass or completely dependent on a secondary food item (legumes).

Microbiome composition varied across geographic area but not with season (ADONIS Weighted Unifrac: Reserve; df = 155, $F_{2,155}$ = 10.0, R_2 = 0.11 p < 0.001, Season; df = 155, $F_{2,155}$ = 1.2, R_2 = 0.007, p = 0.27). Grevy's zebra microbiomes across the ecological gradient was dominated by three phyla: Firmicutes (55%), Bacteriodota (22%) and Verrucomicrobiota (18%) (Figure S6.4). Within Firmicutes, the Lachnospiraceae family, involved in primary digestion of cellulose made up 21%. Almost all Verrucomicrobiota, were of the order WCHB1-41 (17.9%). Bacteriodota contained various microbial families, the three most abundant were Rikenellaceae (6.3%), Prevotellaceae (6.1%), and p-251-o5 (5%).

Individuals with more dissimilar diets had more dissimilar microbiomes (Weighted Unifrac, Mantel statistic r = 0.18, p = 0.001, n = 149). Microbiome composition was influenced by proportion of grass (Poaceae) (ADONIS Weighted Unifrac: df = 148, R_2 = 0.06, $F_{2,155}$ = 10.1, p < 0.001). The first principal component of microbiome β diversity, which explained 12.7% of variation (Figure 6.6), was negatively correlated with proportion of grass in the diet (β= -2.41, s.e = 0.28, t = -8.6, df = 147, R^2 = 0.33, p < 0.001) and positively associated with proportion of legumes in the diet (β= 2.5, s.e = 0.30, t = 8.3, df = 147, R^2 = 0.32, p < 0.001). PC2 was not associated with any dietary items. *Ruminiclostridium* family involved in the secretion of short-chained fatty acids and stabilising the intestinal environment were associated with legume-rich diets. Campylobacteraceae including *Campylobacter spp*, *Escherichia-Shigella spp* and *Lysinibacillus spp,* all of which have the potential to cause disease in humans, were also associated with legume-rich diets. Finally, Bacteroidales BS11 gut group and Bacteroidales RF16 group which are involved in carbohydrate degradation were also associated with legume-rich diets. Increased grass in the diet was associated with increased Firmicutes to Bacteriodota ratio (β= 0.52, s.e = 0.18, t =2.94, df = 144, R^2 = 0.05, p = 0.004). Grass-rich diets were associated with Lachnospiraceae species which are known cellulolytic degraders (Figure S6.7). However, functional analysis demonstrates there was no net change in relative abundance of communities involved in fermentation or cellulolysis (Figure S6.9). Nonetheless, the proportion of grass (Poaecae) in the diet was associated with increased xylanolysis, nitrite respiration and ammonification (Figure S6.9). The proportion of legumes (Fabaecae) was related to increases in nitrate respiration and reduction, sulfate and sulfur compound respiration, increases in aromatic compound degradation and an increased likelihood of harbouring human pathogens (Figure S6.9).

Beta dispersion of microbiome was not associated with proportion of grass in the diet (Grasses^1: β = -30.08, s.e = 16.6, t = - -1.81, p = 0.07, Grasses^2*:* β = -22.3, s.e = 16.6, t = -1.34, p = 0.181, R^2= 0.02, n = 143).

Nemabiome composition varied across the ecological gradient but not with season (ADONIS Weighted Unifrac: Reserve; df = 155, $F_{2,155}$ = 21.6, R_2 = 0.22, *p < 0.001, Season;* df = 155, $F_{2,155}$ = 1.2, R_2 = 0.006, *p = 0.29*). Nemabiome composition across the ecological gradient was dominated by the Strongylidae order *(98.8%)* (Figure S6.5). Trichostrongylidae was the second most abundant order constituting 0.1%. *Strongylus* were the most abundant family (19.6%) followed by *Cylicocyclus* (11.8%), *Cylicostephanus* (8%) and *Cyathostomum* (5%).

Individuals with more dissimilar diets had more dissimilar nemabiomes (Weighted Unifrac, Mantel statistic r = 0.18, p = 0.001, n = 148). Nemabiome composition was influenced by proportion of grass (Poaceae) (ADONIS Weighted Unifrac: df = 148, R_2 = 0.11, $F_{2,155}$ = 17.4, p < 0.001). The first principal component of Nemabiome β diversity was negatively correlated with proportion of grass in the diet (β= -0.97, s.e = 0.31, *t* = -3.2, df = 145, R^2 = 0.06, *p* = 0.002). PC1 was positively correlated with proportion of legumes in the diet (β= 1.0, s.e = 0.32, *t* = 3.02, df = 145, R^2 = 0.05, *p* = 0.003). The helminths associated with grass-rich diets (loaded negatively onto PC1) were *Triodontophorus nipponicus*, *Cylicocyclus auriculatus*, *Cylicocyclus minutus*, *Cylicocyclus adersi*, *Cylicostephanus bidentatus* and *Cythanostomum pateranum (*Figure 6.7*).* The helminth species that loaded positively on PC1 (i.e., more abundant in legume-rich diets) were *Strongylus edentatus*, *Strongylus equinus*, *Strongylus vulgaris* and *Trichostrongylus axei*. The relative abundance of *Strongylus spp* (β= -0.2, s.e = 0.05, *t* = -3.95, df = 145, R^2 = 0.09, *p* < 0.001) and *Cylicostephanus spp* (β= -0.11, s.e = 0.03, *t* = -4.02, df = 145, R^2 = 0.09, *p* < 0.001) declined with increasing grass consumption.

Beta dispersion of nemabiome increased with increasing proportion of grass in the diet **(**Grasses: β = 25.04, s.e = 5.60, t = 4.5, p <0.001, Grasses^2*:* β = -5.90, s.e = 5.60, t = -1.05, p =

0.29, R^2= 0.12, *n = 144;* Figure 6.5*).* Hence, individuals with high grass diets had greater among individual variation while individuals eating legume-rich diets had more similar nemabiome composition between individuals. We also found individuals with more dissimilar microbiomes had more dissimilar nemabiomes (Weighted Unifrac, Mantel statistic r = 0.07, p = 0.02, n = 154).

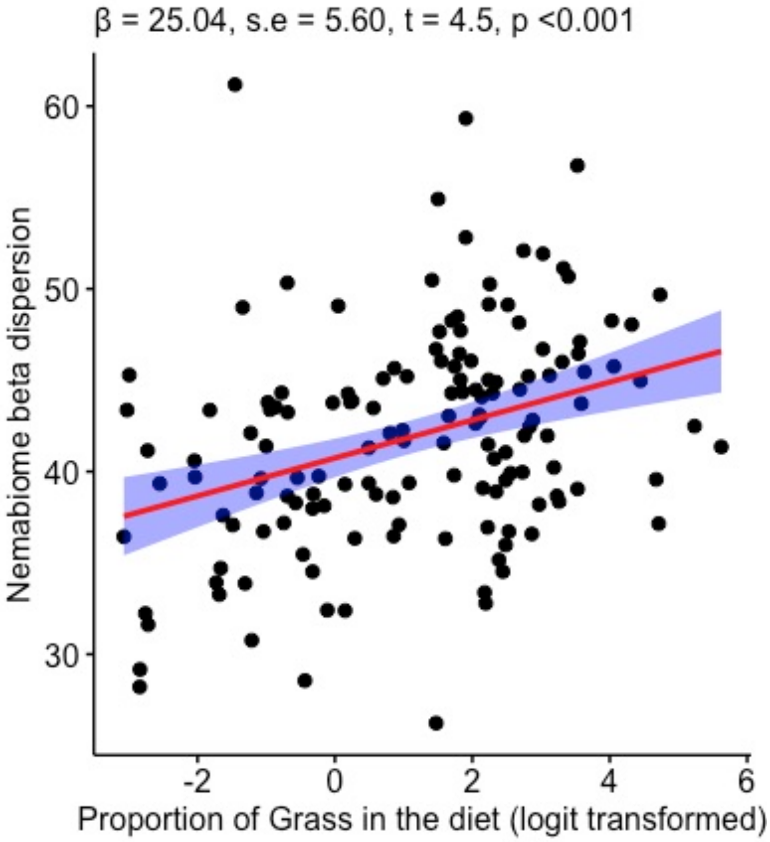


Figure 6.5) Nemabiome beta dispersion increases with increasing proportion of grass in the diet. Above are the results of linear regression. Blue represents 95% confidence interval and red line denotes regression line.

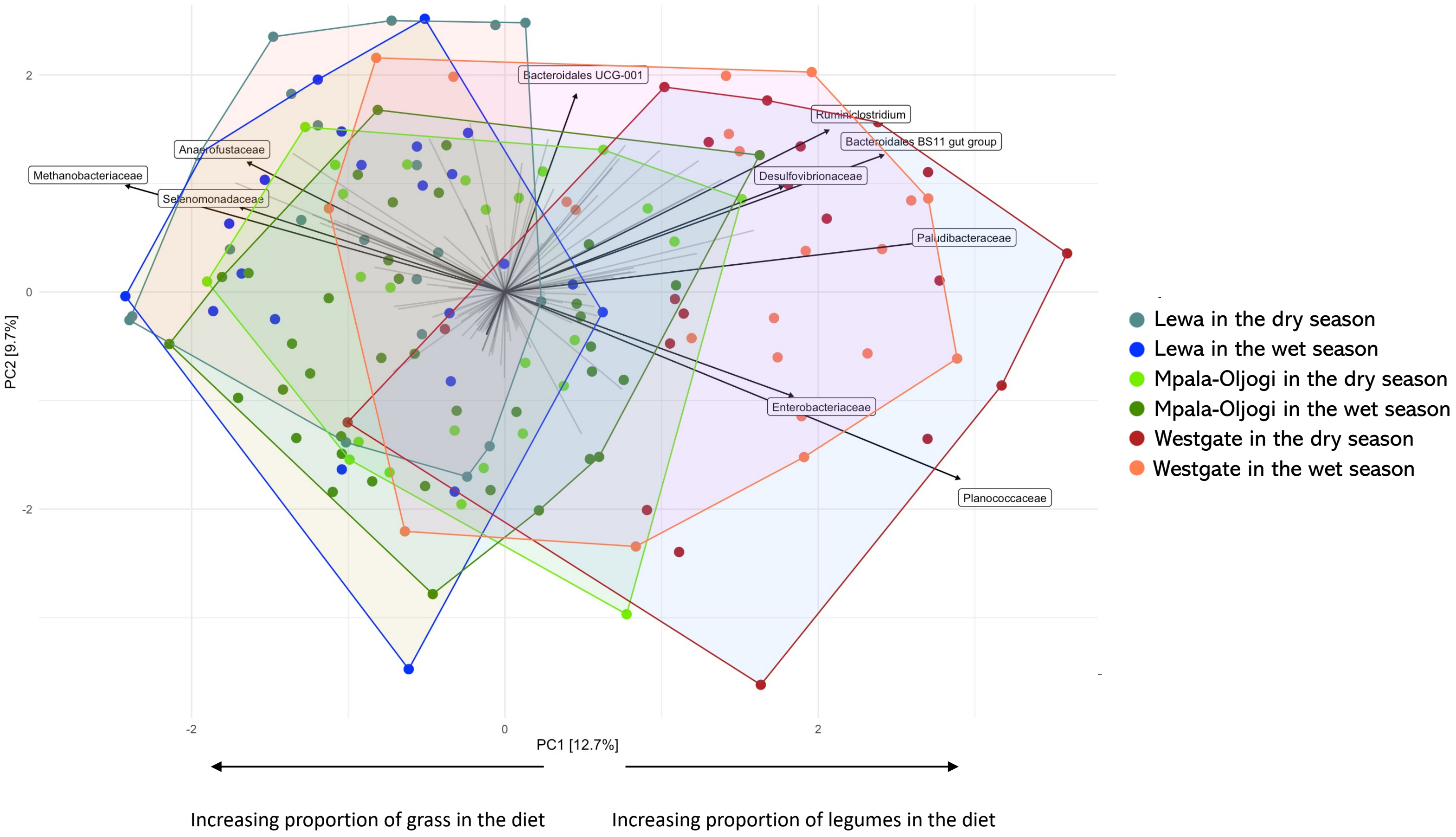


Figure 6.6) Grevy’s zebra microbiome beta diversity varies across the ecological gradient due to dietary items. PC1 negatively correlated with proportion of grass (Poaecae) in the diet and positively correlated with proportion of legumes in the diet (Fabaceae). No measured dietary variable could be linked to PC2. Arrows depict microbial groups loading onto PC axis. Arrow length indicate relative loading scores. Loading scores PCA axis are available in Tables S6.5

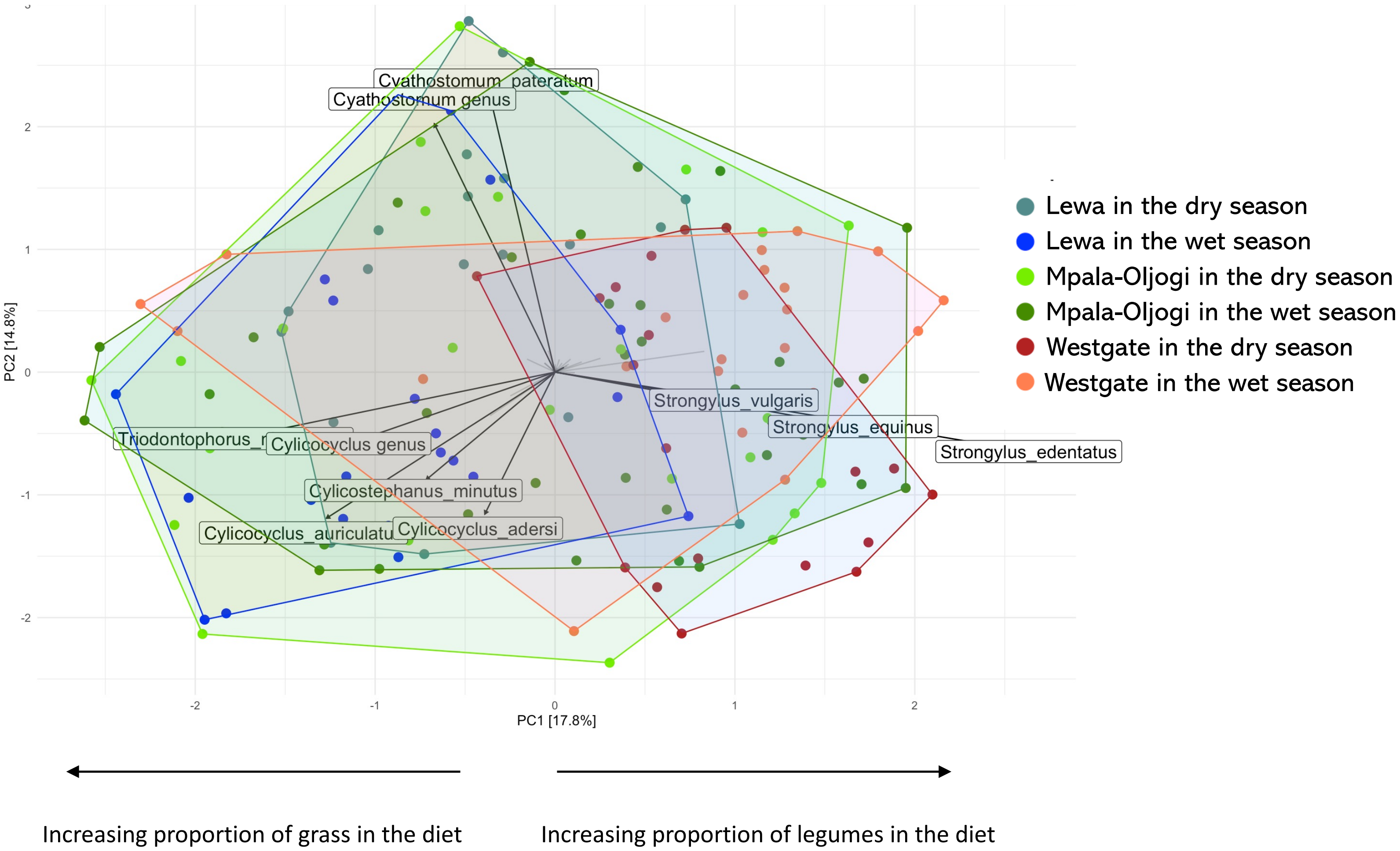


Figure 6.7) Grevy's zebra nemabiome beta diversity varies across the ecological gradient due to dietary items. PCA ordination of nemabiome beta diversity (CLR-transformed agglomerated at species level). PC1 negatively correlates with proportion of grass (Poaecae) in the diet and positively correlates with proportion of legumes in the diet (Fabaceae). No measured variable could be linked to PC2. Arrows depict helminth species loading onto PC axis. Arrow length indicates relative loading scores. Loading scores for each PCA axis are available in Tables S6.6

6.6 Discussion

Our study highlights that Grevy's zebra diet varies across an ecological (rainfall and productivity) gradient and between seasons. Our study is novel in assessing seasonal changes of diet, microbiome and nemabiome in conjunction across an ecological gradient within part of a Grevy's zebra metapopulation. Variation in rainfall across the landscape explained a greater proportion of dietary composition than season. Grevy's zebra in Westgate displayed little seasonal variability in diet and were dependent on legumes (Fabaecae). Grevy's zebra in Mpala-Oljogi and Lewa maintained a high proportion of grass in the diet across seasons but did demonstrate some dietary switching to legumes in the dry season. Seasonal dietary changes in East African megafauna, including Grevy's zebra, have been studied previously but are usually restricted to a single population (Kartzinel et al., 2015; 2019; Kartzinel & Pringle, 2020; Pansu et al., 2019).

Across the ecological gradient we found the extent of seasonal dietary switching varies with rainfall and productivity. During the dry season, Grevy's zebra included legumes in their diet especially *Indigofera spp*. However, individuals in Westgate never transition to a majority grass diet. Diet switching often occurs seasonally between high and low productivity periods (i.*e*. summer/winter or wet/dry) in mixed feeders *e.g.* elephants (*Loxodonta africanus*) (Vogel et al., 2020) and moose (*Alces alces*) (Jesmer et al., 2020). Heavy reliance on alternative foods occurs in other species such as Bison (*Bison bison*) (Hecker et al., 2021), cattle (*Bos taurus africanus*) (Radloff et al., 2013) and Przewalski's horse (*Equus ferus przewalskii*) and may reflect populations being confined to ecologically marginal conditions (Kaczensky et al., 2017). High reliance on a single alternative food source could increase intraspecific and interspecific competition for resources (Macarthur & Levins, 1967; Pringle et al., 2019). Livestock which feed on *Indigofera spp* such as goats (Lengarite et al., 2013) may exacerbate this interspecific competition (Low et al., 2009). Due to poor rainfall, the habitat quality of Westgate (using proportion of grass in the diet as a proxy) in our study period remained poor across seasons. Overall, our results strongly suggest Westgate may be a marginal population where individuals experience prolonged periods of food limitation, while Lewa and Mpala-OlJogi may represent core habitats in the Grevy's zebra range.

Optimal foraging theory predicts that animals should have the lowest dietary breadth when the availability of preferred food items is high (MacArthur & Pianka, 1966). We would predict dietary breadth to decrease in the rainy season as quantity and quality of herbaceous and deciduous plants improves (Adole et al., 2018). Lewa followed this prediction with a decrease in total niche width during the rainy season. However, contrary to predictions, individuals in Westgate increased dietary breadth by incorporating grasses into their diet during the rainy season compared to near complete reliance on legumes during the dry season. Mpala-OlJogi animals had a high total niche width across both seasons. Across populations and seasons, Grevy's zebra dietary breadth showed a negative quadratic relationship with the abundance of grasses (Poaeceae). This quadratic relationship reflects individuals in Westgate being highly dependent on *Indigofera spp,* and increasing their dietary breadth by supplementing their diet with grasses when they become available. It also reflects the common predictions of optimal foraging theory, that animals increase dietary breadth by including alternative items in their diet when primary food sources become scarce.

Population level dietary breadth increased due to increases in within-individual diet diversity *i.e.* individuals ate a greater variety of items, and between-individual diet diversity *i.e.* individuals ate different dietary items from each other. Increases in within-individual diet diversity likely occurred as individuals supplemented their diet with *Indigofera spp* during scarcity (Jesmer et al., 2020). Increases in population level between-individual diet diversity likely occur when animals transitioned from *Indigofera spp* dominated diets to eating diverse Poaceae genera.

Diet influenced Grevy's microbiome composition across ecological gradients at a similar degree to that found in other East African herbivore species (Kartzinel et al., 2019). Increased Poaecae in the diet was associated with increased Firmicutes and decreased Bacteroidetes. Firmicutes abundance is associated with body condition and weight gain in domestic horses (*Equus ferus caballeus*) (Biddle et al., 2018; Langner et al., 2020; Morrison et al., 2018). Diet has also been shown to influence microbiome composition of small mammals, (Ingala et al., 2019; H. Li et al., 2016; Weinstein et al., 2021), primates (Baniel et al., 2021; Hicks et al., 2018), large herbivorous mammals (Kartzinel et al., 2019) and fish

(Bolnick et al., 2014). We also found functional changes in microbiome associated with diet. We found legume-rich diets were associated with *Ruminiclostridium* and Campylobacteraceae including *Campylobacter spp, Escherichia-Shigella spp, Lysinibacillus spp*. *Ruminiclostridium* are crucial in maintaining the stability of the intestinal environment. Members of this family can secrete short-chain fatty acids, which are conducive to maintaining the functionality and morphology of intestinal epithelial cells (Tan et al., 2014). Some members of Campylobacteraceae include *Campylobacter spp, Escherichia-Shigella spp, Lysinibacillus spp* are potential pathogens to humans and animals (Debruyne et al., 2008). Grass poor diets may facilitate greater colonisation by pathogenic bacterial species. Individuals with diets dominated by *Indigofera spp* had microbial communities associated with increased breakdown of aromatic compounds. This functional shift may reflect the increased need to break down ingested toxic compounds (Kohl et al., 2014) in *Indigofera spp* that can cause hepatotoxicity and embryo-lethal effects (Fletcher et al., 2015). However, although *Indigofera spp* contains toxic secondary plant metabolites, these only have lethal effect in extreme high doses. *Indigofera spp* is one of the most heavily eaten plant genera by all large herbivore species in Laikipia (Kartinzel *et al*, 2015) and is known by pastoralists to be a useful plant for fattening cattle (Robert Pringle, personal communciation). Diets comprising very large quantities of Indigofera may be suboptimal for Grevy's zebra over a long time period but may also be an adequate subsititue or supplement in the short term when grass abundance is low. Furthermore, individuals with diets dominated with Poaecae had microbial communities associated with the breakdown of xylan, which is a major hemicellulose found in plant cell walls (25-35% of grass secondary cell wall dry matter) (Gao et al., 2020). Increases in xylan breakdown likely reflect improved digestive efficiency for Poaecae.

Multiple strongyles were found in Grevy's zebra faeces including *Strongylinae* (including genera: *Strongylus, Triodontophorus,* and *Craterostomum*) and *Cyathostominae*. In contrast to recent findings (Tombak *et al*, 2021), Strongylus species *(Strongylus vulgaris*, S. e*dentatus*, and *S. equinus)* were identified to species level and highly abundant within the gastrointestinal system of Grevy's zebra. Individuals with a high proportion of Fabaceae in their diet had greater relative abundance of *Strongylus spp, Cylicostephanus spp and Coronocyclus spp*. *Strongylus spp* are highly pathogenic in equids and are a potential threat to host health (Duncan & Pirie, 1975; McCraw & Slocombe, 1985). *Cylicostephanus spp and Coronocyclus spp* are small Strongylids associated with weight loss and reduced body condition in equids (Love et al., 1999). We also found grass-rich were associated with greater between individual diversity of helminths. Therefore, individuals in marginal areas consuming a high proportion of legumes have more similar compositions of pathogenic helminths. Poor quality diets are associated with decreased immunocompetence to parasites (Fair & Whitaker, 2008; Navarro-Gonzalez et al., 2011; Budischak et al., 2015). Individuals in marginal habitat may become immunocompromised due to poor diet quality and experience negative physiological effects of helminth infection (Beldomenico & Begon, 2010; Navarro-Gonzalez et al., 2011). An alternative explanation is that the aridity of Westgate may result in fewer water bodies and a concentration of zebra dung around them. Dung density would increase exposure risk to the faecal-oral transmitted strongylid species (Tombak et al., 2022), increasing transmission rates between individuals and decreasing between-individual diversity.

Species responses to ecological gradients determine their realised niche, range dynamics and limits (Pearman et al., 2008; Sexton et al., 2009). Resource availability likely limits large herbivore population sizes and distributions (Fryxell, 1987; Sinclair et al., 1985). Areas with greater food resources facilitate increased density, survival and reproduction in large herbivores (Lea et al., 2016; McNaughton, 1988; Mduma et al., 1999). Although we do not have the spatial resolution in demographic data to evaluate performance in our study, Grevy’s zebra populations vary in their recruitment (Rubenstein et al., 2018). Arid regions in Samburu are associated with a reduced proportion of juveniles and infants (Rubenstein et al., 2018). Grevy’s zebra track permanent water sources and NDVI throughout Laikipia (Crego et al., 2021). Grass availability determines habitat selection (Sundaresan et al., 2008) and likely determines seasonal dispersal dynamics across the metapopulation. Grass-rich reserves in Lewa and Mpala-OlJogi are likely “core” habitats where individual physiological status is bolstered from grass-rich diets through time. These conditions may contribute to higher recruitment or carrying capacities in these areas. Furthermore, these areas may provide immigrants to more marginal areas like Westgate. Currently, the Laikipia-Samburu landscape is under pressure of fragmentation from fencing (O’Neill et al., 2022). Conservancies in the south and the west are being disconnected from those in the central and northeastern regions (Crego et al., 2021). If dispersal to and from Westgate became limited, the population may become vulnerable due to the *intrinsic* ecological marginality from its position in the rainfall gradient.

Importantly, our analyses have not been able to account for intraspecific effects such as the composition of sympatric wildlife and presence of livestock. Compositional differences in sympatric wildlife or variation in livestock composition and density could influence diet, microbiome and nemabiome. Phylogenetically similar sympatric wildlife and livestock likely compete with Grevy's zebra for similar resources which may influence resource availability and dietary composition. Futhermore, Grevy's zebra may encounter faeces from phylogenetically similar sympatric wildlife and livestock which could transmit bacteria or nematodes leading to composition alterations. We therefore cannot discount the potential influence of biotic interactions on the trends presented.

It should not be assumed that the diet a species is observed to be eating in a particular area is preferred or optimal (Kerley et al., 2012; Moolman et al., 2019). Research in limited parts of a species' range, or metapopulation, may lead to misleading and biased views of species ecologies (Britnell et al., 2021). For example, suppose studies only occur in marginal habitats, where preferred foods are rarely or never available, in these cases, alternative foods may be assumed to be preferred food items. These interpretations are especially concerning if species have experienced widespread range contraction, and species ecology can only be studied in part of its historic range (Britnell et al., 2021). Misconceptions about the species preferred food sources can lead to inefficient conservation efforts which prioritise less resilient populations.

6.7 Conclusion

Metapopulations occur along ecological gradients from high quality habitats to marginal ones. Marginal habitats are associated with low population density, poor reproduction and survivorship and poor long-term resilience. We have shown that Grevy's zebra demonstrate dietary switching to legumes during food limitation. Legume rich-diets are associated with poor rainfall. These dietary changes have knock-on physiological effects on microbiome structure and function, and nemabiome composition. Marginal populations may become dependent on secondary food items, even within relatively unfragmented landscapes, and suffer negative physiological consequences through more virulent parasite assemblages. In addition, diet influences microbiome function, with dietary switching to toxic food items associated with increased breakdown of aromatic compounds. Furthermore, legume rich diets were associated with increase prevalence of human pathogens which may constitute a disease risk. Our results reveal a potential driver of population dynamics across three major Grevy's zebra populations in Laikipia, Samburu and Meru Counties, Kenya.

6.8 References

Adole, T., Dash, J., & Atkinson, P. M. (2018). Large-scale prerain vegetation green-up across Africa. *Global Change Biology*, *24*(9), 4054–4068.

Antwis, R. (2021). A boom-or-bust approach—The 'Glass Cannon' hypothesis in host microbiomes. *Journal of Animal Ecology*, *90*(5), 1024–1026.

Antwis, R. E., Edwards, K. L., Unwin, B., Walker, S. L., & Shultz, S. (2019). Rare gut microbiota associated with breeding success, hormone metabolites and ovarian cycle phase in the critically endangered eastern black rhino. *Microbiome*, *7*(1), 1–12.

Avramenko, R. W., Redman, E. M., Lewis, R., Yazwinski, T. A., Wasmuth, J. D., & Gilleard, J. S. (2015). Exploring the gastrointestinal "nemabiome": Deep amplicon sequencing to quantify the species composition of parasitic nematode communities. *PLoS ONE*, *10*(12), e0143559.

Baniel, A., Amato, K. R., Beehner, J. C., Bergman, T. J., Mercer, A., Perlman, R. F., Petrullo, L., Reitsema, L., Sams, S., Lu, A., & Snyder-Mackler, N. (2021). Seasonal shifts in the gut microbiome indicate plastic responses to diet in wild geladas. *Microbiome*, *9*(1), 1–20.

Barboza, P. S., Bennett, A., Lignot, J. H., Mackie, R. I., McWhorter, T. J., Secor, S. M., Skovgaard, N., Sundset, M. A., & Wang, T. (2010). Digestive challenges for vertebrate animals: Microbial diversity, cardiorespiratory coupling, and dietary specialization. *Physiological and Biochemical Zoology*, *83*(5), 764–774.

Barnett, D., Arts, I., & Penders, J. (2021). microViz: an R package for microbiome data visualization and statistics. *Journal of Open Source Software*, *6*(63), 3201.

Barranco, C. (2021). Microbiome-targeted diets that alter immune status. *Nature Reviews Gastroenterology & Hepatology 2021 18:9*, *18*(9), 594–594.

Bauer, I. E., McMorrow, J., & Yalden, D. W. (1994). The Historic Ranges of Three Equid Species in North-East Africa: A Quantitative Comparison of Environmental Tolerances. *Journal of Biogeography*, *21*(2), 169.

Beldomenico, P. M., & Begon, M. (2010). Disease spread, susceptibility and infection intensity: vicious circles? *Trends in Ecology and Evolution*, *25*(1), 21–27.

Biddle, A. S., Tomb, J. F., & Fan, Z. (2018). Microbiome and blood analyte differences point to community and metabolic signatures in lean and obese horses. *Frontiers in Veterinary Science*, *5*, 225.

Bison, M., Ibanez, S., Redjadj, C., Boyer, F., Coissac, E., Miquel, C., Rioux, D., Said, S., Maillard, D., Taberlet, P., Yoccoz, N. G., & Loison, A. (2015). Upscaling the niche variation hypothesis from the intra- to the inter-specific level. *Oecologia*, *179*(3), 835–842.

Bocherens, H., Hofman-Kamińska, E., Drucker, D. G., Schmölcke, U., & Kowalczyk, R. (2015). European Bison as a Refugee Species? Evidence from Isotopic Data on Early Holocene Bison and Other Large Herbivores in Northern Europe. *PLOS ONE*, *10*(2), e0115090.

Bolnick, D. I., Snowberg, L. K., Hirsch, P. E., Lauber, C. L., Knight, R., Caporaso, J. G., & Svanbäck, R. (2014). Individuals' diet diversity influences gut microbial diversity in two freshwater fish (threespine stickleback and Eurasian perch). *Ecology Letters*, *17*(8), 979–987.

Bolnick, D. I., Svanbäck, R., Araújo, M. S., & Persson, L. (2007). Comparative support for the niche variation hypothesis that more generalized populations also are more heterogeneous. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(24), 10075–10079.

Bolnick, D. I., Yang, L. H., Fordyce, J. A., Davis, J. M., & Svanback, R. (2002). Measuring Individual-Level Resource Specialization. *Ecology*, *83*(10), 2936.

Britnell, J. A., Lewis, R. N., Elsner-Gearing, F., Harvey, N., Stanbrook, E., & Shultz, S. (2021). Species stereotypes as a result of unconscious research biases compromise conservation efficacy. *Biological Conservation*, *261*, 109275.

Budischak, S. A., Sakamoto, K., Megow, L. C., Cummings, K. R., Urban, J. F., & Ezenwa, V. O. (2015). Resource limitation alters the consequences of co-infection for both hosts and parasites. *International Journal for Parasitology*, *45*(7), 455–463.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583.

Cattadori, I. M., Haydon, D. T., & Hudson, P. J. (2005). Parasites and climate synchronize red grouse populations. *Nature, 433*(7027), 737–741.

Couch, C. E., Stagaman, K., Spaan, R. S., Combrink, H. J., Sharpton, T. J., Beechler, B. R., & Jolles, A. E. (2021). Diet and gut microbiome enterotype are associated at the population level in African buffalo. *Nature Communications 2021 12:1*, *12*(1), 1–11.

Crego, R. D., Wells, H. B. M., Ndung'u, K. S., Evans, L., Njeri Nduguta, R., Chege, M. A., Brown, M. B., Ogutu, J. O., Ojwang, G. O., Fennessy, J., O'Connor, D., Stacy-Dawes, J., Rubenstein, D. I., Martins, D. J., Leimgruber, P., & Stabach, J. A. (2021). Moving through the mosaic: identifying critical linkage zones for large herbivores across a multiple-use African landscape. *Landscape Ecology*, *36*(5), 1325–1340.

Debruyne, L., Gevers, D., & Vandamme, P. (2008). Taxonomy of the family Campylobacteraceae. *Campylobacter*, 1-25.

Demment, M. W., & van Soest, P. J. (1985). A nutritional explanation for body-size patterns of ruminant and nonruminant herbivores. *American Naturalist*, *125*(5), 641–672.

Dixon, P. (2003). VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science*, *14*(6), 927–930.

Duncan, J. L., & Pirie, H. M. (1975). The pathogenesis of single experimental infections with *Strongylus vulgaris* in foals. *Research in Veterinary Science*, *18*(1), 82–93.

Duvallet, C., Gibbons, S. M., Gurry, T., Irizarry, R. A., & Alm, E. J. (2017). Meta-analysis of gut microbiome studies identifies disease-specific and shared responses. *Nature Communications*, *8*(1).

Ezenwa, V. O. (2004). Interactions among host diet, nutritional status and gastrointestinal parasite infection in wild bovids. *International Journal for Parasitology*, *34*(4), 535–542.

Fletcher, M. T., Jassim, R. A. M. al, & Cawdell-Smith, A. J. (2015). The Occurrence and Toxicity of Indospicine to Grazing Animals. *Agriculture 5*(3), 427–440.

Franz, T. E., Caylor, K. K., Nordbotten, J. M., Rodríguez-Iturbe, I., & Celia, M. A. (2010). An ecohydrological approach to predicting regional woody species distribution patterns in dryland ecosystems. *Advances in Water Resources*, *33*(2), 215–230.

Freeland, W. J., & Janzen, D. H. (1974). Strategies in Herbivory by Mammals: The Role of Plant Secondary Compounds. *The American Naturalist*, *108*(961), 269–289.

Fryxell, J. M. (1987). Food limitation and demography of a migratory antelope, the white-eared kob. *Oecologia*, *72*(1), 83–91.

Gao, Y., Lipton, A. S., Wittmer, Y., Murray, D. T., & Mortimer, J. C. (2020). A grass-specific cellulose–xylan interaction dominates in sorghum secondary cell walls. *Nature Communications 11*(1), 1–10.

Gill, B. A., Musili, P. M., Kurukura, S., Hassan, A. A., Goheen, J. R., Kress, W. J., Kuzmina, M., Pringle, R. M., & Kartzinel, T. R. (2019). Plant DNA-barcode library and community phylogeny for a semi-arid East African savanna. *Molecular Ecology Resources*, *19*(4), 838–846.

Ginsberg JR. 1987. Social organization and mating strategies of an arid-adapted equid:the Grevy’s zebra. Ph.D. thesis, Princeton University, Princeton, New Jersey

Glendinning, L., Nausch, N., Free, A., Taylor, D. W., & Mutapi, F. (2014). The microbiota and helminths: sharing the same niche in the human host. *Parasitology*, *141*(10), 1255–1271.

Gloor, G. B., Macklaim, J. M., Pawlowsky-Glahn, V., & Egozcue, J. J. (2017). Microbiome datasets are compositional: And this is not optional. *Frontiers in Microbiology*, *8*, 2224.

Gould, A.L., Zhang, V., Lamberti, L., Jones, E.W., Obadia, B., Korasidis, N., Gavryushkin, A., Carlson, J.M., Beerenwinkel, N. and Ludington, W.B., (2018). Microbiome interactions shape host fitness. *Proceedings of the National Academy of Sciences*, *115*(51),E11951-E11960.

Grueter, C. C., Li, D., Ren, B., Wei, F., Xiang, Z., & van Schaik, C. P. (2009). Fallback foods of temperate-living primates: A case study on snub-nosed monkeys. *American Journal of Physical Anthropology*, *140*(4), 700–715.

Hanning, I., & Diaz-Sanchez, S. (2015). The functionality of the gastrointestinal microbiome in non-human animals. *Microbiome* 3(1), 51.

Hanski, I. (2012). Eco-evolutionary dynamics in a changing world. *Annals of the New York Academy of Sciences*, *1249*(1), 1-17.

Hecker, L. J., Edwards, M. A., & Nielsen, S. E. (2021). Assessing the nutritional consequences of switching foraging behavior in wood bison. *Ecology and Evolution*, *11*(22), 16165–16176.

Hicks, A. L., Lee, K. J., Couto-Rodriguez, M., Patel, J., Sinha, R., Guo, C., Olson, S. H., Seimon, A., Seimon, T. A., Ondzie, A. U., Karesh, W. B., Reed, P., Cameron, K. N., Lipkin, W. I., & Williams, B. L. (2018). Gut microbiomes of wild great apes fluctuate seasonally in response to diet. *Nature Communications*, *9*(1).

Holt, R. D. (2009). Bringing the Hutchinsonian niche into the 21st century: Ecological and evolutionary perspectives. *Proceedings of the National Academy of Sciences*, *106*(Supplement_2), 19659–19665.

Ingala, M. R., Becker, D. J., Bak Holm, J., Kristiansen, K., & Simmons, N. B. (2019). Habitat fragmentation is associated with dietary shifts and microbiota variability in common vampire bats. *Ecology and Evolution*, *9*(11), ece3.5228.

Jesmer, B. R., Kauffman, M. J., Murphy, M. A., & Goheen, J. R. (2020). A test of the Niche Variation Hypothesis in a ruminant herbivore. *Journal of Animal Ecology*, *89*(12), 2825–2839.

Kaczensky, P., Burnik Šturm, M., Sablin, M. v., Voigt, C. C., Smith, S., Ganbaatar, O., Balint, B., Walzer, C., & Spasskaya, N. N. (2017). Stable isotopes reveal diet shift from pre-extinction to reintroduced Przewalski's horses. *Scientific Reports*, *7*(1), 1–9.

Karasov, W. H., Martinez del Rio, C., & Caviedes-Vidal, E. (2011). Ecological physiology of diet and digestive systems. *Annual review of physiology*, *73*, 69-93.

Kartzinel, T. R., Chen, P. A., Coverdale, T. C., Erickson, D. L., Kress, W. J., Kuzmina, M. L., Rubenstein, D. I., Wang, W., & Pringle, R. M. (2015). DNA metabarcoding illuminates dietary niche partitioning by African large herbivores. *Proceedings of the National Academy of Sciences*, *112*(26), 8019–8024.

Kartzinel, T. R., Hsing, J. C., Musili, P. M., Brown, B. R. P., & Pringle, R. M. (2019). Covariation of diet and gut microbiome in African megafauna. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(47), 23588–23593.

Kartzinel, T. R., & Pringle, R. M. (2020). Multiple dimensions of dietary diversity in large mammalian herbivores. *Journal of Animal Ecology*, *89*(6), 1482–1496.

Kauffman, M. J., Aikens, E. O., Esmaeili, S., Kaczensky, P., Middleton, A., Monteith, K. L., Morrison, T. A., Mueller, T., Sawyer, H., & Goheen, J. R. (2021). Causes, Consequences, and Conservation of Ungulate Migration. I*Annual Review of Ecology, Evolution, and Systematics 52*, 453–478).

Kawecki, T. J. (2008). Adaptation to Marginal Habitats. *Annual Review of Ecology, Evolution, and Systematics*, *39*(1), 321–342. https://doi.org/10.1146/annurev.ecolsys.38.091206.095622

Kerley, G. I. H., Beest, M., Cromsigt, J. P. G. M., Pauly, D., & Shultz, S. (2020). The Protected Area Paradox and refugee species: The giant panda and baselines shifted towards conserving species in marginal habitats. *Conservation Science and Practice*, *2*(5).

Kerley, G. I. H., Kowalczyk, R., & Cromsigt, J. P. G. M. (2012). Conservation implications of the refugee species concept and the European bison: King of the forest or refugee in a marginal habitat? *Ecography*, *35*(6), 519–529.

Kerley, G. I. H., Landman, M., & de Beer, S. (2010). How do small browsers respond to resource changes? Dietary response of the Cape grysbok to clearing alien Acacias. *Functional Ecology*, *24*(3), 670–675.

Kitaysky, A. S., Kitaiskaia, E. v, Piatt, J. F., & Wingfield, J. C. (2006). A mechanistic link between chick diet and decline in seabirds? *Proceedings of the Royal Society B: Biological Sciences*, *273*(1585), 445–450.

Kitaysky, A. S., Piatt, J. F., Hatch, S. A., Kitaiskaia, E. v., Benowitz-Fredericks, Z. M., Shultz, M. T., & Wingfield, J. C. (2010). Food availability and population processes: severity of nutritional stress during reproduction predicts survival of long-lived seabirds. *Functional Ecology*, *24*(3), 625–637.

Klingel, H. (1974). Soziale Organisation und Verhalten des Grevy-Zebras (*Equus grevyi*). *Zeitschrift Für Tierpsychologie*, *36*(1–5), 37–70.

Kohl, K. D., Weiss, R. B., Cox, J., Dale, C., & Dearing, M. D. (2014). Gut microbes of mammalian herbivores facilitate intake of plant toxins. *Ecology Letters*, *17*(10), 1238–1246.

Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., & Schloss, P. D. (2013). Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the miseq illumina sequencing platform. *Applied and Environmental Microbiology*, *79*(17), 5112–5120.

Langner, K., Blaue, D., Schedlbauer, C., Starzonek, J., Julliand, V., & Vervuert, I. (2020). Changes in the faecal microbiota of horses and ponies during a two-year body weight gain programme. *PLOS ONE*, *15*(3), e0230015.

Lea, J. M., Kerley, G. I., Hrabar, H., Barry, T. J., & Shultz, S. (2016). Recognition and management of ecological refugees: A case study of the Cape mountain zebra. *Biological Conservation*, *203*, 207–215.

Lee, S. C., Tang, M. S., Lim, Y. A. L., Choy, S. H., Kurtz, Z. D., Cox, L. M., Gundra, U. M., Cho, I., Bonneau, R., Blaser, M. J., Chua, K. H., & Loke, P. (2014). Helminth Colonization Is Associated with Increased Diversity of the Gut Microbiota. *PLOS Neglected Tropical Diseases*, *8*(5), e2880.

Lengarite, M. I., Mbugua, P. N., Gachuiri, C. K., & Kabuage, L. W. (2013). Mineral intake of sheep and goats grazing in the arid rangelands of northern Kenya. *Livestock Research for Rural Development*, *25*(10).

Levins, R. (1969). Some Demographic and Genetic Consequences of Environmental Heterogeneity for Biological Control. *Bulletin of the Entomological Society of America*, *15*(3), 237–240.

Li, H., Li, T., Beasley, D. A. E., Heděnec, P., Xiao, Z., Zhang, S., Li, J., Lin, Q., & Li, X. (2016). Diet diversity is associated with beta but not alpha diversity of pika gut microbiota. *Frontiers in Microbiology*, *7*, 1169.

Li, R. W., Wu, S., Li, W., Huang, Y., & Gasbarre, L. C. (2011). Metagenome Plasticity of the Bovine Abomasal Microbiota in Immune Animals in Response to *Ostertagia Ostertagi* Infection. *PLOS ONE*, *6*(9), e24417.

Liu, C., Cui, Y., Li, X. and Yao, M., 2021. microeco: an R package for data mining in microbial community ecology. *FEMS microbiology ecology*, *97*(2), p.fiaa255.

Louca, S., Parfrey, L. W., & Doebeli, M. (2016). Decoupling function and taxonomy in the global ocean microbiome. *Science. 353*(6305), 1272–1277.

Love, S., Murphy, D., & Mellor, D. (1999). Pathogenicity of cyathostome infection. *Veterinary Parasitology*, *85*(2–3), 113–122.

Low, B., Sundaresan, S. R., Fischhoff, I. R., & Rubenstein, D. I. (2009). Partnering with local communities to identify conservation priorities for endangered Grevy’s zebra. *Biological Conservation*, *142*(7), 1548–1555.

Lozupone, C., & Knight, R. (2005). UniFrac: a New Phylogenetic Method for Comparing Microbial Communities. *Applied and Environmental Microbiology*, *71*(12), 8228.

MacArthur, R. H., & Pianka, E. R. (1966). On Optimal Use of a Patchy Environment. *The American Naturalist*, *100*(916), 603–609.

Macarthur, R., & Levins, R. (1967). The Limiting Similarity, Convergence, and Divergence of Coexisting Species. *The American Naturalist*, *101*(921), 377–385.

Marshall, A. J., Boyko, C. M., Feilen, K. L., Boyko, R. H., & Leighton, M. (2009). Defining fallback foods and assessing their importance in primate ecology and evolution. *American Journal of Physical Anthropology*, *140*(4), 603–614.

Marston, C. G., Wilkinson, D. M., Reynolds, S. C., Louys, J., & O'Regan, H. J. (2019). Water availability is a principal driver of large-scale land cover spatial heterogeneity in sub-Saharan savannahs. *Landscape Ecology*, *34*(1), 131–145.

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal*, *17*(1), 10–12.

McCraw, B. M., & Slocombe, J. O. D. (1985). *Strongylus equinus:* development and pathological effects in the equine host. *Canadian Journal of Comparative Medicine*, *49*(4), 372.

McMurdie, P. J., & Holmes, S. (2013). phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLOS ONE*, *8*(4), e61217.

McNaughton, S. J. (1988). Mineral nutrition and spatial concentrations of African ungulates. *Nature*, *334*(6180), 343–345.

Mduma, S. A. R., Sinclair, A. R. E., & Hilborn, R. (1999). Food regulates the Serengeti wildebeest: A 40-year record. *Journal of Animal Ecology*, *68*(6), 1101–1122.

Meshesha, D. T., Ahmed, M. M., Abdi, D. Y., & Haregeweyn, N. (2020). Prediction of grass biomass from satellite imagery in Somali regional state, eastern Ethiopia. *Heliyon*, *6*(10), e05272.

Moolman, L., Ferreira, S. M., Gaylard, A., Zimmerman, D., & Kerley, G. I. H. (2019). The decline of the Knysna elephants: Pattern and hypotheses. *South African Journal of Science*, *115*(3/4), 1–7.

Morrison, P. K., Newbold, C. J., Jones, E., Worgan, H. J., Grove-White, D. H., Dugdale, A. H., Barfoot, C., Harris, P. A., & Argo, C. M. G. (2018). The equine gastrointestinal microbiome: Impacts of age and obesity. *Frontiers in Microbiology*, *9*, 3017.

Mumo, L., Yu, J., & Ayugi, B. (2019). Evaluation of spatiotemporal variability of rainfall over Kenya from 1979 to 2017. *Journal of Atmospheric and Solar-Terrestrial Physics*, *194*, 105097.

Navarro-Gonzalez, N., Verheyden, H., Hoste, H., Cargnelutti, B., Lourtet, B., Merlet, J., Daufresne, T., Lavín, S., Hewison, A. J. M., Morand, S., & Serrano, E. (2011). Diet quality and immunocompetence influence parasite load of roe deer in a fragmented landscape. *European Journal of Wildlife Research*, *57*(3), 639–645.

Olff, H., Ritchie, M. E., & Prins, H. H. (2002). Global environmental controls of diversity in large herbivores. *Nature*, *415*(6874), 901-904.

O'Neill, H. M. K., Durant, S. M., Strebel, S., & Woodroffe, R. (2022). Fencing affects African wild dog movement patterns and population dynamics. *Oryx*, *56*(1), 128–136.

Pansu, J., Guyton, J. A., Potter, A. B., Atkins, J. L., Daskin, J. H., Wursten, B., Kartzinel, T. R., & Pringle, R. M. (2019). Trophic ecology of large herbivores in a reassembling African ecosystem. *Journal of Ecology*, *107*(3), 1355–1376.

Peachey, L. E., Jenkins, T. P., & Cantacessi, C. (2017). This Gut Ain't Big Enough for Both of Us. Or Is It? Helminth–Microbiota Interactions in Veterinary Species. *Trends in Parasitology*, *33*(8), 619–632.

Pearman, P. B., Guisan, A., Broennimann, O., & Randin, C. F. (2008). Niche dynamics in space and time. *Trends in Ecology & Evolution*, *23*(3), 149–158.

Price, M. N., Dehal, P. S., & Arkin, A. P. (2009). FastTree: Computing Large Minimum Evolution Trees with Profiles instead of a Distance Matrix. *Molecular Biology and Evolution*, *26*(7), 1641.

Pringle, R. M., Kartzinel, T. R., Palmer, T. M., Thurman, T. J., Fox-Dobbs, K., Xu, C. C. Y., Hutchinson, M. C., Coverdale, T. C., Daskin, J. H., Evangelista, D. A., Gotanda, K. M., A. Man in 't Veld, N., Wegener, J. E., Kolbe, J. J., Schoener, T. W., Spiller, D. A., Losos, J. B., & Barrett, R. D. H. (2019). Predator-induced collapse of niche structure and species coexistence. *Nature*, *570*(7759), 58–64.

Pulliam, H. R. (1988). Sources, Sinks, and Population Regulation. *The American Naturalist*, *132*(5), 652–661.

Pulliam, H. R., & Danielson, B. J. (1991). Sources, Sinks, and Habitat Selection: A Landscape Perspective on Population Dynamics. *The American Naturalist*, *137*, S50–S66.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, *41*(D1), D590–D596.

Radloff, F. G. T., Waal, C. van der, & Bond, A. L. (2013). Extensive browsing by a conventional grazer? Stable carbon isotope analysis reveals extraordinary dietary flexibility among Sanga cattle of North Central Namibia. *Isotopes in Environmental and Health Studies*, *49*(3), 318–324.

R Core Team, (2013). R: A language and environment for statistical computing.

Revell, L. J. (2012). phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, *3*(2), 217–223.

Ripple, W.J., Newsome, T.M., Wolf, C., Dirzo, R., Everatt, K.T., Galetti, M., Hayward, M.W., Kerley, G.I., Levi, T., Lindsey, P.A. and Macdonald, D.W., 2015. Collapse of the world's largest herbivores. *Science advances*, *1*(4), p.e1400103.

Roughgarden, J. (1974). Niche Width: Biogeographic Patterns Among Anolis Lizard Populations. *The American Naturalist*, *108*(962), 429–442.

Rubenstein, D.I., (1989). Life history and social organization in arid adapted ungulates. *Journal of Arid Environments*, *17*(2), pp.145-156.

Rubenstein, D., Low Mackey, B., Davidson, ZD, Kebede, F. & King, S.R.B. 2016. *Equus grevyi. The IUCN Red List of Threatened Species* 2016: e.T7950A89624491.

Sargeant, B. L. (2007). Individual foraging specialization: niche width versus niche overlap. *Oikos*, *116*(9), 1431–1437. X

Schliep, K. P. (2011). phangorn: Phylogenetic analysis in R. *Bioinformatics*, *27*(4), 592–593.

Sexton, J.P., McIntyre, P.J., Angert, A.L. and Rice, K.J., 2009. Evolution and ecology of species range limits. *Annu. Rev. Ecol. Evol. Syst.*, *40*,.415-436.

Sinclair, A. R. E., Dublin, H., & Borner, M. (1985). Population regulation of Serengeti Wildebeeest: a test of the food hypothesis. *Oecologia*, *65*(2), 266–268.

Sommer, F., & Bäckhed, F. (2013). The gut microbiota-masters of host development and physiology. *Nature Reviews Microbiology 11*(4), 227–238.

Stephens, D. W., & Krebs, J. R. (1986). Foraging Theory. In *Foraging Theory*. Princeton University Press.

Street, G. M., Rodgers, A. R., Avgar, T., & Fryxell, J. M. (2015). Characterizing demographic parameters across environmental gradients: A case study with Ontario moose (*Alces alces*). *Ecosphere*, *6*(8), 1–13.

Sundaresan, S. R., Fischhoff, I. R., Hartung, H. M., Akilong, P., & Rubenstein, D. I. (2008). Habitat choice of Grevy's zebras (*Equus grevyi*) in Laikipia, Kenya. *African Journal of Ecology*, *46*(3), 359–364.

Sundaresan, S. R., Fischhov, I. R., Dushov, J., & Rubenstein, D. I. (2007). Network metrics reveal differences in social organization between two fission-fusion species, Grevy's zebra and onager. *Oecologia*, *151*, 140–149.

Tan, J., McKenzie, C., Potamitis, M., Thorburn, A. N., Mackay, C. R., & Macia, L. (2014). The role of short-chain fatty acids in health and disease. *Advances in immunology*, *121*, 91-119.

Tinker, M. T., Bentall, G., & Estes, J. A. (2008). Food limitation leads to behavioral diversification and dietary specialization in sea otters. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(2), 560–565.

Tombak, K. J., Easterling, L. A., Lindsay Martinez, |, Seng, M. S., Liana, |, Wait, F., Daniel, |, & Rubenstein, I. (2022). Divergent water requirements partition exposure risk to parasites in wild equids. *Ecology and Evolution*, *12*(3), e8693.

Tombak, K. J., Hansen, C. B., Kinsella, J. M., Pansu, J., Pringle, R. M., & Rubenstein, D. I. (2021). The gastrointestinal nematodes of plains and Grevy's zebras: Phylogenetic relationships and host specificity. *International Journal for Parasitology: Parasites and Wildlife*, *16*, 228–235.

van Valen, L. (1965). Morphological Variation and Width of Ecological Niche. *The American Naturalist*, *99*(908), 377–390.

Vogel, S. M., Blumenthal, S. A., de Boer, W. F., Masake, M., Newton, I., Songhurst, A. C., McCulloch, G., Stronza, A., Henley, M. D., & Coulson, T. (2020). Timing of dietary switching by savannah elephants in relation to crop consumption. *Biological Conservation*, *249*, 108703.

Walshe, N., Duggan, V., Cabrera-Rubio, R., Crispie, F., Cotter, P., Feehan, O., & Mulcahy, G. (2019). Removal of adult cyathostomins alters faecal microbiota and promotes an inflammatory phenotype in horses. *International Journal for Parasitology*, *49*(6), 489–500.

Walshe, N., Mulcahy, G., Hodgkinson, J., & Peachey, L. (2020). No Worm Is an Island; The Influence of Commensal Gut Microbiota on Cyathostomin Infections. *Animals, 10*(12), 2309.

Wastyk, H. C., Fragiadakis, G. K., Perelman, D., Dahan, D., Merrill, B. D., Yu, F. B., Topf, M., Gonzalez, C. G., van Treuren, W., Han, S., Robinson, J. L., Elias, J. E., Sonnenburg, E. D., Gardner, C. D., & Sonnenburg, J. L. (2021). Gut-microbiota-targeted diets modulate human immune status. *Cell*, *184*(16), 4137-4153.e14.

Weinstein, S. B., Martinez-Mota, R., Stapleton, T. E., Klure, D. M., Greenhalgh, R., Orr, T. J., Dale, C., Kohl, K. D., & Denise Dearing, M. (2021). Microbiome stability and structure is governed by host phylogeny over diet and geography in woodrats (*Neotoma spp*.). *Proceedings of the National Academy of Sciences of the United States of America*, *118*(47).

White, T. (2008). The role of food, weather and climate in limiting the abundance of animals. *Biological Reviews*, *83*(3), 227-248.

Williams, D. S. (1998). *Grevy's zebra: ecology in a heterogeneous environment*. *PhD Thesis*

Wolf, C., & Ripple, W. J. (2016). Prey depletion as a threat to the world ' s large carnivores. *Royal Society Open Science*, *3*(8), 160252. https://doi.org/10.1098/rsos.160252

Workentine, M. L., Chen, R., Zhu, S., Gavriliuc, S., Shaw, N., Rijke, J. de, Redman, E. M., Avramenko, R. W., Wit, J., Poissant, J., & Gilleard, J. S. (2020). A database for ITS2 sequences from nematodes. *BMC Genetics*, *21*(1), 1–4.

Wright, E. S. (2020). RNAconTest: comparing tools for noncoding RNA multiple sequence alignment based on structural consistency. *RNA*, *26*(5), 531–540.

WWF (2020) Living Planet Report 2020 -Bending the curve of biodiversity loss. Almond, R.E.A., Grooten M. and Petersen, T. (Eds). WWF, Gland, Switzerland.

Xiang, Z., Tong, W., Guo, Z., Xu, Y., Guo, J., Ruan, Y., & Zhao, P. (2019). Rat H1 parvovirus infection leads to alterations in gut microbiota. *Pathogens and Disease*, *77*(5), 58.

Xing, Y., Liu, J., Lu, F., Wang, L., Li, Y., & Ouyang, C. (2019). Dynamic distribution of gallbladder microbiota in rabbit at different ages and health states. *PLOS ONE*, *14*(2), e0211828.

Zaneveld, J. R., McMinds, R., & Thurber, R. V. (2017). Stress and stability: Applying the Anna Karenina principle to animal microbiomes. *Nature Microbiology* 2(9), 17121.

6.9 Supplementary information

Table S6.1) perMANOVA with 10000 permutations of weighted Unifrac dissimilairity in diet composition across geographic areas (Ol-Jogi-Mpala, Westgate and Lewa) and seasons.

Variable	Df	SumOfSquares	R2	F
Geographic areas (“Reserve”)	2	10.03	0.63	182.39
Season	1	1.02	0.06	37.02
Reserve:Season	2	0.81	0.051	14.67
Residual	145	4.00	0.25	
Total	150	15.84	1	

Table S6.2) perMANOVA with 10000 permutations for weighted Unifrac dissimilairity in diet composition across ecological gradient varying with environmental variables. NDVI denotes normalised difference vegetation index as a proxy of grass availability and forage palatability.

Variable	Df	SumOfSquares	R2	F	Pr(>F)
Rainfall	1	7.259	0.458	133.17	<0.001
NDVI	1	0.515	0.033	9.46	<0.001
Residual	148	8.068	0.509		
Total	150	15.842	1.000		

Table S6.3) Model results of proportion of grass explained by NDVI and rainfall (RRA_poacecae was logit transformed)

Parameter	df	Slope	s.e	t	*F*	*p*	Delta AIC
Intercept	1, 150	-6.3	1.30	-4.9			
NDVI	1, 150	0.044	0.002	4.29	53.4	<0.001	48.193
Rainfall	1, 150	0.014	0.01	7.3	18.4	<0.001	15.7

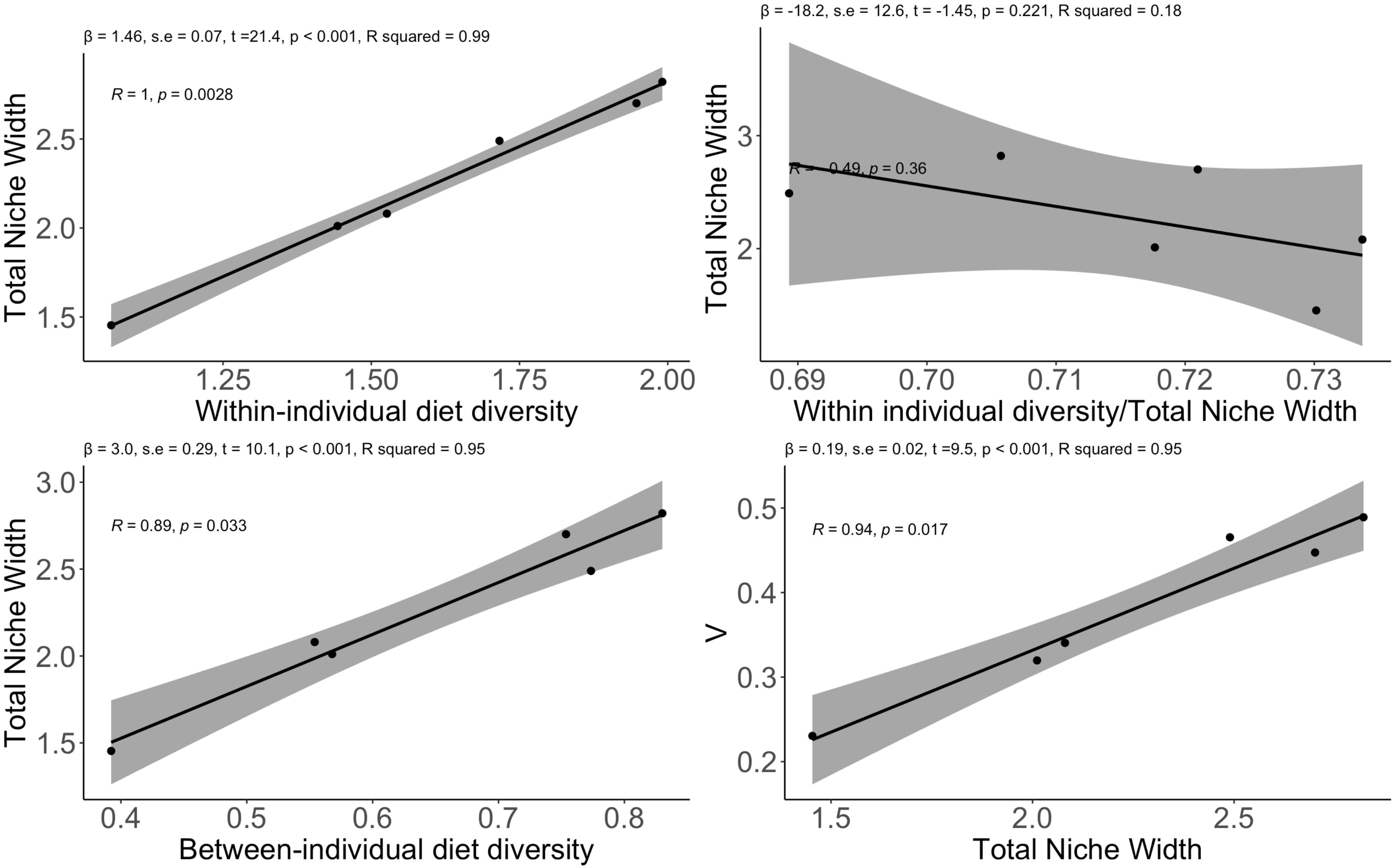
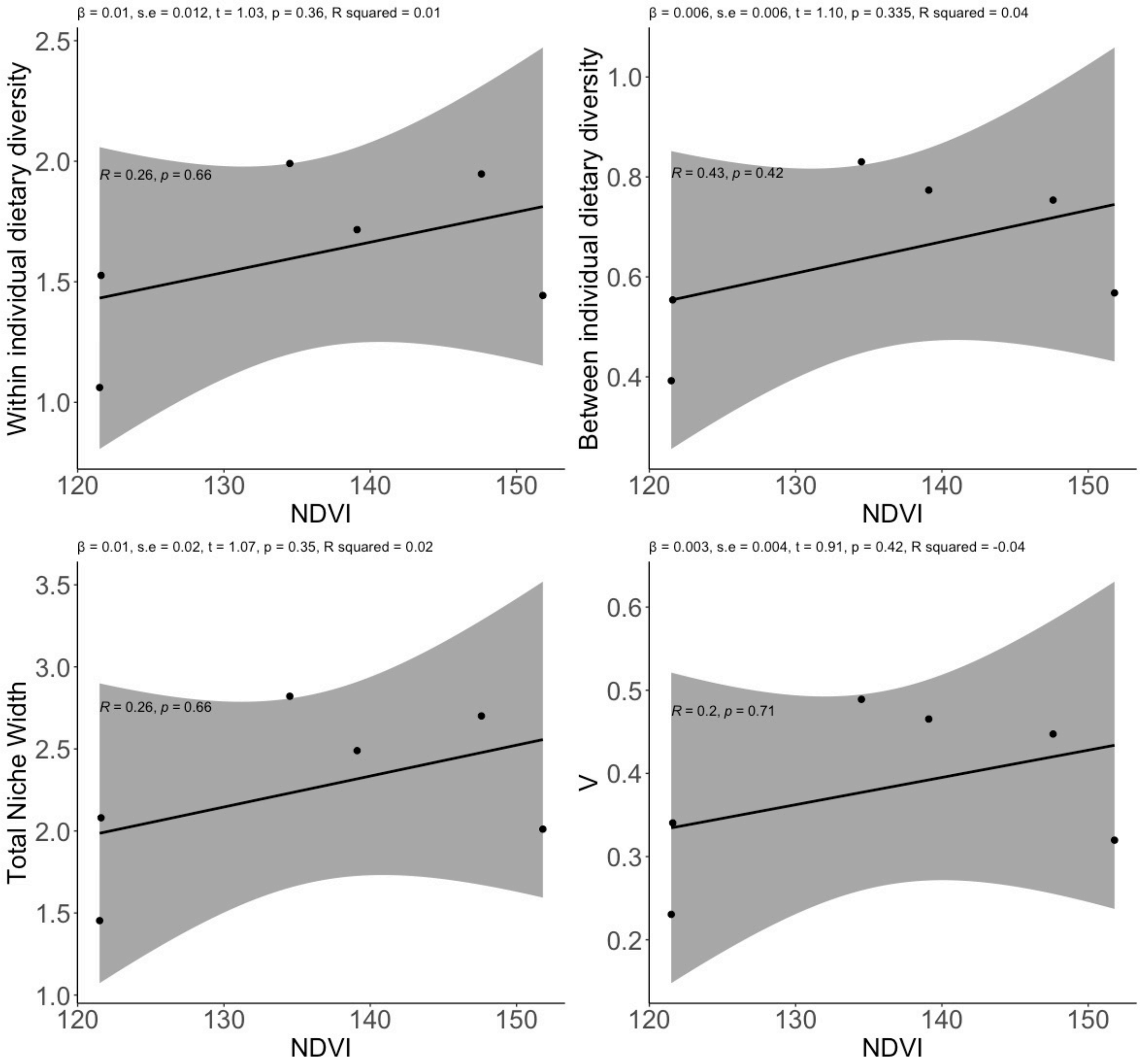


Figure S6.1) Population level specialisation metrics at population level across populations and seasons. Increases in total niche width are due to increases in both between individual and within individual components of total niche width. Results from linear regression are displayed above each graph. R is calculated from spearman’s rank correlation coefficient.



β = 0.01, s.e = 0.012, t = 1.03, p = 0.36, R squared = 0.01

β = 0.006, s.e = 0.006, t = 1.10, p = 0.335, R squared = 0.04

β = 0.01, s.e = 0.02, t = 1.07, p = 0.35, R squared = 0.02

β = 0.003, s.e = 0.004, t = 0.91, p = 0.42, R squared = -0.04

Figure S6.2) Population level individual diet specialisation metrics of Grevy’s zebra with NDVI. Increases in dietary diversity did not significantly correlate with changes in proportion of grass in the diet. Results from linear regression are displayed above each graph. R is calculated from spearman’s rank correlation coefficient.

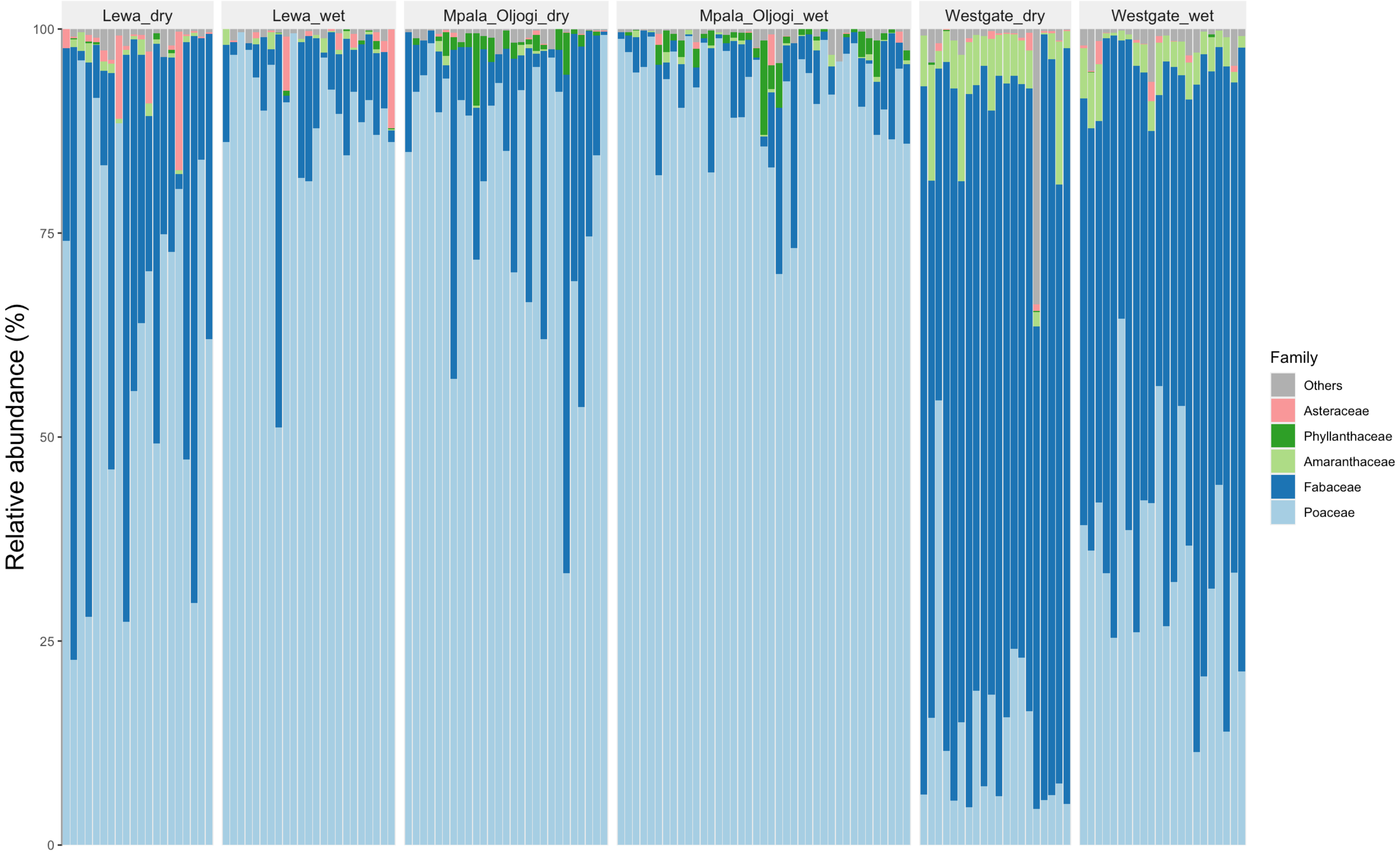


Figure S6.3) Grevy’s zebra diet composition across geographic areas split by reserve and season. Analysed at Family level

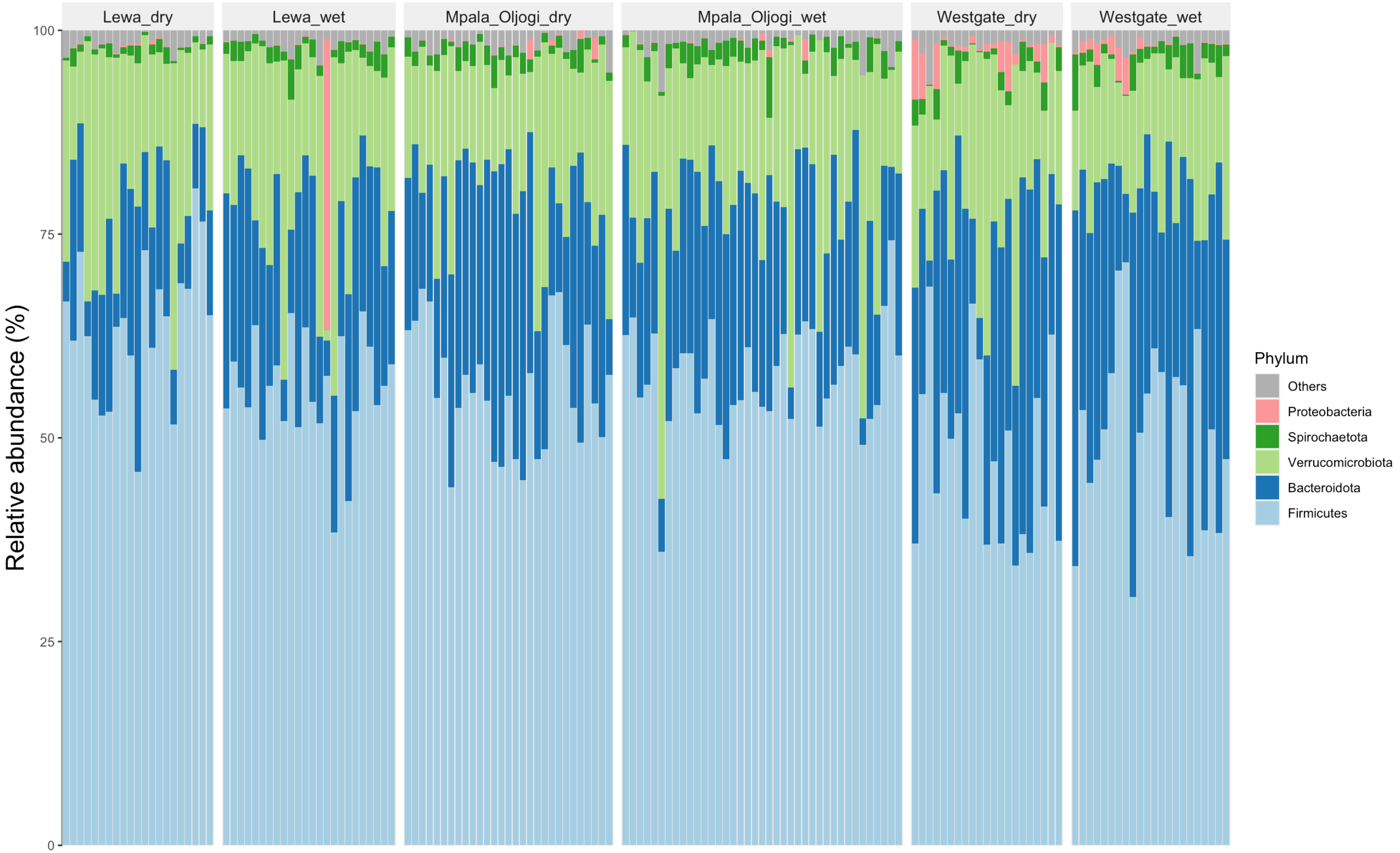


Figure S6.4) Grevy’s zebra microbiome composition across geographic areas split by reserve and season. Analysed at Phylum level

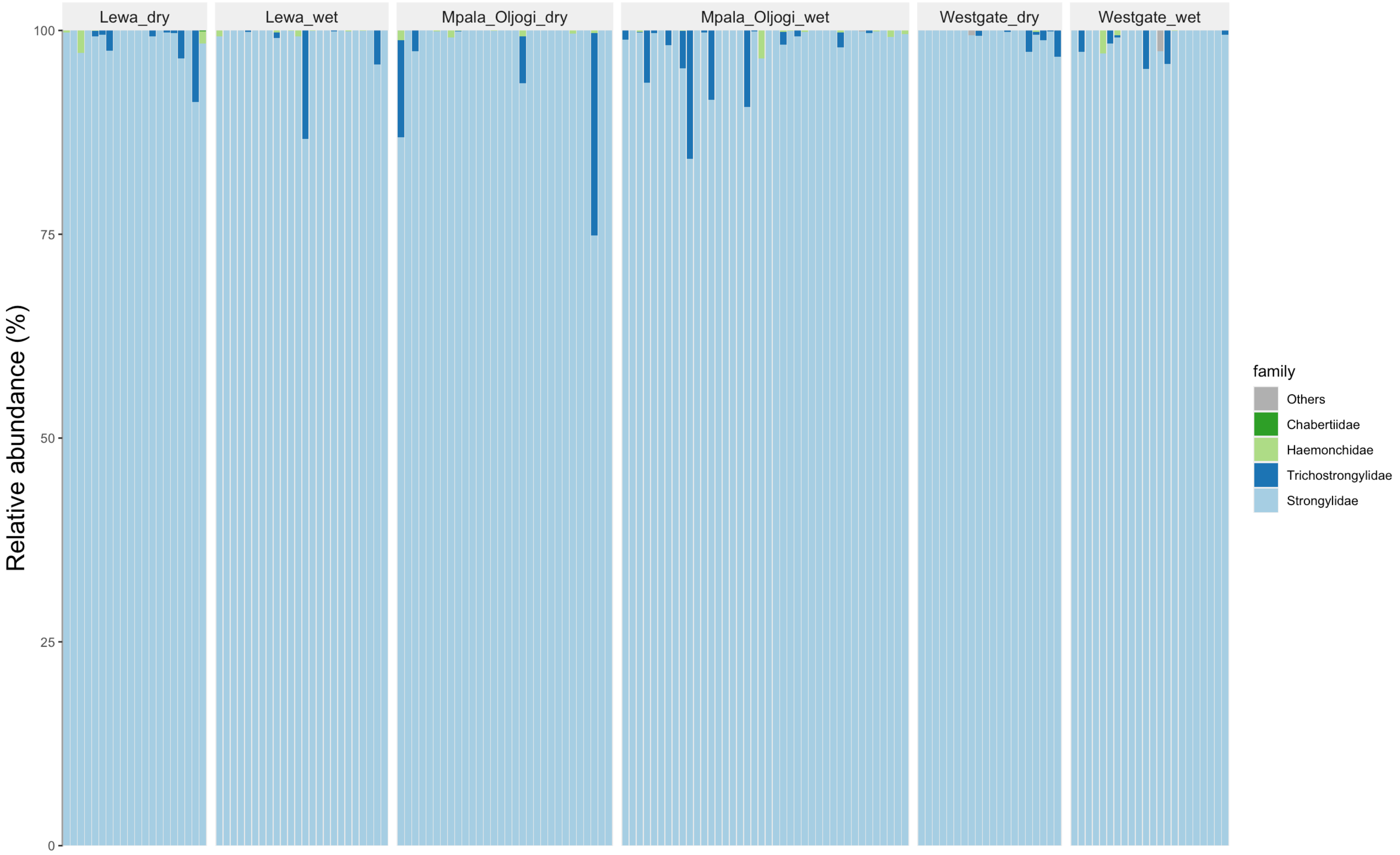


Figure S6.5) Grevy's zebra Nemabiome composition across geographic areas split by reserve and season. Analysed at Family level

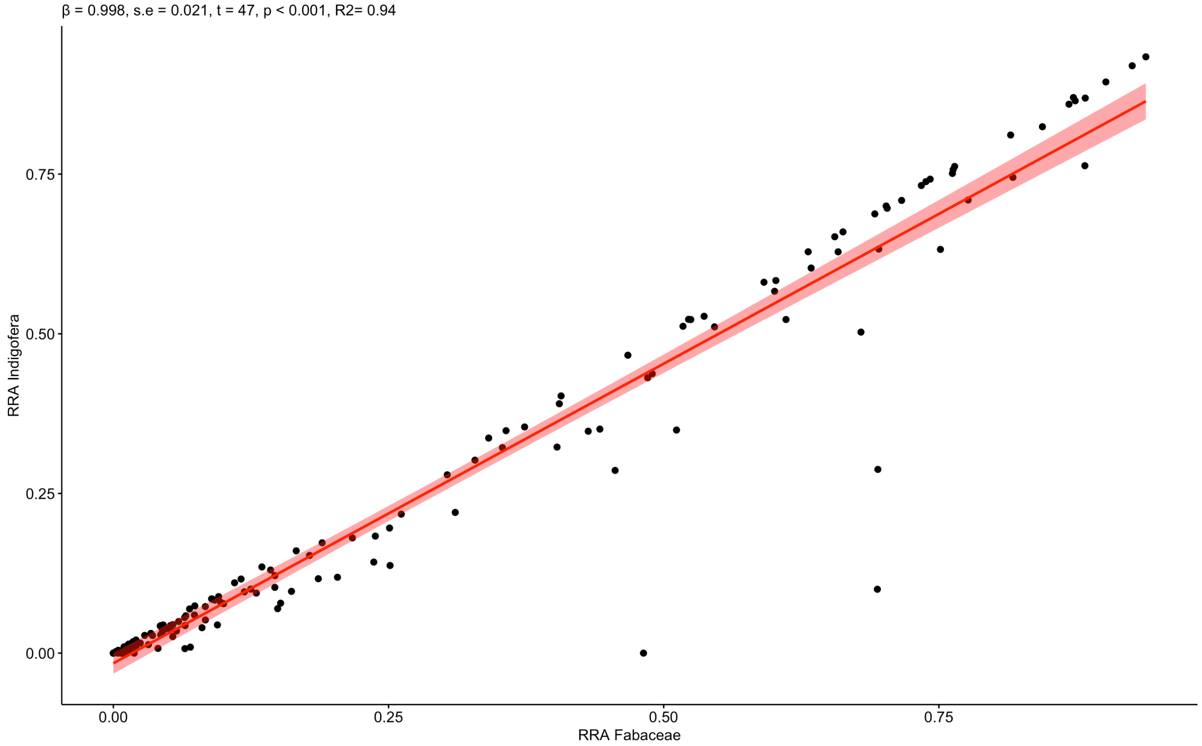


Figure S6.6) Indigofera is the most abundant legume (Fabaceae) in Grevy’s zebra diets across geographic space. Correlation between Relative read abundance (RRA) of Fabaceae and Indigofera. Results from linear mixed effect mode are seen above.

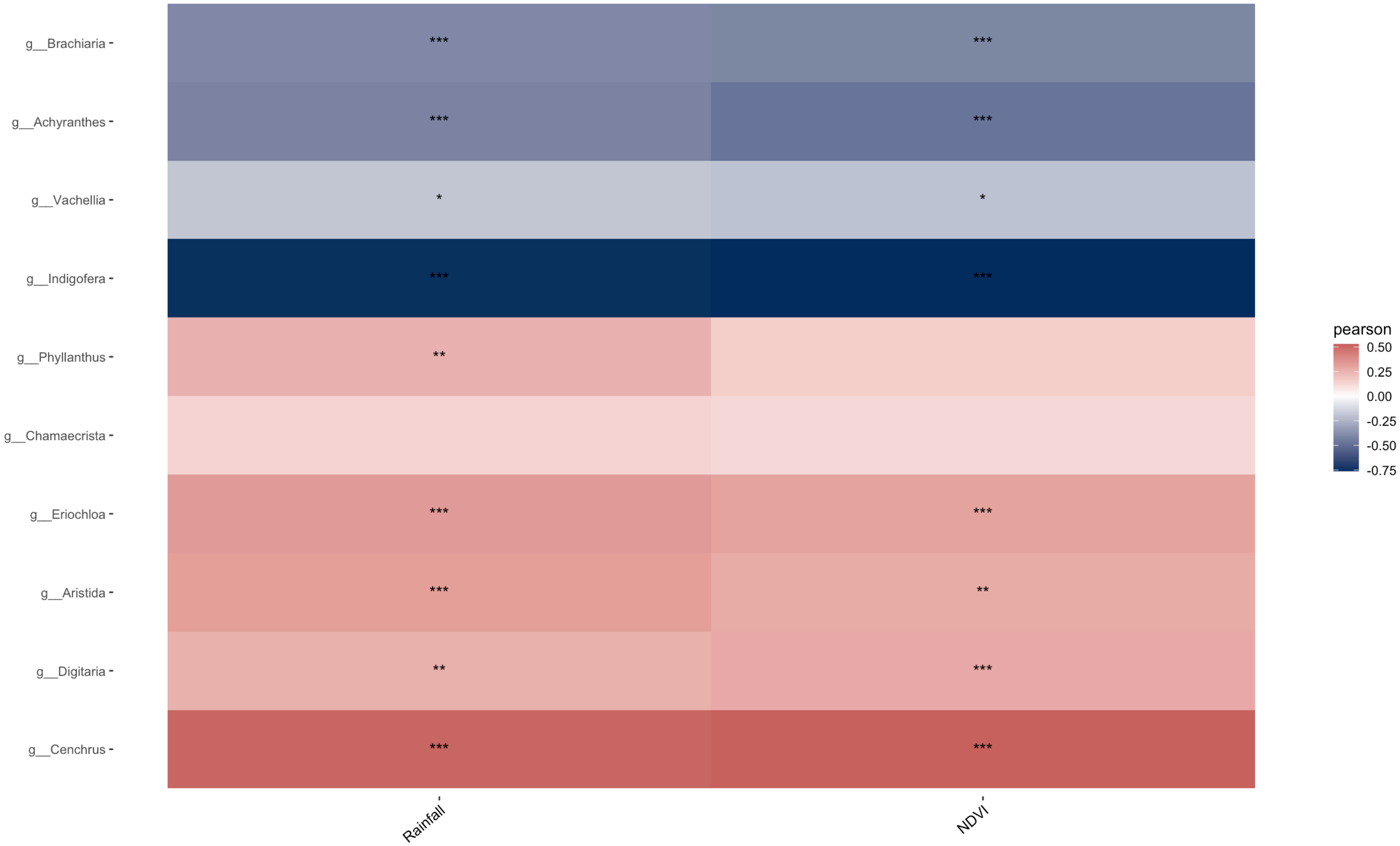


Figure S6.7) Correlation heatmap between top ten most abundant dietary genera from environmental variables – Rainfall and NDVI.

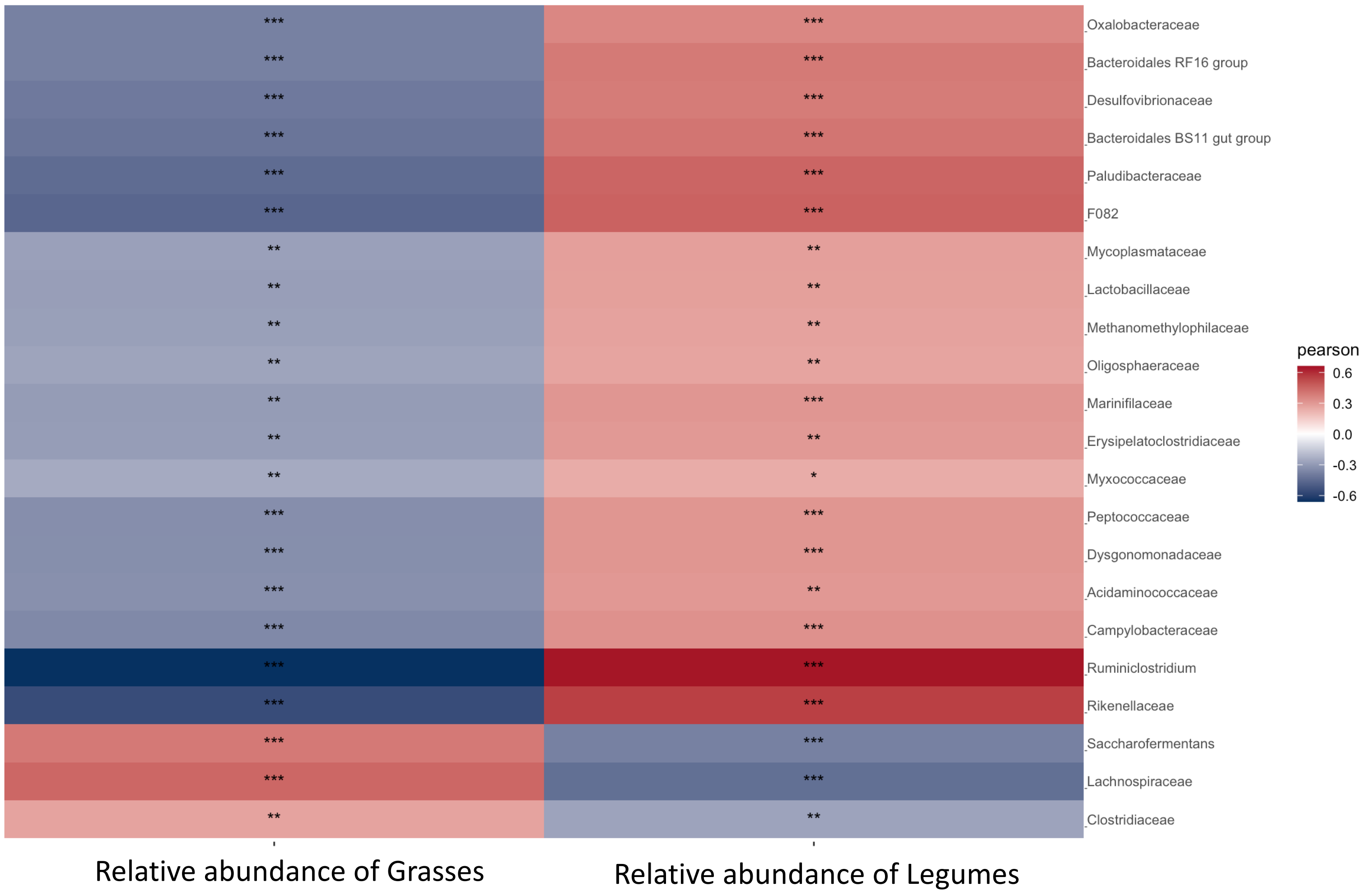


Figure S6.8) Significant correlation heatmap between Relative abundance of grasses and legumes and microbial families

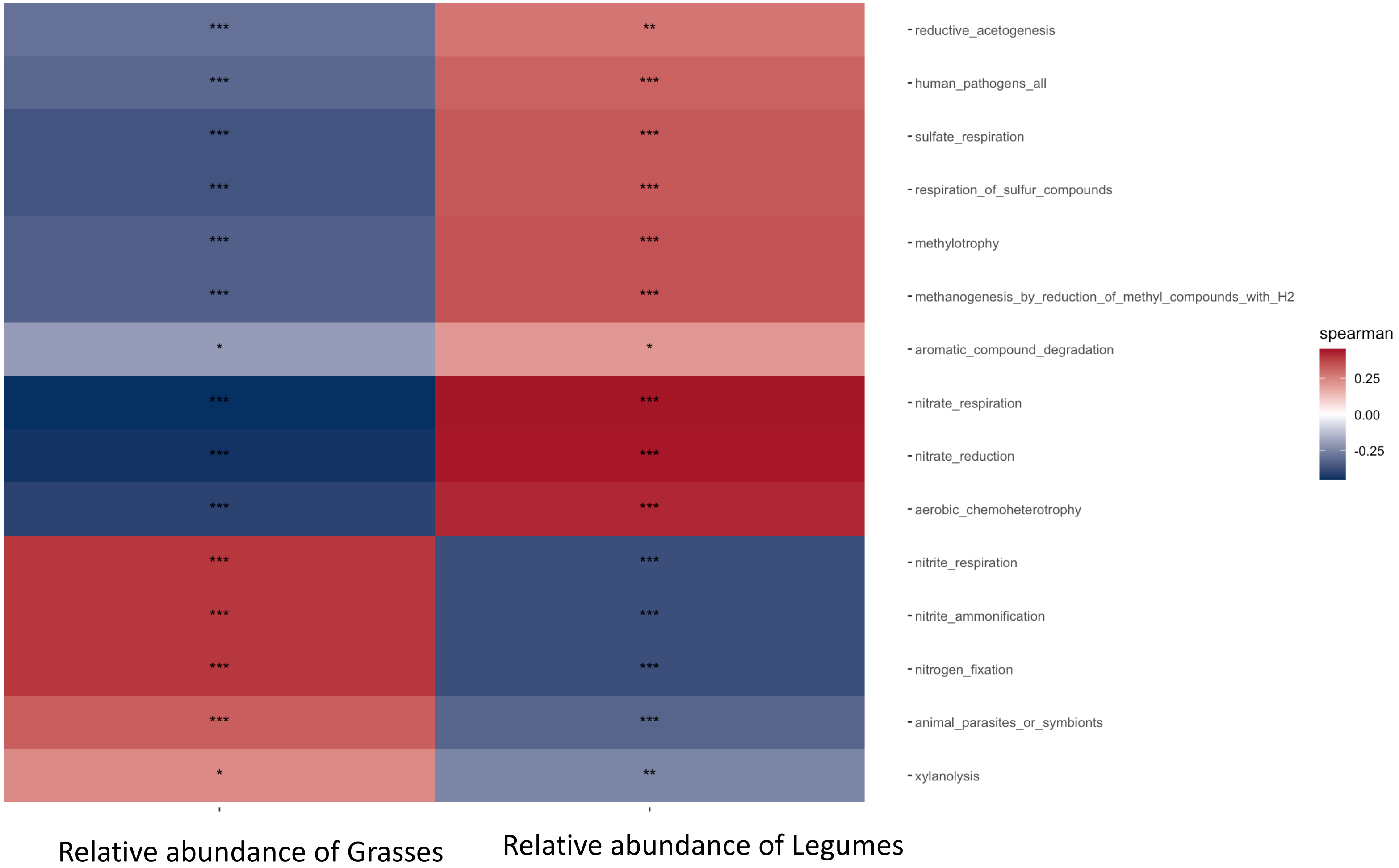


Figure S6.9) Functional analysis of microbiome heatmap from environmental variables including relative read abundance of grasses and legumes using functional analysis using FAPROTAX

Table S6.4) Loading scores of dietary genera on the first principal component of microbiome beta diversity**.** Positive loadings correspond to high rainfall, high NDVI environments and negative loadings correspond to low rainfall low NDVI environments. Only loading scores >0.4 or <-0.4 are displayed.

Plant Genera	Loading score
Cenchrus	3.45087252
Digitaria	2.06795976
Phyllanthus	1.11009059
Themeda	0.92664295
Aristida	0.86398889
Eriochloa	0.70425026
Chamaecrista	0.50991802
Amaranthaceae Family	-1.8210577
Brachiaria	-1.8030702
Achyranthes	-1.6729371
Indigofera	-1.4998671
Vachellia	-1.4866285
Malvaceae Family	-0.9502362
Tribulus	-0.6580243
Commicarpus	-0.5902004
Zaleya	-0.5090982
Tragus	-0.490231

Table S6.5) Loading scores of Microbial families on the first principal component of microbiome beta diversity**.** Positive loadings correspond to legume rich diets and negative loadings correspond to grass rich diets. Only loading scores >0.4 or <-0.4 are displayed.

Microbiome family	**Loading score**
Planococcaceae	1.82509729
Paludibacteraceae	1.67627569
Bacteroidales BS11 gut group	1.51944551
Ruminiclostridium	1.29922528
Enterobacteriaceae	1.15567614
Desulfovibrionaceae	1.11653931
Bacteroidales RF16 group	1.00040789
Campylobacteraceae	0.87114368
Oxalobacteraceae	0.79491891
Hungateiclostridiaceae Order	0.77749604
MVP-15 Class	0.77099676
Bacteria Kingdom	0.62173363
Acidaminococcaceae	0.5273001
Marinifilaceae	0.51724877
Dysgonomonadaceae	0.50748214
Firmicutes Phylum	0.48323346
Micrococcaceae	0.47042108
Erysipelatoclostridiaceae	0.4587014
Bradymonadales Order	0.43514235
Oligosphaeraceae	0.43118628
Methanobacteriaceae	-1.5212105
Coriobacteriales	-1.0818454
Selenomonadaceae	-1.0639087
Anaerofustaceae	-1.0325841
Eggerthellaceae	-0.8516174
Muribaculaceae	-0.7738051
Eubacteriaceae	-0.7428599
Akkermansiaceae	-0.7110899
Erysipelotrichaceae	-0.6858355
Armatimonadota Phylum	-0.6789685
Clostridia UCG-014 Order	-0.6596015
Defluviitaleaceae	-0.6413965
Clostridia Class	-0.5547494
Saccharofermentans	-0.5238476

Anaerolineaceae	-0.4858943
Clostridiaceae	-0.4498808
Christensenellaceae	-0.4447543
Monoglobaceae	-0.4287484
RF39 Order	-0.3937986

Table S6.6) Loading scores of nemabiome species on the first principal component of nemabiome beta diversity**.** Positive loadings correspond to legume rich diets and negative loadings correspond to grass rich diets. Only loading scores >0.4 or <-0.4 are displayed.

Nemabiome species	Loading scores
Strongylus_edentatus	3.59619678
Strongylus_equinus	2.26401454
Strongylus_vulgaris	1.35762095
Trichostrongylus_axei	1.24642619
Triodontophorus_nipponicus	-2.4423416
Cylicocyclus_auriculatus	-1.9092342
Cylicocyclus genus	-1.5664702
Cylicostephanus_minutus	-1.0752517
Cyathostomum genus	-1.0076969
Cylicocyclus_adersi	-0.5860927
Cylicostephanus_bidentatus	-0.5417798
Cyathostomum_pateratum	-0.5226224

Table S6.7) perMANOVA with 10000 permutations of weighted Unifrac dissimilarity in microbiome composition across geographic areas (Ol-Jogi-Mpala, Westgate and Lewa) and seasons.

Variable	**Df**	**SumOfSquares**	**R2**	**F**	**Pr(>F)**
Geographic areas (“Reserve”)	2	0.69	0.11	9.98	<0.001
Season	1	0.04	0.01	1.18	0.27
Reserve:Season	2	0.37	0.06	2.18	<0.001
Residual	145	5.05	0.82		
Total	150	6.15	1.00		

Table S6.8) perMANOVA with 10000 permutations for weighted Unifrac dissimilarity in microbiome composition across ecological gradient with diet

Variable	Df	SumOfSquares	R2	F	Pr(>F)
Proportion of grass in the diet	1	0.38	0.06	10.07	<0.001
Residual	147	5.53	0.94		
Total	148	5.91	1.00		

Table S6.9) perMANOVA with 10000 permutations of weighted Unifrac dissimilarity in nemabiome composition across geographic areas (Ol-Jogi-Mpala, Westgate and Lewa) and seasons.

Variable	Df	SumOfSquares	R2	F	*p*
Geographic areas (“Reserve”)	2.00	3.49	0.22	21.56	0.00
Season	1.00	0.09	0.01	1.12	0.29
Reserve:Season	5.00	0.52	0.03	1.30	0.19
Residual	147.00	11.91	0.74		
Total	155.00	16.01	1.00		

Table S6.10) perMANOVA with 10000 permutations for weighted Unifrac dissimilarity in nemabiome composition across ecological gradient varying with environmental variables. NDVI denotes normalised difference vegetation index as a proxy of grass availability and forage palatability.

Variable	Df	SumOfSquares	R2	F	Pr(>F)
Proportion of grass in the diet	1	1.6	0.11	17.4	<0.001
Residual	145	13.28	0.89		
Total	146	14.88	1		

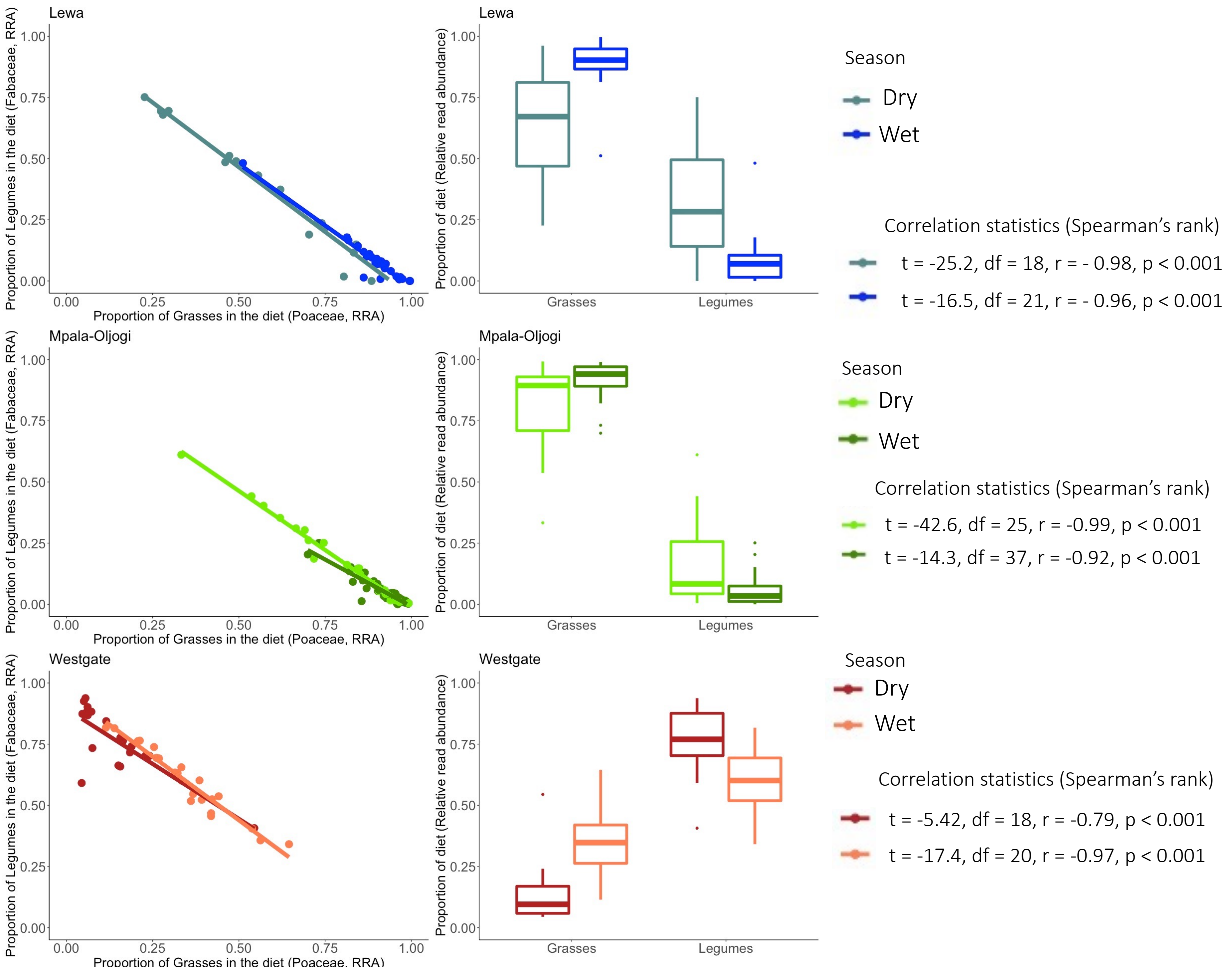


Figure S6.10) Grevy's zebra incorporate legumes into their diet when they cannot saturate their diet with grasses across the ecological gradient and across seasons. Boxplots show the relative read abundance (RRA) in the sampling ranges of zebra in all three populations. Top) Lewa, Middle) Mpala-Ol-jogi, Bottom) Westgate. Statistics depict the corresponding correlation estimated using Spearman's rank correlation coefficient.

Chapter 7) Discussion

This thesis focused on evaluating the scope, drivers and dynamics of ecological marginality and marginalisation. I did this by analysing ecological marginality at three scales: a global scale across terrestrial mammal species, a species range scale in Cape mountain zebra (*Equus zebra zebra*) and across an ecological gradient within part of the metapopulation of Grevy's zebra (*Equus grevyi*). This approach revealed ecological marginalisation to be a common consequence of range contraction and a global conservation threat to terrestrial mammals. Furthermore, it provides evidence of the physiological consequences of ecological marginalisation in two species of conservation importance.

The third chapter demonstrates that ecological marginalisation increases extinction risk across mammals and is a global and widespread phenomenon. Our results demonstrate species ranges and niche spaces have become smaller and more homogenous due to range contraction. The remaining niche space is situated in ecologically or climatically extreme portions of species historic niches. With increasing displacement from the historic niche centroid, species experience greater extinction risk independent of range contraction. Large-bodied species and species with small geographic range size were under greatest threat. This increased vulnerability from *intrinsic* and niche characteristics is currently not integrated into conservation initiatives. Section 7.1 evaluates whether IUCN species assessments account for ecological marginalisation and whether they perceive marginalisation as a threat to the species. Section 7.2 outlines the threats that marginalisation may pose to conservation efforts. I discuss how ecological marginality reveals potential flaws in contemporary conservation practices. In section 7.2.1, I discuss how widespread contraction, ecological marginalisation and biases in study areas can form species stereotypes which may undermine conservation efforts. In section 7.3, I outline how additional physiological and comparative analyses can be used to identify species in marginal conditions and how to incorporate these into species assessments.

The fourth, fifth and sixth chapters use physiological biomarkers to evaluate marginality and understand performance and dynamics. Chapter 4 highlights the importance of validating physiological biomarkers while revealing novel drivers of HPA axis activity across a species range. We discuss how single-biomarker studies, such as this, could be improved by using and analysing multiple physiological biomarkers in section 7.2.1. We demonstrate the utility and effectiveness of multiple biomarkers studies in chapters 5 and 6. In chapters 5 and 6, I demonstrate that marginal habitats with poor resource availability led to alterations in microbiome composition and function, and nemabiome composition and burden.

Limitations for the research presented are outlined in section 7.5. Specifically, I outline how assumptions about species distributions and response curves may not hold for all species. Finally, I conclude in section 7.6 by suggesting further work that builds on the research findings presented in this thesis.

7.1. Is marginality being implemented into conservation practices?

In Chapter 3, I demonstrate that ecological marginalisation is a widespread threat to terrestrial mammals globally. Given that 1) ecological marginalisation is a common consequence of range contraction, 2) refugee species or partial refugees may be found globally (Kerley et al., 2020) and 3) ecological marginalisation increases extinction risk, it is crucial to evaluate whether conservation initiatives are incorporating marginality into assessments. As a case study, I investigate the IUCN species descriptions of a vulnerable group Endangered (EN) and Critically Endangered (CR) ungulates (groups of *Perissodactyla, Bovidae, Cervidae, Suidae, Hippopotomidae* and *Giraffidae*). I applied the identifying factors of refugee species outlined by Kerley et al., 2012 – range contraction, occupancy of a reduced diversity of habitats compared to the historic range, anomalous resource use and low densities. I also evaluate whether species descriptions acknowledge: 1) “lack of access to high quality habitats”, 2) “poor habitat suitability throughout the realized range”, 3) “confinement to refugia” or 4) the species were named explicitly as a “candidate-” or “partial refugees” or “refugee species”.

EN and CR ungulates provide a model study group as they are: 1) a large, well-studied group and hence have good data on the contemporary and historic ranges to establish appropriate baselines, 2) the group has a relatively uniform ecology as all species are herbivores, 3) due to this uniform ecology, we would predict that ungulates compete with humans and livestock for access to productive habitats, 4) the group have experienced contemporary and historic persecution due to hunting thus we can be predict they should show retreat from these pressures, 5) a high proportion of the remaining populations are in protected areas and 6) are vulnerable to ecological marginalisation as demonstrated in Chapter 2.

All species had experienced extensive range contraction (identifying factor 1). Many were described as occupying a less diverse niche (identifying factor 2) or occurring at low densities within protected areas (identifying factor 3). None of the species were described as a having a biological mismatch with their habitat despite existing literature demonstrating such mismatches, for example*,* Huemul (*Hippocamelus bisulcus*) (Flueck, 2021). Despite, 25% (12 of 49) of IUCN assessments describing a potential inability to access high-quality habitat or sufficient resources, only 6% (3 out of 49) of these assessments acknowledged marginality as a threat to the species.

Despite existing literature demonstrating that some EN and CR ungulates are confirmed or candidate refugees and are actively protected in marginal environments *e.g.* Hirola (*Beatragus hunteri*) (Ali et al., 2017, 2018) and Huemul (*Hippocamelus bisulcus*) (Flueck, 2021), none were referred to as a candidate, partial or refugee species within their species assessments. The most recent IUCN red list assessment for European bison (*Bison bonasus*) acknowledges its refugee status (Plumb *et al*, 2020) but this, to my knowledge, is the first to explicitly apply the term. Although I only evaluate ungulates as a single case study, marginality is likely poorly implemented into species assessments generally or not perceived as a compounding threat to species extinction risk**.**

Recognition for marginality within conservation planning is also poorly evaluated in the wider conservation literature. Despite, acknowledgement from Ripple and colleagues (Ripple, 2015) of the threat of marginalisation in ungulates, many representative accounts of Mammal Conservation such as (Littlewood et al., 2020) do not mention refugee species or the possibility that populations may be confined to sub-optimal environments.

7.2. How does ecological marginalisation impact conservation efforts?

7.2.1 Marginality and shifted baselines

Occupation of marginal environments is particularly deleterious when the marginal habitat is not recognised as such (Kerley et al., 2012; Bilney, 2014). When range contraction occurs over many generations, each generation may accept their perception of the environment as a new norm, a form of generational amnesia (Kahn and Friedman, 1995). As each generation accepts their new reference point as the "natural" state, the true magnitude of decline or shift is mis-estimated (Soga & Gaston, 2018). This phenomenon is termed shifting baseline syndrome (Pauly, 1995).

Shifting baseline syndrome is traditionally applied to perceptual changes of species abundance. However, shifting baselines may also apply to our perception of species ecologies. As humans have caused an unprecedented truncation of species niche space, species can become protected or maintained in marginal habitat types. In the restricted niche, the species ecology, such as the habitat currently inhabited, the behaviours performed, the preferred diet or the maximum rate of population growth, can be misinterpreted as the optimal or "natural" state for the species (Figure 7.1). I term this misestimation a species stereotype (Britnell et al., 2021; Appendix 2). Many confirmed, and candidate refugee species display a shifting baseline in the perception of what their optimal habitat is *i.e.* where the species "belongs" or should be protected.

7.2.2 Marginality, shifted baselines and formation of species stereotypes

In Britnell et al, 2021 (Appendix 2), I discuss how widespread range contraction, biases in study areas and ecological marginality can lead to three significant problems: 1) misestimating historic or potential niche breath, 2) forming a partial understanding of the species biology and 3) forming misconceptions about community ecology and potential.

Extensive range contraction restricts species to a small subset of historic niche conditions and habitats. This restricted range is unlikely to represent the full historic habitat diversity (Scheele et al., 2017, Chapter 3). Under human pressures, animals may retreat into areas of reduced threats which are marginal refugia (Chapter 3). Alternatively, species may be facilitated by human pressures, broadening their historical niche (Mcgeoch & Latombe, 2016; Pineda-Munoz et al., 2021). In either instance, the species' current realised niche is altered from an historical baseline. If contemporary populations are studied without historic context, the new realized niche, and the behaviours and physiology exhibited within it, may be considered a "natural" state for the species. In the case of range contraction, stereotypes may limit our conservation efforts as the species' entire potential range is not accurately identified. In the case of range expansion, novel exaptations to anthropogenic resources, may be misconstrued as "evolved" traits.

Where range contraction has left species in multiple historic habitat types, a fuller picture of a species' biology can be obtained by simply studying more populations. However, in the worst-case scenario, species have been completely extirpated from all but one historic habitat type. In these instances, studying the more contemporary populations in the remaining similar niche conditions can reinforce 'stereotypes' as the contraction or shift in occupied niche space is not recognised or acknowledged.

Alongside widespread range contraction, systematic biases exist in ecological research impacting where we study species and which species are studied (Martin et al., 2012; Titley et al., 2017). For example, large, charismatic, diurnal mammals are better studied than other groups (Dos Santos et al., 2020). Thus, our knowledge of specific populations becomes comprehensive while our understanding about ecological flexibility, resilience and variation between populations is poor. These biases can lead to species stereotypes even if based on ample extremely rich data sets. Due to sampling ease, dense populations are usually prioritised over sparsely populated marginal ones (Stamps, 2011). However, as I have demonstrated in Chapters 5 and 6, marginal populations can show varying dietary ecology and physiology to "core" populations. Recognising and addressing these biases has helped highlight priority areas for research regarding underrepresented taxon groups (Conde et al., 2019) and habitats (Martin et al., 2012).

Finally, marginality across habitat types can lead to an incomplete understanding of species interactions at the community level. Species interactions depend on both biotic and abiotic factors and are likely habitat-specific (Pellissier et al., 2018). As discussed, land-use patterns and human persecution have shaped species assemblages (Ellis et al., 2021) impacting ecological network structure and function (Takemoto & Kajihara, 2016). Many species now occupy areas with incomplete trophic guild assemblages (Wolf & Ripple, 2017). Community composition change through introduction, extirpation or extinction results in missing and altered functional processes such as vegetation clearing (Guyton *et al*, 2020), nutrient cycling (Doughty et al., 2016) and resource extraction, modification and maintenance (Lundgren et al., 2018). For example, equids dig for wells in desert habitats, increasing water availability for other species (Lundgren et al., 2021). Importantly, equids do not show the same behaviours in all habitats (Lundgren et al., 2021; Rubin et al., 2021). Many range restricted species only exist in, and therefore have only been studied in, a limited number of communities. This can lead to a species stereotype of an organism’s functional role within an ecosystem where competitive and mutualistic interactions are assumed to be the same irrespective of habitat or biological community (Robles & Martin, 2014; Pellissier et al., 2018)

Species stereotypes can become so extreme that a refugee species can be promoted for protection of its marginal environment. The Audouin’s Gull (*Ichthyaetus audouinii*) was used as a flagship species to promote conservation of the small marginal archipelagos that it never historically occupied. It also converts these islets to an unsuitable breeding area with its nitrogen-rich faeces (Martínez-Abraín & Jiménez, 2016; Martínez-Abraín et al., 2019). The misconception of its “belonging” in this environment only increases the species' extinction risk as it performs poorly (Martínez-Abraín & Jiménez, 2016). Therefore, unidentified shifting baselines can result in conservation complacency (Bilney, 2014), the acceptance of an inadequate conservation effort as sufficient, and confinement of species into marginal habitats (Britnell et al., 2021, Appendix 2)

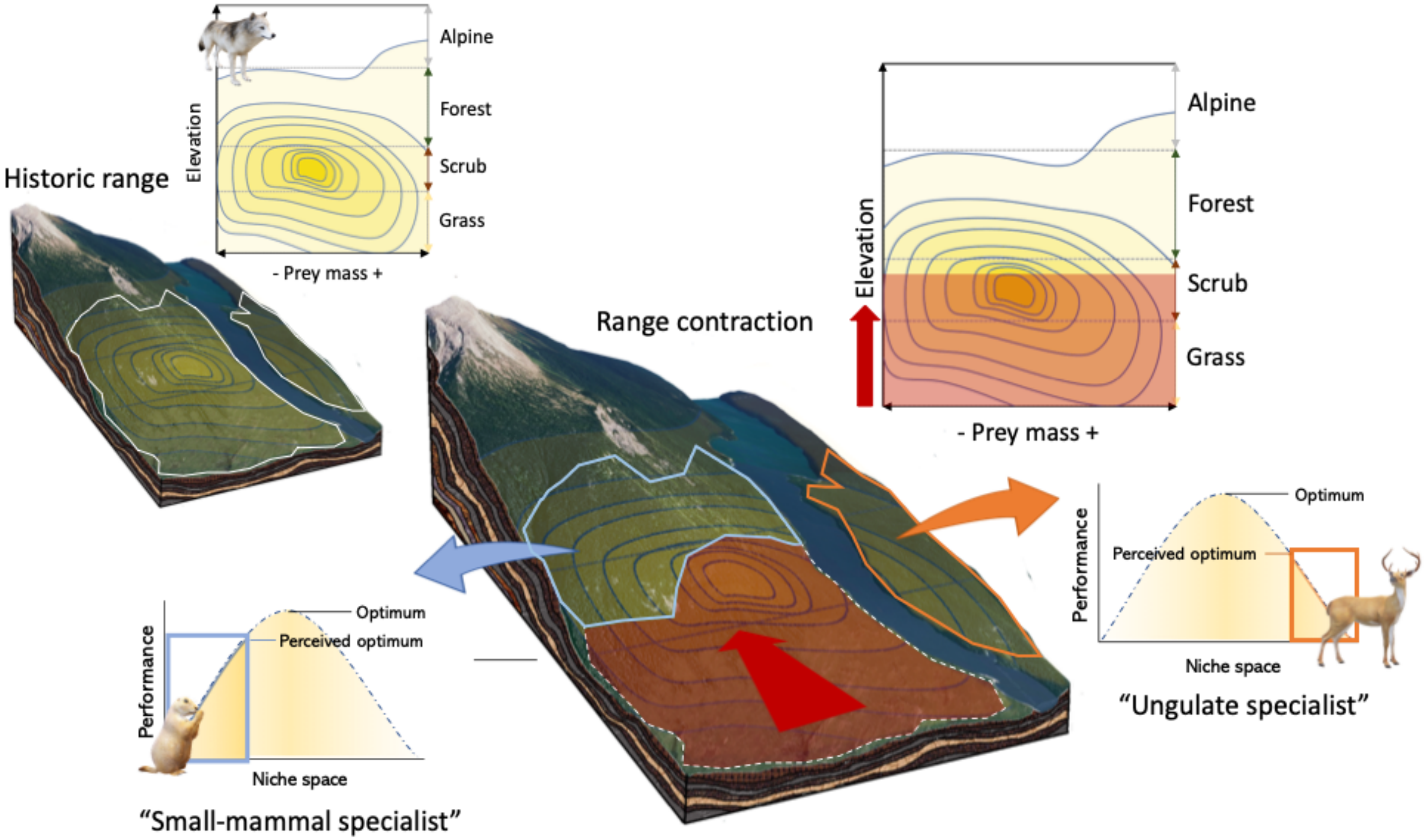


Figure 7.1) Formation of species stereotypes due to widespread range contraction. Historic range of a hypothetical carnivore (wolf) across a theoretical niche space based on environmental factors (elevation and prey mass). (Right) The hypothetical carnivore’s range contraction where species is excluded (red) from part of its range and corresponding niche space. Hence the carnivore is restricted to suboptimal range in forest and alpine areas at higher elevation. If research is conducted in the blue outlined area or orange outlined areas only, the carnivore could be stereotyped as a small-mammal specialist (blue) or ungulate specialist (orange). (Taken from Britnell *et al*, 2021)

7.2.3 Marginality and unreliable inferences from shifted models and niches

Anthropogenic pressures have shaped species distribution, niches, and population structure. These impacts are likely to increase with continuing habitat conversion and formation of novel habitats (Seddon et al., 2014). Descriptive, quantitative, or conceptual models that use data collected from a heavily modified system can produce unreliable inferences (Kerley & Monserrat*,* 2022). Models that do not account for historical shift and potential stereotypes are termed shifted models (Kerley & Monserrat, 2022). Shifted models are attempts to describe and model the ecology of a species (or system) using anthropogenically altered ecological parameters.

Within conservation biology, predictive modelling is often used as a tool to inform conservation initiatives. Currently, modelling practices assume species occupy their optimal environments (Braunisch et al., 2008). Ecologically marginalised populations (Cromsigt et al., 2012) and ecological traps (Titeux et al., 2020) invalidate this assumption. Furthermore, many models are built using current presence-only data, *i.e.* accounts of where the species currently resides (Phillips et al., 2009; von Takach et al., 2020). If historical data is incorporated into models, it can radically alter inferences. For example, in South African mammals, incorporating historical occurrence records to account for shifted models changed niche breadth and habitat suitability estimates of over a third of the species assessed (Monsarrat et al., 2019)

Incorporating historical data and demographic metrics from, or inferred from, contemporary datasets, can improve model accuracy (Howard et al., 2014; Monsarrat, et al., 2019; Veloz et al., 2015). Historical records such as fossil records (Bilney, 2014), museum collections and written accounts (Boshoff & Kerley, 2010; Boshoff et al., 2016) are available for many species across ecosystems. Nüchel and colleagues (2018) incorporated historic data to investigate range and niche contraction in Snub-nosed monkey species in genus *Rhinopithecus*. All *Rhinopithecus spp* are Endangered or Critically Endangered and Nüchel and colleagues (2018) reveal all species were displaced into higher elevation, colder and drier areas of their historic niche due to range contraction (Nüchel et al., 2018). Their ecological marginalisation to the extremes of their historic niche likely contributes to their extinction risk (Nüchel et al., 2018). Historical records are also used to estimate historic communities through co-occurrence (Bilney, 2014) although this is contested (Blanchet et al., 2020).

However, it is essential to consider that demographic or historical records may be absent or biased for some species (Monsarrat and Kerley, 2018; Monsarrat, Boshoff and Kerley, 2019). Demographic and historic data is often biased towards charismatic, culturally or economically important species (Monsarrat & Kerley, 2018) and towards more accessible areas (Monsarrat, Boshoff, et al., 2019). Therefore, it is crucial to account for potential biases in historic datasets to contextualise their effects on correlative ecological models (Monsarrat et al., 2019).

Furthermore, simply because animals are abundant in an area does not imply the area is optimal. As a species' population size and growth is determined by both habitat availability and quality (Griffen & Drake, 2008), a species occupying a sub-optimal habitat may still be abundant, provided the habitat is large enough (Kerley et al., 2012). There are many instances where species optima do not coincide to where they are most abundant, for example: transitory states, a heterogenous spatial structure of suitability, allee effects (Osorio-Olvera et al., 2019), asymmetric niche shapes, spatial variation in density dependence and alternative mechanistic drivers of population dynamics (Holt, 2020).

7.2.4 Marginality, Reintroductions and missed conservation opportunities

A vital question in current conservation thought is how we can re-establish and protect species throughout their (adequately estimated) historic ranges (Grace *et al.*, 2019). Large scale reintroduction programs are taking place to try and restore species throughout their historical range. However, a lack of appreciation of marginality and shifting baselines might lead to reintroducing a potentially viable population into a marginal area, and therefore reducing its feasibility of successful establishment. For example, the Stitchbird *(Notiomystis* *cincta)* was reintroduced into a predator-free island lacking adequate food sources for the species (Armstrong & Perrott, 2000; Armstrong et al., 2002). Other species which have already been reintroduced into marginal environments within their historic range include Cape mountain zebra (Lea *et al.*, 2016), Przewalski's horse (*Equus ferus przwalskii)* (van Dierendonck & Wallis De Vries, 1996), and the Hawaiian nēnē goose (*Branta sandvicensis*) (Black et al., 1997). However, translocation into ecologically marginal environments remains a threat to species recovery and establishment (Gippoliti et al., 2021). These threats become severe in Critically Endangered species with low population numbers.

Establishing historical baselines allows us to identify ecological marginalisation and investigate potential habitat types for assisted recolonisation and reintroduction (Grace et al., 2019). The Kakapo (*Strigops habroptilus*) is impacted by multiple anthropogenic threatening processes including habitat loss and predation from invasive species. It is recognised as a refugee species (Lentini et al., 2018). Fossil evidence demonstrated the species' preferred habitats may have included mountain beech, Hall's totara or broadleaf forest in areas with high rainfall and moderate winters (Lentini et al., 2018). Reintroduction into high quality areas increases the likelihood of successful establishment of the species and therefore increases efficiency of conservation efforts.

As stereotypes may underestimate species' potential to thrive in various communities and ecosystems, it is important to account for their possibility when planning reintroductions. If a relationship between two species is erroneously considered obligate, conservation efforts may be unnecessarily restricted to situations where the two species co-occur. For example, reintroduction efforts for the black-footed ferret (*Mustela nigripes)* were limited based on their assumed obligate associations with prairie dogs (*Cynomys* sp.). However, fossil evidence suggests this is not an obligate relationship, which increases the number of potential release sites (Owen et al., 2000)

As human impacts have changed a large proportion of habitats, conservation efforts continue to try and restore habitats to higher habitat quality. Lack of understanding of marginality might lead to ineffective or potentially deleterious restoration strategies. In contrast, incorporating a good understanding of marginality means that those systems can be manipulated to meet desired ecological conditions (Hobbs et al., 2014; Martinez-Abrain et al, 2018). Shifting baselines can cause restoration efforts to continually attempt to restore habitat to a poorer, or unattainable, state than it was historically (Duarte et al, 2009). Regardless of effective restoration, if a species is ecologically trapped it may choose to occupy the ecological trap over good quality habitat (Delibes et al., 2001; Hale & Swearer, 2016)

A vital contribution to improving reintroduction and restoration efforts is considering species demographic rates in “non-traditional” habitat types and communities. Currently, whether a species is introduced into a protected area or considered of conservation importance in a landscape may be due to its “nativeness” (Wallach et al., 2018). Perceptions of “traditional” habitat can be an artefact from recent human interference. They may change radically due to recolonisation following declines in human pressures (Silliman et al., 2018). In many instances, introductions of a non-native species can fill a missing functional role, improving the performance of many species in the area (Lundgren et al., 2018). Within contemporary landscapes, animals can perform better in many novel or human induced habitat types than in their “traditional” habitat types (Martínez-Abraín and Jiménez 2018; Martínez-Abraín & Jiménez, 2016). By emphasizing nativeness over performance or functionality, we may miss conservation opportunities. Although, these instances can be dismissed as invasive species or alien assemblages, a strict definition of a native species range has proved challenging to formalise (Pereyra, 2020).

7.2.5 Ecological marginality and protected area placement - preventing a protected area paradox

Protected areas may be ineffective and potentially detrimental when they are situated in marginal habitat (Kerley, Kowalczyk and Cromsigt, 2012). Ecologically marginalised populations may become protected when information about resource availability, habitat requirements or species assemblages are lost through shifting baseline syndrome (Pauly, 1995) (Figure 7.2). If historical data is not available, there can be limited ability to assess shifting baselines or species stereotypes leading to conservation complacency (Bilney et al., 2010; Bilney, 2014; Appendix 2). Conservation efforts then actively protect species in areas where they perform poorly due to *intrinsic* niche characteristics.

Confinement to marginal habitat results in populations with reduced reproduction and survival. As the birth rate and death rate are significant factors dictating the viability of a population, any small perturbation of a marginal habitat can lead to large variations in viability (Scheele *et al.*, 2017). Therefore, protected areas in marginal habitats would have reduce resilience to other threatening processes such as offtake from poaching, resource extraction, or climatic change (Kerley, Kowalczyk and Cromsigt, 2012; Scheele *et al.*, 2017). Whereas, in core environments minimal intensity pressure may not impact viability or performance. For example, ectotherms in high-altitude environments display slower growth rates and longer times to reach sexual maturity compared to ectotherms at low altitude (Morrison & Hero, 2003). Therefore, an equal intensity of threat, *i.e.* adult mortality, has a more significant impact on high-altitude ectotherms than lowland ectotherms of the same species causing greater extirpation rates of high-altitude populations (Muths et al., 2011)

In the case of larger species, they generally require large areas to persist (Ripple et al, 2015; Wolf & Ripple, 2016). These land requirements would be even greater within marginal environments to support functional populations. This larger area requirement in marginal habitats increases the susceptibility to fragmentation and the conservation challenges that affect small populations (Ripple et al., 2015). As such, the lack of engagement with marginality could lead to a protected area paradox whereby many small, unproductive and ineffective protected areas are formed that do not represent high-quality land or do not protect a sufficient amount of land for the species to persist in large numbers.

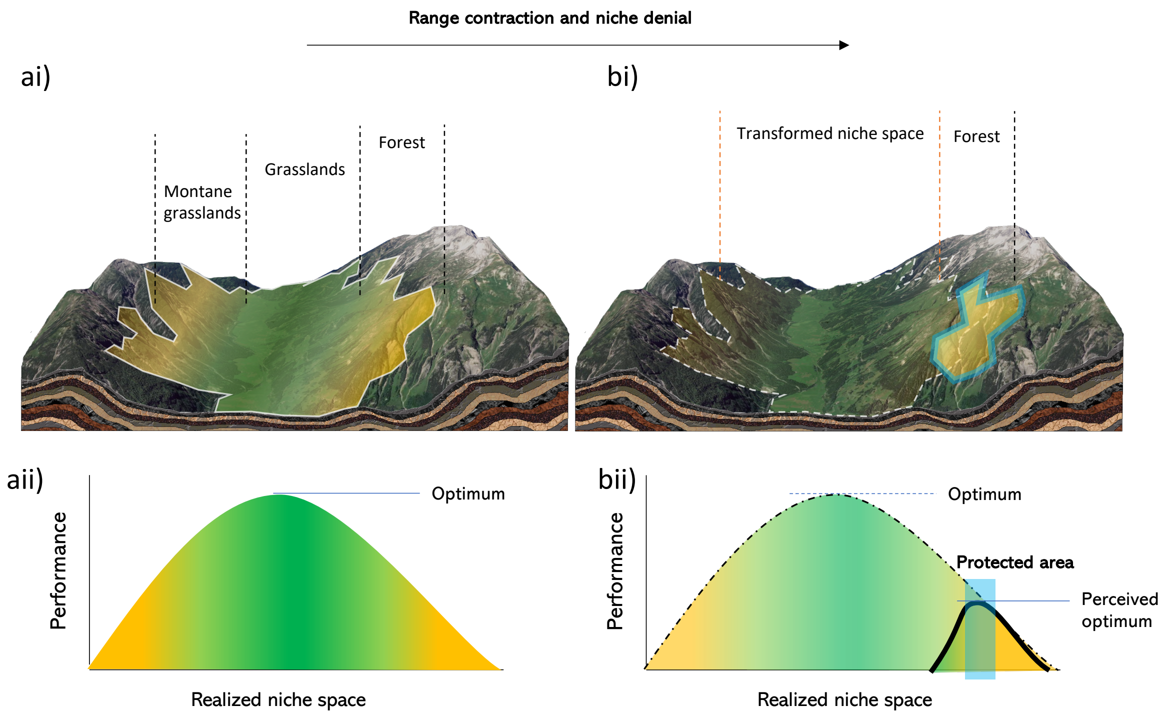
The current distribution of global protected areas is biased and unrepresentative. Many protected areas are situated where wild animals remained *i.e.* assumed “pristine” or “untouched” habitats (Joppa & Pfaff, 2009) or in “last remaining” habitats (Caughley, 1994). However, large-scale habitat transformation, widespread range contraction towards range limits (Channell et al., 2000; Chapter 3) and ecological marginalisation (Pineda-Munoz et al., 2021, Chapter 3) occurred in many species long before the establishment of protected areas. Protection plans do not adequately represent global niche conditions, with 93.1% of amphibians, 89.5% of birds, and 90.9% of terrestrial mammal species' potential niche space unprotected (Hanson et al., 2020). For many species, protected areas do not cover a sufficient proportion of their range to guarantee long-term survival (Venter et al., 2014). In some extreme cases these do not cover any of their contemporary range so called "gap species" (Rodrigues et al. 2004).

Although, many protected areas assist in preventing extinction (Watson et al., 2014), some continue to harbour populations in decline (Rada et al., 2019). These declines may be partially explained by protecting marginal niche conditions and sub-optimal portions of the species range (Kerley *et al.*, 2020). Areas of low human intensity are more likely to become protected areas, *i.e.,* high elevation areas, steeper slope, and poor accessibility (Joppa & Pfaff, 2009; Venter et al., 2018). As a result the establishment of protected areas is biased towards areas of low productivity (Luck, 2007).

As performance rates are impacted by habitat size and quality, the size of protected areas is another critical component. Protected area size is heavily dependent on productive capacity. The size of National parks in Africa and Australia strongly negatively correlates with population density (Ransom & Kaczensky, 2016) and human density and NPP, respectively (Luck, 2007). Highly productive habitats, which could promote high diversity and abundance, are often small and surrounded by dense human settlements (Luck, 2007). These high-productivity protected areas may be too small to maintain large functional populations. In contrast, the large parks, situated in low NPP areas, may be too marginal. These high-productivity small parks are also under greater threat of invasive species (Turbelin et al., 2017), novel conditions (Ellis, 2011), edge effects (Kiffner et al., 2013) and ecological traps (Battin, 2004) due to their proximity to human settlements. Therefore, when highly productive land is protected, fragments are usually small, isolated and prone to effects that reduce vital/performance rates.

Traditional views of improving protected areas revolve around expanding protected areas or restoring habitat connectivity between them. Although these strategies can be beneficial in many instances, they may not solve the marginality problem. If a habitat is in marginal conditions, increasing the size of parks may have a minimal effect on population size compared to protecting various habitat types. If a species is restricted to marginal areas across its' range, greater connection between them would still result in low demographic and performance rates compared to the high quality areas. Possible methods to counter this risk include experimental translocations into historic and potential habitat types (Seddon, 2010). This should be paired with a strictly experimental approach for testing under which conditions measures improve (e.g. Wilson et al., 2019). It is vital to note that translocation into parts of the historic range does not guarantee successful reestablishment.

In some cases, conservation efforts can misestimate the resilience of ecologically marginalised species within protected areas. For example, in Cape Mountain zebra (*Equus zebra zebra,* CMZ), most sub-populations are re-stocked from a single relict source population due to poor performance in other relict populations. Fencing confines many populations, including poorly performing relict populations, to grass-poor areas and others, including the source population, to grass-rich areas leading to considerable variation in fecundity, sub-population number and growth rates between populations (Lea et al., 2016). As a result, the sub-species number is increasing. At the same time, many populations remain small and isolated or declining (Lea et al., 2016) and genetic diversity of key relict populations continues to decline (Kotzé et al., 2019).



]

Figure 7.2) The formation of marginal protected areas due to shifting baselines and range contraction. ai) Untransformed landscape with free movement of individuals between optimal and marginal habitats. aii) Response curve of untransformed landscapes such that organisms occupy both optimal and marginal portions to their potential niche. Green is a source optimal population with high reproductive rate and high survivorship, yellow represents a marginal sink population of comparatively poor reproductive rate and low survivorship. bi) A transformed landscapes where only small proportion of marginal populations are protected and occupation of previous optimal habitat is prevented. bii) Corresponding response curve of transformed landscapes. Organisms have been pushed to occupy marginal habitats and. due to shifting baselines syndrome ,are now perceived to occupy high quality areas. Protected area may be formed in the best possible habitat available of these last remaining areas but are marginal compared to historically occupied areas. Protection takes place in sub-optimal area. The blue circles represent protected areas.

7.3 Incorporating marginality in conservation planning

Conservation planning and assessments can account for ecological marginality and refugee species by incorporating metrics or methods to evaluate: 1) mismatch with environment or anomalous resource use, 2) demographic variability, 3) niche shifts and 4) physiological challenge and 5) assessing species responses to heterogeneous landscapes

7.3.1 Comparative morphological, physiological and behavioural metrics across closely related species and ecologies

Comparing morphological, physiological or behavioural features of a species to close related species or species with similar ecologies can help identify mismatch with environment or anomalous resource use (Kerley, Kowalczyk and Cromsigt, 2012). Widespread range contraction has occurred in a relatively short evolutionary period, and morphology is slow to adapt to changing ecological pressures. Therefore, organisms can be pushed into areas with a mismatch between their morphology and their restricted niche. For example, the proportions of Hypsodont (high-crowned) teeth indicate feeding preferences and habitat selection in ungulates (Mendoza and Palmqvist, 2008). Using 134 species Mendoza and Palmqvist (2008) clustered hypsodonty metrics into open field feeders and closed canopy feeders. When plotted against other species, European Bison (*Bison bonasus*) and Lowland Anoa represent outliers (Mendoza and Palmqvist, 2008), confined to closed canopy as morphological open field feeders.

Assuming the function of the morphological or physiological trait is related to an ecologically important variable, traits can display convergence (Feilich & López-Fernández, 2019). This convergence can be used to compare morphological or physiological traits of multiple species to demonstrate an outlier. For example, Giant panda (*Ailuropoda melanoleuca*) exist only in montane bamboo forests (Loucks et al., 2001). They are believed to be specialist bamboo feeders (Kerley et al., 2020). However, the species historically occupied a wider ecological niche (Li et al., 2020). The macronutrient absorption rates (Nie et al., 2019), gastrointestinal morphology, microbiome composition and structure are more similar to carnivores than herbivores (Xue et al., 2015; Guo et al., 2018; Han et al., 2019). Modern dietary specialisation on bamboo could result from range contraction and marginalisation rather than an evolved preference (Kerley et al., 2020).

As undertaken in Chapters 4, 5 and 6, comparative physiological measures, between populations or closely related species, can also be used to assess anomalous resource use through time. Physiology is involved in shaping the limits of where organisms persist (Bozinovic et al., 2011). In the absence of dispersal limitation, animal distribution limits are associated with individuals' physiological limits (Parsons, 1990). As resources vary over time and space, ecological gradients are formed. The functional marginality hypothesis states that physiological responses should vary across ecological gradients, with marginal populations showing a 'less optimal' physiological response than high quality populations (Shultz et al., 2021, Appendix 1)

Ecological gradients can arise from spatial variation in resource availability. Evaluating physiological status across ecological gradients allows potential resource limitation to be identified. I demonstrate in Chapter 5 that Cape mountain zebra become heavily dependent on food items other than grass in their contemporary range. Although this may not constitute anomalous resource use, browse consumption may be sub-optimal for a species adapted to eating grass. Physiological markers have also been used in other species; Gobush et al, (2014) assessed the potential impacts of food limitation by measuring endocrinological levels of faecal thyroid hormone (fT3) and faecal glucocorticoid concentration (fGCMs) in Hawaiian monk seals (*Neomonachus schauinslandi*) across an ecological gradient. On the main Hawaiian Islands (MHI) the population was growing while the populations on the Northwestern Hawaiian Islands (NWHI) were in decline. Declining subpopulations exhibited elevation of fGCMs and reduced fT3. fGCMs were highest at a site in NWHI while fT3 was relatively low indicating possible food limitation in the population. High fGCMs were associated with poorer survival rates and lower intrinsic population growth rates (Gobush et al., 2014).

Environmental stochasticity results in temporal variation in resources. I demonstrate that Grevy's zebra (Chapter 6) become dependent on legumes during the dry season. Some populations were dependent throughout the study period. Areas across a species range may vary inter-annually in their ability to sustain large populations. As protected areas are geographically static, evaluating how populations respond to environmental changes within PAs may be an essential indicator of habitat suitability. For example, Wessling et al*,* 2018 compared dehydration, energetic status, and glucocorticoid levels of Chimpanzees (*Pan troglodytes*) due to seasonal variation across two populations: Fongoli, a savannah-mosaic habitat at the margins of the range and Taï National Park, a lowland rainforest. Both populations displayed range limits due to dehydration in the dry season, but Fongoli Chimpanzees demonstrated elevated cortisol levels, potentially implying a more considerable seasonal cost and poorer resilience to water limitation (Wessling et al., 2018).

7.3.2 Establishing historical baselines, historical niche space and shifts

Understanding range contraction and historical niche space is key to assessing the magnitude of niche loss, shift, and conservation success (Grace et al., 2019). To supplement, fossil records, museum collections and written accounts, the historical ecology of the species can be investigated *via* stable isotope analysis.

Stable isotope analysis is a powerful technique to describe the ecological niches of contemporary and historical populations. Variance in stable isotope ratios in animal tissues or bones compares trophic niches among species (Bearhop et al., 2004). An “isotopic niche,” a proxy of the population’s ecological niche, can provide quantitative information on resource and habitat use (Newsome et al., 2007). Comparing isotopic niches through time (historic vs. contemporary) can assess shifts in resource and habitat use within and between species (Jackson et al., 2011). For example, before extinction in the wild, Przewalski’s horse (*Equus ferus przewalskii)* was heavily hunted. Hunting marginalized the species, pushing them to adopt a marginal grass-browse diet in the winter (Kaczensky et al., 2017). A sympatric species, Khulan (*Equus hemionus hemionus*), which were not persecuted, display a minimal shift in diet across seasons (Kaczensky et al., 2017).

Importantly, assessing whether adequate historic information on the species is available to define historic baselines and niches is crucial to prevent the formation of species stereotypes. Actions plans would benefit from inclusion of a *bias* assessment to evaluate how at risk a species is for bias formation (Britnell *et* al, 2021; Figure 7.3; Appendix 2)

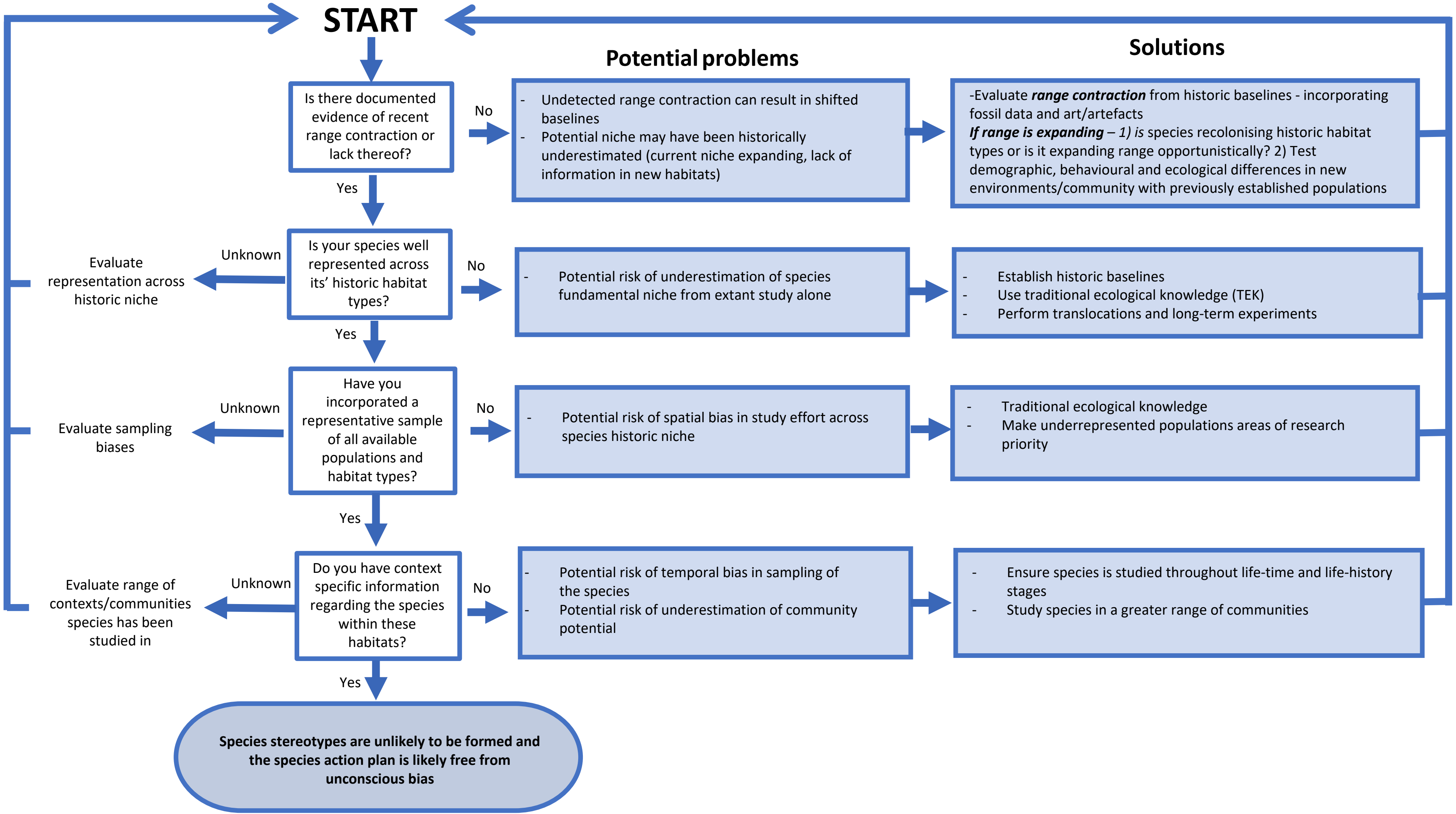


Figure 7.3) Flowchart to incorporate into action plans to detect potential species stereotypes or bias in our current understanding of species.

7.3.3 Demographic metrics for protected area success and species assessments

Increasing PA coverage alone cannot guarantee the prevention of extinction or long-term species resilience. We must move from targets based on coverage to assessing alternative performance metrics for protected areas by incorporating fine-scale comparative demographic metrics as a standard of protected area management. Currently, data on birth, death, immigration and emigration rates, required to establish source-sink status comprehensively, is lacking in conservation and ecological research (Furrer & Pasinelli, 2016; Heinrichs et al., 2019). This lack of data remains a neglected problem in many areas despite its potential benefit for conservation (Gilroy & Edwards, 2017).

Wildlife abundances and population trends, such as density and population changes, can be used as key metrics of protected area effectiveness (Kiffner et al., 2020). However, the lack of or infrequency of wildlife monitoring in many ecosystems means these metrics are sparsely applied (Barnes et al., 2016; Caro, 2016). Wildlife monitoring is currently biased towards high-income countries (Moussy et al., 2022). Therefore, areas with high biomass and diversity in developing countries are underrepresented. Demographic data is sample-intensive and can be challenging to collect over wide spatial scales (Conde et al., 2019). Promisingly, in spite of this, large datasets are increasingly available (Salguero-Gómez et al., 2015, 2016).

7.3.4 Species responses to heterogeneous landscapes

The simplest species response curves assume that fitness proxies decline linearly across an ecological gradient. This assumption leads to a gradual uniform decline in fitness from the highest densities in the centre of a range to the lowest at the periphery (Brown, 1984; Guo et al., 2005; Figure 7.4). Although easy to model, a Gaussian response curve may not accurately reflect species tolerances, especially near range limits (Sagarin et al., 2006). Species and populations can show varying resilience to ecological gradients with some displaying large fitness shifts due to small changes in ecological space while others may be more resilient (Sagarin et al., 2006) (Figure 7.4). Organisms may have a narrow set of ecological conditions across a limited geographical space which promote high performance *i.e.* an skewed niche (Figure 7.4). Therefore, carrying capacity and performance rates may be higher in a very narrow range of conditions. In this case, most protected areas will likely be situated in marginal conditions. Alternatively, a species may be more ecologically flexible and perform well across ecological gradients (Figure 7.4). In this instance, a species may be more resilience to protected area placement and perform well throughout multiple habitats.

If response curves are evaluated and part of conservation assessment, species niches can be better assessed. For example, species with a broad range of habitats in which they perform well – a “response curve generalist”, would benefit from large geographic areas which can encompass a high proportion of their niche breadth and diversity. Alternatively, a species which displays a specialist response curve and declines sharply with small changes in environmental factors will need a more precise protected area placement (Figure 7.4). These response curves may reflect species physiological tolerance limits (Lee et al., 2009) or patterns of dispersal limitation (Sagarin et al., 2006). Depending on a species’ resilience to ecological gradients and species intrinsic responses to environmental change, PA efficacy may be driven mainly by coverage or habitat quality (Figure 7.4). Despite the potential importance for conservation (Fahrig et al., 2022), the impacts of PA size and quality are usually assessed in experimental systems. Moreover, they are rarely applied to large bodied species of conservation concern or applied to heterogenous landscapes or species’ ranges.

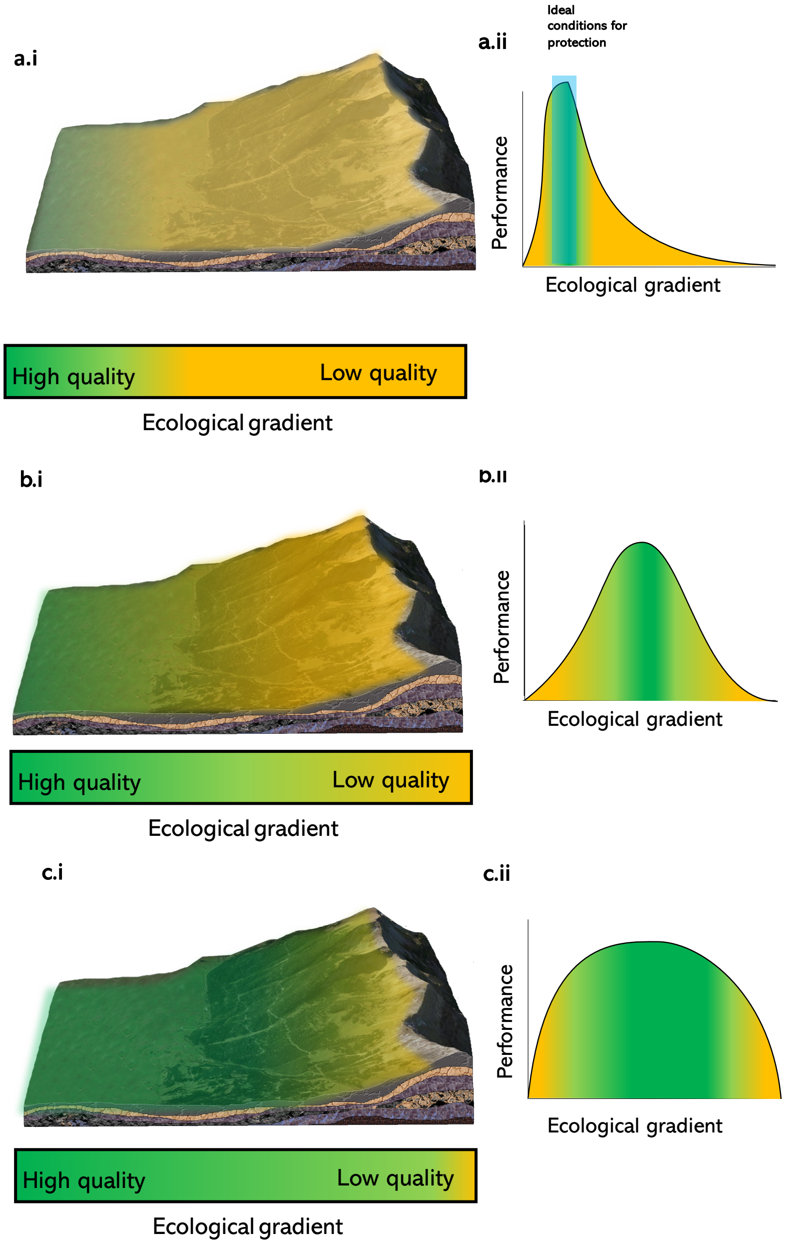


Figure 7.4) Species responses and fitness may decline at different rates as species approach range or ecological limits. ai) The distributional range of habitat available to a hypothetical specialist species with very specialised conditions being optimal , aii) Corresponding species response curve of the hypothetical specialist species where a small change in environmental space leads to a large performance decline and the assumed optimal conditions for protection , bi) The distributional range of habitat available to a hypothetical non-specialist species with a normal distribution response curve model. Bii) The corresponding species response curve of the hypothetical non-specialist species where a performance decline declines linearly towards ecological or geographic edges. ci) The distributional range of habitat available to a generalist species, cii) Corresponding species response curve of the hypothetical generalist species where performance only declines when species is very close to ecological limit.

7.4 Study limitations and improving methodology

7.4.1 Marginality and the use of multiple validated biomarkers to understand population health

Using physiological biomarkers at large scales can help identify vulnerable or declining populations by reference to better performing populations. In Chapter 4, for example, I demonstrate lower CMZ GC levels are associated with higher foal:mare ratio (fecundity). However, as stated in chapter 4, single marker studies can give an incomplete picture of individual condition and population health. Stressors such as resource limitation, social conflict, predation pressure and disease risk can act independently or in combination causing additive, synergistic or antagonistic effects (Beldomenico & Begon, 2010; Todgham & Stillman, 2013). Instead of overanalysing single biomarkers, we could expand the suite of biomarkers used to assess the interplay and impact stressors have on multiple physiological processes.

Thyroid hormone (TH) plays a major role in regulating growth, development, and metabolism (McAninch and Bianco, 2014). TH is the key regulator of metabolic rate within animals (Mullur et al., 2014). Under stable physiological conditions, the hypothalamic-pituitary-thyroid axis maintains stable TH levels and, therefore stable levels of energy homeostasis (Andersen et al., 2003). However, during fasting or times of calorie restriction or deficit, energy expenditure decreases and as such there is a fall in circulating TH levels (Rosenbaum et al., 2005; Vella et al., 2011). Calorie restriction has also been linked to reproductive suppression (Moatt *et al.*, 2016). Levels of T3, a thyroid hormone metabolite found in faeces, correlate with field metabolic rate of equids (Brinkmann *et al.*, 2016). Therefore, T3 provides a non-invasive method to evaluate changes in energy expenditure, potentially attributable to calorie restriction, which may help to elucidate the causes of reproductive failure or suppression.

Although Chapter 5 showed increased parasite burdens are associated with poor fecundity, we do not know the physiological mechanism. Additional biomarkers such as faecal immunoglobulins, may be used to estimate the immunological effect of nemabiome composition and parasite burden (Watt et al., 2016; Sparks et al., 2018), which in turn correlate with survival

7.5 Further work

7.5.1 Marginality and the mechanistic causes of poor fecundity in CMZ

The mechanistic link between habitat quality and reproductive suppression is not well understood. However, variation in habitat quality is a growing problem worldwide and is key to population persistence and extinction risk (Griffen & Norelli, 2015). Many explanations have been suggested for poor fecundity in equids including sperm quality (Van Buiten *et al.*, 1999; Van Eldik *et al.*, 2006), miscarriage and pregnancy rates (van Buiten, Remmen and Colenbrander, 1998; Lucas *et al*, 1991) and reduced juvenile survival (Gray et al., 2012; Smith et al., 2008). In Cape mountain zebra populations, juvenile survival rates are as high as ~80% (Smith *et al.*, 2008). Although, infanticide does occur in cape mountain zebra (Britnell, Vorster, et al., 2021, Appendix 3) , it is uncertain as to the extent it influences population fluctuations. Previous work on CMZ has identified reduced female fecundity rather than survival as the limiting factor of population growth (Lea et al., 2016).

Sperm quality is related to inbreeding in equids (Van Eldik *et al.*, 2006) and has been a suggested mechanism of reproductive failure. However, populations of low genetic diversity do not always exhibit poor population performance (Moodley & Harley, 2005). Currently, the Cation channel sperm (CatSper) genes which play an important role in hyperactivation of sperm during fertilisation are under investigation in Cape mountain zebra (Smith et al., 2021). Mutations in these genes lead to reduced fertility and even infertility.

Poor quality habitat could be reducing the pregnancy or parturition rates. Pregnancy rate is impacted by habitat quality (Joly et al., 2015; Wasser et al., 2017). Foaling rates are more variable in the equid family than many other vertebrate families including *suidae* and *bovidae* (van Buiten, Remmen and Colenbrander, 1998). Within feral horses, ~50% of reproductively unsuccessful years are attributed to foetal loss after 120 days of gestation suggesting that after mid-term miscarriage is a common phenomenon within the equid family (Lucas *et al*, 1991).

7.5.2 Marginality and Social Behaviour

Environmental characteristics should influence individual behaviours and social behaviour such as aggression rates, infanticide rates and social cohesion (Meise et al., 2019; Møller, 2004). In ecologically marginal populations, demographic stochasticity may occur due to small population size leading to biased sex ratios. In equids, biased sex ratios could exacerbate male harassment towards females and foals (Linklater et al., 1999), increasing female mortality and rates of infanticide. This could act as a positive feedback loop, further increasing female mortality, exacerbating the sex ratio imbalance. If the population becomes female-dominated, female infanticide rates (female killing of foals) could also increase as offspring compete for resources.

It is disputed whether infanticide plays an important role in the population dynamics of wild equids (Gray et al., 2012). A long-term study of wild plains zebra (*Equus quagga*) found no evidence that infanticide take place in wild conditions (Vitet et al., 2021). However, infanticide does occur in Cape mountain zebra in wild conditions (see Appendix 3, Britnell, Vorster, et al., 2021) and can be relatively frequent. Within three years (2018–2020) in Sanbona Wildlife Reserve, 5 of 24 foals (approx. 21%) were found dead with infanticide consistent injuries (Britnell, Vorster, et al., 2021, Appendix 3). Although populations in this study did not have biased sex ratios, one was food-limited, with individuals relying heavily on artificial supplementary food sources.

Furthermore, marginality may influence group cohesion and population stability and structure through resource limitation (Rubenstein et al., 2015; Meise et al., 2019). Male-biased sex ratios could exacerbate competition for females, increase turnover events, increase the frequency and intensity of harassment from bachelor groups and increase the risk of male infanticide. Population structuring also has consequences for maintaining genetic diversity and disease/pathogen spread. Ecological marginality, therefore, may have vital consequences for behaviour of individuals, population structuring and reveal a link between population performance and environmental variables.

7.5.3 Marginality, species composition and species interactions

In this thesis, I only evaluated single species and did not account for interspecific interactions in marginal habitats. Marginal habitats may have severe consequences for interspecific interactions. Firstly, marginality may structure food webs and dietary ecology. Increased resource limitation may increase overlap in dietary items between species increasing interspecific competition. Although species display niche separation in many studies, few studies have evaluated niche separation across ecological gradients or during times of resource limitation. As herbivore numbers and density are reduced in marginal habitats, predators may experience increased intraspecific competition and attempt to minimise this by specialising in specific prey items (Svanbäck & Bolnick, 2005, 2007).

Secondly, ecological marginality may structure abundance and diversity throughout trophic chains. Animal diversity and abundance rely on sufficient biomass at lower trophic chains to maintain population sizes sufficiently large to survive perturbation (Huston, 2005). As energy is lost with each trophic level, the biomass and abundance of large animals at the higher trophic levels is limited by the energy in lower levels (Wright, 1983). Consequently, the total biomass of herbivores that can be supported is dependent on the availability and biomass of palatable plants. The total biomass of carnivores will be dependent on the availability and biomass of herbivores. This energetic limitation means that the largest populations of animals throughout trophic levels, and potentially, the largest number of animal species, may be dependent on the environmental conditions relating to primary productivity (Huston, 2005) and the productive capacity of the landscape following human use (Huston, 1993). Hence, ecologically marginalised populations may act as an early warning system of extinction risk. Within ecological networks, extinction of poorly performing species are usually preceded by the extinction of other species (Säterberg et al., 2013). Once vital rates, such as survival, decrease there can be loss of ecological functions leading to habitat shift (e.g. Ali et al., 2017) and widespread extinctions due to loss of obligate relationships (Brodie et al., 2014) . This is particularly problematic for long-lived large-bodied species which can only experience small changes in mortality or abundance before becoming functionally extinct (Säterberg et al., 2013).

7.6 Conclusion

This thesis has explored the importance of ecological marginalisation at three scales: globally, across a species range and across an ecological gradient. At each scale, I have demonstrated that ecological marginality influences species performance or physiology. Ecological marginalisation is a common consequence of range contraction and increases extinction risk across terrestrial mammals. Secondly, I have shown ecological marginalisation impacts the species physiology of two equid species and have revealed potential proximate drivers of poor performance in the Cape mountain zebra. I have argued that ecological marginalisation is an unappreciated and widespread conservation threat that is not accounted for in contemporary conservation planning. I have discussed ways to incorporate marginalisation into conservation assessments and outlined the potential deleterious consequences if it is ignored. Marginalisation, especially ecological marginalisation, is key to understanding species distributions and range dynamics and in contextualising their application to conservation science. Marginalisation must be seen as a hitherto unrecognised and widespread phenomenon that will play a critical role in forming effective conservation initiatives.

7.7 References

Ali, A. H., Ford, A. T., Evans, J. S., Mallon, D. P., Hayes, M. M., King, J., Amin, R., & Goheen, J. R. (2017). Resource selection and landscape change reveal mechanisms suppressing population recovery for the world’s most endangered antelope. *Journal of Applied Ecology*, *54*(6), 1720–1729.

Ali, A. H., Kauffman, M. J., Amin, R., Kibara, A., King, J., Mallon, D. P., Musyoki, C., & Goheen, J. R. (2018). Demographic drivers of a refugee species: Large-scale experiments guide strategies for reintroductions of hirola. *Ecological Applications*, *28*(2), 275–283.

Andersen, S., Bruun, N. H., Pedersen, K. M., & Laurberg, P. (2003). Biologic Variation is Important for Interpretation of Thyroid Function Tests. *Thyroid*, *13*(11), 1069–1078.

Armstrong, D. P., Davidson, R. S., Dimond, W. J., Perrott, J. K., Castro, I., Ewen, J. G., Griffiths, R., Taylor, J., Journal, S., Issue, S., Biotas, I., & Jun, M. (2002). Population Dynamics of Reintroduced Forest Birds on New Zealand Islands. *Journal of Biogeography*, *29*(5), 609–621.

Armstrong, D. P., & Perrott, J. K. (2000). An experiment testing whether condition and survival are limited by food supply in a reintroduced Hihi population. *Conservation Biology*, *14*(4), 1171–1181.

Barnes, M. D., Craigie, I. D., Harrison, L. B., Geldmann, J., Collen, B., Whitmee, S., Balmford, A., Burgess, N. D., Brooks, T., Hockings, M., & Woodley, S. (2016). Wildlife population trends in protected areas predicted by national socio-economic metrics and body size. *Nature Communications 7*(1), 1–9.

Battin, J. (2004). When good animals love bad habitats: Ecological traps and the conservation of animal populations. *Conservation Biology*, *18*(6), 1482–1491.

Bearhop, S., Adams, C. E., Waldron, S., Fuller, R. A., & Macleod, H. (2004). Determining trophic niche width: a novel approach using stable isotope analysis. *Journal of Animal Ecology*, *73*(5), 1007–1012.

Beldomenico, P. M., & Begon, M. (2010). Disease spread, susceptibility and infection intensity: vicious circles? *Trends in Ecology and Evolution*, *25*(1), 21–27.

Bilney, R. J. (2014). Poor historical data drive conservation complacency: The case of mammal decline in south-eastern Australian forests. *Austral Ecology*, *39*(8), 875–886.

Bilney, R. J., Cooke, R., & White, J. G. (2010). Underestimated and severe: Small mammal decline from the forests of south-eastern Australia since European settlement, as revealed by a top-order predator. *Biological Conservation*, *143*(1), 52–59.

Black, J. M., Marshall, A. P., Gilburn, A., Santos, N., Hoshide, H., Medeiros, J., Mello, J., Hodges, C. N., & Katahira, L. (1997). Survival, Movements, and Breeding of Released Hawaiian Geese: An Assessment of the Reintroduction Program. *The Journal of Wildlife Management*, *61*(4), 1161.

Blanchet, F. G., Cazelles, K., & Gravel, D. (2020). Co-occurrence is not evidence of ecological interactions. *Ecology Letters*, *23*(7), 1050–1063.

Boshoff, A. F., & Kerley, G. I. H. (2010). Historical mammal distribution data: How reliable are written records? *South African Journal of Science*, *106*(1–2), 26–33.

Boshoff, A., Landman, M., & Kerley, G. (2016). Filling the gaps on the maps: Historical distribution patterns of some larger mammals in part of southern Africa. *Transactions of the Royal Society of South Africa*, *71*(1), 23–87.

Bozinovic, F., Calosi, P., & Spicer, J. I. (2011). Physiological Correlates of Geographic Range in Animals. *Annual Review of Ecology, Evolution and Systematics*, *42*. 155-179

Braunisch, V., Bollmann, K., Graf, R. F., & Hirzel, A. H. (2008). Living on the edge—Modelling habitat suitability for species at the edge of their fundamental niche. *Ecological Modelling*, *214*(2–4), 153–167.

Brinkmann, L., Gerken, M., Hambly, C., Speakman, J. R., & Riek, A. (2016). Thyroid hormones correlate with field metabolic rate in ponies, Equus ferus caballus. *Journal of Experimental Biology*, *219*(16), 2559-2566.

Britnell, J. A., Lewis, R. N., Elsner-Gearing, F., Harvey, N., Stanbrook, E., & Shultz, S. (2021). Species stereotypes as a result of unconscious research biases compromise conservation efficacy. *Biological Conservation*, *261*, 109275.

Britnell, J. A., Vorster, L., & Shultz, S. (2021). Evidence of infanticide in the Cape mountain zebra (*Equus zebra zebra*). *Behaviour*, *158*(11), 1043–1055.

Brodie, J. F., Aslan, C. E., Rogers, H. S., Redford, K. H., Maron, J. L., Bronstein, J. L., & Groves, C. R. (2014). Secondary extinctions of biodiversity. *Trends in Ecology and Evolution 29(*12), 664–672.

Brown, J. H. (1984). On the Relationship between Abundance and Distribution of Species. *The American Naturalist*, *124*(2), 255–279.

Caro, T., (2016). Guidelines for wildlife monitoring: savannah herbivores. *Tropical Conservation Science*, *9*(1), 1-15.

Channell, R., Lomolino, M. v., & Lomollno, M. v. (2000). Dynamic biogeography and conservation of endangered species. *Nature*, *403*(6765), 84–86.

Conde, D. A., Staerk, J., Colchero, F., da Silva, R., Schöley, J., Maria Baden, H., Jouvet, L., Fa, J. E., Syed, H., Jongejans, E., Meiri, S., Gaillard, J. M., Chamberlain, S., Wilcken, J., Jones, O. R., Dahlgren, J. P., Steiner, U. K., Bland, L. M., Gomez-Mestre, I., … Vaupel, J. W. (2019). Data gaps and opportunities for comparative and conservation biology. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(19), 9658–9664.

Cromsigt, J. P. G. M., Kerley, G. I. H., & Kowalczyk, R. (2012). The difficulty of using species distribution modelling for the conservation of refugee species - the example of European bison. *Diversity and Distributions*, *18*(12), 1253–1257.

Delibes, M., Gaona, P., & Ferreras, P. (2001). Effects of an attractive sink leading into maladaptive habitat selection. *American Naturalist*, *158*(3), 277–285.

dos Santos, J. W., Correia, R. A., Malhado, A. C., Campos-Silva, J. V., Teles, D., Jepson, P., & Ladle, R. J. (2020). Drivers of taxonomic bias in conservation research: a global analysis of terrestrial mammals. *Animal Conservation*, *23*(6), 679-688.

Doughty, C. E., Roman, J., Faurby, S., Wolf, A., Haque, A., Bakker, E. S., Malhi, Y., Dunning, J. B., & Svenning, J. C. (2016). Global nutrient transport in a world of giants. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(4), 868–873.

Duarte, C. M., Conley, D. J., Carstensen, J., & Sánchez-Camacho, M. (2009). Return to Neverland: shifting baselines affect eutrophication restoration targets. *Estuaries and Coasts*, *32*(1), 29-36.

Ellis, E. C. (2011). Anthropogenic transformation of the terrestrial biosphere. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, *369*(1938), 1010–1035.

Ellis, E. C., Gauthier, N., Goldewijk, K. K., Bird, R. B., Boivin, N., Díaz, S., Fuller, D. Q., Gill, J. L., Kaplan, J. O., Kingston, N., Locke, H., McMichael, C. N. H., Ranco, D., Rick, T. C., Rebecca Shaw, M., Stephens, L., Svenning, J. C., & Watson, J. E. M. (2021). People have shaped most of terrestrial nature for at least 12,000 years. *Proceedings of the National Academy of Sciences of the United States of America*, *118*(17),

Ezenwa, V. O., Stefan Ekernas, L., & Creel, S. (2012). Unravelling complex associations between testosterone and parasite infection in the wild. *Functional Ecology*, *26*(1), 123–133.

Fahrig, L., Watling, J. I., Arnillas, C. A., Arroyo-Rodríguez, V., Jörger-Hickfang, T., Müller, J., Pereira, H. M., Riva, F., Rösch, V., Seibold, S., Tscharntke, T., & May, F. (2022). Resolving the SLOSS dilemma for biodiversity conservation: a research agenda. *Biological Reviews*, *97*(1), 99–114.

Feilich, K. L., & López-Fernández, H. (2019). When Does Form Reflect Function? Acknowledging and Supporting Ecomorphological Assumptions. *Integrative and Comparative Biology*, *59*(2), 358–370.

Flueck, W. T. (2021). Functional limb anatomy in a refugee species: The endangered Patagonian huemul deer (*Hippocamelus bisulcus*). *Anatomia, Histologia, Embryologia*, *50*(2), 411–416.

Furrer, R. D., & Pasinelli, G. (2016). Empirical evidence for source-sink populations: a review on occurrence, assessments and implications. In *Biological reviews of the Cambridge Philosophical Society 91*(3) 782–795.

Gilroy, J. J., & Edwards, D. P. (2017). Source-Sink Dynamics: a Neglected Problem for Landscape-Scale Biodiversity Conservation in the Tropics. *Current Landscape Ecology Reports*, *2*(1), 51–60.

Gippoliti, S., Robovský, J., & Angelici, F. M. (2021). Taxonomy and Translocations of African Mammals: A Plea for a Cautionary Approach. *Conservation 1*(2), 121–136.

Gobush, K. S., Booth, R. K., & Wasser, S. K. (2014). Validation and application of noninvasive glucocorticoid and thyroid hormone measures in free-ranging Hawaiian monk seals. *General and Comparative Endocrinology*, *195*, 174–182.

Grace, M., Resit Akçakaya, H., Bennett, E., Hilton-Taylor, C., Long, B., Milner-Gulland, E. J., Young, R., & Hoffmann, M. (2019). Using historical and palaeoecological data to inform ambitious species recovery targets. In *Philosophical Transactions of the Royal Society B: Biological Sciences 374*(1788) p. 20190297.

Gray, M. E., Cameron, E. Z., Peacock, M. M., Thain, D. S., & Kirchoff, V. S. (2012). Are low infidelity rates in feral horses due to infanticide? *Behavioral Ecology and Sociobiology*, *66*(4), 529–537.

Griffen, B. D., & Drake, J. M. (2008). Effects of habitat quality and size on extinction in experimental populations. *Proceedings of the Royal Society B: Biological Sciences*, *275*(1648), 2251–2256.

Griffen, B. D., & Norelli, A. P. (2015). Spatially variable habitat quality contributes to within-population variation in reproductive success. *Ecology and Evolution*, *5*(7), 1474–1483.

Guo, Q., Taper, M., Schoenberger, M., & Brandle, J. (2005). Spatial-temporal population dynamics across species range: from centre to margin. *Oikos*, *108*(1), 47–57.

Guo, W., Mishra, S., Zhao, J., Tang, J., Zeng, B., Kong, F., Ning, R., Li, M., Zhang, H., Zeng, Y., Tian, Y., Zhong, Y., Luo, H., Liu, Y., Yang, J., Yang, M., Zhang, M., Li, Y., Ni, Q., … Li, Y. (2018). Metagenomic study suggests that the gut microbiota of the giant panda

(Ailuropoda melanoleuca) may not be specialized for fiber fermentation. *Frontiers in Microbiology*, *9*, 229.

Hale, R., & Swearer, S. E. (2016). *Ecological traps: current evidence and future directions*. *283*(1824), 20152647.

Han, H., Wei, W., Hu, Y., Nie, Y., Ji, X., Yan, L., Zhang, Z., Shi, X., Zhu, L., Luo, Y., Chen, W., & Wei, F. (2019). Diet Evolution and Habitat Contraction of Giant Pandas via Stable Isotope Analysis. *Current Biology*, *29*(4), 664-669.e2.

Hanson, J. O., Rhodes, J. R., Butchart, S. H. M., Buchanan, G. M., Rondinini, C., Ficetola, G. F., & Fuller, R. A. (2020). Global conservation of species' niches. *Nature*, *580*(7802), 232–234.

Heinrichs, J. A., Walker, L. E., Lawler, J. J., Schumaker, N. H., Monroe, K. C., & Bleisch, A. D. (2019). Recent Advances and Current Challenges in Applying Source-Sink Theory to Species Conservation. *Current Landscape Ecology Reports*, *4*(3), 51–60.

Hobbs, R. J., Higgs, E., Hall, C. M., Bridgewater, P., Chapin, F. S., Ellis, E. C., Ewel, J. J., Hallett, L. M., Harris, J., Hulvey, K. B., Jackson, S. T., Kennedy, P. L., Kueffer, C., Lach, L., Lantz, T. C., Lugo, A. E., Mascaro, J., Murphy, S. D., Nelson, C. R., … Yung, L. (2014). Managing the whole landscape: Historical, hybrid, and novel ecosystems. *Frontiers in Ecology and the Environment*, *12*(10), 557–564.

Holt, R. D. (2020). Reflections on niches and numbers. *Ecography*, *43*(3), 387–390.

Howard, C., Stephens, P. A., Pearce-Higgins, J. W., Gregory, R. D., & Willis, S. G. (2014). Improving species distribution models: The value of data on abundance. *Methods in Ecology and Evolution*, *5*(6), 506–513.

Huston, M. (1993). Biological Diversity, Soils, and Economics. *Science*, *262*(5140), 1676–1680.

Huston, M. A. (2005). The three phases of land-use change: Implications for biodiversity. In *Ecological Applications 15*(6), 1864–1878.

Jackson, A. L., Inger, R., Parnell, A. C., & Bearhop, S. (2011). Comparing isotopic niche widths among and within communities: SIBER – Stable Isotope Bayesian Ellipses in R. *Journal of Animal Ecology*, *80*(3), 595–602.

Joly, K., Wasser, S. K., & Booth, R. (2015). Non-Invasive Assessment of the Interrelationships of Diet, Pregnancy Rate, Group Composition, and Physiological and Nutritional Stress of Barren-Ground Caribou in Late Winter. *PloS One*, *10*(6).

Joppa, L. N., & Pfaff, A. (2009). High and far: Biases in the location of protected areas. *PLoS ONE*, *4*(12), e8273.

Kaczensky, P., Burnik Šturm, M., Sablin, M. v., Voigt, C. C., Smith, S., Ganbaatar, O., Balint, B., Walzer, C., & Spasskaya, N. N. (2017). Stable isotopes reveal diet shift from pre-extinction to reintroduced Przewalski's horses. *Scientific Reports*, *7*(1), 1–9.

Kahn Jr, P. H., & Friedman, B. (1995). Environmental views and values of children in an inner-city black community. *Child development*, *66*(5), 1403-1417.

Kerley, G. I. H., Beest, M., Cromsigt, J. P. G. M., Pauly, D., & Shultz, S. (2020). The Protected Area Paradox and refugee species: The giant panda and baselines shifted towards conserving species in marginal habitats. *Conservation Science and Practice*, *2*(5).

Kerley, G. I. H., Kowalczyk, R., & Cromsigt, J. P. G. M. (2012). Conservation implications of the refugee species concept and the European bison: King of the forest or refugee in a marginal habitat? *Ecography*, *35*(6), 519–529.

Kerley G. I. H. & Monsarrat S., Shifted models cannot be used for predicting responses of biodiversity to global change: the African elephant as an example. (2022) *African Zoology.*

Kiffner, C., Binzen, G., Cunningham, L., Jones, M., Spruiell, F., & Kioko, J. (2020). Wildlife population trends as indicators of protected area effectiveness in northern Tanzania. *Ecological Indicators*, *110*, 105903.

Kiffner, C., Stoner, C., & Caro, T. (2013). Edge effects and large mammal distributions in a national park. *Animal Conservation*, *16*(1), 97–107.

Kotzé, A., Smith, R. M., Moodley, Y., Luikart, G., Birss, C., van Wyk, A. M., Grobler, J. P., & Dalton, D. L. (2019). Lessons for conservation management: Monitoring temporal changes in genetic diversity of Cape mountain zebra (*Equus zebra zebra*). *PLOS ONE*, *14*(7), e0220331.

Lea, J. M., Kerley, G. I., Hrabar, H., Barry, T. J., & Shultz, S. (2016). Recognition and management of ecological refugees: A case study of the Cape mountain zebra. *Biological Conservation*, *203*, 207–215.

Lee, J. E., Janion, C., Marais, E., van Vuuren, B. J., & Chown, S. L. (2009). Physiological tolerances account for range limits and abundance structure in an invasive slug. *Proceedings of the Royal Society B: Biological Sciences*, *276*(1661), 1459.

Lentini, P. E., Stirnemann, I. A., Stojanovic, D., Worthy, T. H., & Stein, J. A. (2018). Using fossil records to inform reintroduction of the kakapo as a refugee species. *Biological Conservation*, *217*, 157–165.

Li, S., McShea, W. J., Wang, D., Gu, X., Zhang, X., Zhang, L., & Shen, X. (2020). Retreat of large carnivores across the giant panda distribution range. *Nature Ecology and Evolution*, *4*(10), 1327–1331.

Linklater, W. L., Cameron, E. Z., Minot, E. O., & Stafford, K. J. (1999). Stallion harassment and the mating system of horses. *Animal Behaviour*, *58*(2), 295–306.

Littlewood, N. A., Rocha, R., Smith, R. K., Martin, P. A., Lockhart, S. L., Schoonover, R. F., Wilman, E., Bladon, A. J., Sainsbury, K. A., Pimm, S., & Sutherland, W. J. (2020). Terrestrial Mammal Conservation. *Terrestrial Mammal Conservation*. Open Book Publishers.

Loucks, C. J., Lu, Z., Dinerstein, E., Wang, H., Olson, D. M., Zhu, C., & Wang, D. (2001). Giant pandas in a changing landscape. *Science 294*(5546), 1465.

Lucas, Z., Raeside, J. I., & Betteridge, K. J. (1991). Non-invasive assessment of the incidences of pregnancy and pregnancy loss in the feral horses of Sable Island. *Journal of Reproduction and Fertility. 44*, 479-488.

Luck, G. W. (2007). The relationships between net primary productivity, human population density and species conservation. *Journal of Biogeography*, *34*(2), 201–212.

Lundgren, E. J., Ramp, D., Ripple, W. J., & Wallach, A. D. (2018). Introduced megafauna are rewilding the Anthropocene. *Ecography*, *41*(6), 857–866.

Lundgren, E. J., Ramp, D., Stromberg, J. C., Wu, J., Nieto, N. C., Sluk, M., Moeller, K. T., & Wallach, A. D. (2021). Equids engineer desert water availability. *Science*, *372*(6541), 491–495.

Lundgren, E. J., Ramp, D., Wu, J., Sluk, M., Moeller, K. T., Stromberg, J. C., & Wallach, A. D. (2021). Response. *Science*, *373*(6558), 973–974.

Martin, L. J., Blossey, B., & Ellis, E. (2012). Mapping where ecologists work: Biases in the global distribution of terrestrial ecological observations. *Frontiers in Ecology and the Environment 10*(4), 195–201).

Martínez-Abraín, A., & Galán, P. (2018). A test of the substitution–habitat hypothesis in amphibians. *Conservation Biology*, *32*(3), 725-730.

Martínez-Abraín, A., & Jiménez, J. (2016). Anthropogenic areas as incidental substitutes for original habitat. *Conservation Biology*, *30*(3), 593–598.

Martínez-Abraín, A., Jiménez, J., & Oro, D. (2019). Pax Romana: 'refuge abandonment' and spread of fearless behavior in a reconciling world. *Animal Conservation 22(*1),3–13.

McAninch, E. A., & Bianco, A. C. (2014). Thyroid hormone signaling in energy homeostasis and energy metabolism. *Annals of the New York Academy of Sciences*, *1311*(1), 77-87.

Mcgeoch, M. A., & Latombe, G. (2016). Characterizing common and range expanding species. *Journal of Biogeography*, *43*(2), 217–228.

Meise, K., Franks, D. W., & Bro-Jørgensen, J. (2019). Using social network analysis of mixed-species groups in African savannah herbivores to assess how community structure responds to environmental change. *Philosophical Transactions of the Royal Society B*, *374*(1781).

Møller, A. P. (2004). Rapid temporal change in frequency of infanticide in a passerine bird associated with change in population density and body condition. *Behavioral Ecology*, *15*(3), 462–468.

Moatt, J. P., Nakagawa, S., Lagisz, M., & Walling, C. A. (2016). The effect of dietary restriction on reproduction: a meta-analytic perspective. *BMC Evolutionary Biology*, *16*(1), 1-9.

Monsarrat, S., Boshoff, A. F., & Kerley, G. I. H. (2019). Accessibility maps as a tool to predict sampling bias in historical biodiversity occurrence records. *Ecography*, *42*(1), 125–136.

Monsarrat, S., Jarvie, S., & Svenning, J.C. C. (2019). Anthropocene refugia: Integrating history and predictive modelling to assess the space available for biodiversity in a human-dominated world. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *374*(1788),

Monsarrat, S., & Kerley, G. I. H. (2018). Charismatic species of the past: Biases in reporting of large mammals in historical written sources. *Biological Conservation*, *223*, 68–75.

Monsarrat, S., Novellie, P., Rushworth, I., & Kerley, G. (2019). Shifted distribution baselines: neglecting long-term biodiversity records risks overlooking potentially suitable habitat for conservation management. *Philosophical Transactions of the Royal Society B*,

Moodley, Y., & Harley, E. H. (2005). Population structuring in mountain zebras (Equus zebra): The molecular consequences of divergent demographic histories. *Conservation Genetics*, *6*(6), 953–968.

Moolman, L., Ferreira, S. M., Gaylard, A., Zimmerman, D., & Kerley, G. I. H. (2019). The decline of the Knysna elephants: Pattern and hypotheses. *South African Journal of Science*, *115*(3/4), 1–7.

Morrison, C., & Hero, J. M. (2003). Geographic variation in life-history characteristics of amphibians: A review. *Journal of Animal Ecology*, *72*(2), 270–279.

Moussy, C., Burfield, I. J., Stephenson, P. J., Newton, A. F. E., Butchart, S. H. M., Sutherland, W. J., Gregory, R. D., McRae, L., Bubb, P., Roesler, I., Ursino, C., Wu, Y., Retief, E. F., Udin, J. S., Urazaliyev, R., Sánchez-Clavijo, L. M., Lartey, E., & Donald, P. F. (2022). A quantitative global review of species population monitoring. *Conservation Biology*, *36*(1), e13721.

Mullur, R., Liu, Y. Y., & Brent, G. A. (2014). Thyroid hormone regulation of metabolism. *Physiological reviews*, *94*(2), 355-382.

Muths, E., Scherer, R. D., & Pilliod, D. S. (2011). Compensatory effects of recruitment and survival when amphibian populations are perturbed by disease. *Journal of Applied Ecology*, *48*(4), 873–879.

Newsome, S. D., Rio, C. M. del, Bearhop, S., & Phillips, D. L. (2007). A niche for isotopic ecology. *Frontiers in Ecology and the Environment*, *5*(8), 429–436.

Nie, Y., Wei, F., Zhou, W., Hu, Y., Senior, A. M., Wu, Q., Yan, L., & Raubenheimer, D. (2019). Giant Pandas Are Macronutritional Carnivores. *Current Biology*, *29*(10), 1677-1682.e2.

Nüchel, J., Bøcher, P. K., Xiao, W., Zhu, A. X., & Svenning, J. C. (2018). Snub-nosed monkeys (Rhinopithecus): potential distribution and its implication for conservation. *Biodiversity and Conservation*, *27*(6), 1517–1538.

O’Dwyer, K., Dargent, F., Forbes, M. R., & Koprivnikar, J. (2020). Parasite infection leads to widespread glucocorticoid hormone increases in vertebrate hosts: A meta-analysis. *Journal of Animal Ecology*, *89*(2), 519–529.

Osorio-Olvera, L., Soberón, J., & Falconi, M. (2019). On population abundance and niche structure. *Ecography*, *42*(8), 1415–1425.

Owen, P. R., Bell, C. J., & Mead, E. M. (2000). Fossils, diet, and conservation of black-footed ferrets (*Mustela Nigripes*). *Journal of Mammalogy*, *81*(2), 422–433.

Parsons, P. A. (1990). The metabolic cost of multiple environmental stresses: Implications for climatic change and conservation. *Trends in Ecology & Evolution*, *5*(9), 315–317.

Pauly, D. (1995). Anecdotes and the shifting baseline syndrome of fisheries. *Trends in Ecology & Evolution 10*(10) 430.

Pellissier, L., Albouy, C., Bascompte, J., Farwig, N., Graham, C., Loreau, M., Maglianesi, M. A., Melián, C. J., Pitteloud, C., Roslin, T., Rohr, R., Saavedra, S., Thuiller, W., Woodward, G., Zimmermann, N. E., & Gravel, D. (2018). Comparing species interaction networks along environmental gradients. *Biological Reviews*, *93*(2), 785–800.

Pereyra, P. J. (2020). Rethinking the native range concept. *Conservation Biology*, *34*(2), 373–377.

Phillips, S. J., Dudík, M., Elith, J., Graham, C. H., Lehmann, A., Leathwick, J., & Ferrier, S. (2009). Sample selection bias and presence-only distribution models: implications for background and pseudo-absence data. *Ecological Applications*, *19*(1), 181–197.

Pineda-Munoz, S., Wang, Y., Lyons, S. K., Tóth, A. B., & McGuire, J. L. (2021). Mammal species occupy different climates following the expansion of human impacts. *Proceedings of the National Academy of Sciences*, *118*(2).

Plumb, G., Kowalczyk, R. & Hernandez-Blanco, J.A. (2020). *Bison bonasus*. *The IUCN Red List of Threatened Species* 2020: e.T2814A45156279.

Rada, S., Schweiger, O., Harpke, A., Kühn, E., Kuras, T., Settele, J., & Musche, M. (2019). Protected areas do not mitigate biodiversity declines: A case study on butterflies. *Diversity and Distributions*, *25*(2), 217–224.

Ransom, J. I., & Kaczensky, P. (Eds.). (2016). *Wild equids: Ecology, management, and conservation*. JHU Press.

Ripple, W. J., Newsome, T. M., Wolf, C., Dirzo, R., Everatt, K. T., Galetti, M., ... & Van Valkenburgh, B. (2015). Collapse of the world’s largest herbivores. *Science advances*, *1*(4), e1400103.

Robles, H., & Martin, K. (2014). Habitat-mediated variation in the importance of ecosystem engineers for secondary cavity nesters in a nest web. *PLoS ONE*, *9*(2).

Rodrigues, A. S., Andelman, S. J., Bakarr, M. I., Boitani, L., Brooks, T. M., Cowling, R. M., ... & Yan, X. (2004). Effectiveness of the global protected area network in representing species diversity. *Nature*, *428*(6983), 640-643.

Rosenbaum, M., Goldsmith, R., Bloomfield, D., Magnano, A., Weimer, L., Heymsfield, S., Gallagher, D., Mayer, L., Murphy, E., & Leibel, R. L. (2005). Low-dose leptin reverses skeletal muscle, autonomic, and neuroendocrine adaptations to maintenance of reduced weight. *Journal of Clinical Investigation*, *115*(12), 3579–3586.

Rubenstein, D. I., Sundaresan, S. R., Fischhoff, I. R., Tantipathananandh, C., & Berger-Wolf, T. Y. (2015). Similar but different: Dynamic social network analysis highlights fundamental differences between the fission-fusion societies of two equid species, the onager and Grevy's zebra. *PLoS ONE*, *10*(10).

Rubin, E. S., Conrad, D., Jones, A. S., & Hervert, J. J. (2021). Feral equids' varied effects on ecosystems. *Science*, *373*(6558), 973.

Sagarin, R. D., Gaines, S. D., & Gaylord, B. (2006). Moving beyond assumptions to understand abundance distributions across the ranges of species. *Trends in Ecology & Evolution*, *21*(9), 524–530.

Salguero-Gómez, R., Jones, O. R., Archer, C. R., Bein, C., de Buhr, H., Farack, C., Gottschalk, F., Hartmann, A., Henning, A., Hoppe, G., Römer, G., Ruoff, T., Sommer, V., Wille, J., Voigt, J., Zeh, S., Vieregg, D., Buckley, Y. M., Che-Castaldo, J., … Vaupel, J. W. (2016). COMADRE: A global data base of animal demography. *Journal of Animal Ecology*, *85*(2), 371–384.

Salguero-Gómez, R., Jones, O. R., Archer, C. R., Buckley, Y. M., Che-Castaldo, J., Caswell, H., Hodgson, D., Scheuerlein, A., Conde, D. A., Brinks, E., de Buhr, H., Farack, C., Gottschalk, F., Hartmann, A., Henning, A., Hoppe, G., Römer, G., Runge, J., Ruoff, T., … Vaupel, J. W. (2015). The compadre Plant Matrix Database: An open online repository for plant demography. *Journal of Ecology*, *103*(1), 202–218.

Säterberg, T., Sellman, S., & Ebenman, B. (2013). High frequency of functional extinctions in ecological networks. *Nature*, *499*(7459), 468–470.

Scheele, B. C., Foster, C. N., Banks, S. C., & Lindenmayer, D. B. (2017). Niche Contractions in Declining Species: Mechanisms and Consequences. *Trends in Ecology and Evolution*, *32*(5), 346–355.

Seddon, P. J. (2010). From Reintroduction to Assisted Colonization: Moving along the Conservation Translocation Spectrum. *Restoration Ecology*, *18*(6), 796–802.

Seddon, P. J., Griffiths, C. J., Soorae, P. S., & Armstrong, D. P. (2014). Reversing defaunation: restoring species in a changing world. *Science*, *345*(6195), 406-412.

Shultz, S., Britnell, J. A., & Harvey, N. (2021). Untapped potential of physiology, behaviour and immune markers to predict range dynamics and marginality. *Ecology and Evolution*, *11*(23), 16446–16461.

Silliman, B. R., Hughes, B. B., Gaskins, L. C., He, Q., Tinker, M. T., Read, A., Nifong, J., & Stepp, R. (2018). Are the ghosts of nature's past haunting ecology today? *Current Biology*, *28*(9), R532–R537.

Smith, R. K., Marais, A., Chadwick, P., Lloyd, P. H., & Hill, R. A. (2008). Monitoring and management of the endangered Cape mountain zebra Equus zebra zebra in the Western Cape, South Africa. *African Journal of Ecology*, *46*(2), 207–213.

Smith, R. M., Kotzé, A., Grobler, J. P., & Dalton, D. L. (2021). Identifying sequence variation in cation channel sperm associated genes in Cape mountain zebra (*Equus zebra zebra*). *South African Journal of Animal Science*, *50*(6), 807–813.

Soga, M., & Gaston, K. J. (2018). Shifting baseline syndrome: causes, consequences, and implications. *Frontiers in Ecology and the Environment 16*(4), 222–230.

Sparks, A. M., Watt, K., Sinclair, R., Pilkington, J. G., Pemberton, J. M., Johnston, S. E., McNeilly, T. N., & Nussey, D. H. (2018). Natural selection on antihelminth antibodies in a wild mammal population. *American Naturalist*, *192*(6), 745–760.

Stamps, J. A. (2011). Density bias in behavioral ecology. *Behavioral Ecology*, *22*(2), 231-232.

Svanbäck, R., & Bolnick, D. I. (2005). Intraspecific competition affects the strength of individual specialization: An optimal diet theory method. *Evolutionary Ecology Research*, *7*(7), 993–1012.

Svanbäck, R., & Bolnick, D. I. (2007). Intraspecific competition drives increased resource use diversity within a natural population. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1611), 839–844.

Takemoto, K., & Kajihara, K. (2016). Human Impacts and Climate Change Influence Nestedness and Modularity in Food-Web and Mutualistic Networks. *PLOS ONE*, *11*(6), e0157929.

Titeux, N., Aizpurua, O., Hollander, F. A., Sardà-Palomera, F., Hermoso, V., Paquet, J. Y., Mestdagh, X., Settele, J., Brotons, L., & van Dyck, H. (2020). Ecological traps and species distribution models: a challenge for prioritizing areas of conservation importance. *Ecography*, *43*(3), 365–375.

Titley, M. A., Snaddon, J. L., & Turner, E. C. (2017). Scientific research on animal biodiversity is systematically biased towards vertebrates and temperate regions. *PLoS ONE*, *12*(12).

Todgham, A. E., & Stillman, J. H. (2013). Physiological responses to shifts in multiple environmental stressors: Relevance in a changing world. *Integrative and Comparative Biology*, *53*(4), 539–544.

Turbelin, A. J., Malamud, B. D., & Francis, R. A. (2017). Mapping the global state of invasive alien species: patterns of invasion and policy responses. *Global Ecology and Biogeography*, *26*(1), 78–92.

Van Buiten, A., Remmen, J. L. A. M., & Colenbrander, B. (1998). Fertility of Shetland pony stallions used in different breeding systems: a retrospective study. *Veterinary quarterly*, *20*(3), 100-103.

Van Buiten, A., Van den Broek, J., Schukken, Y. H., & Colenbrander, B. (1999). Validation of non-return rate as a parameter for stallion fertility. *Livestock production science*, *60*(1), 13-19.

van Dierendonck, M. C., & Wallis De Vries, M. F. (1996). Ungulate Experiences with Reintroductions : the Takhi or Przewalski Horse (*Equus ferus przewalskii*) in Mongolia. *Conservation Biology*, *10*(3), 728–740.

Van Eldik, P., Van Der Waaij, E. H., Ducro, B., Kooper, A. W., Stout, T. A. E., & Colenbrander, B. (2006). Possible negative effects of inbreeding on semen quality in Shetland pony stallions. *Theriogenology*, *65*(6), 1159-1170.

Vella, K. R., Ramadoss, P., Lam, F. S., Harris, J. C., Ye, F. D., Same, P. D., O'Neill, N. F., Maratos-Flier, E., & Hollenberg, A. N. (2011). NPY and MC4R signaling regulate thyroid hormone levels during fasting through both central and peripheral pathways. *Cell Metabolism*, *14*(6), 780–790.

Veloz, S., Salas, L., Altman, B., Alexander, J., Jongsomjit, D., Elliott, N., & Ballard, G. (2015). Improving effectiveness of systematic conservation planning with density data. *Conservation Biology*, *29*(4), 1217–1227.

Venter, O., Fuller, R. A., Segan, D. B., Carwardine, J., Brooks, T., Butchart, S. H., ... & Watson, J. E. (2014). Targeting global protected area expansion for imperiled biodiversity. *PLoS biology*, *12*(6), e1001891.

Venter, O., Magrach, A., Outram, N., Klein, C. J., Possingham, H. P., Marco, M. di, Watson, J. E. M., di Marco, M., & Watson, J. E. M. (2018). Bias in protected-area location and its effects on long-term aspirations of biodiversity conventions. *Conservation Biology*, *32*(1), 127–134.

Vitet, C., Duncan, P., Ganswindt, A., Mabika, C., & Chamaillé-Jammes, S. (2021). Do infanticides occur in harem-forming equids? A test with long-term sociodemographic data in wild plains zebras. *Animal Behaviour*, *177*, 9–18.

von Takach, B., Scheele, B. C., Moore, H., Murphy, B. P., & Banks, S. C. (2020). Patterns of niche contraction identify vital refuge areas for declining mammals. *Diversity and Distributions*, *26*(11), 1467–1482.

Wallach, A. D., Lundgren, E. J., Ripple, W. J., & Ramp, D. (2018). Invisible megafauna. *Conservation Biology*, *32*(4), 962–965.

Wasser, S. K., Lundin, J. I., Ayres, K., Seely, E., Giles, D., Balcomb, K., Hempelmann, J., Parsons, K., & Booth, R. (2017). Population growth is limited by nutritional impacts on pregnancy success in endangered Southern Resident killer whales (*Orcinus orca*). *PLOS ONE*, *12*(6), e0179824.

Watson, J. E. M., Dudley, N., Segan, D. B., & Hockings, M. (2014). The performance and potential of protected areas. *Nature 515*(7525), 67–73.

Watt, K. A., Nussey, D. H., Maclellan, R., Pilkington, J. G., & McNeilly, T. N. (2016). Fecal antibody levels as a noninvasive method for measuring immunity to gastrointestinal nematodes in ecological studies. *Ecology and Evolution*, *6*(1), 56–67.

Wessling, E. G., Deschner, T., Mundry, R., Pruetz, J. D., Wittig, R. M., & Kühl, H. S. (2018). Seasonal Variation in Physiology Challenges the Notion of Chimpanzees (*Pan troglodytes verus*) as a Forest-Adapted Species. *Frontier in Ecology and Evolution 6*, 1–21.

Wilson, E. C., Shipley, A. A., Zuckerberg, B., Peery, M. Z., & Pauli, J. N. (2019). An experimental translocation identifies habitat features that buffer camouflage mismatch in snowshoe hares. *Conservation Letters 12*(2) e12614.

Wolf, C., & Ripple, W. J. (2016). Prey depletion as a threat to the world ' s large carnivores. *Royal Society Open Science*, *3*(8), 160252.

Wolf, C., & Ripple, W. J. (2017). Range contractions of the world's large carnivores. *Royal Society Open Science*, *4*(7), 170052.

Wright, D. H. (1983). Species-Energy Theory: An Extension of Species-Area Theory. *Oikos*, *41*(3), 496.

Xue, Z., Zhang, W., Wang, L., Hou, R., Zhang, M., Fei, L., Zhang, X., Huang, H., Bridgewater, L. C., Jiang, Y., Jiang, C., Zhao, L., Pang, X., & Zhang, Z. (2015). The bamboo-eating giant panda harbors a carnivore-like gut microbiota, with excessive seasonal variations. *MBio*, *6*(3), 1–12.

Appendix 1) Untapped potential of physiology, behaviour and immune markers to predict range dynamics and marginality

Published in Ecology and Evolution as Shultz, S., Britnell, J. A., & Harvey, N. (2021). Untapped potential of physiology, behaviour and immune markers to predict range dynamics and marginality. *Ecology and evolution*, *11*(23), 16446-16461.

Received: 4 March 2021 | Revised: 9 September 2021 | Accepted: 14 October 2021

DOI: 10.1002/ece3.8331

REVIEW ARTICLE

Ecology and Evolution Open Access WILEY

Untapped potential of physiology, behaviour and immune markers to predict range dynamics and marginality

Susanne Shultz[1] | **Jake A. Britnell**[1,2] | **Nicholas Harvey**[1,2]

[1]School of Earth and Environmental Sciences, University of Manchester, Manchester, UK

[2]Chester Zoo, Upton-By-Chester, UK

Correspondence
Susanne Shultz, Department of Earth and Environmental Sciences, University of Manchester, Manchester M13 9PT, UK.
Email: susanne.shultz@manchester.ac.uk

Funding information
Royal Society, Grant/Award Number: UF160725; Natural Environment Research Council, Grant/Award Number: NE/L002469/1

Abstract

Linking environmental conditions to the modulators of individual fitness is necessary to predict long-term population dynamics, viability, and resilience. Functional physiological, behavioral, and reproductive markers can provide this mechanistic insight into how individuals perceive physiological, psychological, chemical, and physical environmental challenges through physiological and behavioral responses that are fitness proxies. We propose a *Functional Marginality* framework where relative changes in allostatic load, reproductive health, and behavior can be scaled up to evidence and establish causation of macroecological processes such as local extirpation, colonization, population dynamics, and range dynamics. To fully exploit functional traits, we need to move beyond single biomarker studies to develop an integrative approach that models the interactions between extrinsic challenges, physiological, and behavioral pathways and their modulators. In addition to providing mechanistic markers of range dynamics, this approach can also serve as a valuable conservation tool for evaluating individual- and population-level health, predicting responses to future environmental change and measuring the impact of interventions. We highlight specific studies that have used complementary biomarkers to link extrinsic challenges to population performance. These frameworks of integrated biomarkers have untapped potential to identify causes of decline, predict future changes, and mitigate against future biodiversity loss.

KEYWORDS

conservation, endocrinology, glucocorticoids, gut health, macrophysiology, microbiome, social networks, thyroid hormone

1 | INTRODUCTION

One in five vertebrate species is classified by the IUCN as vulnerable, endangered, or critically endangered (Hoffmann et al., 2010). These declines and losses are largely attributed to anthropocentric changes in the environment such as land conversion, climate change, and unsustainable natural resource harvesting and extraction (Brook et al., 2008). Ultimately, range contraction and biodiversity loss are the end product of extrinsic or intrinsic challenges leading to population decline, emigration, and local extirpation. Across a species' range, populations occur

---

along ecological gradients from optimal, or central, habitats, where conditions and resources lead to high population density or maximal reproduction and survival, to marginal habitats where population density, reproduction, and/or survival are much lower (Holt, 2009; Kawecki, 2008). Identifying and mitigating the causes of reduced reproduction, compromised survivorship, and emigration are key for predicting and arresting biodiversity loss (Chown & Gaston, 2008).

The simplest species models assume that fitness follows a unimodal distribution with high density and growth rates in the center of a range and low density or poorly performing marginal populations found at the range periphery (Guo et al., 2005). However, environmental characteristics and species' responses are much patchier than this, such that geographic and ecological marginality are not equivalent (Pironon et al., 2017; Santini et al., 2019). Variation in carrying capacity across environmental gradients can lead to high-density "source" populations producing emigrants that disperse to low-density "sinks" in marginal habitats (Pulliam & Danielson, 1991); however, if all populations reach carrying capacity, dispersal is likely to be balanced between high- and low-density populations (Fretwell, 1969, 1972). In natural populations, however, environmental and demographic stochasticity result in dynamic reproduction, survival, and dispersal rates (Holt, 2003), which can cause low-density populations to be less resilient, with higher rates of local extirpation and recolonization, than high-density populations. Moreover, environmental change can either increase carrying capacity, leading to population growth and colonization, or result in decreased carrying capacity, population decline, local extirpation, and range contraction (Gaillard et al., 2000). Where ecological conditions are extreme for a species, local extirpation occurs faster than recolonization, limiting viable ranges. Range contraction occurs where previously resilient populations become unviable as growth and immigration rates no longer sustain the population. Identifying and predicting these dynamics in marginal populations provides key insight into long-term dynamics.

Responses to environmental change can be predicted using climate envelope, population viability, and mechanistic distribution models. Climate envelope, or habitat suitability, models relate species occurrence to environmental variables to explain or predict species distribution (Pearson & Dawson, 2003) and can predict occupancy changes under different environmental scenarios. While they are widely applicable because they require limited information, climate envelope models have limited capacity to predict local occupancy change as they do not incorporate population specific dynamics, carrying capacities, species interactions, or dispersal potential. Climate envelope models based solely on occupancy are often poor predictors of habitat suitability and patterns of population abundance across ranges (Osorio-Olvera et al., 2019). Population viability models, by contrast, can reliably predict future trends for specific populations (Brook et al., 2000) but require accurate vital rates, which are labor- and time-intensive to collect. Thus, their ability to predict resilience and viability across taxa and at large scales is limited. A middle ground is combining range and population dynamics for large-scale assessments of occupancy based on factors associated with local resilience or vulnerability. Mechanistic population and distribution models can provide this link between local population viability and range dynamics (Kearney & Porter, 2009) by using key behavioral and ecophysiological factors as functional indicators of resilience. Such models are more widely applicable than population viability analyses, are more robust, can be extrapolated to other populations, and have more predictive value than climate envelope models. They can also provide rapid and large-scale population assessments of marginal habitats to produce spatially explicit, predictive distribution maps across ecological gradients.

Interpreting the relevance of functional indicators across ecological gradients requires understanding their relationship with population responses (Bonier et al., 2009). Here, we advocate a *Functional Marginality* framework using physiological and behavioral indicators to assess population resilience. First, we describe functional physiological and behavioral indicators in the context of key stressors and explain methods to incorporate multiple indicators in predictive models. Second, we describe how functional marginality can be used to identify predictive hypotheses for occupancy changes, range dynamics, responses to environmental change, and evaluate the efficacy of management interventions. Although we primarily focus on mammals, this approach could be applied to many other taxa.

1.1 | Functional indicators

Functional traits are morphological, physiological, or behavioral traits that are fitness proxies via their effects on growth, reproduction, and survival (Violle et al., 2007), and indicate how a species perceives and responds to its environment (McGill et al., 2006). Positive functional indicators include relaxed time budgets, positive energy balance, and surplus energy stores manifesting in good body condition, good reproductive performance, and sound immune function or low disease burden. Negative indicators are those associated with a decline in condition in response to four types of challenges or stressors: physiological, psychological, chemical, and physical (Pottinger, 2003). Physiological stressors include resource, nutrient, or water restriction and disease. Psychological stressors include conflict, predation risk, and disturbance or persecution. Chemical stressors include altered pH, low dissolved oxygen, and exposure to pollutants, contaminants, or toxins. Finally, physical stressors encompass climate extremes and substrate as well as damage incurred by predation, conflict, or injury. Each class of stressor is associated with characteristic physiological and behavioral responses tied to pathways that maintain homeostasis (Madliger et al., 2018). Here, we discuss how physiological, psychological, chemical, and physical stressors can be manifest in physiological and behavioral indicators.

1.1.1 | Physiological stressors

Energetic and metabolic stress

Fitness is inextricably tied to maintaining sufficient energy reserves to support metabolism, invest in reproduction, and allow individuals a buffer during periods of scarcity or in response to challenges or disease (Burger et al., 2019). Responses to stressors often incur an energetic cost, which can compromise reproduction or growth (Christiansen et al., 2013). In response to a decline in resource availability an organism can change its behavior to increase energy availability by increasing foraging rate, feeding time, or travel distances, before mobilizing energy reserves or down regulating metabolism (Reneerkens et al., 2002). Thus, environmental change due either to climate or land use can have direct impacts on resource availability and seasonality. Behavioral changes can indicate energy budget challenges. For example, animals can adjust time budgets to spend more time traveling and feeding, and less time resting, to meet energy needs (Dunbar et al., 2009). Changes in habitat use or diet can also indicate energetic stress. For example, browsers becoming more dependent on grazing (Landman et al., 2013), or conversely grazers becoming more reliant on browse (Faith, 2012) suggesting a forced shift from preferred foods. The extent of temporary seasonal switching versus prolonged dependence on less preferred "fallback foods" can indicate significant resource stress in marginal habitats (Grueter et al., 2009). This is especially true when animals are pushed from an optimal diet to consume items that they are not physiologically adapted to handle (Ingala et al., 2019; Kitaysky et al., 2006). Thus, changes in the dynamics of seasonal and prolonged dietary shifts within and between populations could be used as a proxy for energetic stress. For terrestrial vertebrates, in addition to food limitations, water stress caused by abstraction or seasonality can lead to changes in space use, increased aggregations, and distance traveled.

Energy stress is also manifest in physiological responses. The hypothalamus–pituitary–thyroid axis (HPT) regulates metabolic rate by changing the amount of circulating thyroid hormone in response to metabolic requirements and responds to both thermal stress and food availability (Costa-e-Sousa & Hollenberg, 2012). Thyroid hormones and metabolic rate measures can identify how quickly animals are mobilizing and using energy; however, opposing responses to thermal and nutritional challenges can lead to a difficult to interpret metabolic trade-off between energy use and acquisition (Cristóbal-Azkarate et al., 2016). Large, longer-term differences in energy balance can be evaluated through changes in body condition, as the loss of muscle and fat reserves suggests a negative energy budget. Body condition scoring is routinely used in the management of wild mammals and standardized schemes have been developed for several species including black rhinos (*Diceros bicornis*) (Reuter & Adcock, 1998) and African buffalo (*Syncerus caffer*) (Ezenwa et al., 2009). Despite thyroid hormones offering a window into an individual's energy balance (Behringer et al., 2018), fewer studies utilize thyroid hormones as biomarkers to assess the impact of environmental factors on fitness than those that use glucocorticoids, which indicate acute fluctuations in energy mobilization.

The impact of resource driven dietary shifts and external stressors can also be manifest within the gut, where microbial communities perform key functional roles in the host and contribute significantly to host health (Gilbert et al., 2018; Sommer & Bäckhed, 2013). Diet changes can lead to changes of key microbiota, which impact on gut function (Borbón-García et al., 2017). Beyond diet, microbiome communities are influenced by a range of factors including habitat, social network properties, and climatic conditions (Trevelline et al., 2019). Furthermore, primary and secondary acute stress responses such as glucocorticoids modulate the microbiome (Noguera et al., 2018). An imbalance of the microbial community, known as dysbiosis, can reduce digestive efficiency, increase inflammation, and susceptibility to infection (Amato et al., 2013; Dethlefsen et al., 2007; Gilbert et al., 2016). Signatures of dysbiosis will vary across hosts, as microbiome composition is sensitive to both diet and vertical transmission; however, dysbiosis or atypical microbiomes can be characterized by the degree of a displacement from a core microbiome composition (Zaneveld et al., 2017). Although the fitness consequences of changes in microbial community are poorly understood, microbiome composition has been linked to reproductive performance (Antwis et al., 2019) and cellular inflammation (Walshe et al., 2019).

Acute challenges: predation, disturbance, and social instability

A primary response to acute stressors such as predation, persecution, or disturbance is the activation of the hypothalamic–pituitary–adrenal (HPA) axis in birds and mammals or the hypothalamic–pituitary–interrenal (HPI) axis in fish, amphibians, and reptiles, which leads to the release of glucocorticoids (GCs) and catecholamines (Beehner & Bergman, 2017; Sopinka et al., 2016). Thus, the HPA/HPI axes and epinephrine stress responses are coupled with metabolism and metabolic rates, as both increase the body's ability to mobilize energy for acute challenges. GCs have been used as an indicator of stress, commonly under the assumption that chronic elevation compromises health and ultimately fitness (Millspaugh & Washburn, 2004). However, short-term activation of the HPA is an adaptive response to allow individuals to effectively respond to acute challenges such that relationships between GCs and fitness are not straightforward (Moberg, 2000). The relationship between GC levels, GC reaction potential, and individual fitness (Bonier et al., 2009; Breuner et al., 2008) is context-dependent, such that during good conditions a high GC responsiveness is associated with poor survivorship and recruitment, whereas during poor conditions the relationship may be reversed when individuals in poor condition become unable to mount significant GC responses (Blas et al., 2007). There is so much variation in how individuals and species respond to chronic stress that there is no consistent profile to identify chronic stress across species (Dickens & Romero, 2013). What GCs do provide is evidence for a perceived challenge or stressor.

Combining physiological responses with other functional traits can help identify where these responses may lead to reduced fitness. For example, behavioral and endocrine profiles can be supplemented with direct physiological measures such as blood pressure, heart, and respiratory rate, if these are feasible for the study species, or proxies for these metrics if they are not (Madliger et al., 2018; Sopinka et al., 2016). Social instability also interacts with physiology (Gersick & Rubenstein, 2017; Seebacher & Krause, 2017) and is associated with elevated GCs in spotted hyenas (*Crocuta crocuta*) (Van Meter et al., 2009), Barbary macaques (*Macaca Sylvanus*) (Edwards et al., 2013), olive baboons (*Papio anubis*) (Sapolsky, 1992), and horses (Nuñez et al., 2014). Human disturbance can also disrupt the normal behavior of animals such as flight responses or changes in space use and time budgets (Wong & Candolin, 2015). The key to understanding the impact of all these markers is how they impact on fitness proxies such as energy reserves, reproductive, and survival rates.

Disease burdens

Heavy disease or parasite burden have fitness consequences (Pedersen & Fenton, 2007) including survival and fecundity that directly impact on population dynamics (Hillegass et al., 2010; Hudson, 1986; Hudson et al., 1998). Gastrointestinal nematode communities, or the nemabiome, can directly affect host fitness but also have the potential to influence resistance and susceptibility to other infecting species (Supali et al., 2010). However, parasite infections are not universally harmful, removing helminths induces a strong inflammatory response (Walshe et al., 2019) and can potentially trigger autoimmune diseases (McKay, 2009). In addition to direct transmission risk, widespread anthropogenic disturbance can exacerbate disease risk through stress-induced immunosuppression. Although there is limited causal evidence between human impacts, stress, and disease occurrence, it is widely assumed that stress may be a major cause of increased susceptibility to wildlife disease (Hing et al., 2016). This may be due to the suppression of reproduction and immune function by the HPA axis as evidence for direct relationships between elevated GCs and parasite burden is well established (O'Dwyer et al., 2020).

Immune responses are also molecular indicators of physiological challenge or stress (Celi et al., 2019; Madliger et al., 2018; Sopinka et al., 2016). Immunoglobulins, or "antibodies" (e.g., IgA, IgG, IgM), form a critical part of the immune response by recognizing, binding to and neutralizing antigens, such as bacteria or viruses (Schroeder & Cavacini, 2010). Fecal antibody assays have been used to measure the immune response to parasites (Watt et al., 2016), which in turn correlate with survival (Sparks et al., 2018). Additional biomarkers that are associated with short-term and long-term responses to external challenges and stressors are blood parameters such as hematocrit levels and white blood cell counts (Madliger et al., 2018; Sopinka et al., 2016). Reduced hematocrit levels in birds are associated with a range of challenges including disease burden and nutritional status (Fair et al., 2007). Heterophil or neutrophil to lymphocyte ratios can indicate chronic stress, whereas eosinophil levels can indicate infectious disease (Davis et al., 2008). Inflammation markers can provide evidence of infectious and noninfectious processes. Calprotectin, lipocalin, and lactoferrin are inflammation markers that limit bacterial growth (Mao et al., 2012) and are used to diagnose inflammatory bowel disease in humans (Van Rheenen et al., 2010). Such biomarkers, which are gaining traction in human clinical practice, have untapped potential for use in wildlife monitoring. Increased metabolism results in the production of chemically reactive metabolic by-products known as reactive oxygen species (ROS) (Sies, 1991). Typically, ROS are removed from the body by antioxidants, but if they are generated in excess, oxygen radicals build up and bind to a range of biological molecules. This oxidative stress results in cellular and DNA damage, reduced defense mechanism, and accelerated aging (Finkel & Holbrook, 2000). Chronically elevated GC production is associated with oxidative stress across species (Costantini et al., 2011).

1.1.2 | Chemical and physical stressors

In addition to natural stressors, organic compounds, trace elements, and pharmaceuticals have all been responsible for catastrophic species declines (Rowe, 2008). Chemicals that are persistent and can bioaccumulate in food webs are particularly dangerous as they can have destabilizing effects on ecosystems. Major environmental contaminants are pesticides, perfluorinated compounds, and pharmaceuticals. Bioaccumulation of persistent organochlorines, such as DDT and associated compounds, has been implicated as major environmental contaminants, which cause catastrophic bird declines and are implicated in endocrine disruption in humans (Blus, 2011). Tributyltin (TBT) is an antifoulant that is well known for its endocrine disruptive effects. Although DDT and TBT are now banned globally, their persistence means that they still occur at appreciable levels in the environment. Perfluoroalkylated compounds are commonly used in various forms of manufacturing. They are persistent in the environment and are linked to endocrine disruption, fertility, and metabolism (Jensen & Leffers, 2008). These compounds also affect human health, for example, increasing cancer risk, and declines in reproductive health, and longevity. The widespread use of antibiotics for human and veterinary health is linked to environmental bioaccumulation that, in turn, is implicated in the spread of antimicrobial resistance (Singer et al., 2016). Anti-inflammatory drugs also bioaccumulate with detrimental effects. The unregulated veterinary use of the anti-inflammatory drug diclofenac resulted in catastrophic declines of Asian vulture populations (Green et al., 2004; Shultz et al., 2004). Marine predators are especially vulnerable due to biomagnification and coastal habitats are particularly vulnerable to bioaccumulation due to sewage, run-off, and sedimentation. For this reason, seabirds have been touted as sentinels for estuarine and continental shelf habitats (Burger & Gochfeld, 2004). Thus, the potential role and impact of chemical contaminants on fitness should be evaluated in unexplained population collapse and range contraction, especially where changes in resource availability, disease, or acute stressors do not appear sufficient to explain declines.

Physical stressors such as injury, particularly when associated with pain, are associated with increased glucocorticoid levels in free ranging mammals (Ganswindt et al., 2010; Rolland et al., 2017; Tripp et al., 2011; Wolf et al., 2018) and birds (Scheun et al., 2021). In some species, physical injury is a major cause of morbidity and mortality. For example, marine mammals including whales, seals, and manatees are particularly vulnerable to anthropogenic injury. In fact, >95% of Florida manatee adults show evidence of boat strike injury (Bassett et al., 2020). Seabirds are also subject to high rates of anthropogenic injury (Dias et al., 2019). Critically, in addition to direct mortality, injury is associated with reproductive suppression and delayed mortality in birds (Fajardo et al., 2000; Parsons et al., 2018), fish (Mueller et al., 2020), and reptiles (Sack et al., 2017) and can lead to population decline associated with high levels of physical injury. Thus, although physical injury is not necessarily a widespread problem, in some species both the acute and chronic impacts of injury are significant conservation challenges.

1.2 | Interpreting and integrating indicators

Using functional markers at large scales to identify vulnerable or declining populations requires reference or benchmark values from well performing populations. Where this is not possible, for example, in a species undergoing widespread declines and range collapse, it may be possible to use benchmarks from historical records or use congeners as a reference population (Britnell et al., 2021; Bocherens et al., 2015; Kerley et al., 2012). The expectation is that individuals from populations in marginal habitats or under challenges will have either single or multiple functional indicators that diverge from an optimal benchmark. Negative indicators will increase and positive indicators will decrease with the distance from central or optimal habitats (either geographically or in terms of niche hypervolume).

Single marker studies can give an incomplete or even misleading picture of individual condition and population health as multiple stressors can act independently or in tandem causing additive, synergistic, or antagonistic effects (Beldomenico & Begon, 2010; Todgham & Stillman, 2013). Few studies employ multitool approaches to evaluate the impact of stress on multiple physiological pathways (Madliger et al., 2018) and studies, which investigate stressors, physiology, and demography together are even more scarce (Beehner & Bergman, 2017). Functional responses to multiple environmental challenges can be measured using the concept of allostatic load, which is the cumulative physiological impact of challenges, when the body can no longer buffer challenges this becomes allostatic overload (McEwen & Wingfield, 2003). Allostatic overload leads to loss of condition, immune, and reproductive suppression and disease.

Concurrently evaluating a suite of biomarkers can provide information about how the different pathways interconnect and impact fitness in relation to environmental stressors or challenges (Figure 1, Table 1). The relationship between multiple biomarkers and population performance can be evaluated with a multivariate model selection approach (Johnson & Omland, 2004), a growth curve model or similar structural equation modeling approaches (Schlotz et al., 2011), or multidimensional data analysis. Thus, a set of indicators can be used to set up alternative hypotheses to determine primary challenges causing poor performance (Figure 2). For example, acute stressors such as disturbance, predation, or persecution may be associated with space use or time budget changes (use of refuges or cover, increased vigilance, movement, and decreased feeding and/or resting) and increased HPA activation. Resource limitation should be associated with increased foraging effort, diet changes, and decreased metabolic rate. Diet changes can be manifest by either increased switching to low-quality "fall back" foods during times of

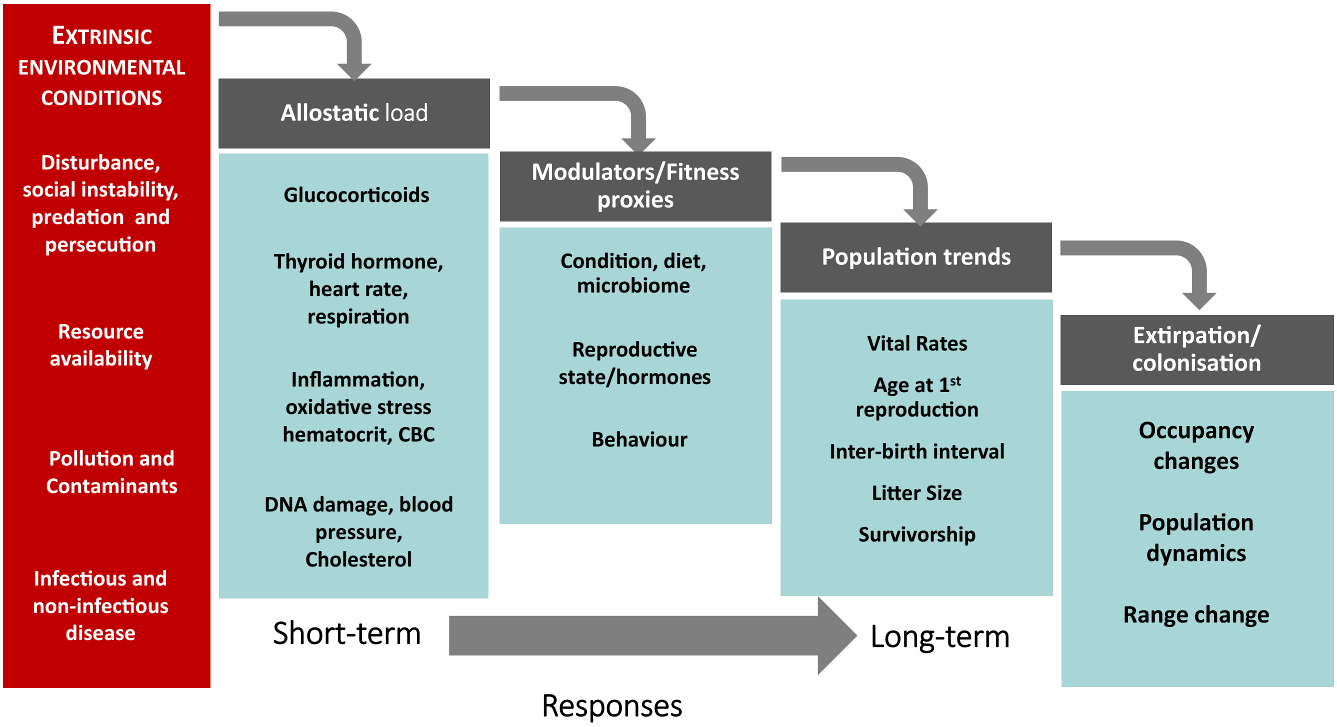


FIGURE 1 A conceptual diagram showing the different biomarkers available that can be integrated into studies using the footprints and pathway approach

TABLE 1 Example studies linking environmental stressors with physiological responses and demographic/population consequences

Class	Species	Challenge	Functional indicators	Population/fitness proxies	Reference
Physiological- Resource	Killer whales *Orcinus orca*	Fish abundance Vessel density	fGCs, fT3	Pregnancy ↓ Population growth ↓	Wasser et al. (2017)
	African elephants *Loxodonta africana*	Rainfall	fGCs fPG	Reproductive function ↓	Foley et al. (2001)
	Shetland ponies *Equus caballus*	Seasonality	Heart rate Locomotion T3 ↑	Field metabolic rate ↑	Brinkmann et al. (2016)
	Soay sheep *Ovis aries*	Maternal effects Genetic variation	Ig proteins Fecal egg count	Survival ↓	Sparks et al. (2018)
	Roe deer *Capreolus capreolus*	Primary productivity	fA, fPG, Estradiol fGCs, fN, IgA	Reproductive condition ↓	Escribano-Avila et al. (2013)
	Cape Mountain zebra *Equus zebra zebra*	Season	GCs ↑ Diet shifts	population growth rate ↓	Lea et al. (2018)
	Barbary macaques	Seasonality-food availability	T3 ↑		Cristóbal-Azkarate et al. (2016)
	White-tailed deer *Odocoileus virginianus*	Seasonality-food availability	T3/T4		Bahnak et al. (1981)
	European badger *Taxidea taxus*	Food availability	T3		Harlow and Seal (1981)
	Chimpanzee *Pan troglodytes*	Habitat quality	Creatinine, GCs		Wessling et al. (2018)
	Common vampire bats *Desmodus rotundus*	Habitat conversion	Diet, behavior, microbiome	Immune function	Ingala et al. (2019)
	Black howler monkey *Alouatta pigra*	Logging and deforestation	Diet and microbiome diversity		Amato et al. (2013)
	Primates	Habitat quality	Microbiome diversity		Stumpf et al. (2016)
	Western fence lizard	Central-peripheral populations	GCs, plasma protein, hematocrit	Body weight	Dunlap (1995), Dunlap and Wingfield (1995)
	Maned wolf *Chrysocyon brachyurus*	Transformed landscapes	GCs ↑, T3↑, PG↓	Suggested reduced reproduction	Vynne et al. (2014)
	Primates (*Pan* spp, *Ateles* spp, *Papio*)	Habitat quality	Time budgets	Range and occupancy dynamics	Bettridge et al. (2010), Dunbar et al. (2009), Korstjens and Dunbar (2007), Korstjens et al. (2010)
Physiological- Acute Stress	Guadalupe fur seals	Capture	Aldosterone ↑ return to baselines		DeRango et al. (2019)
	Bottle-nosed dolphin	Beaching	Aldosterone ↑		Champagne et al. (2018)
	Stingrays	Tourist activity	ROS ↑		Semeniuk et al. (2009)
	Damselfly *Enallagma cyathigerum*	Predation	Stress proteins O^2 consumption Enzyme activity Oxidative stress	Growth rates ↓	Slos and Stoks (2008)

TABLE 1 (Continued)

Class	Species	Challenge	Functional indicators	Population/fitness proxies	Reference
	Black grouse *Tetrao tetrix*	Human disturbance	Feeding times ↑	Energy expenditure ↑	Arlettaz (2015) #1790
	Great tit *Parus major*	Artificial light	Corticosterone ↑	Fledging ↓	Ouyang et al. (2015)
	Eastern black rhinos	Captive environment	PG↓ androgens↓	Reproduction↓	Antwis et al. (2019), Edwards et al. (2015)
	Florida manatee *Trichechus manatus latirostris*	Release from rehabilitation; injury and disease	Serum and urinary creatinine ↑, creatine kinase ↑, urea nitrogen, GCs↑, lymphocyte proliferation ↓		Manire et al. (2003), Tripp et al. (2011)
	African elephants *Loxodonta africana*	Translocation	GCs ↑		Jachowski et al. (2013), Viijoen et al. (2008)
	Chimpanzee Pan troglodytes	Human Disturbance	GCs ↑		McLennan et al. (2019)
Disease	Red grouse	Nematodes		Fecundity ↓	Hudson (1986), Hudson et al. (1998)
	Seychelles warblers (*Acrocephalus sechellensis*)	Parasitism, habitat quality	ROS ↑	Survival and fecundity↓	van de Crommenacker et al. (2011), van de Crommenacker et al. (2012), van de Crommenacker et al. (2017)
	Soay sheep	Parasitism	IgA	Survival ↓	Sparks et al. (2018), Watt et al. (2016)
Chemical and Physical	Fathead minnow *Pimephales promelas*	Environmental estrogen EE2		Survival and fecundity↓	Schwindt et al. (2014)
	Little auk	Mercury exposure		Body condition↓growth rate ↓	Amélineau et al. (2019)
	Black legged kittiwakes	Perfluorinated carboxylates	GC ↓	Body condition↓ Hatching↓	Tartu et al. (2014)
	Monk seals	lethal injury	GC, T3	Body condition↓	Gobush et al. (2014)
	African elephants *Loxodonta africana*	Foot injury	fGC↑	Body condition↓	Ganswindt et al. (2010)

Note: We highlight studies that link environmental challenges with multiple biomarkers and fitness proxies in terms of health, condition, or reproduction.

			Acute challenges	Resource limitation	Disease	Chemical	Physical
Behaviour	Time Budget	*Vigilance*	++	-	N.C.	N.C.	N.C./-
		Feeding	-	++	+	N.C.	N.C./-
		Resting	-	--	+	+	++
	Space Use	*Refuges/Risk Avoidance*	++	-	+	+	+
	Social network	*Cohesion*	+	--	N.C.	N.C.	N.C./-
Physiology	Endocrine	*Glucorticoids*	++	N.C.	+	+	+
		Thyroid/Metabolism	+	--	-	-	-
		Reproductive hormones	N.C./-	-	-	-	-
	Inflammatory and Immune	*ROS (Oxidative Stress)*	N.C./+	N.C./+	+	+	+
		Immune markers: e.g. immunoglobulin, leukocytes	N.C./-	N.C./-	++	N.C./+	+
		Inflammatory markers: cytokines, calprotectin, lactoferrin	N.C./-	N.C./+	++	N.C./+	++
		Haematocrit	N.C./-	N.C./-	-	-	-
	Direct physiological measures	*Heart and respiratory rate, blood pressure*	++	N.C.	+	V	++
Diet	Metabarcoding/pellt analysis	*Diet shift*	N.C./+	++/--	N.C.	N.C.	N.C.
		Diet quality	N.C./-	--	N.C.	N.C.	N.C.
		Microbiome stability	N.C./-	--	N.C.	N.C.	N.C.
Condition		*Body scoring*	N.C.	--	-	-	-

FIGURE 2 Conceptual framework for testing alternative hypotheses for different stressors. +/− indicates potential direction of change. ++/−− indicators are expected to show large magnitude responses. N.C. indicates no consistent/predictable response

scarcity, or in extreme cases, the diet being completely comprised of low-quality items. Loss of condition and fitness costs, such as decreases in survival and fecundity, that are not associated with diet or behavioral change will likely be caused by disease when there are clear inflammatory responses, and by contaminants or chemical stressors when there are not. As each vulnerable population may face a unique set of challenges, a predefined set of testable hypotheses can be used to identify most likely candidates. A contingency table of expected responses can act as a starting point for formulating testable hypotheses based on the Functional Marginality Framework (Figure 2).

Multivariate modeling approaches can tease apart the relative importance of extrinsic factors. The role of environmental traits and functional indicators of health outcomes (i.e., reproductive failure, elevated mortality) can be evaluated using a model selection approach (Deelen et al., 2019). Multiple markers can also effectively evaluate the extent of "dysfunction" as a measure of deviation, such as Mahalanobis distance, from a multivariate central tendency (Milot et al., 2014). Evaluating the model weight for different factors (Johnson & Omland, 2004) can identify functional indicators that best predict fitness variance or population resilience, which can be used as key population health markers and focused on in future research. Clearly, a challenge with this macrophysiological approach is identifying markers that can be rapidly and noninvasively collected such as demography, behavior (association patterns, space use, and time budgets), and noninvasive biological samples (e.g., fecal and urine). Using model species, where noninvasive samples can be easily collected from a large number of individuals and tied to reproductive, survivorship, or population growth rate outcomes (Lea et al., 2018), is a key priority for developing a macrophysiological approach.

1.3 | Theoretical frameworks

Mechanistic distribution models use functional traits to link environmental variation with individual- and population-level performance (Buckley et al., 2010) as variation in physiological biomarkers of stress, health, and reproduction can act as these heuristic indicators of population viability (Chown & Gaston, 2008; Ellis et al., 2012; Gaston et al., 2009). Thus, they can predict likely population performance and range dynamics including the probability of colonization and extirpation under changing conditions (Figure 3a). For example, range-wide land transformation and climate changes can lead to an increased allostatic load (e.g., oxidative stress, inflammation, and disease) and a decline in positive biomarkers (reproductive function, metabolism, hematocrit levels) in adversely affected populations, with a net reduction in functional condition (Figure 3b). Functional traits can also evaluate patterns of range contraction, where the expanding edge will be associated with improved functional traits and

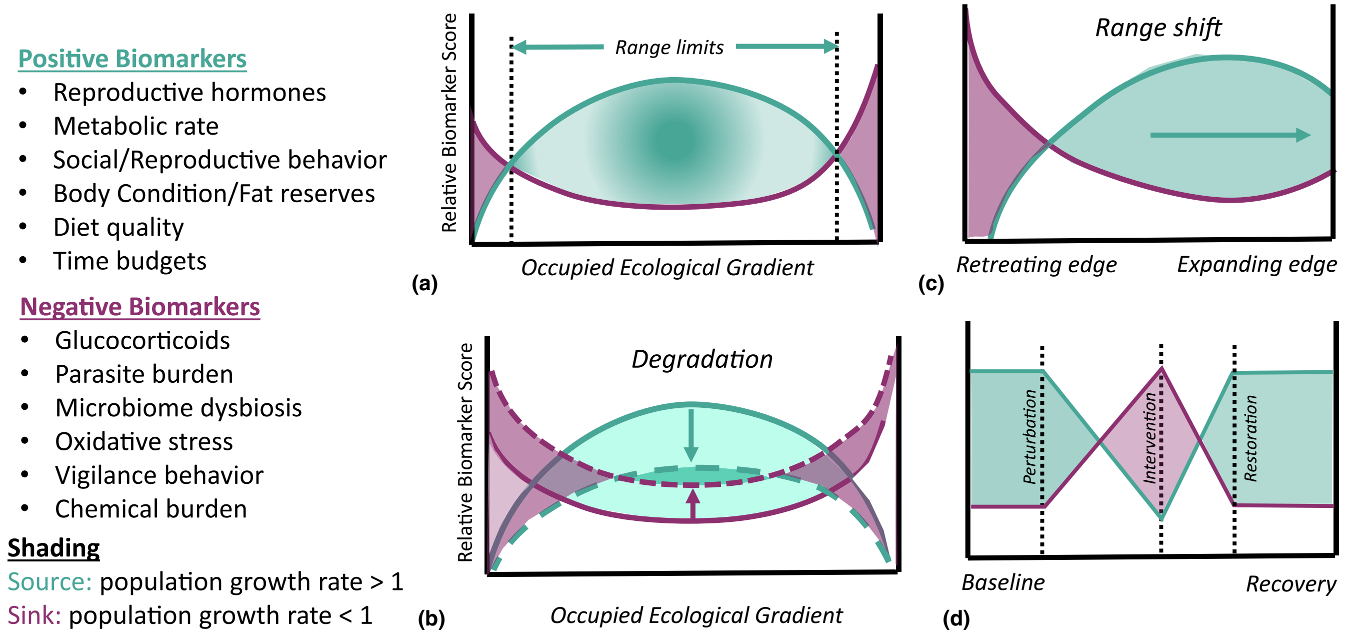


FIGURE 3 Conceptual diagram of the *Functional Marginality* Framework. (a) Viable populations are determined by good functional condition leading to sustainable growth rates, range limits are determined by an increased burden of negative functional traits relative to positive ones. (b) Range shifts will be associated with improving functional condition on the expanding edge and declining condition on the retreating edge. (c) Habitat degradation leads to a net decline in functional condition (balance of positive indicators and negative allostatic load) across occupied habitat resulting in more sink populations and fewer source populations. (d) Functional condition can be tracked over time by repeatedly measuring positive and negative functional traits, and will exhibit characteristic profiles during periods of threat and recovery

the retreating edge associated with declines in positive indicators and increasing burden of negative indicators (Figure 3c). Moreover, if range contraction is characterized by retreat into suboptimal refuges away from encroaching threats (Scheele et al., 2017), then in additional to poor functional condition in the contracting edge, remaining populations should have poor functional condition as compared to populations in optimal habitats, or where baselines are not available, relative to closely related species (Figure 3c). If environmental change causes species to retreat into optimal habitats (Channell & Lomolino, 2000), then functional traits in the remaining habitats should be consistent with those in optimal habitats or source populations. We would also expect a truncated distribution of markers toward less optimal states across the remaining range (Figure 3c).

Although variation in resource availability across landscapes is widely appreciated, metabolic costs also vary in terms of slopes, substrates, and thermal stresses (Shepard et al., 2013). Incorporating spatial patterns of energy availability and costs in "energetic landscapes" can provide a step change in our understanding of how environmental conditions impact on fitness at the individual and population level. Spatial variation in threats from predation, disturbance, and disease risk can be used to create predictive models of "landscapes of fear" and "landscapes of disgust" (Gallagher et al., 2017; Laundré et al., 2001; Weinstein et al., 2018). In particular, spatial variation in predation risk has consequences on physiology, reproduction, immune function, and behavior (Clinchy et al., 2011, 2013). These spatial models can be integrated to create "landscapes of stress," where physiological or behavioral trade-offs can be directly incorporated into population or habitat use models (Koprivnikar & Penalva, 2015). For example, brown bears (*Ursus arctos)* near human settlements have lower heart rate variability, a cardiovascular indicator of stress, and they move further during increased human activity, which is expected to have an energetic cost (Støen et al., 2015). Similarly, landscape features, movement, and disturbance together predict physiological state in grizzly bears (Wilson et al., 2021).

We can also use indicators to test intervention success or the functional recovery of individuals or a population, which can provide insights into efficacy of restoration, colonization, and reintroduction. As humans have extensively changed and degraded habitats, conservation efforts often try to restore habitats or populations to reflect a historical state or ecological baseline (Britnell et al., 2021). A mechanistic approach can provide the evidence about how best to restore or manipulate degraded systems and how to establish whether an intervention has had the desired response (Hobbs et al., 2014). Successful interventions should increase population growth rates and nudge a population from being a sink to being self-sustaining or a source. Following an intervention, negative biomarkers should decrease, and positive biomarkers increase, relative to pre-intervention levels (Figure 3d; Cooke & Suski, 2008). The relationship between functional indicators and fitness can be assessed through changes in vital rates, for example, by monitoring changes in pregnancy rates of vertebrates before and after restoration or policy implementation (Pallin et al., 2018).

Planning for, and the short- and long-term effects of, active management such as handling, translocation, and reintroduction can also be evaluated with functional indicators. Factors such as climate suitability are predictors of translocation failure (Bellis et al., 2020), and mechanistic distribution models can increase the robustness of habitat suitability predictions. Translocation success is also linked to stress responses and resilience, which occur during the translocation event and the establishment phase immediately after release (Dickens et al., 2010). Following an intervention, positive and negative biomarkers should return to pretranslocation levels after intervention and recovery. Conducting physiological monitoring before, during, and after release can improve our understanding of translocations, and the factors required for them to be a success. For example, a study using the Florida manatee *(Trichechus manatus latirostris)* in a simulated release process found that decreased food intake and changes in water salinity led to serum creatinine elevations and impaired immune function, indicated by lymphocyte proliferation assays (Manire et al., 2003). This is beneficial from a conservation perspective, as it increases the probability of future reintroduction success, and from a welfare perspective, as it allows methods to be refined to avoid stress and reduces the number of animals required (Tarszisz et al., 2014).

1.4 | Examples

There is now a small but growing number of studies that have used functional traits to understand range dynamics and differences between central and peripheral populations. For examples, GCs, blood parameters, and body condition vary between central and marginal populations of Western fence lizards (*Sceloporus occidentalis*) (Dunlap, 1995; Dunlap & Wingfield, 1995). Combining variation in time budgets with species distribution models in primates is an excellent example of using behavior patterns to understand drivers of population and range dynamics (Bettridge et al., 2010; Dunbar et al., 2009; Korstjens & Dunbar, 2007; Korstjens et al., 2010). There is also extensive evidence for how pollution and contaminants affect fitness proxies and functional indicators in birds (Rattner et al., 1984), although this approach has not been widely used to evaluate spatial range dynamics in a macroecology context. Despite this, there are limited examples of studies that evidence links between environmental stressors, physiology, behavior, and fitness measures to predict both individual- and population-level responses to challenges (Beehner & Bergman, 2017; Cooke et al., 2013). We have summarized a range of studies that have used functional markers to assess the impact of challenges and population or fitness consequences (Table 1). There are, however, a few key studies that have evaluated links between environmental challenges, functional markers, and population-level variation in resilience and viability. Physiological and behavioral biomarkers have been used to identify marginal or “refugee” populations in Cape mountain zebra (*Equus zebra zebra*) (Lea et al., 2018) where poor reproduction performance is associated with elevated androgens and glucocorticoids as a consequence of resource limitation and skewed population sex ratios. Functional markers including elevated creatinine, C-peptide, and glucocorticoids were used to evidence thermal stress in chimpanzees (*Pan troglodytes*) inhabiting a savannah-mosaic habitat at the margins of their range (Wessling et al., 2018). Hawaiian monk seals (*Neomonachus schauinslandi*) have experienced declines since the 1950s that have been variously attributed to poor juvenile survival due to resource limitations, injury, and disease (Harting et al., 2021). Declining subpopulations were associated with chronic elevation of fGCMs and low fT3, especially in immature individuals and had, on average, poorer survival rates and lower intrinsic population growth rates (Gobush et al., 2014). In better performing populations, multiple markers still highlighted how anthropogenic injury and disease relative to malnutrition affected intrinsic growth rates (Harting et al., 2021). This modeling approach that identifies how local stressors affect growth rates could be applied to most conservation scenarios.

2 | CONCLUSION

In recent decades, much research has been carried out to develop biomarkers, which provide an indication of how the environment affects the physiological and behavioral state of an organism and ultimately on fitness. This is a difficult task as physiology is extremely complex. Physiological responses are the result of multiple interconnecting pathways, which can respond to the same stressors and interact with each other, making the change in a single biomarker difficult to relate to fitness. We propose that the establishment of complementary and integrated biomarkers to indicate population health, properly validated and applied to testable hypotheses, would be a major advance for large-scale ecology and conservation. Validation, the discovery of relevant biomarkers or combination thereof, is a key part of this approach. The approaches we describe can be used to show which biomarkers are useful at predicting future changes in fitness measures associated with population changes. Once established, these biomarkers can be the basis for investigating the causes of poor individual health and changes in survival and reproduction and testing ecological and conservation hypotheses. This information can help to uncover the causes of distributional limits and predict future changes, estimate resilience of populations to novel threats, assess the efficacy of conservation efforts, and reveal macroecological trends and processes. This approach provides conservation biologists and practitioners the ability to produce evidence for the causal mechanisms underlying conservation problems and macro- or evolutionary ecologists the ability to investigate the physiological mechanisms underlying long-term and large-scale processes. Advances in these fields can contribute toward the calls for evidence-based conservation and help to alleviate the threat of species extinctions and ecological collapse.

ACKNOWLEDGMENTS

The authors would like to thank anonymous reviewers for constructive feedback that significantly improved this manuscript. We thank Danielle Hinchcliffe and Susan Walker for discussions about the approach in earlier versions of this manuscript. SS is supported by a Royal Society URF (UF160725). NH and JB are both supported by the NERC EAO DTP program (NE/L002469/1) and Chester Zoo.

CONFLICT OF INTEREST

We declare there are no conflicts of interest associated with this article.

AUTHOR CONTRIBUTIONS

Susanne Shultz: Conceptualization (equal); Project administration (lead); Supervision (lead); Writing-original draft (equal); Writing-review & editing (lead). **Jake A. Britnell:** Conceptualization (equal); Writing-original draft (equal); Writing-review & editing (supporting). **Nicholas Harvey:** Writing-original draft (supporting); Writing-review & editing (supporting).

DATA AVAILABILITY STATEMENT

There are no primary data presented in this manuscript. All studies discussed are referenced in the manuscript.

ORCID

Susanne Shultz https://orcid.org/0000-0002-7135-4880
Jake A. Britnell https://orcid.org/0000-0003-1527-7013
Nicholas Harvey https://orcid.org/0000-0001-7824-0045

REFERENCES

Amato, K. R., Yeoman, C. J., Kent, A., Righini, N., Carbonero, F., Estrada, A., Rex Gaskins, H., Stumpf, R. M., Yildirim, S., Torralba, M., Gillis, M., Wilson, B. A., Nelson, K. E., White, B. A., & Leigh, S. R. (2013). Habitat degradation impacts black howler monkey (*Alouatta pigra*) gastrointestinal microbiomes. *The ISME Journal*, *7*, 1344–1353. https://doi.org/10.1038/ismej.2013.16

Amélineau, F., Grémillet, D., Harding, A. M., Walkusz, W., Choquet, R., & Fort, J. (2019). Arctic climate change and pollution impact little auk foraging and fitness across a decade. *Scientific Reports*, *9*, 1–15. https://doi.org/10.1038/s41598-018-38042-z

Antwis, R. E., Edwards, K. L., Unwin, B., Walker, S. L., & Shultz, S. (2019). Rare gut microbiota associated with breeding success, hormone metabolites and ovarian cycle phase in the critically endangered eastern black rhino. *Microbiome*, *7*, 27.

Bahnak, B. R., Holland, J. C., Verme, L. J., & Ozoga, J. J. (1981). Seasonal and nutritional influences on growth hormone and thyroid activity in white-tailed deer. *The Journal of Wildlife Management*, *45*, 140–147. https://doi.org/10.2307/3807882

Bassett, B. L., Hostetler, J. A., Leone, E., Shea, C. P., Barbeau, B. D., Lonati, G. L., Panike, A. L., Honaker, A., & Ward-Geiger, L. I. (2020). Quantifying sublethal Florida manatee watercraft interactions by examining scars on manatee carcasses. *Endangered Species Research*, *43*, 395–408. https://doi.org/10.3354/esr01075

Beehner, J. C., & Bergman, T. J. (2017). The next step for stress research in primates: To identify relationships between glucocorticoid secretion and fitness. *Hormones and Behavior*, *91*, 68–83. https://doi.org/10.1016/j.yhbeh.2017.03.003

Behringer, V., Deimel, C., Hohmann, G., Negrey, J., Schaebs, F. S., & Deschner, T. (2018). Applications for non-invasive thyroid hormone measurements in mammalian ecology, growth, and maintenance. *Hormones and Behavior*, *105*, 66–85. https://doi.org/10.1016/j.yhbeh.2018.07.011

Beldomenico, P. M., & Begon, M. (2010). Disease spread, susceptibility and infection intensity: Vicious circles? *Trends in Ecology & Evolution*, *25*, 21–27.

Bellis, J., Bourke, D., Maschinski, J., Heineman, K., & Dalrymple, S. (2020). Climate suitability as a predictor of conservation translocation failure. *Conservation Biology*, *34*, 1473–1481. https://doi.org/10.1111/cobi.13518

Bettridge, C., Lehmann, J., & Dunbar, R. (2010). Trade-offs between time, predation risk and life history, and their implications for biogeography: A systems modelling approach with a primate case study. *Ecological Modelling*, *221*, 777–790. https://doi.org/10.1016/j.ecolmodel.2009.11.017

Blas, J., Bortolotti, G. R., Tella, J. L., Baos, R., & Marchant, T. A. (2007). Stress response during development predicts fitness in a wild, long lived vertebrate. *Proceedings of the National Academy of Sciences of the United States of America*, *104*, 8880–8884. https://doi.org/10.1073/pnas.0700232104

Blus, L. J. (2011). DDT, DDD, and DDE in birds. In W. N. Beyer, G. H. Heinz, & A. W. Redmon-Norwood (Eds.), *Environmental contaminants in biota* (pp. 425–446). CRC Press.

Bocherens, H., Hofman-Kamińska, E., Drucker, D. G., Schmölcke, U., & Kowalczyk, R. (2015). European bison as a refugee species? Evidence from isotopic data on Early Holocene bison and other large herbivores in northern Europe. *PLoS One*, *10*, e0115090. https://doi.org/10.1371/journal.pone.0115090

Bonier, F., Moore, I. T., Martin, P. R., & Robertson, R. J. (2009). The relationship between fitness and baseline glucocorticoids in a passerine bird. *General and Comparative Endocrinology*, *163*, 208–213. https://doi.org/10.1016/j.ygcen.2008.12.013

Borbón-García, A., Reyes, A., Vives-Flórez, M., & Caballero, S. (2017). Captivity shapes the gut microbiota of Andean bears: Insights into health surveillance. *Frontiers in Microbiology*, *8*, 1316. https://doi.org/10.3389/fmicb.2017.01316

Breuner, C. W., Patterson, S. H., & Hahn, T. P. (2008). In search of relationships between the acute adrenocortical response and fitness. *General and Comparative Endocrinology*, *157*, 288–295. https://doi.org/10.1016/j.ygcen.2008.05.017

Brinkmann, L., Gerken, M., Hambly, C., Speakman, J. R., & Riek, A. (2016). Thyroid hormones correlate with field metabolic rate in ponies, Equus ferus caballus. *Journal of Experimental Biology*, *219*, 2559–2566.

Britnell, J. A., Lewis, R. N., Elsner-Gearing, F., Harvey, N., Stanbrook, E., & Shultz, S. (2021). Species stereotypes as a result of unconscious research biases compromise conservation efficacy. *Biological Conservation*, *261*, 109275. https://doi.org/10.1016/j.biocon.2021.109275

Brook, B. W., O'Grady, J. J., Chapman, A. P., Burgman, M. A., Akcakaya, H. R., & Frankham, R. (2000). Predictive accuracy of population viability analysis in conservation biology. *Nature*, *404*, 385–387. https://doi.org/10.1038/35006050

Brook, B. W., Sodhi, N. S., & Bradshaw, C. J. (2008). Synergies among extinction drivers under global change. *Trends in Ecology & Evolution*, *23*, 453–460. https://doi.org/10.1016/j.tree.2008.03.011

Buckley, L. B., Urban, M. C., Angilletta, M. J., Crozier, L. G., Rissler, L. J., & Sears, M. W. (2010). Can mechanism inform species' distribution models? *Ecology Letters*, *13*, 1041–1054. https://doi.org/10.1111/j.1461-0248.2010.01479.x

Burger, J., & Gochfeld, M. (2004). Marine birds as sentinels of environmental pollution. *EcoHealth*, *1*, 263–274. https://doi.org/10.1007/s10393-004-0096-4

Burger, J. R., Hou, C., & Brown, J. H. (2019). Toward a metabolic theory of life history. *Proceedings of the National Academy of Sciences*, *116*(52), 26653–26661. https://doi.org/10.1073/pnas.1907702116

Celi, P., Verlhac, V., Calvo, E. P., Schmeisser, J., & Kluenter, A.-M. (2019). Biomarkers of gastrointestinal functionality in animal nutrition and health. *Animal Feed Science and Technology*, *250*, 9–31. https://doi.org/10.1016/j.anifeedsci.2018.07.012

Champagne, C. D., Kellar, N. M., Trego, M. L., Delehanty, B., Boonstra, R., Wasser, S. K., Booth, R. K., Crocker, D. E., & Houser, D. S. (2018). Comprehensive endocrine response to acute stress in the bottlenose dolphin from serum, blubber, and feces. *General and Comparative Endocrinology*, *266*, 178–193. https://doi.org/10.1016/j.ygcen.2018.05.015

Channell, R., & Lomolino, M. V. (2000). Trajectories to extinction: Spatial dynamics of the contraction of geographical ranges. *Journal of Biogeography*, *27*, 169–179. https://doi.org/10.1046/j.1365-2699.2000.00382.x

Chown, S. L., & Gaston, K. J. (2008). Macrophysiology for a changing world. *Proceedings of the Royal Society B: Biological Sciences*, *275*, 1469–1478. https://doi.org/10.1098/rspb.2008.0137

Christiansen, F., Rasmussen, M. H., & Lusseau, D. (2013). Inferring activity budgets in wild animals to estimate the consequences of disturbances. *Behavioral Ecology*, *24*, 1415–1425. https://doi.org/10.1093/beheco/art086

Clinchy, M., Sheriff, M. J., & Zanette, L. Y. (2013). Predator-induced stress and the ecology of fear. *Functional Ecology*, *27*, 56–65. https://doi.org/10.1111/1365-2435.12007

Clinchy, M., Zanette, L., Charlier, T. D., Newman, A. E., Schmidt, K. L., Boonstra, R., & Soma, K. K. (2011). Multiple measures elucidate glucocorticoid responses to environmental variation in predation threat. *Oecologia*, *166*, 607–614. https://doi.org/10.1007/s00442-011-1915-2

Cooke, S. J., Sack, L., Franklin, C. E., Farrell, A. P., Beardall, J., Wikelski, M., & Chown, S. L. (2013). What is conservation physiology? Perspectives on an increasingly integrated and essential science. *Conservation Physiology*, *1*, cot001. https://doi.org/10.1093/conphys/cot001

Cooke, S. J., & Suski, C. D. (2008). Ecological restoration and physiology: An overdue integration. *BioScience*, *58*, 957–968. https://doi.org/10.1641/B581009

Costa-e-Sousa, R. H., & Hollenberg, A. N. (2012). Minireview: The neural regulation of the hypothalamic-pituitary-thyroid axis. *Endocrinology*, *153*, 4128–4135. https://doi.org/10.1210/en.2012-1467

Costantini, D., Marasco, V., & Møller, A. P. (2011). A meta-analysis of glucocorticoids as modulators of oxidative stress in vertebrates. *Journal of Comparative Physiology B*, *181*, 447–456. https://doi.org/10.1007/s00360-011-0566-2

Cristóbal-Azkarate, J., Maréchal, L., Semple, S., Majolo, B., & MacLarnon, A. (2016). Metabolic strategies in wild male Barbary macaques: Evidence from faecal measurement of thyroid hormone. *Biology Letters*, *12*, 20160168. https://doi.org/10.1098/rsbl.2016.0168

Davis, A., Maney, D., & Maerz, J. (2008). The use of leukocyte profiles to measure stress in vertebrates: A review for ecologists. *Functional Ecology*, *22*, 760–772. https://doi.org/10.1111/j.1365-2435.2008.01467.x

Deelen, J., Kettunen, J., Fischer, K., van der Spek, A., Trompet, S., Kastenmüller, G., Boyd, A., Zierer, J., van den Akker, E. B., Ala-Korpela, M., Amin, N., Demirkan, A., Ghanbari, M., van Heemst, D., Ikram, M. A., van Klinken, J. B., Mooijaart, S. P., Peters, A., Salomaa, V., … Slagboom, P. E. (2019). A metabolic profile of all-cause mortality risk identified in an observational study of 44,168 individuals. *Nature Communications*, *10*, 1–8. https://doi.org/10.1038/s41467-019-11311-9

DeRango, E. J., Greig, D. J., Gálvez, C., Norris, T. A., Barbosa, L., Elorriaga-Verplancken, F. R., & Crocker, D. E. (2019). Response to capture stress involves multiple corticosteroids and is associated with serum thyroid hormone concentrations in Guadalupe fur seals (Arctocephalus philippii townsendi). *Marine Mammal Science*, *35*, 72–92.

Dethlefsen, L., McFall-Ngai, M., & Relman, D. A. (2007). An ecological and evolutionary perspective on human–microbe mutualism and disease. *Nature*, *449*, 811–818. https://doi.org/10.1038/nature06245

Dias, M. P., Martin, R., Pearmain, E. J., Burfield, I. J., Small, C., Phillips, R. A., Yates, O., Lascelles, B., Borboroglu, P. G., & Croxall, J. P. (2019). Threats to seabirds: A global assessment. *Biological Conservation*, *237*, 525–537. https://doi.org/10.1016/j.biocon.2019.06.033

Dickens, M. J., Delehanty, D. J., & Romero, L. M. (2010). Stress: An inevitable component of animal translocation. *Biological Conservation*, *143*, 1329–1341. https://doi.org/10.1016/j.biocon.2010.02.032

Dickens, M. J., & Romero, L. M. (2013). A consensus endocrine profile for chronically stressed wild animals does not exist. *General and Comparative Endocrinology*, *191*, 177–189. https://doi.org/10.1016/j.ygcen.2013.06.014

Dunbar, R. I., Korstjens, A. H., & Lehmann, J. (2009). Time as an ecological constraint. *Biological Reviews*, *84*, 413–429. https://doi.org/10.1111/j.1469-185X.2009.00080.x

Dunlap, K. D. (1995). External and internal influences on indices of physiological stress: II. Seasonal and size-related variations in blood composition in free-living lizards, Sceloporus occidentalis. *Journal of Experimental Zoology*, *272*, 85–94.

Dunlap, K. D., & Wingfield, J. (1995). External and internal influences on indices of physiological stress. I. Seasonal and population variation in adrenocortical secretion of free-living lizards, *Sceloporus occidentalis*. *Journal of Experimental Zoology*, *271*, 36–46.

Edwards, K. L., Shultz, S., Pilgrim, M., & Walker, S. L. (2015). Irregular ovarian activity, body condition and behavioural differences are associated with reproductive success in female eastern black rhinoceros (Diceros bicornis michaeli). *General and Comparative Endocrinology*, *214*, 186–194. https://doi.org/10.1016/j.ygcen.2014.07.026

Edwards, K. L., Walker, S. L., Bodenham, R. F., Ritchie, H., & Shultz, S. (2013). Associations between social behaviour and adrenal activity in female Barbary macaques: Consequences of study design. *General and Comparative Endocrinology*, *186*, 72–79. https://doi.org/10.1016/j.ygcen.2013.02.023

Ellis, R. D., McWhorter, T. J., & Maron, M. (2012). Integrating landscape ecology and conservation physiology. *Landscape Ecology*, *27*, 1–12. https://doi.org/10.1007/s10980-011-9671-6

Escribano-Avila, G., Pettorelli, N., Virgós, E., Lara-Romero, C., Lozano, J., Barja, I., Cuadra, F. S., & Puerta, M. (2013). Testing Cort-Fitness and Cort-Adaptation hypotheses in a habitat suitability gradient for roe deer. *Acta Oecologica*, *53*, 38–48. https://doi.org/10.1016/j.actao.2013.08.003

Ezenwa, V. O., Jolles, A. E., & O'Brien, M. P. (2009). A reliable body condition scoring technique for estimating condition in African buffalo. *African Journal of Ecology*, *47*, 476–481. https://doi.org/10.1111/j.1365-2028.2008.00960.x

Fair, J., Whitaker, S., & Pearson, B. (2007). Sources of variation in haematocrit in birds. *Ibis*, *149*, 535–552. https://doi.org/10.1111/j.1474-919X.2007.00680.x

Faith, J. T. (2012). Palaeozoological insights into management options for a threatened mammal: Southern Africa's Cape mountain zebra (*Equus zebra zebra*). *Diversity and Distributions*, *18*, 438–447. https://doi.org/10.1111/j.1472-4642.2011.00841.x

Fajardo, I., Babiloni, G., & Miranda, Y. (2000). Rehabilitated and wild barn owls (*Tyto alba*): Dispersal, life expectancy and mortality in Spain. *Biological Conservation*, *94*, 287–295. https://doi.org/10.1016/S0006-3207(00)00003-3

Finkel, T., & Holbrook, N. J. (2000). Oxidants, oxidative stress and the biology of ageing. *Nature*, *408*, 239.

Foley, C. A. H., Papageorge, S., & Wasser, S. K. (2001). Noninvasive stress and reproductive measures of social and ecological pressures in free-ranging African elephants. *Conservation Biology*, *15*, 1134–1142. https://doi.org/10.1046/j.1523-1739.2001.0150041134.x

Fretwell, S. D. (1969). On territorial behavior and other factors influencing habitat distribution in birds. *Acta Biotheoretica*, *19*, 45–52. https://doi.org/10.1007/BF01601955

Fretwell, S. (1972). Populations in a seasonal environment. *Monographs in Population Biology*, *5*, 1–217.

Gaillard, J. M., Festa-Bianchet, M., Yoccoz, N. G., Loison, A., & Toigo, C. (2000). Temporal variation in fitness components and population dynamics of large herbivores. *Annual Review of Ecology and Systematics*, *31*, 367–393. https://doi.org/10.1146/annurev.ecolsys.31.1.367

Gallagher, A. J., Creel, S., Wilson, R. P., & Cooke, S. J. (2017). Energy landscapes and the landscape of fear. *Trends in Ecology & Evolution*, *32*, 88–96. https://doi.org/10.1016/j.tree.2016.10.010

Ganswindt, A., Münscher, S., Henley, M., Palme, R., Thompson, P., & Bertschinger, H. (2010). Concentrations of faecal glucocorticoid metabolites in physically injured free-ranging African elephants *Loxodonta africana*. *Wildlife Biology*, *16*, 323–332. https://doi.org/10.2981/09-081

Gaston, K. J., Chown, S. L., Calosi, P., Bernardo, J., Bilton, D. T., Clarke, A., Clusella-Trullas, S., Ghalambor, C. K., Konarzewski, M., Peck, L. S., Porter, W. P., Pörtner, H. O., Rezende, E. L., Schulte, P. M., Spicer, J. I., Stillman, J. H., Terblanche, J. S., & van Kleunen, M. (2009). Macrophysiology: A conceptual reunification. *The American Naturalist*, *174*, 595–612. https://doi.org/10.1086/605982

Gersick, A. S., & Rubenstein, D. I. (2017). Physiology modulates social flexibility and collective behaviour in equids and other large ungulates. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1727), 20160241. https://doi.org/10.1098/rstb.2016.0241

Gilbert, J. A., Blaser, M. J., Caporaso, J. G., Jansson, J. K., Lynch, S. V., & Knight, R. (2018). Current understanding of the human microbiome. *Nature Medicine*, *24*, 392. https://doi.org/10.1038/nm.4517

Gilbert, J. A., Quinn, R. A., Debelius, J., Xu, Z. Z., Morton, J., Garg, N., Jansson, J. K., Dorrestein, P. C., & Knight, R. (2016). Microbiome-wide association studies link dynamic microbial consortia to disease. *Nature*, *535*, 94–103. https://doi.org/10.1038/nature18850

Gobush, K., Booth, R., & Wasser, S. (2014). Validation and application of noninvasive glucocorticoid and thyroid hormone measures in free-ranging Hawaiian monk seals. *General and Comparative Endocrinology*, *195*, 174–182. https://doi.org/10.1016/j.ygcen.2013.10.020

Green, R. E., Newton, I., Shultz, S., Cunningham, A. A., Gilbert, M., Pain, D. J., & Prakash, V. (2004). Diclofenac poisoning as a cause of vulture population declines across the Indian subcontinent. *Journal of Applied Ecology*, *41*, 793–800. https://doi.org/10.1111/j.0021-8901.2004.00954.x

Grueter, C. C., Li, D., Ren, B., Wei, F., Xiang, Z., & van Schaik, C. P. (2009). Fallback foods of temperate-living primates: A case study on snub-nosed monkeys. *American Journal of Physical Anthropology*, *140*, 700–715. https://doi.org/10.1002/ajpa.21024

Guo, Q., Taper, M., Schoenberger, M., & Brandle, J. (2005). Spatial-temporal population dynamics across species range: From centre to margin. *Oikos*, *108*, 47–57. https://doi.org/10.1111/j.0030-1299.2005.13149.x

Harlow, H. J., & Seal, U. S. (1981). Changes in hematology and metabolites in the serum and urine of the badger, *Taxidea taxus*, during food deprivation. *Canadian Journal of Zoology*, *59*, 2123–2128.

Harting, A. L., Barbieri, M. M., Baker, J. D., Mercer, T. A., Johanos, T. C., Robinson, S. J., Littnan, C. L., Colegrove, K. M., & Rotstein, D. S. (2021). Population-level impacts of natural and anthropogenic causes-of-death for Hawaiian monk seals in the main Hawaiian Islands. *Marine Mammal Science*, *37*, 235–250. https://doi.org/10.1111/mms.12742

Hillegass, M. A., Waterman, J. M., & Roth, J. D. (2010). Parasite removal increases reproductive success in a social African ground squirrel. *Behavioral Ecology*, *21*, 696–700. https://doi.org/10.1093/beheco/arq041

Hing, S., Narayan, E. J., Thompson, R. C. A., & Godfrey, S. S. (2016). The relationship between physiological stress and wildlife disease: Consequences for health and conservation. *Wildlife Research*, *43*, 51–60. https://doi.org/10.1071/WR15183

Hobbs, R. J., Higgs, E., Hall, C. M., Bridgewater, P., Chapin, F. S., Ellis, E. C., Ewel, J. J., Hallett, L. M., Harris, J., Hulvey, K. B., Jackson, S. T., Kennedy, P. L., Kueffer, C., Lach, L., Lantz, T. C., Lugo, A. E., Mascaro, J., Murphy, S. D., Nelson, C. R., … Yung, L. (2014). Managing the whole landscape: Historical, hybrid, and novel ecosystems. *Frontiers in Ecology and the Environment*, *12*, 557–564. https://doi.org/10.1890/130300

Hoffmann, M., Hilton-Taylor, C., Angulo, A., Böhm, M., Brooks, T. M., Butchart, S. H. M., Carpenter, K. E., Chanson, J., Collen, B., Cox, N. A., Darwall, W. R. T., Dulvy, N. K., Harrison, L. R., Katariya, V., Pollock, C. M., Quader, S., Richman, N. I., Rodrigues, A. S. L., Tognelli, M. F., … Stuart, S. N. (2010). The impact of conservation on the status of the world's vertebrates. *Science*, *330*, 1503–1509. https://doi.org/10.1126/science.1194442

Holt, R. D. (2003). On the evolutionary ecology of species' ranges. *Evolutionary Ecology Research*, *5*, 159–178.

Holt, R. D. (2009). Bringing the Hutchinsonian niche into the 21st century: Ecological and evolutionary perspectives. *Proceedings of the National Academy of Sciences of the United States of America*, *106*, 19659–19665. https://doi.org/10.1073/pnas.0905137106

Hudson, P. J. (1986). The effect of a parasitic nematode on the breeding production of red grouse. *The Journal of Animal Ecology*, *55*(1), 85–92. https://doi.org/10.2307/4694

Hudson, P. J., Dobson, A. P., & Newborn, D. (1998). Prevention of population cycles by parasite removal. *Science*, *282*, 2256–2258. https://doi.org/10.1126/science.282.5397.2256

Ingala, M. R., Becker, D. J., Bak Holm, J., Kristiansen, K., & Simmons, N. B. (2019). Habitat fragmentation is associated with dietary shifts and microbiota variability in common vampire bats. *Ecology and Evolution*, *9*, 6508–6523. https://doi.org/10.1002/ece3.5228

Jachowski, D., Slotow, R., & Millspaugh, J. (2013). Delayed physiological acclimatization by African elephants following reintroduction. *Animal Conservation*, *16*, 575–583. https://doi.org/10.1111/acv.12031

Jensen, A. A., & Leffers, H. (2008). Emerging endocrine disrupters: Perfluoroalkylated substances. *International Journal of Andrology*, *31*, 161–169. https://doi.org/10.1111/j.1365-2605.2008.00870.x

Johnson, J. B., & Omland, K. S. (2004). Model selection in ecology and evolution. *Trends in Ecology & Evolution*, *19*, 101–108. https://doi.org/10.1016/j.tree.2003.10.013

Kawecki, T. J. (2008). Adaptation to marginal habitats. *Annual Review of Ecology, Evolution, and Systematics*, *39*, 321–342. https://doi.org/10.1146/annurev.ecolsys.38.091206.095622

Kearney, M., & Porter, W. (2009). Mechanistic niche modelling: Combining physiological and spatial data to predict species' ranges. *Ecology Letters*, *12*, 334–350. https://doi.org/10.1111/j.1461-0248.2008.01277.x

Kerley, G., Kowalczyk, R., & Cromsigt, J. (2012). Conservation implications of the refugee species concept and the European bison: King of the forest or refugee in a marginal habitat? *Ecography*, *35*, 519–529. https://doi.org/10.1111/j.1600-0587.2011.07146.x

Kitaysky, A. S., Kitaiskaia, E. V., Piatt, J. F., & Wingfield, J. C. (2006). A mechanistic link between chick diet and decline in seabirds? *Proceedings of the Royal Society B: Biological Sciences*, *273*, 445–450. https://doi.org/10.1098/rspb.2005.3351

Koprivnikar, J., & Penalva, L. (2015). Lesser of two evils? Foraging choices in response to threats of predation and parasitism. *PLoS One*, *10*, e0116569. https://doi.org/10.1371/journal.pone.0116569

Korstjens, A. H., & Dunbar, R. I. M. (2007). Time constraints limit group sizes and distribution in red and black-and-white Colobus. *International Journal of Primatology*, *28*, 551–575. https://doi.org/10.1007/s10764-007-9148-2

Korstjens, A. H., Lehmann, J., & Dunbar, R. (2010). Resting time as an ecological constraint on primate biogeography. *Animal Behaviour*, *79*, 361–374. https://doi.org/10.1016/j.anbehav.2009.11.012

Landman, M., Schoeman, D. S., & Kerley, G. I. H. (2013). Shift in black rhinoceros diet in the presence of elephant: Evidence for competition? *PLoS One*, *8*, e69771. https://doi.org/10.1371/journal.pone.0069771

Laundré, J. W., Hernández, L., & Altendorf, K. B. (2001). Wolves, elk, and bison: Reestablishing the "landscape of fear" in Yellowstone National Park, USA. *Canadian Journal of Zoology*, *79*, 1401–1409. https://doi.org/10.1139/z01-094

Lea, J. M. D., Walker, S. L., Kerley, G. I. H., Jackson, J., Matevich, S. C., & Shultz, S. (2018). Non-invasive physiological markers demonstrate link between habitat quality, adult sex ratio and poor population growth rate in a vulnerable species, the Cape mountain zebra. *Functional Ecology*, *32*, 300–312. https://doi.org/10.1111/1365-2435.13000

Madliger, C. L., Love, O. P., Hultine, K. R., & Cooke, S. J. (2018). The conservation physiology toolbox: Status and opportunities. *Conservation Physiology*, *6*, coy029. https://doi.org/10.1093/conphys/coy029

Manire, C. A., Walsh, C. J., Rhinehart, H. L., Colbert, D. E., Noyes, D. R., & Luer, C. A. (2003). Alterations in blood and urine parameters in two Florida manatees (*Trichechus manatus latirostris*) from simulated conditions of release following rehabilitation. *Zoo Biology*, *22*, 103–120. https://doi.org/10.1002/zoo.10074

Mao, R., Xiao, Y.-L., Gao, X., Chen, B.-L., He, Y., Yang, L., Hu, P.-J., & Chen, M.-H. (2012). Fecal calprotectin in predicting relapse of inflammatory bowel diseases: A meta-analysis of prospective studies. *Inflammatory Bowel Diseases*, *18*, 1894–1899. https://doi.org/10.1002/ibd.22861

McEwen, B. S., & Wingfield, J. C. (2003). The concept of allostasis in biology and biomedicine. *Hormones and Behavior*, *43*, 2–15. https://doi.org/10.1016/S0018-506X(02)00024-7

McGill, B. J., Enquist, B. J., Weiher, E., & Westoby, M. (2006). Rebuilding community ecology from functional traits. *Trends in Ecology & Evolution*, *21*, 178–185. https://doi.org/10.1016/j.tree.2006.02.002

McKay, D. M. (2009). The therapeutic helminth? *Trends in Parasitology*, *25*, 109–114. https://doi.org/10.1016/j.pt.2008.11.008

McLennan, M. R., Howell, C. P., Bardi, M., & Heistermann, M. (2019). Are human-dominated landscapes stressful for wild chimpanzees (*Pan troglodytes*)? *Biological Conservation*, *233*, 73–82. https://doi.org/10.1016/j.biocon.2019.02.028

Millspaugh, J. J., & Washburn, B. E. (2004). Use of fecal glucocorticoid metabolite measures in conservation biology research: Considerations for application and interpretation. *General and Comparative Endocrinology*, *138*, 189–199. https://doi.org/10.1016/j.ygcen.2004.07.002

Milot, E., Cohen, A. A., Vézina, F., Buehler, D. M., Matson, K. D., & Piersma, T. (2014). A novel integrative method for measuring body condition in ecological studies based on physiological dysregulation. *Methods in Ecology and Evolution*, *5*, 146–155. https://doi.org/10.1111/2041-210X.12145

Moberg, G. P. (2000). Biological response to stress: Implications for animal welfare. In G. P. Moberg & J. A. Mench (Eds.), *The biology of animal stress: Basic principles and implications for animal welfare* (vol. *1*, pp. 21). CABI Publishing.

Mueller, M., Sternecker, K., Milz, S., & Geist, J. (2020). Assessing turbine passage effects on internal fish injury and delayed mortality using X-ray imaging. *PeerJ*, *8*, e9977. https://doi.org/10.7717/peerj.9977

Noguera, J. C., Aira, M., Pérez-Losada, M., Domínguez, J., & Velando, A. (2018). Glucocorticoids modulate gastrointestinal microbiome in a wild bird. *Royal Society Open Science*, *5*, 171743. https://doi.org/10.1098/rsos.171743

Nuñez, C. M. V., Adelman, J. S., Smith, J., Gesquiere, L. R., & Rubenstein, D. I. (2014). Linking social environment and stress physiology in feral mares (*Equus caballus*): Group transfers elevate fecal cortisol levels. *General and Comparative Endocrinology*, *196*, 26–33. https://doi.org/10.1016/j.ygcen.2013.11.012

O'Dwyer, K., Dargent, F., Forbes, M. R., & Koprivnikar, J. (2020). Parasite infection leads to widespread glucocorticoid hormone increases in vertebrate hosts: A meta-analysis. *Journal of Animal Ecology*, *89*, 519–529. https://doi.org/10.1111/1365-2656.13123

Osorio-Olvera, L., Soberón, J., & Falconi, M. (2019). On population abundance and niche structure. *Ecography*, *42*, 1415–1425. https://doi.org/10.1111/ecog.04442

Ouyang, J. Q., de Jong, M., Hau, M., Visser, M. E., van Grunsven, R. H., & Spoelstra, K. (2015). Stressful colours: Corticosterone concentrations in a free-living songbird vary with the spectral composition of experimental illumination. *Biology Letters*, *11*, 20150517. https://doi.org/10.1098/rsbl.2015.0517

Pallin, L. J., Baker, C. S., Steel, D., Kellar, N. M., Robbins, J., Johnston, D. W., Nowacek, D. P., Read, A. J., & Friedlaender, A. S. (2018). High pregnancy rates in humpback whales (Megaptera novaeangliae) around the Western Antarctic Peninsula, evidence of a rapidly growing population. *Royal Society Open Science*, *5*, 180017.

Parsons, N. J., Vanstreels, R. E., & Schaefer, A. M. (2018). Prognostic indicators of rehabilitation outcomes for adult African penguins (*Spheniscus demersus*). *Journal of Wildlife Diseases*, *54*, 54–65. https://doi.org/10.7589/2017-06-146

Pearson, R. G., & Dawson, T. P. (2003). Predicting the impacts of climate change on the distribution of species: Are bioclimate envelope models useful? *Global Ecology and Biogeography*, *12*, 361–371. https://doi.org/10.1046/j.1466-822X.2003.00042.x

Pedersen, A. B., & Fenton, A. (2007). Emphasizing the ecology in parasite community ecology. *Trends in Ecology & Evolution*, *22*, 133–139. https://doi.org/10.1016/j.tree.2006.11.005

Pironon, S., Papuga, G., Villellas, J., Angert, A. L., García, M. B., & Thompson, J. D. (2017). Geographic variation in genetic and demographic performance: New insights from an old biogeographical paradigm. *Biological Reviews*, *92*, 1877–1909. https://doi.org/10.1111/brv.12313

Pottinger, T. G. (2003). Interactions of endocrine-disrupting chemicals with stress responses in wildlife. *Pure and Applied Chemistry*, *75*, 2321–2333. https://doi.org/10.1351/pac200375112321

Pulliam, H. R., & Danielson, B. J. (1991). Sources, sinks, and habitat selection: A landscape perspective on population dynamics. *The American Naturalist*, *137*, S50–S66.

Rattner, B. A., Eroschenko, V. P., Fox, G. A., Fry, D. M., & Gorsline, J. (1984). Avian endocrine responses to environmental pollutants. *Journal of Experimental Zoology*, *232*, 683–689. https://doi.org/10.1002/jez.1402320337

Reneerkens, J., Piersma, T., & Ramenofsky, M. (2002). An experimental test of the relationship between temporal variability of feeding opportunities and baseline levels of corticosterone in a shorebird. *Journal of Experimental Zoology*, *293*, 81–88. https://doi.org/10.1002/jez.10113

Reuter, H. O., & Adcock, K. (1998). Standardised body condition scoring system for black rhinoceros (*Diceros bicornis*). *Pachyderm*, *26*, 116–121.

Rolland, R. M., McLellan, W. A., Moore, M. J., Harms, C. A., Burgess, E. A., & Hunt, K. E. (2017). Fecal glucocorticoids and anthropogenic injury and mortality in North Atlantic right whales *Eubalaena glacialis*. *Endangered Species Research*, *34*, 417–429. https://doi.org/10.3354/esr00866

Rowe, C. L. (2008). "The calamity of so long life": Life histories, contaminants, and potential emerging threats to long-lived vertebrates. *BioScience*, *58*, 623–631. https://doi.org/10.1641/B580709

Sack, A., Butler, E., Cowen, P., & Lewbart, G. A. (2017). Morbidity and mortality of wild turtles at a North Carolina wildlife clinic: A 10-year retrospective. *Journal of Zoo and Wildlife Medicine*, *48*, 716–724. https://doi.org/10.1638/2016-0053.1

Santini, L., Pironon, S., Maiorano, L., & Thuiller, W. (2019). Addressing common pitfalls does not provide more support to geographical and ecological abundant-centre hypotheses. *Ecography*, *42*, 696–705. https://doi.org/10.1111/ecog.04027

Sapolsky, R. M. (1992). Cortisol concentrations and the social significance of rank instability among wild baboons. *Psychoneuroendocrinology*, *17*, 701–709. https://doi.org/10.1016/0306-4530(92)90029-7

Scheele, B. C., Foster, C. N., Banks, S. C., & Lindenmayer, D. B. (2017). Niche contractions in declining species: Mechanisms and consequences. *Trends in Ecology & Evolution*, *32*, 346–355. https://doi.org/10.1016/j.tree.2017.02.013

Scheun, J., Ludynia, K., Snyman, A., & Ganswindt, A. (2021). Non-invasive hormone monitoring as a robust method for determining adrenocortical activity in injured, emaciated and oil-contaminated African penguins undergoing rehabilitation. *General and Comparative Endocrinology*, *303*, 113703.

Schlotz, W., Hammerfald, K., Ehlert, U., & Gaab, J. (2011). Individual differences in the cortisol response to stress in young healthy men: Testing the roles of perceived stress reactivity and threat appraisal using multiphase latent growth curve modeling. *Biological Psychology*, *87*, 257–264. https://doi.org/10.1016/j.biopsycho.2011.03.005

Schroeder, H. W. Jr., & Cavacini, L. (2010). Structure and function of immunoglobulins. *Journal of Allergy and Clinical Immunology*, *125*, S41–S52. https://doi.org/10.1016/j.jaci.2009.09.046

Schwindt, A. R., Winkelman, D. L., Keteles, K., Murphy, M., & Vajda, A. M. (2014). An environmental oestrogen disrupts fish population dynamics through direct and transgenerational effects on survival and fecundity. *Journal of Applied Ecology*, *51*, 582–591. https://doi.org/10.1111/1365-2664.12237

Seebacher, F., & Krause, J. (2017). Physiological mechanisms underlying animal social behaviour. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1727), 20160231. https://doi.org/10.1098/rstb.2016.0231

Semeniuk, C. A., Bourgeon, S., Smith, S. L., & Rothley, K. D. (2009). Hematological differences between stingrays at tourist and non-visited sites suggest physiological costs of wildlife tourism. *Biological Conservation*, *142*, 1818–1829. https://doi.org/10.1016/j.biocon.2009.03.022

Shepard, E. L. C., Wilson, R. P., Rees, W. G., Grundy, E., Lambertucci, S. A., & Vosper, S. B. (2013). Energy landscapes shape animal movement ecology. *The American Naturalist*, *182*, 298–312. https://doi.org/10.1086/671257

Shultz, S., Baral, H. S., Charman, S., Cunningham, A. A., Das, D., Ghalsasi, G. R., Goudar, M. S., Green, R. E., Jones, A., Nighot, P., Pain, D. J., & Prakash, V. (2004). Diclofenac poisoning is widespread in declining vulture populations across the Indian subcontinent. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *271*, S458–S460. https://doi.org/10.1098/rsbl.2004.0223

Sies, H. (1991). Oxidative stress: From basic research to clinical application. *The American Journal of Medicine*, *91*, S31–S38. https://doi.org/10.1016/0002-9343(91)90281-2

Singer, A. C., Shaw, H., Rhodes, V., & Hart, A. (2016). Review of antimicrobial resistance in the environment and its relevance to environmental regulators. *Frontiers in Microbiology*, *7*, 1728. https://doi.org/10.3389/fmicb.2016.01728

Slos, S., & Stoks, R. (2008). Predation risk induces stress proteins and reduces antioxidant defense. *Functional Ecology*, *22*, 637–642. https://doi.org/10.1111/j.1365-2435.2008.01424.x

Sommer, F., & Bäckhed, F. (2013). The gut microbiota—masters of host development and physiology. *Nature Reviews Microbiology*, *11*, 227–238. https://doi.org/10.1038/nrmicro2974

Sopinka, N. M., Donaldson, M. R., O'Connor, C. M., Suski, C. D., & Cooke, S. J. (2016). Stress indicators in fish. *Fish physiology*, *35*, 405–462.

Sparks, A. M., Watt, K., Sinclair, R., Pilkington, J. G., Pemberton, J. M., Johnston, S. E., McNeilly, T. N., & Nussey, D. H. (2018). Natural selection on antihelminth antibodies in a wild mammal population. *The American Naturalist*, *192*, 745–760. https://doi.org/10.1086/700115

Støen, O.-G., Ordiz, A., Evans, A. L., Laske, T. G., Kindberg, J., Fröbert, O., Swenson, J. E., & Arnemo, J. M. (2015). Physiological evidence for a human-induced landscape of fear in brown bears (*Ursus arctos*). *Physiology & Behavior*, *152*, 244–248. https://doi.org/10.1016/j.physbeh.2015.09.030

Stumpf, R. M., Gomez, A., Amato, K. R., Yeoman, C. J., Polk, J., Wilson, B. A., Nelson, K. E., White, B., & Leigh, S. R. (2016). Microbiomes, metagenomics, and primate conservation: New strategies, tools, and applications. *Biological Conservation*, *199*, 56–66. https://doi.org/10.1016/j.biocon.2016.03.035

Supali, T., Verweij, J. J., Wiria, A. E., Djuardi, Y., Hamid, F., Kaisar, M. M. M., Wammes, L. J., Lieshout, L. V., Luty, A. J. F., Sartono, E., & Yazdanbakhsh, M. (2010). Polyparasitism and its impact on the immune system. *International Journal for Parasitology*, *40*, 1171–1176. https://doi.org/10.1016/j.ijpara.2010.05.003

Tarszisz, E., Dickman, C. R., & Munn, A. J. (2014). Physiology in conservation translocations. *Conservation Physiology*, *2*, cou054. https://doi.org/10.1093/conphys/cou054

Tartu, S., Gabrielsen, G. W., Blévin, P., Ellis, H., Bustnes, J. O., Herzke, D., & Chastel, O. (2014). Endocrine and fitness correlates of long-chain perfluorinated carboxylates exposure in Arctic breeding black-legged kittiwakes. *Environmental Science & Technology*, *48*, 13504–13510. https://doi.org/10.1021/es503297n

Todgham, A. E., & Stillman, J. H. (2013). Physiological responses to shifts in multiple environmental stressors: Relevance in a changing world. *Integrative and Comparative Biology*, *53*, 539–544. https://doi.org/10.1093/icb/ict086

Trevelline, B. K., Fontaine, S. S., Hartup, B. K., & Kohl, K. D. (2019). Conservation biology needs a microbial renaissance: A call for the consideration of host-associated microbiota in wildlife management practices. *Proceedings of the Royal Society B: Biological Sciences*, *286*, 20182448.

Tripp, K. M., Verstegen, J. P., Deutsch, C. J., Bonde, R. K., Wit, M. D., Manire, C. A., Gaspard, J., & Harr, K. E. (2011). Evaluation of adrenocortical function in Florida manatees (*Trichechus manatus latirostris*). *Zoo Biology*, *30*, 17–31.

van de Crommenacker, J., Hammers, M., van der Woude, J., Louter, M., Santema, P., Richardson, D. S., & Komdeur, J. (2017). Oxidative status and fitness components in the Seychelles warbler. *Functional Ecology*, *31*, 1210–1219. https://doi.org/10.1111/1365-2435.12861

van de Crommenacker, J., Komdeur, J., Burke, T., & Richardson, D. S. (2011). Spatio-temporal variation in territory quality and oxidative status: A natural experiment in the Seychelles warbler (*Acrocephalus sechellensis*). *Journal of Animal Ecology*, *80*, 668–680. https://doi.org/10.1111/j.1365-2656.2010.01792.x

van de Crommenacker, J., Richardson, D. S., Koltz, A. M., Hutchings, K., & Komdeur, J. (2012). Parasitic infection and oxidative status are associated and vary with breeding activity in the Seychelles warbler. *Proceedings of the Royal Society B: Biological Sciences*, *279*, 1466–1476. https://doi.org/10.1098/rspb.2011.1865

Van Meter, P. E., French, J. A., Dloniak, S. M., Watts, H. E., Kolowski, J. M., & Holekamp, K. E. (2009). Fecal glucocorticoids reflect socio-ecological and anthropogenic stressors in the lives of wild spotted hyenas. *Hormones and Behavior*, *55*, 329–337. https://doi.org/10.1016/j.yhbeh.2008.11.001

Van Rheenen, P. F., Van de Vijver, E., & Fidler, V. (2010). Faecal calprotectin for screening of patients with suspected inflammatory bowel disease: Diagnostic meta-analysis. *BMJ*, *341*, c3369. https://doi.org/10.1136/bmj.c3369

Viijoen, J. J., Ganswindt, A., du Toit, J. T., & Langbauer, W. R. (2008). Translocation stress and faecal glucocorticoid metabolite levels in free-ranging African savanna elephants. *South African Journal of Wildlife Research*, *38*, 146–152. https://doi.org/10.3957/0379-4369-38.2.146

Violle, C., Navas, M. L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., & Garnier, E. (2007). Let the concept of trait be functional! *Oikos*, *116*, 882–892. https://doi.org/10.1111/j.0030-1299.2007.15559.x

Vynne, C., Booth, R. K., & Wasser, S. K. (2014). Physiological implications of landscape use by free-ranging maned wolves (*Chrysocyon brachyurus*) in Brazil. *Journal of Mammalogy*, *95*, 696–706.

Walshe, N., Duggan, V., Cabrera-Rubio, R., Crispie, F., Cotter, P., Feehan, O., & Mulcahy, G. (2019). Removal of adult cyathostomins alters faecal microbiota and promotes an inflammatory phenotype in horses. *International Journal for Parasitology*, *49*, 489–500. https://doi.org/10.1016/j.ijpara.2019.02.003

Wasser, S. K., Lundin, J. I., Ayres, K., Seely, E., Giles, D., Balcomb, K., Hempelmann, J., Parsons, K., & Booth, R. (2017). Population growth is limited by nutritional impacts on pregnancy success in endangered Southern Resident killer whales (*Orcinus orca*). *PLoS One*, *12*, e0179824. https://doi.org/10.1371/journal.pone.0179824

Watt, K. A., Nussey, D. H., Maclellan, R., Pilkington, J. G., & McNeilly, T. N. (2016). Fecal antibody levels as a noninvasive method for measuring immunity to gastrointestinal nematodes in ecological studies. *Ecology and Evolution*, *6*, 56–67. https://doi.org/10.1002/ece3.1858

Weinstein, S. B., Buck, J. C., & Young, H. S. (2018). A landscape of disgust. *Science*, *359*, 1213–1214. https://doi.org/10.1126/science.aas8694

Wessling, E. G., Deschner, T., Mundry, R., Pruetz, J. D., Wittig, R. M., & Kühl, H. S. (2018). Seasonal variation in physiology challenges the notion of chimpanzees (*Pan troglodytes* verus) as a forest-adapted species. *Frontiers in Ecology and Evolution*, *6*, 60. https://doi.org/10.3389/fevo.2018.00060

Wilson, A. E., Wismer, D., Stenhouse, G., Coops, N. C., & Janz, D. M. (2021). Landscape condition influences energetics, reproduction, and stress biomarkers in grizzly bears. *Scientific Reports*, *11*, 1–16. https://doi.org/10.1038/s41598-021-91595-4

Wolf, T. E., Valades, G. B., Simelane, P., Bennett, N. C., & Ganswindt, A. (2018). The relationship between physical injury, body condition and stress-related hormone concentrations in free-ranging giraffes. *Wildlife Biology*, *1*, 1–6.

Wong, B., & Candolin, U. (2015). Behavioral responses to changing environments. *Behavioral Ecology*, *26*, 665–673. https://doi.org/10.1093/beheco/aru183

Zaneveld, J. R., McMinds, R., & Thurber, R. V. (2017). Stress and stability: Applying the Anna Karenina principle to animal microbiomes. *Nature Microbiology*, *2*, 1–8. https://doi.org/10.1038/nmicrobiol.2017.121

How to cite this article: Shultz, S., Britnell, J. A., & Harvey, N. (2021). Untapped potential of physiology, behaviour and immune markers to predict range dynamics and marginality. *Ecology and Evolution*, 11, 16446–16461. https://doi.org/10.1002/ece3.8331

Appendix 2) Species stereotypes as a result of unconscious research biases compromise conservation efficacy

Published in Biological Conservation as J Britnell, J. A., Lewis, R. N., Elsner-Gearing, F., Harvey, N., Stanbrook, E., & Shultz, S. (2021). Species stereotypes as a result of unconscious research biases compromise conservation efficacy. *Biological Conservation*, *261*, 109275.

Biological Conservation 261 (2021) 109275

Contents lists available at ScienceDirect

Biological Conservation

journal homepage: www.elsevier.com/locate/biocon

Perspective

Species stereotypes as a result of unconscious research biases compromise conservation efficacy

J.A. Britnell [a,b], R.N. Lewis [a,b], F. Elsner-Gearing [a,b], N. Harvey [a,b], E. Stanbrook [a], S. Shultz [a,*]

[a] Department of Earth and Environmental Sciences, University of Manchester, Manchester, United Kingdom
[b] North of England Zoological Society (Chester Zoo), Caughall Road, Upton-by-Chester, CH2 1LH, United Kingdom

ARTICLE INFO

Keywords:
Research-bias
Shifting-baselines
Niche-space
Overgeneralisation
Range-contraction
Extinction

ABSTRACT

Taxonomic and geographic biases in ecological research are widely recognised. In addition, information bias *within* a species can impact our understanding of their biology. This can lead to an underestimation of potential intra- or inter-population level variation and plasticity, and incomplete inferences about species response curves across environmental gradients. The consequences of these 'species stereotypes' are misestimation of the potential niche and narrow, potentially biased, views of habitat and diet preferences. For example, species may be characterised as ecologically static, or a habitat, diet, or prey 'specialist'. Several factors can contribute to the formation of a 'stereotype', including a focus on extant populations, or a subset of them, that only partially represent the full historical distribution of a species, and an emphasis on species interactions derived from a small number of potential communities. Such species stereotypes are likely widespread and impact on many taxa. These misconceptions can have knock-on effects for conservation programmes and lead to ineffective or harmful conservation interventions such as actively managing species in marginal habitats, not identifying key threats and incorrect predictions of vulnerability to environmental change. Recognising biases is vital to addressing these potential problems and providing accurate information for conservation programmes. Biases can be identified by evaluating historical distributions, translocations within historical distributions, developing mechanistic distribution models and assessing traditional ecological knowledge. We suggest that explicit assessment of biases and potential stereotypes are included in red listing or species assessments, biodiversity action plans, and protected area network design and evaluation.

1. Species stereotypes may explain poor conservation effectiveness

Despite the increase in global protected areas, species continue to decline (Tittensor et al., 2014). Although this 'protected area paradox' is a consequence of protected areas encompassing only a small proportion of most species' geographical ranges, another contributing factor may be that conservation strategies can be based on limited, inaccurate or biased conceptions about species' basic ecology and fundamental niche space (Kerley et al., 2020). Whilst research biases towards certain species (Clark and May, 2002) or habitats (Martin et al., 2012) are well discussed, biases relating to how we study populations *within* a species have only recently gained more attention (Christie et al., 2020). Within-species biases have the potential to influence our understanding of the species as a whole. For example, within-population sampling biases due to STRANGE characteristics (Social status, Trappability, Rearing, Acclimation, Natural changes, Genetics and Experience) impact on the conclusions of behavioural experiments (Webster and Rutz, 2020). Additionally, if information about a species is limited to a small, biased sample of populations, a narrow perception of species' basic biology may be assumed to represent the entire species' ecological breadth (Mengüllüoğlu et al., 2018). Incomplete information can lead to a 'species stereotype', a misestimation of intra- or inter-population level variation and response curves, and an erroneous, narrow or incomplete characterisation of the species' biology.

We propose that species stereotypes are pervasive, underappreciated, and can have serious consequences for the effectiveness of conservation strategies. In this review, we discuss three causes of species stereotypes: 1) recent range contraction, 2) within-species research bias and 3) limited evidence of species interactions. We discuss specific examples where these errors have impacted on conservation interventions in terms of misestimating potential niche space and resilience, failing to

---

* Corresponding author.
E-mail address: susanne.shultz@manchester.ac.uk (S. Shultz).

https://doi.org/10.1016/j.biocon.2021.109275
Received 30 November 2020; Received in revised form 13 July 2021; Accepted 16 July 2021

identify key threats, and poor assessment of community dynamics and potential. If resilience and available niche space are overestimated, extinction risk can be underappreciated, whereas if they are underestimated potential opportunities for conservation intervention can be missed. Finally, we outline solutions to identify species stereotypes and discuss how to incorporate assessments of biases into the conservation planning process. This review focusses on vertebrates, however, species stereotypes are likely to impact all taxonomic groups including invertebrates (*e.g.* Semmens et al., 2016) and plants (*e.g.* Jensen et al., 2020).

1.1. Cause 1: population decline, range contraction and shifts leading to misestimating historic and potential niche breadth

Population decline, range contraction and range shifts are widespread across taxa. Extensive range contraction leaves species restricted to a fraction of their historical ranges, which is unlikely to represent their formerly realised or potential niche breadth (Scheele et al., 2017) (Fig. 1). Moreover, many species are likely to move away from anthropogenic landscapes and persecution into areas of reduced threats (Scheele et al., 2017), which may be suboptimal refuges rather than oases of optimal habitats (Kerley et al., 2020). Alternatively, species may adopt a commensal lifestyle, which can broaden a historical niche (McGeoch and Latombe, 2016). The consequences of anthropogenic impacts and range contraction can lead to a species' current realised niche being significantly reduced and shifted from an historical baseline (Fig. 1). The importance of restoring species to 'full ecological functionality' across their ecological niche has recently gained traction (Grace et al., 2019), however, unaddressed stereotypes may limit these efforts where a species' full potential niche is not identified. In these cases, a full picture of a species' biology cannot be obtained by simply studying more extant populations. In fact, additional data from relict or refugee populations can serve to reinforce 'stereotypes' where the contraction or shift in occupied niche space has not been recognised or acknowledged. An understanding of a species' biology, ecology and conservation status based primarily on altered contemporary states results in 'shifted baselines' (Pauly, 1995).

It is probable that stereotypes are pervasive across taxa, especially in areas with high human population density associated with widespread habitat loss. However, in many cases enough populations have been extirpated to make it difficult to assess relative population performance. Therefore, the specific examples we highlight where range contraction has led to niche space being misestimated are cases where conservation interventions have been demonstrably suboptimal (Table 1). Most of these species have been mistakenly identified as specialists with narrow habitat preferences or relicts who have been backed into an evolutionary cul-de-sac. However, historical records or changes in fitness and performance in more biologically or taxonomically appropriate habitats suggest that their apparent specialisms are a product of chance rather than adaptation or preference. Such issues may be exacerbated in endangered species of conservation concern, as rarity is likely to reduce sampling opportunities and increase the potential for biases in available data.

In extreme circumstances, range contraction may result in the complete extirpation of species from the wild. In these circumstances the only available contemporary populations may be found in captivity. Stereotypes based on captive populations may contribute to a skewed understanding of behaviour and biology. The reintroduction of Przewalski's horse (*Equus ferus przewalski*) into their historical range is particularly challenging, as there is little information on the ecology or behaviour of wild populations pre-extirpation. Furthermore, what information we do have is biased, as the last remaining populations were ecological refugees found in arid, browse-dominated habitats with very low human density (Kaczensky et al., 2017). Thus, much of our understanding about their social behaviour and life history is necessarily based on captive populations (Boyd and Houpt, 1994). Vital rates from

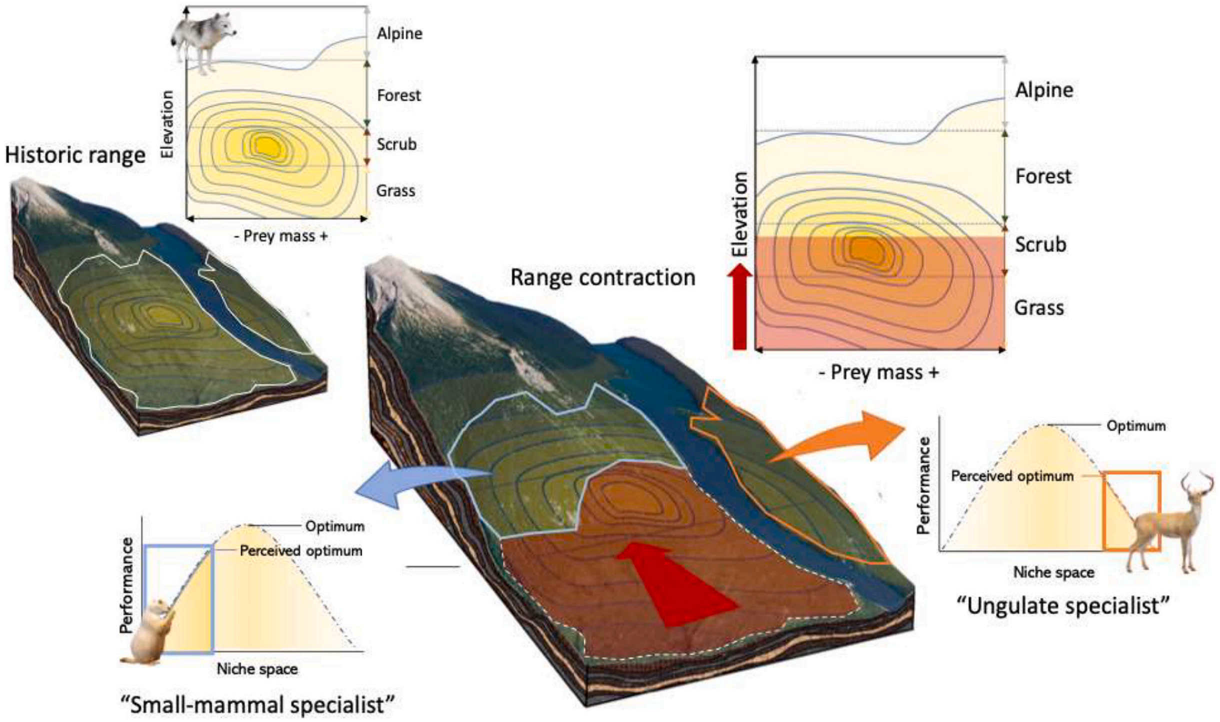


Fig. 1. Historic range of a hypothetical carnivore (left) with theoretical niche space based on two axis (elevation and prey mass). The same range (right) under range contraction where the carnivore is excluded from the red part of its range and corresponding niche space. Contracted niche space confines the carnivore to suboptimal range in forest and alpine areas at higher elevation. If research were confined to either the blue outlined, or orange outlined study sites in the contracted range, the carnivore could be stereotyped as a small-mammal specialist (blue) or ungulate specialist (orange). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

Examples of species stereotypes. Species stereotypes identified from scientific literature where recent information challenges commonly held views of habitat or dietary preferences due to mis- or underestimation of niche space, and the impact of these stereotypes on conservation efficacy for the species.

Class	Species	Stereotyped as:	Evidence for bias	Conservation implications	Bias acknowledged in IUCN species assessment
Aves	Nēnē (*Branta sandvicensis*)	High altitude preferred habitats.	Historical occupancy and movement data (Hess et al., 2012); lower mortality in and emigration to lowland release sites (Black et al., 1997)	Selection of suboptimal (high altitude) release sites	No (BirdLife International, 2017)
	Cantabrian Capercaillie (*Tetrao urogallus cantabricus)*	Adapted to Euro-siberia and unsuited to Mediterranean biogeographic regions	Recently found in Mediterranean oak forests (González et al., 2010)	Failure to conserve across full range of suitable habitats; underestimation of ecological flexibility	No (BirdLife International, 2016)
Mammalia	Cheetah *(Acinonyx jubatus)*	Savannah specialists	Occupies a range of habitats; broader prey spectra, higher cub survival, lower kleptoparasitism in thickets (Bissett and Bernard, 2007)	Underestimation of ecological flexibility; failure to conserve across all suitable habitats	No (Durant et al., 2015)
	Giant panda (*Ailuropoda melanoleuca)*	Bamboo specialist	Distribution of fossil/archaeological record; gastrointestinal microbiota; (Kerley et al., 2020; Han et al., 2019)	Failure to conserve suitable habitat; underestimation of ecological flexibility; potential reintroduction sites overlooked	No (Swaisgood et al., 2016)
	European Bison (*Bison bonasus)*	Forest specialist	Phylogeny; dental and digestive morphology; neonatal behaviour; microhabitat selection (Kerley et al., 2012); isotopic shift in skeletal remains (Bocherens et al., 2015)	Reintroduction to suboptimal (forest) habitats (Kerley et al., 2012)	Yes (Plumb et al., 2020)
	Sea otters (*Enhydra lutris)*	Obligate kelp beds habitat	Substantial predator protection and increased food in seagrasses and salt marshes (Silliman et al., 2018)	Failure to conserve across full range of suitable habitats; incorrect assumptions used in species distribution models	No (Doroff and Burdin, 2015)
	Cape mountain zebra (*Equus zebra zebra)*	Mountain specialist	Distribution of fossil record (Faith, 2012); reduced population performance (Lea et al., 2016)	Maintained in suboptimal (fynbos) habitat (Lea et al., 2016)	No (Gosling et al., 2019)
	Eurasian otters (*Lutra lutra)*	Preferentially occupy river heads	Higher reproductive success in middle river stretches; higher abundance of preferred prey in low-elevation rivers (Martínez-Abraín and Jiménez, 2016)	Failure to conserve in optimal habitat; incorrect assumptions used in species distribution models	No (Roos et al., 2015)
	Mediterranean monk seal (*Monachus monachus)*	Cave-breeding specialist	Offspring survival greater on open beaches; offspring have black fur (Martínez-Abraín and Jiménez, 2016)	Failure to conserve in optimal habitat; reduced population performance	No (Karamanlidis and Dendrinos, 2015)
	North Borneo orangutan *(Pongo pygmeaus morio)*	Primary old growth forest specialist	Relatively high density in low intensity logging concessions compared to some primary forest (Ancrenaz et al., 2010)	Failure to incorporate habitat outside protected areas into conservation management plans (Meijaard, 2017)	No (Ancrenaz et al., 2016)
	Tapanuli orangutan (*Pongo tapanuliensis*)	High altitude specialist	Historical resources indicate broader past distribution, covering a wider range of habitat types including lowland areas (Meijaard et al., 2021)	Failure to conserve across full range of suitable habitats; underestimation of ecological flexibility	No habitat preferences; Yes wider historical distribution (Nowak et al., 2017)
	Javan rhinoceros (*Rhinoceros sondaicus*)	Solitary, lowland forest specialist (Nardelli, 2016)	Historical accounts (Groves and Leslie, 2011) and habitat preferences (Santosa et al., 2013) suggest more widespread distribution and gregarious	Only conserved in small populations at low density in dense primary forest	Yes habitat; No behaviour (Ellis and Talukdar, 2020)
	Saiga (*Saiga tatarica*)	Restricted to semi/complete deserts in Central Asia	Stable isotope analysis of fossil specimens (Jürgensen et al., 2017) and poor habitat suitability within current range (Cui et al., 2017)	Underestimation of ecological flexibility; suitable habitats for reintroduction overlooked	No (IUCN SSC antelope group, 2018)
Reptilia	American Alligator (*Alligator mississippiensis)*	Freshwater specialists	Higher relative abundance in salt marshes, seagrasses, and mangroves, compared to freshwater habitats (Nifong and Silliman, 2017)	Failure to conserve across full range of suitable habitats; impact on food chain dynamics	No (Elsey et al., 2019)

captive animals can underestimate reproductive rates and overestimate adult survivorship, which, in turn, impacts on population viability models (Slotta-Bachmayr et al., 2004). Due to these differences, management interventions based solely on data from captivity may not be as useful as initially expected.

1.2. Cause 2: research biases leading to partial understanding of species biology

Systematic biases, in terms of study locations and species, are widely recognised in ecological research (Titley et al., 2017). European and North American habitats are better studied than those in the tropics; large, charismatic, diurnal mammals are better studied than other groups. Recognising these biases has helped both researchers and conservation practitioners identify underrepresented taxon groups (Conde et al., 2019) and habitats (Martin et al., 2012) and highlight priority areas for research. However, research biases also occur within species and may include geographic or temporal biases. Habitats or time periods where animals are easier to find and observe are more attractive for researchers and remote, inaccessible sites with little infrastructure are underrepresented. These biases can be perpetuated where research funding is preferentially directed towards low risk, long-term and well-established sites. Funding bias and the time and resource-intensive nature of these projects is recognised as a barrier to addressing biases in evidence-based conservation for primates (Junker et al., 2020). Thus, our knowledge of specific populations becomes very deep but our understanding about ecological flexibility, resilience and variation between populations is poor. These biases can lead to species stereotypes even where extremely rich data are available.

In some cases, preconceived or popular views of a species' ecology lead to bias in study populations, reinforcing already held stereotypes even in extensively studied species of conservation concern. For example, the majority of long-term, data-rich studies of cheetah (*Acinonyx jubatus*) have taken place in open habitats, such as the Serengeti, despite well-performing, resilient populations in more closed habitats (Bissett and Bernard, 2007) (Table 1). In these cases, species stereotypes can be addressed more easily than information biases caused by range contraction since targeted research across the habitats occupied by extant populations can identify biases and confirm habitats associated with resilient populations.

Species stereotypes can also arise from an unappreciation of temporal variation in niche breadth, which may occur across the lifespan of an organism (*e.g.* seasonal migration) or across life history stages of a species. If only data from well-studied time periods or life history stages are used in a species' management across its lifespan (Drenner et al., 2012) key habitat requirements can be missed and important conservation areas may be overlooked. This is especially common in species with long migrations (Morreale et al., 2007), larval stages (Leis et al., 2011), pelagic or deep-water living (Scott and Chivers, 2009), or a combination of these. A review of migratory bird conservation status (Kirby et al., 2008) highlights that key threats can occur at breeding, over-wintering sites, and along migratory routes, with important sites varying by group and region.

Stereotypes can disproportionately affect species with larval stages because we generally know far less about early life stages than more easily identifiable adult individuals, especially when larvae form large heterospecific assemblages. Therefore, conservation efforts are often based on the more accessible adult form despite effective conservation being dependent on meeting the species' needs across its lifespan. The European eel (*Anguilla anguilla*) faces threats at every life stage, which has led to a population reduction of over 95% since the 1980s (Åström and Dekker, 2007). In particular, there has been a marked decline in recruitment of glass eels to continental waters, provisionally attributed to the impacts of climate change on larval survival and migration (Jacoby et al., 2015). Despite this, research is still largely restricted to the post-larval (fresh water) phases and our understanding of the marine larval stage remains severely limited (Jacoby et al., 2015).

1.3. Cause 3. Limited evidence of species interactions and community structuring

The third cause of species stereotypes is an incomplete understanding of species interactions at the community level. Species interactions depend on both biotic and abiotic factors and are likely to be habitat specific (Pellissier et al., 2018). Species that have only been studied in a small portion of their potential habitats, and in a limited number of communities, can lead to a narrow perception of an organism's functional role within an ecosystem (Robles and Martin, 2014). If only a subset of communities is assessed, some interactions may be mistaken for obligate biological relationships. Community assemblages can be highly dependent on human-mediated species introductions and removals, which form novel ecological interactions (Morse et al., 2014). Such novel interactions can also emerge due to climate change and land use change (Hobbs et al., 2006). Communities in human-dominated landscapes are particularly likely to lead to stereotypes due to novel interactions and the loss of species interactions. Conversely, limiting basic species research to 'pristine' communities with little anthropogenic influence may underestimate species resilience or potential for commensality.

1.4. Example conservation implications of species stereotypes and shifted baselines

Stereotypes about species biology, population dynamics or habitat preferences derived from shifted baselines can impact on the efficacy of conservation interventions (Papworth et al., 2009). Where narrow habitat occupancy or diet is a result of historical environmental change or persecution, continued management in the margins of a species' potential niche space can constrain them to continued poor performance despite intensive interventions (Kerley et al., 2020). This may be the case, even in flagship species, that have received extensive conservation investment. For example, ecological 'refugees', such as in the European Bison *(Bison bonasus)* may be confined to, and managed in, suboptimal habitats with reduced survival, reproduction, and resilience (Fig. 1, Kerley et al., 2012). In addition, there are examples of species (Table 1) recolonising and thriving outside of relict habitats due to assumed behavioural adaptation or reduced human threat, suggesting the relict populations represent a narrow window of the species niche potential (Martínez-Abraín et al., 2019 for European examples; Silliman et al., 2018 for North America and Asia).

Generalising from a subset of available data can limit conservation programmes and misinform management decisions, including missing opportunities to establish new populations or implementing overly uniform or inappropriate management strategies (Table 1). If research and conservation action is focused on only one part of the annual range or captures only one temporal stage, major threats in others can be missed. For example, the sociable lapwing (*Vanellus gregarius*), underwent a 90% population decline in the 20th century (BirdLife International, 2019). This species breeds on the steppes of Kazakhstan and South Russia, follows two different migratory routes through conflict zones in Central Asia and overwinters in South Asia, the Middle East, and North Africa (Donald et al., 2016). As research was focused on known breeding and wintering groups, high mortality from illegal hunting along the migration route was overlooked and is now recognised as an important factor in the recorded declines (Brochet et al., 2017). Detailed studies of Eurasian lynx (*Lynx lynx*), focused on Central and Eastern European populations reported that lynx preferentially prey on medium to large ungulates (Mengüllüoğlu et al., 2018). However, recent research suggests their diet can be far more varied depending on prey communities across the range. Thus, rather than limiting conservation interventions to populations with access to large-bodied prey, in some parts of the Eurasian lynx's range encouraging lagomorph populations may be a more appropriate strategy (Mengüllüoğlu et al., 2018). Species stereotypes from a single life stage or habitat can result in misestimation of the species' ability to withstand predicted challenges. Consequently, conservation needs will be missed, and necessary interventions may not be enacted. Fishing quotas for Atlantic bluefin tuna *(Thunnus thynnus)* were established based on key movement and dispersal patterns from studies focusing solely on eastern stock. However, because fish from the western stock were more likely to migrate to the eastern stock area than *vice versa*, the western stock may be more vulnerable to overfishing because of lower recruitment rates (Cadrin et al., 2018).

Ecological research biased towards undisturbed or 'pristine' habitats may overlook suitable habitats that may increase the number and extent of viable populations. For decades orangutans (*Pongo pygmaeus*) have primarily been studied in undisturbed old growth forest within national parks. On the basis of those studies it was assumed they would perform poorly in disturbed habitats, such as those generated by selective timber harvesting (Meijaard, 2017). In fact, North Bornean orangutan (*Pongo pygmaeus morio*) occur at relatively high density in some logging concessions, suggesting they are more resilient and ecologically flexible than assumed (Ancrenaz et al., 2010; Meijaard et al., 2010). Importantly, the assumption that they required old growth primary forest led to delayed implementation of conservation measures outside national parks (Meijaard, 2017). The closely related Critically Endangered Tapanuli orangutan (*Pongo tapanuliensis*) was assumed to be a high altitude specialist. Historical evidence, however, suggests a much wider distribution (Meijaard et al., 2021) and there is the possibility that low intensity logging habitats could also maintain sustainable populations.

Stereotypes may underestimate species' potential to thrive in a range of communities and ecosystems. For example, if a relationship between two species is thought to be obligate but is not, then conservation efforts may be unnecessarily restricted to situations where the two species co-occur. Reintroduction efforts for the black-footed ferret (*Mustela nigripes)* were canalised based on extant communities' dependence on prairie dogs (*Cynomys* sp.) as prey. However, fossil evidence suggests this is not an obligate relationship, which increases the number of potential release sites (Owen et al., 2000). Conversely, if a particular interaction or its cascading effects are not known, then conservation programmes may fail to include species vital to the survival of the target species or the functioning of the ecosystem. This is most notable in systems where keystone species have been extirpated or reduced to low densities. The reintroduction of wolves (*Canis lupus)* to Yellowstone National Park which predate elk *(Cervus elaphus),* has led to decreased browsing pressure and increased populations of beavers *(Caster canadensis)*, bison *(Bison bison)*, and passerines (Ripple and Beschta, 2012). Conservation of this ecosystem would be much more difficult without knowledge of the cascading impacts of wolves.

Range contraction of large herbivores may mean we have studied systems that are missing key functionality such as nutrient cycling (Doughty et al., 2016) and seed dispersal (Pires et al., 2014). Moreover, the loss of megaherbivore ecosystem engineers can lead to the loss of mosaic habitats and encroaching woodland (Ripple et al., 2015). These newly closed habitats may then be deemed as inappropriate for large grazing species, even if part of the historic range. Species stereotypes based on limited understanding of species' ecosystem roles can give rise to unexpected ecosystem trajectories. An example of an unforeseen interaction is the protection of Audouin's gull (*Larus audouinii*) on small, coastal islet habitats (Martínez-Abraín and Jiménez, 2016). The nitrogen-rich guano from these colonies changes island vegetation communities making habitats unsuitable for future breeding, highlighting the need for alternative conservation strategies.

Finally, misunderstandings about a species' biology can feed into predictive models of species distribution and responses to anthropogenic change, which are widely used to both identify suitable habitat and to predict future responses to climate and other anthropogenic impacts. The accuracy of such models depends on the quality and completeness of data (Stockwell and Peterson, 2002); one persistent limitation is biased sampling of species occurrences (Anderson, 2012). Although the quality of models has consistently improved over time, incomplete sampling and extrapolation based on assumptions about a species biology remain a concern (Araújo et al., 2019). These biases in sampling can be both geographic and environmental, such that the resolution of occurrence data is not the same across a species range. Where biases in the underlying data are not recognised and accounted for, models may underestimate suitable ranges and poorly predict responses to environmental change.

1.5. Solution 1: establishing accurate historical baselines

There is no 'one size fits all' approach across species and conservation programmes. However, we highlight a set of tools that can be used to identify and evaluate the potential for existing biases, as it is impossible to act on biases without recognising them. The most challenging aspect to addressing species stereotypes is that information biases are unlikely to be recognised without an objective evaluation of species historical niche occupancy *versus* the ecological space our contemporary understanding is based on. To identify shifted baselines (Pauly, 1995), we can evaluate range contraction, assemblage change, or habitat shifts by evaluating fossil and archaeological assemblages, reconstructing palaeo-environments, comparing traits between historical and modern-day populations, and evaluating occupancy changes (Bonebrake et al., 2010). Fossil and occurrence records can be paired with historic climate, habitat, and human activity intensity data to improve habitat suitability and species distribution models (Grace et al., 2019; Monsarrat et al., 2019). Moreover, these historical data can evidence past changes in realised niche space, habitat occupancy, and behavioural and ecological observations before widespread declines. For example, fossils of Cape mountain zebra *(Equus zebra zebra)* revealed a previous association with extensive grasslands (Faith, 2012), a clear contrast to the mountain fynbos habitats where extant populations underperform (Lea et al., 2016). Similarly, isotope ratio changes between fossil and modern European Bison highlighted recent shifts from grazing to browsing (Bocherens et al., 2015). Combining fossil assemblages and isotope ratio changes has demonstrated current giant panda distribution is more restricted to montane bamboo forests, with their diet more specialised on bamboo, than historical populations that were once widespread across a range of habitats in China and South East Asia with a wider ecological niche and broader diet (Han et al., 2019).

Historical occupancy records based on museum collections of specimens and other art/artefacts are also available for many species (Mcclenachan et al., 2012) and can reveal changes in niche space. Historical records of snub-nosed monkeys (*Rhinopithecus sp.*) reveal that the genus was extirpated from lowland areas, refuting the stereotype of the species as a highland specialist (Nüchel et al., 2018). In South African mammals, combining extant ranges with historical occurrence records changes the estimate of niche breadth and habitat occupancy in over a third of evaluated species (Monsarrat et al., 2019). Incorporating historical data into biogeographical studies and, in turn, using appropriate historical baselines in conservation management, can allow us to select suitable habitats for protection, identify suitable release sites, and potentially determine causes of extirpation.

An important caveat for all historical records (written, collection, and fossil) is that there are geographical and taxonomic biases in reporting and a significant challenge in detecting absence from pseudo-absence in records (Isaac and Pocock, 2015). Written accounts are more likely to come from near well-travelled routes and to be more accurate for large, charismatic, easily identified species than for reclusive or poorly described taxa (Monsarrat and Kerley, 2018). Museum collections are biased by collectors' interests, geographical access, and curation (Wehi et al., 2012). Fortunately, accessibility of historical references is improving, as many early natural history sources are increasingly accessible as museum collections digitise and provide translations for their archives. Although there are some concerns regarding reliability of these records, frameworks exist to assess how record quality may be impacted by the type of record, where, when and why the record was collected, by whom and in which context (Pooley, 2018). Similarly, whilst fossils provide useful long-term information, they are inherently biased due to taphonomy and researchers should be careful to assess completeness and bias in sampling (Benton et al., 2011).

1.6. Solution 2: exploring potential niche space with translocations, mechanistic distribution models and comparative methods

Due to range contractions, contemporary populations alone may not provide a complete picture of a species' fundamental niche. Several streams of information could help fill these gaps: mechanistic distribution models, evaluating previous translocation outcomes, releasing individuals within the species' historical range, assisted colonization and community construction and moving individuals outside of any recorded range for that species (Seddon, 2010).

Evaluating variation in fitness proxies, physiology, and demography across populations can be used to construct response curves across ecological gradients. The shape of these curves (*i.e.* unimodal, truncated, or skewed) can suggest that current distributions do not capture the full response curve or that habitats thought to be optimal are not necessarily associated with the best population performance (Fig. 1). Moreover, these data can be fed into mechanistic distribution models to predict viable habitats by interpolation or extrapolation to identify biases in data or sampling (Cabral et al., 2017). Valuable information about species niche potential can also be gleaned from the behaviour and ecology of closely related species. Evolutionary trajectories of species are not independent, such that the less time since divergence the more similar species should be, particularly in terms of traits with a high degree of inertia (*e.g.* locomotion, diet and life history). Thus, where we have incomplete information, we can look to closely related species with similar basic biology to evaluate where their performance and fitness is optimal. Inferences from congeners has been used to evaluate likely diet and habitat preferences (Butchart and Bird, 2010) but not explicitly to identify potential shifted baselines or anthropogenically constrained niche occupancy.

Within historic ranges, habitat modification and climatic changes since extirpation can reduce suitability for reintroduction (Cui et al., 2017), especially if the initial reason for decline has not been mitigated. However, where extant populations appear to have low reproductive or high mortality rates relative to what would be predicted, reintroduction into previously occupied habitats could be transformative for the species' viability. The potential for using historic data to identify suitable translocation and release sites was recently explored for the kakapo (*Strigops habroptilus*) (Lentini et al., 2018). Once widespread across New Zealand, the kakapo is now confined to a number of small offshore islands where it is intensively managed. Modeling past distribution suggested that kakapo may have been present in a wider range of habitats than currently occupied and allowed for identification of suitable contemporary areas for population establishment.

Whilst the concept of native ranges is being questioned (Pereyra, 2020), the uncertainty involved and the precautionary principle dictate that assisted colonization and the movement of species outside of their recorded ranges should not take place (Ricciardi and Simberloff, 2009). But avoiding translocations places conservation in a paradox where we do not move species for fear of unexpected consequences but cannot predict these consequences due to the lack of translocations studies if historical records or comparative populations are not available (Bilney, 2014). If fencing or discontinuous habitats prevent species from shifting their ranges in response to environmental change, conservation paralysis may commit the species to extinction. Under certain situations experimental translocations, whether inside or outside of historical ranges, may be the only way to expand our knowledge of a species' biology and determine whether a particular habitat is suitable. In some cases, a functional role within the ecosystem must be filled for ecological functionality to be restored (Lundgren et al., 2018). Soft releases into fenced areas can reduce risk and can be followed by gradual releases into the wider habitat. Information gained from these approaches can identify ecological and behavioural flexibility (or lack thereof) of species in novel conditions, performance capacity in different environments, and the effect of reintroduced organisms on variation in existing communities. Translocations should be coupled with monitoring to increase understanding of the fundamental niche of the species (Radeloff et al., 2015). Although these interventions could provide invaluable data for future conservation efforts, they require careful consideration of a range of complex issues, including funding/resources, ethical evaluation and measures of success, particularly in a changing environment.

1.7. *Solution 3: traditional ecological knowledge*

Local human populations co-existing with the biodiversity of the area can be a source of knowledge about species biology. Many communities depend on natural systems to provide them with food, medicine, and other resources. This traditional ecological knowledge (TEK) often spans broad temporal and spatial scales (Gilchrist et al., 2005), is passed down across generations, and represents the cumulative body of knowledge, belief, and practice relating to interactions between living beings and their environment (Charnley et al., 2007). While TEK itself is subject to bias and incorrect inferences, it can be a valuable source for providing new predictions or inferences about species biology and historical ecosystems that can be tested with evidence, experiments, or historical references. Considering this, TEK can help identify species stereotypes in ecological research. Integrating wildlife science with TEK can provide more complete information about changes over time and space than either can alone. For example, in studies of large varanid lizards, indigenous rangers consistently found more docile lizards and those harder to see, than non-indigenous scientists, which changes our understanding of behaviour and population status (Ward-Fear et al., 2019). TEK is increasingly featured in both international and national conservation policy; however, there is less progress in including indigenous peoples' knowledge in conservation decision-making (McCarthy et al., 2018).

1.8. *Solution 4: ecological restoration, increasing connectivity and rewilding*

In addition to experimental conservation translocations for individual species, a more landscape-scale approach may prove useful. Large-scale ecological restoration, or rewilding (Lorimer et al., 2015), to increase habitat connectivity and encourage dispersal could help address species stereotypes and the niche representation of protected area networks in two ways. First, it would allow populations to settle in suitable areas of their own volition, circumventing the risks associated with human-mediated movement, allowing effective conservation of species without knowing their exact optimal habitat. Establishing connectivity provides opportunities for organisms to migrate to preferred areas in the future. Second, it could provide vital research opportunities to better understand these species as they recover. Coupling restoration with close monitoring could highlight optimal habitats for population performance and recreate historical population dynamics (Radeloff et al., 2015). Systematic monitoring of population performance and physiology across environmental gradients as species migrate could be useful in creating mechanistic models to assess their, and closely related species', responses to future climactic change. Pursuing this strategy with well-planned experimental designs would allow us to redress species stereotypes in ecological research whilst conserving species, habitats, and ecosystem function.

1.9. *Solution 5: incorporating an assessment of bias and shifted baselines into conservation assessment and action plans*

Given the potential implications of misestimating habitat suitability for conservation efficacy, we suggest that biodiversity action plans should evaluate risks of shifted baselines and ensure a historical perspective on species biology and habitat preservation. The IUCN Green List acknowledges and attempts to prevent shifting baselines (Grace et al., 2019). However, for most assessed species highlighted within this review, the possibility of shifted baselines, skewed or limited knowledge, or an incomplete view of the species ecology and performance capacities is not discussed in IUCN Red List assessments, despite existing literature demonstrating this to be a threat (Table 1). This occurs because assessments are based on existing data, knowledge, and expert opinions. If there are biases in the evidence used for the assessment, the Red List may inadvertently perpetuate stereotypes and suboptimal recommendations. Biases and shifted baselines are most likely with the following criteria: extensive range contraction, reduction or shifts in contemporary habitat occupancy compared to historical occupancy, and species behavioural ecology being based on few studies that do not reflect the range of habitats previously or currently occupied. Where the risk of bias is high, we recommend flagging this in the Red List assessment and an action plan and research program that incorporates the solutions mentioned above: assessment of historical evidence of niche shift and contraction, use of isotopes to evaluate diet and habitat changes over longer time periods, survey methods to ascertain more recent shifts in occurrence and occupancy, identification of key habitats that were part of the historical range, and closely monitored experimental translocations where appropriate (Fig. 2).

At a larger scale, biodiversity action plans are compiled by expert

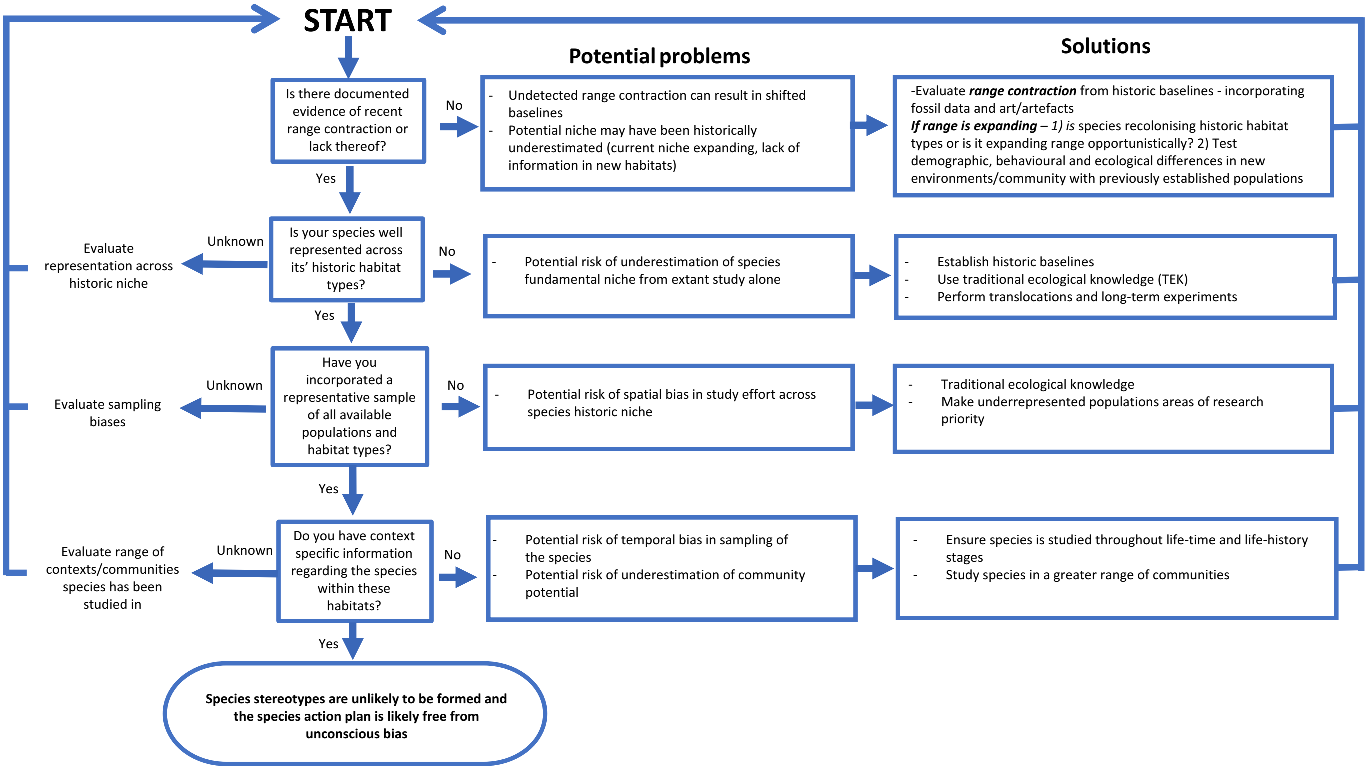


Fig. 2. Incorporating bias assessment into species' action plans. Decision tree evaluating whether a species stereotypes and unconcious biases may be present in our current understanding of a species and within our current assessments/action plans for a species of conservation concern. Here we highlight a stepwise approach of where potential problems may arise and identify solutions to these problems that can be incorporated into action plans or conservation assessments.

working groups to identify threats and conservation priorities at the species, community, and landscape level. Species action plans document the priorities for habitat protection, threat mitigation, and necessary research and monitoring programmes needed to fill in knowledge gaps for specific species. These plans often promote increased survey and monitoring efforts to further understanding of extant populations. A similar strategy, but with higher-level objectives, is a focal species approach whereby the conservation requirements of vulnerable species are assessed as a surrogate for landscape level conservation. The assumption of this approach is that species occupancy and response curves are nested, such that protecting the optimal habitat of one species will, by default, protect optimal habitat for community members (Patterson, 1987). However, sympatric species may co-occur but not be nested in niche space, such that the optimal habitat for one is marginal for another (Lindenmayer et al., 2002). Moreover, incorrect assumptions about community composition, functional roles, and ecological baselines may constrain management recommendations.

A key example of landscape level decision making is the Natura 2000 protected area network in Europe. Sites are designated based on two criteria: 1) their conservation value in terms of distribution patterns, fragmentation, isolation and anthropogenic pressures of threatened species; 2) ensuring representation of habitat types within a region (McLeod et al., 2005). Currently, how well the historical niche occupancy of a species is represented by extant distributions is not explicitly addressed, nor is the potential for shifted baselines in terms of habitat assessment and niche potential of the species. Incorporating an explicit analysis of current *versus* historical niche occupancy, traditional knowledge and closely related species biology could identify where conservation opportunities may be missed. Moreover, this process could highlight existing protected areas within the Natura 2000 network that provide opportunities for translocation of species that have undergone niche contraction into previously occupied habitats.

1.10. Conclusion

The formation of species stereotypes has serious implications for our understanding of the natural world and may lead us to misestimate species' ecological and behavioural flexibility. There are likely many more species where we continue to implement sub-optimal interventions and have missed conservation opportunities due to a stereotypic view. We have highlighted a number of examples where these biases have impacted on conservation decisions and demonstrate that these stereotypes are not acknowledged in conservation guidance. These stereotypes fall into three main groups: 1) perceiving a species as a specialist based on available information, 2) inaccurate assessments of suitable habitats due to a shifted baseline, 3) incomplete understanding of community interactions and potential. If inappropriate stereotypes are not recognised, then unsuitable conservation programmes may be implemented. We provided specific examples of active management in suboptimal habitats, appropriate release sites going unidentified and unutilised, the creation of unsustainable communities, and the implementation of ineffective management strategies. Critically, this problem often cannot be solved by collecting more data on extant populations if they are a subset of the ecological ranges a species can occupy. Identifying and remedying bias requires an integrated study of species' biology incorporating historical data, traditional ecological knowledge, and rigorous experimental design for future interventions to help to combat stereotypes and improve future conservation programmes. We suggest that an explicit assessment of potential bias and incomplete information on niche and community potential is incorporated into species assessments, management interventions, and protected area evaluation.

Author contributions

All authors conceived of the manuscript, all authors contributed to the writing and editing of the manuscript and agreed to submission.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The work of JAB, RNL, FE, NH and ES on this project was funded by the Natural Environment Research Council (NERC) Doctoral Training Partnership (grant NE/L002469/1). JAB, RNL, FE and NH are also supported through the Chester Zoo Conservation Scholars programme. SS is funded by a Royal Society URF (UF110641).

Ethical statement

No animals were used in this study.

References

Ancrenaz, M., Ambu, L., Sunjoto, I., Ahmad, E., Manokaran, K., Meijaard, E., Lackman, I., 2010. Recent surveys in the forests of Ulu Segama Malua, Sabah, Malaysia, show that orang-utans (P. p. morio) can be maintained in slightly logged forests. PLoS ONE 5, e11510. https://doi.org/10.1371/journal.pone.0011510.

Ancrenaz, M., Gumal, M., Marshall, A.J., Meijaard, E., Wich, S.A., Husson, S., 2016. *Pongo pygmaeus* (errata version published in 2018). In: The IUCN Red List of Threatened Species 2016, e.T17975A123809220. https://doi.org/10.2305/IUCN.UK.2016-1.RLTS.T17975A17966347.en.

Anderson, R.P., 2012. Harnessing the world's biodiversity data: promise and peril in ecological niche modeling of species distributions. Ann. N. Y. Acad. Sci. 1260, 66–80. https://doi.org/10.1111/j.1749-6632.2011.06440.x.

Araújo, M.B., Anderson, R.P., Barbosa, A.M., Beale, C.M., Dormann, C.F., Early, R., Rahbek, C., 2019. Standards for distribution models in biodiversity assessments. Sci. Adv. 5 (1), eaat4858. https://doi.org/10.1126/sciadv.aat4858.

Åström, M., Dekker, W., 2007. When will the eel recover? A full life-cycle model. ICES J. Mar. Sci. 64 (7), 1491–1498. https://doi.org/10.1093/icesjms/fsm122.

Benton, M.J., Dunhill, A.M., Lloyd, G.T., Marx, F.G., 2011. Assessing the quality of the fossil record: insights from vertebrates. Geol. Soc. 358, 63–94. https://doi.org/10.1144/SP358.6.

Bilney, R.J., 2014. Poor historical data drive conservation complacency: the case of mammal decline in south-eastern Australian forests. Austral. Ecol. 39, 875–886. https://doi.org/10.1111/aec.12145.

BirdLife International, 2016. Tetrao urogallus. In: The IUCN Red List of Threatened Species 2016, e.T22679487A85942729. https://doi.org/10.2305/IUCN.UK.2016-3.RLTS.T22679487A85942729.en.

BirdLife International, 2017. *Branta sandvicensis* (amended version of 2016 assessment). In: The IUCN Red List of Threatened Species 2017, e.T22679929A112386209. https://doi.org/10.2305/IUCN.UK.2017-1.RLTS.T22679929A112386209.en.

BirdLife International, 2019. *Vanellus gregarius* (amended version of 2018 assessment). In: The IUCN Red List of Threatened Species 2019, e.T22694053A155545788. https://doi.org/10.2305/IUCN.UK.2019-3.RLTS.T22694053A155545788.en.

Bissett, C., Bernard, R.T.F., 2007. Habitat selection and feeding ecology of the cheetah (*Acinonyx jubatus*) in thicket vegetation: is the cheetah a savanna specialist? J. Zool. 271, 310–317. https://doi.org/10.1111/j.1469-7998.2006.00217.x.

Black, J.M., Marshall, A.P., Gilburn, A., Santos, N., Hoshide, H., Medeiros, J., Mello, J., Hodges, C.N., Katahira, L., 1997. Survival, movements, and breeding of released Hawaiian geese: an assessment of the reintroduction program. J. Wildl. Manag. 61, 1161–1173.

Bocherens, H., Hofman-Kamińska, E., Drucker, D.G., Schmölcke, U., Kowalczyk, R., 2015. European bison as a refugee species? Evidence from isotopic data on Early Holocene bison and other large herbivores in northern Europe. PLoS One 10, 1–19. https://doi.org/10.1371/journal.pone.0115090.

Bonebrake, T.C., Christensen, J., Boggs, C.L., Ehrlich, P.R., 2010. Population decline assessment, historical baselines, and conservation. Conserv. Lett. 3, 371–378. https://doi.org/10.1111/j.1755-263X.2010.00139.x.

Boyd, L., Houpt, K.A., 1994. Przewalski's Horse: The History and Biology of an Endangered Species. State University of New York Press.

Brochet, A., Van Den Bossche, W., Jones, V., Arnardottir, H., Damoc, D., Demko, M., Driessens, G., Flensted, K., Gerber, M., Ghasabyan, M., Gradinarov, D., Hansen, J., Horváth, M., Karlonas, M., Krogulec, J., Kuzmenko, T., Lachman, L., Lehtiniemi, T., Lorgé, P., Lötberg, U., Lusby, J., Ottens, G., Paquet, J., Rukhaia, A., Schmidt, M., Shimmings, P., Stipnieks, A., Sultanov, E., Vermouzek, Z., Vintchevski, A., Volke, V., Willi, G., Butchart, S., 2017. Illegal killing and taking of birds in Europe outside the Mediterranean: assessing the scope and scale of a complex issue. In: Bird Conservation International, 29, pp. 10–40. https://doi.org/10.1017/S0959270917000533.

Butchart, S.H., Bird, J.P., 2010. Data deficient birds on the IUCN red list: what don't we know and why does it matter? Biol. Conserv. 143, 239–247. https://doi.org/10.1016/j.biocon.2009.10.008.

Cabral, J.S., Valente, L., Hartig, F., 2017. Mechanistic simulation models in macroecology and biogeography: state-of-art and prospects. Ecography 40, 267–280. https://doi.org/10.1111/ecog.02480.

Cadrin, S., Morse, M., Kerr, L., Secor, D., Siskey, M., 2018. Exploratory stock assessment of eastern and western population-of-origin Atlantic Bluefin tuna accounting for stock composition. Sci. Pap. ICCAT 74 (6), 3290–3304.

Charnley, S., Paige Fischer, A., Jones, E.T., 2007. Integrating traditional and local ecological knowledge into forest biodiversity conservation in the Pacific Northwest. For. Ecol. Manag. 246, 14–28. https://doi.org/10.1016/j.foreco.2007.03.047.

Christie, A.P., Amano, T., Martin, P.A., Petrovan, S.O., Shackelford, G.E., Simmons, B.I., Smith, R.K., Williams, D.R., Wordley, C.F.R., Sutherland, W.J., 2020. Poor availability of context-specific evidence hampers decision-making in conservation. Biol. Conserv. 248,108666 https://doi.org/10.1016/j.biocon.2020.108666.

Clark, J.A., May, R.M., 2002. Taxonomic bias in conservation research. Science 297,191b, 192. https://doi.org/10.1126/science.297.5579.191b.

Conde, D.A., et al., 2019. Data gaps and opportunities for comparative and conservation biology. PNAS 116, 9658–9664. https://doi.org/10.1073/pnas.1816367116.

Cui, S., Milner-Gulland, E.J., Singh, N.J., Chu, H., Li, C., Chen, J., Jiang, Z., 2017. Historical range, extirpation and prospects for reintroduction of saigas in China. Sci. Rep. 7, 44200. https://doi.org/10.1038/srep44200.

Donald, P.F., et al., 2016. A Globally Important Migration Staging Site for Sociable Lapwings *Vanellus gregarius* in Turkmenistan and Uzbekistan. Sandgrouse, 38, pp. 82–95.

Doroff, A., Burdin, A., 2015. Enhydra lutris. In: The IUCN Red List of Threatened Species 2015: E.T7750A21939518. https://doi.org/10.2305/IUCN.UK.2015-2.RLTS.T7750A21939518.en.

Doughty, C.E., Roman, J., Faurby, S., Wolf, A., Haque, A., Bakker, E.S., Malhi, Y., Dunning, J.B., Svenning, J.C., 2016. Global nutrient transport in a world of giants. PNAS 113, 868–873. https://doi.org/10.1073/pnas.1502549112.

Drenner, S.M., Clark, T.D., Whitney, C.K., Martins, E.G., Cooke, S.J., Hinch, S.G., 2012. A synthesis of tagging studies examining the behaviour and survival of anadromous salmonids in marine environments. PLoS One 7 (3), e31311. https://doi.org/10.1371/journal.pone.0031311.

Durant, S., Mitchell, N., Ipavec, A., Groom, R., 2015. Acinonyx jubatus. In: The IUCN Red List of Threatened Species 2015, e.T219A50649567. https://doi.org/10.2305/IUCN.UK.2015-4.RLTS.T219A50649567.en.

Ellis, S., Talukdar, B., 2020. Rhinoceros sondaicus. In: The IUCN Red List of Threatened Species 2020, e.T19495A18493900. https://doi.org/10.2305/IUCN.UK.2020-2.RLTS.T19495A18493900.en.

Elsey, R., Woodward, A., Balaguera-Reina, S.A., 2019. Alligator mississippiensis. In: The IUCN Red List of Threatened Species 2019, e.T46583A3009637. https://doi.org/10.2305/IUCN.UK.2019-2.RLTS.T46583A3009637.en.

Faith, J.T., 2012. Palaeozoological insights into management options for a threatened mammal: Southern Africa's Cape mountain zebra (*Equus zebra zebra*). Divers. Distrib. 18, 438–447. https://doi.org/10.1111/j.1472-4642.2011.00841.x.

Gilchrist, G., Mallory, M., Merkel, F., 2005. Can local ecological knowledge contribute to wildlife management? Case studies of migratory birds. Ecol. Soc. 10 (1), 20.

González, M.A., Olea, P.P., Robles, L., Ena, V., 2010. The Mediterranean Quercus pyrenaica oak forest: a new habitat for the Capercaillie? J. Ornithol. 151, 901–906. https://doi.org/10.1007/s10336-010-0528-6.

Gosling, L.M., Muntifering, J., Kolberg, H., Uiseb, K., King, S.R.B., 2019. *Equus zebra* (amended version of 2019 assessment). The IUCN Red List of Threatened Species 2019. https://doi.org/10.2305/IUCN.UK.2019-1.RLTS.T7960A160755590.en (e. T7960A160755590).

Grace, M., Resit Akçakaya, H., Bennett, E., Hilton-Taylor, C., Long, B., Milner-Gulland, E. J., Young, R., Hoffmann, M., 2019. Using historical and palaeoecological data to inform ambitious species recovery targets. Phil. Trans. R. Soc. https://doi.org/10.1098/rstb.2019.0297 (B3742019029720190297).

Groves, C.P., Leslie Jr., D.M., 2011. *Rhinoceros sondaicus* (Perissodactyla: Rhinocerotidae). Mamm. Species 43 (887), 190–208.

Han, H., et al., 2019. Diet evolution and habitat contraction of giant pandas *via* stable isotope analysis. Curr. Biol. 29, 664–669.e2. https://doi.org/10.1016/j.cub.2018.12.051.

Hess, S.C., Leopold, C.R., Misajon, K., Hu, D., Jeffrey, J.J., 2012. Restoration of movement patterns of the Hawaiian Goose. Wilson J. Ornithol. 124, 478–486. https://doi.org/10.1676/12-005.1.

Hobbs, R.J., et al., 2006. Novel ecosystems: theoretical and management aspects of the new ecological world order. Glob. Ecol. Biogeogr. 15, 1–7. https://doi.org/10.1111/j.1466-822X.2006.00212.x.

Isaac, N., Pocock, M., 2015. Bias and information in biological records. Biol. J. Linn. Soc. 115 (3), 522–531. https://doi.org/10.1111/bij.12532.

IUCN SSC Antelope Specialist Group, 2018. Saiga tatarica. In: The IUCN Red List of Threatened Species 2018, e.T19832A50194357. https://doi.org/10.2305/IUCN.UK.2018-2.RLTS.T19832A50194357.en.

Jacoby, D.M.P., et al., 2015. Synergistic patterns of threat and the challenges facing global anguillid eel conservation. Glob. Ecol. Conserv. 4, 321–333. https://doi.org/10.1016/j.gecco.2015.07.009.

Jensen, D.A., Ma, K., Svenning, J.C., 2020. Steep topography buffers threatened gymnosperm species against anthropogenic pressures in China. Ecol. Evol. 10, 1838–1855. https://doi.org/10.1002/ece3.5983.

Junker, J., et al., 2020. A severe lack of evidence limits effective conservation of the world's primates. Bioscience 70 (9), 794–803. https://doi.org/10.1093/biosci/biaa082.

Jürgensen, J., Drucker, D.G., Stuart, A.J., Schneider, M., Buuveibaatar, B., Bocherens, H., 2017. Diet and habitat of the saiga antelope during the late quaternary using stable carbon and nitrogen isotope ratios. Quat. Sci. Rev. 160, 150–161. https://doi.org/10.1016/j.quascirev.2017.01.022.

Kaczensky, P., Burnik Šturm, M., Sablin, M.V., Voigt, C.C., Smith, S., Ganbaatar, O., Balint, B., Walzer, C., Spasskaya, N.N., 2017. Stable isotopes reveal diet shift from pre-extinction to reintroduced Przewalski's horses. Sci. Rep. 7, 5950. https://doi.org/10.1038/s41598-017-05329-6.

Karamanlidis, A., Dendrinos, P., 2015. *Monachus monachus* (errata version published in 2017). In: The IUCN Red List of Threatened Species 2015, e.T13653A117647375. https://doi.org/10.2305/IUCN.UK.2015-4.RLTS.T13653A45227543.en.

Kerley, G.I.H., Kowalczyk, R., Cromsigt, J.P.G.M., 2012. Conservation implications of the refugee species concept and the European bison: King of the forest or refugee in a marginal habitat? Ecography 35, 519–529. https://doi.org/10.1111/j.1600-0587.2011.07146.x.

Kerley, G.I.H., Beest, M., Cromsigt, J.P.G.M., Pauly, D., Shultz, S., 2020. The protected area paradox and refugee species: the giant panda and baselines shifted towards conserving species in marginal habitats. Conserv. Sci. Pract. 2 https://doi.org/10.1111/csp2.203.

Kirby, J.S., Stattersfield, A.J., Butchart, S.H.M., Evans, M.I., Grimmett, R.F.A., Jones, V. R., O'sullivan, J., Tucker, G.M., Newton, I., 2008. Key conservation issues for migratory land- and waterbird species on the world's major flyways. Bird Conserv. Int. 18 (S1), S49–S73. https://doi.org/10.1017/S0959270908000439.

Lea, J.M., Kerley, G.I., Hrabar, H., Barry, T.J., Shultz, S., 2016. Recognition and management of ecological refugees: a case study of the Cape mountain zebra. Biol. Conserv. 203, 207–215. https://doi.org/10.1016/j.biocon.2016.09.017.

Leis, J.M., Siebeck, U., Dixson, D.L., 2011. How nemo finds home: the neuroecology of dispersal and of population connectivity in larvae of marine fishes. Integr. Comp. Biol. 51, 826–843. https://doi.org/10.1093/icb/icr004.

Lentini, P., Stirnemann, I., Stojanovic, D., Worthy, T., Stein, J., 2018. Using fossil records to inform reintroduction of the kakapo as a refugee species. Biol. Conserv. 217, 157–165. https://doi.org/10.1016/j.biocon.2017.10.027.

Lindenmayer, D.B., Manning, A.D., Smith, P.L., Possingham, H.P., Fischer, J., Oliver, I., McCarthy, M.A., 2002. The focal-species approach and landscape restoration: a critique. Conserv. Biol. 16, 338–345. https://doi.org/10.1046/j.1523-1739.2002.00450.x.

Lorimer, J., Sandom, C., Jepson, P., Doughty, C., Barua, M., Kirby, K.J., 2015. Rewilding: science, practice, and politics. Annu. Rev. Environ. Resour. 40, 39–62. https://doi.org/10.1146/annurev-environ-102014-021406.

Lundgren, E.J., Ramp, D., Ripple, W.J., Wallach, A.D., 2018. Introduced megafauna are rewilding the Anthropocene. Ecography 41, 857–866. https://doi.org/10.1111/ecog.03430.

Martin, L.J., Blossey, B., Ellis, E., 2012. Mapping where ecologists work: biases in the global distribution of terrestrial ecological observations. Front. Ecol. Environ. 10, 195–201. https://doi.org/10.1890/110154.

Martínez-Abraín, A., Jiménez, J., 2016. Anthropogenic areas as incidental substitutes for original habitat. Conserv. Biol. 30, 593–598. https://doi.org/10.1111/cobi.12644.

Martínez-Abraín, A., Jiménez, J., Oro, D., 2019. Pax Romana: 'refuge abandonment' and spread of fearless behaviour in a reconciling world. Anim. Conserv. 22, 3–13. https://doi.org/10.1111/acv.12429.

McCarthy, C., Shinjo, H., Hoshino, B., Enkhjargal, E., 2018. Assessing local indigenous knowledge and information sources on biodiversity, conservation and protected area Management at Khuvsgol Lake National Park, Mongolia. Land 7, 117. https://doi.org/10.3390/land7040117.

Mcclenachan, L., Ferretti, F., Baum, J.K., 2012. From archives to conservation: why historical data are needed to set baselines for marine animals and ecosystems. Conserv. Lett. 5, 349–359. https://doi.org/10.1111/j.1755-263X.2012.00253.x.

McGeoch, M.A., Latombe, G., 2016. Characterizing common and range expanding species. J. Biogeogr. 43, 217–228. https://doi.org/10.1111/jbi.12642.

McLeod, C.R., Yeo, M., Brown, A.E., Burn, A.J., Hopkins, J.J., Way, S.F. (Eds.), 2005. The Habitats Directive: selection of Special Areas of Conservation in the UK, 2nd edn. Joint Nature Conservation Committee, Peterborough. www.jncc.gov.uk/SACselection.

Meijaard, E., 2017. How a mistaken ecological narrative could be undermining orangutan conservation. In: Pages 90–97 Effective Conservation Science: Data Not Dogma. Oxford University Press. https://doi.org/10.1093/oso/9780198808978.001.0001.

Meijaard, E., Albar, G., Nardiyono, Rayadin, Y., Ancrenaz, M., Spehar, S., 2010. Unexpected ecological resilience in Bornean orangutans and implications for pulp and paper plantation management. PLoS One 5 (9), e12813. https://doi.org/10.1371/journal.pone.0012813.

Meijaard, E., Ni'matullah, S., Dennis, R., Sherman, J., Onrizal, Wich, S.A., 2021. The historical range and drivers of decline of the Tapanuli orangutan. PLoS One 16 (1), e0238087. https://doi.org/10.1371/journal.pone.0238087.

Mengüllüoğlu, D., Ambarlı, H., Berger, A., Hofer, H., 2018. Foraging ecology of Eurasian lynx populations in Southwest Asia: conservation implications for a diet specialist. Ecol. Evol. 8, 9451–9463. https://doi.org/10.1002/ece3.4439.

Monsarrat, S., Kerley, G.I.H., 2018. Charismatic species of the past: biases in reporting of large mammals in historical written sources. Biol. Conserv. 223, 68–75. https://doi.org/10.1016/j.biocon.2018.04.036.

Monsarrat, S., Novellie, P., Rushworth, I., Kerley, G., 2019. Shifted distribution baselines: neglecting long-term biodiversity records risks overlooking potentially suitable habitat for conservation management. R. Soc. B Biol. Sci. 374, 20190215. https://doi.org/10.1098/rstb.2019.0215.

Morreale, S.J., Plotkin, P.T., Shaver, D.J., Kalb, H.J., 2007. Adult Migration and Habitat Utilization. In Biology and Conservation of Ridley Sea Turtles. Johns Hopkins University Press.

Morse, N., Pellissier, P., Cianciola, E., Brereton, R., Sullivan, M., Shonka, N., Wheeler, T., McDowell, W., 2014. Novel ecosystems in the Anthropocene: a revision of the novel ecosystem concept for pragmatic applications. Ecol. Soc. 19 https://doi.org/10.5751/ES-06192-190212.

Nardelli, F., 2016. Current status and conservation prospects for the Javan rhinoceros *Rhinoceros sondaicus* Desmarest. Int. Zoo News 63 (3), 180–202.

Nifong, J.D., Silliman, B., 2017. Abiotic factors influence the dynamics of marine habitat use by a highly mobile "freshwater" top predator. Hydrobiologia 1–20. https://doi.org/10.1007/s10750-017-3255-7.

Nowak, M.G., Rianti, P., Wich, S.A., Meijaard, E., Fredriksson, G., 2017. Pongo tapanuliensis. In: The IUCN Red List of Threatened Species 2017, e.T120588639A120588662. https://doi.org/10.2305/IUCN.UK.2017-3.RLTS.T120588639A120588662.en.

Nüchel, J., Bøcher, P.K., Xiao, W., Zhu, A.X., Svenning, J.C., 2018. Snub-nosed monkeys (*Rhinopithecus*): potential distribution and its implication for conservation. Biodivers. Conserv. 27, 1517–1538. https://doi.org/10.1007/s10531-018-1507-0.

Owen, P.R., Bell, C.J., Mead, E.M., 2000. Fossils, diet, and conservation of black-footed ferrets (*Mustela Nigripes*). J. Mammal. 81, 422–433. https://doi.org/10.1644/1545-1542(2000)081<0422:FDACOB>2.0.CO;2.

Papworth, S.K., Rist, J., Coad, L., Milner-Gulland, E.J., 2009. Evidence for shifting baseline syndrome in conservation. Conserv. Lett. 2, 93–100. https://doi.org/10.1111/j.1755-263X.2009.00049.x.

Patterson, B.D., 1987. The principle of nested subsets and its implications for biological conservation. Conserv. Biol. 1, 323–334. https://doi.org/10.1111/j.1523-1739.1987.tb00052.x.

Pauly, D., 1995. Anecdotes and the shifting baseline syndrome of fisheries. Trends Ecol. Evol. 10, 430. https://doi.org/10.1016/S0169-5347(00)89171-5.

Pellissier, L., et al., 2018. Comparing species interaction networks along environmental gradients. Biol. Rev. 93, 785–800. https://doi.org/10.1111/brv.12366.

Pereyra, P.J., 2020. Rethinking the native range concept. Conserv. Biol. 34, 373–377. https://doi.org/10.1111/cobi.13406.

Pires, M.M., Galetti, M., Donatti, C.I., Pizo, M.A., Dirzo, R., Guimarães, P.R., 2014. Reconstructing past ecological networks: the reconfiguration of seed-dispersal interactions after megafaunal extinction. Oecologia 175, 1247–1256. https://doi.org/10.1007/s00442-014-2971-1.

Plumb, G., Kowalczyk, R., Hernandez-Blanco, J.A., 2020. Bison bonasus. In: *The IUCN Red List of Threatened Species* 2020: e.T2814A45156279. https://doi.org/10.2305/IUCN.UK.2020-3.RLTS.T2814A45156279.en.

Pooley, S., 2018. Descent with modification: critical use of historical evidence for conservation. Conserv. Lett. 11, e12437 https://doi.org/10.1111/conl.12437.

Radeloff, V.C., et al., 2015. The rise of novelty in ecosystems. Ecol. Appl. 25, 2051–2068. https://doi.org/10.1890/14-1781.1.

Ricciardi, A., Simberloff, D., 2009. Assisted colonization is not a viable conservation strategy. Trends Ecol. Evol. 24, 248–253. https://doi.org/10.1016/j.tree.2008.12.006.

Ripple, W.J., Beschta, R.L., 2012. Trophic cascades in Yellowstone: the first 15 years after wolf reintroduction. Biol. Conserv. 145, 205–213. https://doi.org/10.1016/j.biocon.2011.11.005.

Ripple, W.J., et al., 2015. Collapse of the world's largest herbivores. Sci. Adv. 1 (4), e1400103 https://doi.org/10.1126/sciadv.1400103.

Robles, H., Martin, K., 2014. Habitat-mediated variation in the importance of ecosystem engineers for secondary cavity nesters in a nest web. PLoS ONE 9. https://doi.org/10.1371/journal.pone.0090071.

Roos, A., Loy, A., de Silva, P., Hajkova, P., Zemanová, B., 2015. Lutra lutra. In: The IUCN Red List of Threatened Species 2015, e.T12419A21935287. https://doi.org/10.2305/IUCN.UK.2015-2.RLTS.T12419A21935287.en.

Santosa, Y., Rahmat, U.M., Prasetyo, L.B., Kartono, A.P., 2013. Javan Rhino (Rhinoceros sondaicus Desmarest 1822) utilization distribution and habitat selection in Ujung Kulon National Park. Jurnal Manajemen Hutan Tropika 19 (1), 31–38. https://doi.org/10.7226/jtfm.19.1.31-38.

Scheele, B.C., Foster, C.N., Banks, S.C., Lindenmayer, D.B., 2017. Niche contractions in declining species: mechanisms and consequences. Trends Ecol. Evol. 32,346–355 https://doi.org/10.1016/j.tree.2017.02.013.

Scott, M.D., Chivers, S.J., 2009. Movements and diving behaviour of pelagic spotted dolphins. Mar. Mammal Sci. 25, 137–160. https://doi.org/10.1111/j.1748-7692.2008.00241.x.

Seddon, P.J., 2010. From reintroduction to assisted colonization: moving along the conservation translocation spectrum. Restor. Ecol. 18, 796–802. https://doi.org/10.1111/j.1526-100X.2010.00724.x.

Semmens, B.X., Semmens, D.J., Thogmartin, W.E., Wiederholt, R., López-Hoffman, L., Diffendorfer, J.E., Pleasants, J.M., Oberhauser, K.S., Taylor, O.R., 2016. Quasi-extinction risk and population targets for the eastern, migratory population of monarch butterflies *(Danaus plexippus)*. Sci. Rep. 6, 1–7. https://doi.org/10.1038/srep23265.

Silliman, B.R., Hughes, B.B., Gaskins, L.C., He, Q., Tinker, M.T., Read, A., Nifong, J., Stepp, R., 2018. Are the ghosts of nature past haunting conservation today? Curr. Biol. R532–R537. https://doi.org/10.1016/j.cub.2018.04.002.

Slotta-Bachmayr, L., Boegel, R., Kaczensky, P., Stauffer, C., Walzer, C., 2004. Use of population viability analysis to identify management priorities and success in reintroducing przewalski's horses to southwestern Mongolia. J. Wildl. Manag. 68, 790–798.

Stockwell, D.R., Peterson, A.T., 2002. Effects of sample size on accuracy of species distribution models. Ecol. Model. 148, 1–13. https://doi.org/10.1016/S0304-3800(01)00388-X.

Swaisgood, R., Wang, D., Wei, F., 2016. *Ailuropoda melanoleuca* (errata version published in 2017). In: The IUCN Red List of Threatened Species 2016, e.T712A121745669. https://doi.org/10.2305/IUCN.UK.2016-2.RLTS.T712A45033386.en.

Titley, M.A., Snaddon, J.L., Turner, E.C., 2017. Scientific research on animal biodiversity is systematically biased towards vertebrates and temperate regions. PLoS ONE 12. https://doi.org/10.1371/journal.pone.0189577.

Tittensor, D.P., Walpole, M., Hill, S.L., Boyce, D.G, Britten, G.L, Burgess, N.D., Butchart, S.H., Leadley,, P.W., Regan, E.C., Alkemade, R., Baumung,, R., 2014. A mid-term analysis of progress toward international biodiversity targets. Science 346 (6206), 241–244. https://doi.org/10.1126/science.1257484.

Ward-Fear, G., Rangers, B., Pearson, D., Bruton, M., Shine, R., 2019. Sharper eyes see shyer lizards: collaboration with indigenous peoples can alter the outcomes of conservation research. Conserv. Lett. e12643. https://doi.org/10.1111/conl.12643.

Webster, M.M., Rutz, C., 2020. How STRANGE are your study animals? Nature 582, 337–340. https://doi.org/10.1038/d41586-020-01751-5.

Wehi, P., Whaanga, H., Trewick, S., 2012. Artefacts, biology and bias in museum collection research. Mol. Ecol. 21 (13), 3103–3109. https://doi.org/10.1111/j.1365-294X.2012.05589.x.

Appendix 3) Evidence of infanticide in the Cape mountain zebra (*Equus zebra zebra*)

Published in Behaviour Britnell, J. A., Vorster, L., & Shultz, S. (2021). Evidence of infanticide in the Cape mountain zebra (Equus zebra zebra). *Behaviour*, *158*(11), 1043-1055.

BRILL

Behaviour 158 (2021) 1043–1055

Behaviour
brill.com/beh

Comments/Reflections

Evidence of infanticide in the Cape mountain zebra (*Equus zebra zebra*)

J.A. Britnell [a,b,*,**], **L. Vorster** [c] **and S. Shultz** [a,***]

[a] School of Earth and Environmental Sciences, University of Manchester, Manchester M13 9NT, UK
[b] North of England Zoological Society (Chester Zoo), Caughall Road, Upton-by-Chester CH2 1LH, UK
[c] Sanbona Wildlife Reserve, Barrydale 6720, South Africa
[*]Corresponding author's e-mail address: jake.britnell@postgrad.manchester.ac.uk
[**]ORCID: https://orcid.org/0000-0003-1527-7013
[***]ORCID: https://orcid.org/0000-0002-7135-4880

Received 11 December 2020; initial decision 10 May 2021; revised 9 June 2021; accepted 17 June 2021; published online 20 July 2021

Abstract

Infanticide has been described across mammal species. Infanticide is thought to be a tactic which increases reproductive opportunities or reduces competition over local resources. Species of the genus *Equus* exhibit life history traits such as expensive young, long gestation, lactation and dependency, extended inter-birth interval when there is a foal at foot and strong male reproductive skew. These traits suggest infanticide may be present throughout the genus. However, most documented cases of infanticide attempts come from captive populations and rely heavily on indirect accounts in free-roaming populations. Here, we report an infanticide attempt in Cape mountain zebra (*Equus zebra zebra*). The aggression was perpetrated by multiple bachelor males on two foals belonging to the same family group. The foals were separated from the parent group, chased and harried for a total of 45 minutes before the mothers and herd stallion were able to regain their offspring. We also report three cases of infanticide from necropsy. The injuries sustained by the foals are consistent with infanticide-based injuries documented in other equids species. The timing of these deaths occurs after a stallion turnover. These two cases provide the most conclusive evidence to date that infanticide takes place within mountain zebra.

Keywords

fitness, mortality, equids, aggression, sex ratio, social stability, stallion turnover, usurpation.

DOI 10.1163/1568539X-bja10106

1. Introduction

Infanticide is the act of killing a dependent offspring of the same species, often associated with species with extended parental care, resulting in an increase in access to either resources or reproductive opportunities for the perpetrator (van Schaik & van Noordwijk, 2010). Multiple hypotheses exist to explain infanticidal behaviour (Table 1) (Hrdy, 1979; Ebensperger, 1998). Dependent on the life-history and ecological settings of the species, infanticide can be perpetrated by, and increase fitness of either sex. For males, it terminates maternal investment in unrelated offspring and can return females to reproductive status more quickly (Packer & Pusey, 1982). Male infanticide should be prevalent in species with high competition between males for mating opportunities (Lukas & Huchard, 2014). For females, infanticide of unrelated offspring can increase the availability of resources for their own offspring (Ebensperger, 1998). Female infanticide should be prevalent in species breeding in groups with intense bouts of reproductive output and large maternal energetic investment (Lukas & Huchard, 2019). Many species experiencing infanticide develop counterstrategies to reduce risk such as stronger social bonds between females and more permanent male-female bonds (Van Schaik & Kappeler, 1997; Opie et al., 2013). Finally, both male and female infanticide may be non-adaptive or perpetrated to predate offspring during times of resource limitation (Hrdy, 1979).

Although rare, infanticide has been described across mammals (Lukas & Huchard, 2019). Equids have many life-history strategies which would suggest infanticide as an adaptive behaviour. Many equid species have high male skew in operational sex ratio (Ransom & Kaczensky, 2016). Some species, including horses, plains and mountain zebra, have a social structure where a single male and multiple females form a single-male group. In this structure, breeding opportunities are monopolised by few males. Excess, non-breeding males and females without groups, especially juvenile females, often join bachelor groups (Joubert, 1972; Penzhorn, 1984). This social structure, with high variation in mating opportunities, increases the likelihood of infanticide (Lukas & Huchard, 2014). The loss of a stallion male through death, defection or usurpation, may lead the new stallion male to commit infanticide. In plains zebra, stallion turnovers are frequent especially in groups with reproductive females (Vitet et al., 2021). Furthermore, females have long gestation periods, indicative of large maternal investment (Ransom & Kaczensky, 2016). Equids also display behavioural traits which could serve

Table 1.
Overview of the hypotheses to explain infanticidal behaviour.

Hypothesis	Explanation	Proposed adaptive reason for behaviour	Associated behaviours
Predation/ exploitation	Infanticide as a mechanism to obtain food	Increase food availability	Consumption of infant by perpetrator. More frequent in energy stressed individuals and resource poor areas
Resource competition	Infanticide as a mechanism to obtain increased resources for perpetrator or its offspring	Increase resource availability	More likely in areas of high density or poor food availability
Adoption avoidance	Infanticide as a mechanism to ensure parental care is provided to related offspring	Increase parental certainty and increase resources and parental care to related offspring	Should be committed more often by the sex that bears the primary costs of adoption. More likely to occur after usurpation of male in Single-male groups
Sexual selection	Infanticide as a mechanism to increase their reproductive fitness	Increase chance of mating and siring offspring or decreases fitness of competitors	Infanticidal males should not kill offspring they have sired. The elimination of offspring should bring females into reproductive status quicker and infanticidal males should be more likely to sire subsequent offspring
Social pathology	Behaviour is neutral or pathological. Infanticide occurs due to accident, disturbance or general aggression	Non-adaptive	Result of loss of habitat or high-density areas

as counterstrategies to infanticide such as strong female sociality (Cameron et al., 2009), long-term male-female associations (Rubenstein, 1981), pregnancy blocks (Bartoš et al., 2021) and induced abortions (Berger, 1983).

Within equids, infanticide is documented in feral and domestic horses (*Equus caballus*) (Duncan, 1982; Berger, 1983; Gray, 2009), plains zebra (*Equus quagga*) including most subspecies (Pluháček & Bartoš, 2000, 2005; Pluháček et al., 2006), Hartmann's mountain zebra (*Equus zebra hartmannae*) in captivity (Joubert, 1972; Pluháček & Bartoš, 2000) and Przewalski's horses (*Equus ferus przewalskii*) (Zharkikh, 1999; Chen et al., 2008; Feh & Munkhtuya, 2008; Dorj & Namkhai, 2013). However, apart from *Equus ferus*, infanticide has rarely been witnessed in studies of free-ranging wild equids with many accounts originating from captive populations. In plains zebra, a long-term study spanning 16 years in Hwange National Park, Zimbabwe, observed no infanticides (Vitet et al., 2021). Therefore, whether equid species commit infanticide in free-roaming conditions is questioned. Although, multiple videos are available on the internet or in documentaries regarding infanticide in zebra, the authors are unaware of any which involve Mountain zebra. Furthermore, although these videos provide anecdotal evidence, this is not incorporated into scientific literature.

Mountain zebra (*Equus zebra*) is a single-male band forming polygynous species, where a stallion monopolises breeding of multiple females (Penzhorn, 1984). At approximately one-year old, male offspring are displaced from their single-male bands and join a bachelor group (Penzhorn, 1984, 1985). These bachelor groups consist of non-band holding males and some juvenile females who are unlikely to have parentage of offspring found within single-male groups (Penzhorn, 1984, 1985). Male dominated bachelor groups are known to harass single-male groups. There has been no first-hand evidence of infanticide for the subspecies Cape mountain zebra (*Equus zebra zebra*, CMZ) or the species Mountain zebra (*Equus zebra*) in the wild. Despite this, male and female infanticide have been suggested in CMZ (Penzhorn, 1984; Lloyd & Rasa, 1989). Penzhorn (1984) notes a previous "Nature Conservator" witnessing a fight between a female with a foal and a stallion when he "ventured too close" although he did not witness any aggression towards the foal first-hand. Penzhorn reports two herd stallions allegedly killing new-borns resulting in their removal from the reserve although Penzhorn did not witness the infanticide (Penzhorn, 1984). Multiple counterstrategies to infanticide have been suggested in Mountain zebra including pregnancy block (Penzhorn, 1985). Infanticide has also been suggested due to injuries thought to be from accidental aggression from the mother (Lloyd & Rasa, 1989).

HERE, we provide an eye-witness account of an infanticide attempt on two CMZ foals by a bachelor group in Mountain Zebra National Park. Furthermore, we provide evidence from necropsies of three foals in Sanbona Wildlife Reserve with injuries consistent with infanticide attempts documented in other equid species.

2. Material and methods

2.1. Study areas and animals

Mountain Zebra National Park (MZNP) (32.1410°S, 25.5096°E) is a South African National Park in the Eastern Cape Province. MZNP is one of three relict populations and represents an important source used to restock CMZ into their historic range (Hrabar & Kerley, 2013). Zebra in MZNP are unmanaged and subject to natural predation. MZNP has four biomes: Grassland, Nama Karoo, Thicket and Savannah. The population is approximately 1191 CMZ with a population density of 0.0558 individuals/ha (Lea et al., 2016). The infanticide attempt in MZNP was witnessed by Professor Susanne Shultz. The behaviour was recorded consistently from the onset of the event until the end of the incident.

Sanbona Wildlife Reserve (SWR) (33.8663°S, 20.5284°E) is a 58 000 ha privately-owned reserve within the Western Cape of South Africa. Population size is estimated to be 52 CMZ. CMZ were reintroduced to the area in 2016 with the first infant born in 2018. The population is currently growing at an estimated rate of 20.9% per year. Adult sex ratio to date is approximately 50:50. During this study, CMZ in SWR could access supplementary feed. Some groups would regularly congregate near feeding sites. Feeding sites were positioned in areas central to known groups, minimizing competition between groups for the resource. Sites were mostly frequented by a single CMZ group such that inter-group competition for feeding sites is unlikely. Supplementary feeding sites were established following reintroduction due to extensive drought conditions in the area. Foals have been born to groups which were not known to use supplementary feed. The CMZ occupy a predator-free southern section of the reserve and therefore experience no predation pressure. SWR has two dominate biomes, fynbos and succulent karoo with population density of 0.0017 (individuals/ha). The cause of death of the three foals (SWR6, SWR7, SWR19) in SWR was investigated via necropsy performed by Dr Willem Burger, Veterinarian and Liesl Vorster, Ecologist.

2.2. *Observational methods and definitions*

Observational sampling was performed systematically in SWR by Liesl Vorster. This sampling includes noting of group composition of all CMZ groups in SWR monthly. Zebra were observed in MZNP during population surveys between 2013–2020 by Professor Susanne Shultz, Neinke Alberts, Dr Jessica Lea and Jake Britnell. For each group encountered, we recorded time, GPS location, group composition and distance to other bands. Bands were defined as discrete units where individuals within the bands were closer to each other than to other bands. We used a gambit of group approach, whereby all associated individuals were considered group members. A group was deemed to be unstable if there was a change in individuals (excluding births, deaths or departure of juveniles) between observations. CMZ are thought to have groups which remain stable over the adult lifestage (Penzhorn, 1984).

3. Results

3.1. *Infanticide attempt*

During monitoring in MZNP on the 31st December 2012, we witnessed bachelor males harassing a single-male band with two young foals (<3 months old). The single-male group consisted of one stallion male, two adult females, two female sub-adults and two foals. At around 16:30 the single-male band crossed a clearing and approached a perennial river to drink. Soon after, a band of 15 bachelor males approached the group, split and herded the foals from their group. All bachelors were in a very heightened state. The resident male and the two mothers attempted to regain their offspring by trying to separate the foals and drive them away from the bachelors. The resident male received significant and sustained aggression from bachelors and was driven away from the group. Bachelor males chased, bit at the foals and herded the foals away from the rest of the single-male group. All bachelor males were involved in the encounter, however, with the number of moving animals it was not possible to determine whether all males were involved with aggression towards the foals. In addition to chasing the foals, the bachelors also chased each other, engaged in rearing, lunging and biting. After approximately 20 minutes, one foal escaped the bachelors and returned to two female sub-adults who proceeded to move, with the foal, to a steep slope away from the bachelors. The chasing of the other foal continued. At 17:15, after 45 minutes of consistently herding, lunging at, and chasing the foal, the bachelor group broke into subgroups and the foal was able to return to the single-male group. The returning foal was rapidly ushered away by its mother and rejoined the rest of the single-male group. A single bachelor male continued to chase the single-male group. The foal was distressed and panting but showed no visible physical injury. At 17:25, the single-male group was approached again by two males from the bachelor group, but the stallion aggressively chased them away. The single-male-group stallion was vigilant towards bachelor males once the foals had returned, and the single-male group moved off.

3.2. Infanticide injuries from necropsy

3.2.1. Subject one and two — SWR6 and SWR19

SWR6 was a female foal first seen on 10th January 2019. The foal was approximately four days old, was very lethargic and subsequently collapsed and died 10 minutes after observation began. A necropsy revealed exterior markings of a kick to the left side and exterior damage of bite marks to the ear (Figure 1). Bruising was found within the inside of the abdominal cavity associated with the position of the kick mark. The kick had resulted in broken ribs and a herniated piece of intestine through the abdominal cavity. No interaction was witnessed to know whether the perpetrator was male or female. SWR6 belonged to a group of 9 individuals composed of 6 adult females, 1 adult male, 1 female sub-adult and SWR6. The stallion had been with group since 2017 and SWR6 was likely his offspring.

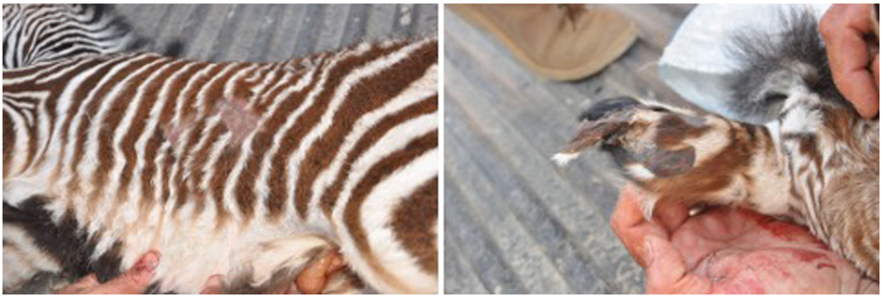


Figure 1. External injuries of the foal. left) displays two kick marks to the left-hand side of the animal which resulted in broken ribs. right) displays bite marks to the ear.

SWR19 was a female foal born in July 2020. This two-day old foal was found dead with broken neck and ribs. SWR19 belonged to a group of 9 individuals, composed of 6 adult females and 1 male and 1 sub-adult female. Both SWR6 and SWR19 belonged to the same single-male group. The single-male group experienced a takeover in January 2020 (after the death of SWR6) and thus SWR19 was likely not the new male's offspring. The single-male group was known to use a feeding site, but no aggression was recorded between groups for access to the resource.

3.2.2. Subject three — SWR7

SWR7 was first seen on 19th January 2019. The female foal was aged 3–4 days old. The foal was first seen lying down at 12:00 being guarded by the mother. The foal was later seen standing for a short period with its head pointed downwards and seemed unable to lift its head (Figure 2). The foal subsequently died and was retrieved on the 20th January 2019. A necropsy revealed the foal had sustained a broken neck and massive internal trauma with broken ribs. Four months prior to the birth of SWR7, the stallion was usurped by a new male. The foal was therefore unlikely to be the offspring of the new male. However, no interaction was witnessed to know whether a male or a female committed the attack. SWR7 belonged to a group of 7 individuals, composed of 5 females and 1 male and SWR7. The single-male group of SWR7 was known to use a supplementary feeding site, no other group is known to permanently use the area.

Figure 2. SWR7 at 15:30 where the foal was seen to be standing for a short period before falling down. Furthermore, the foal had sustained a broken neck, this was determined to be the reason the foal was unable to lift its head during observation. The individuals in the background are members of the breeding group. Photo taken by Liesl Vorster.

4. Discussion

4.1. Consistency of infanticide behaviour across equid species and potential explanations for behaviour

4.1.1. Infanticidal behaviour in MZNP

We witnessed bachelor males harassing and aggressively attacking two foals, however, we did not witness direct injury. Behaviours are consistent with infanticidal attacks in other equids including chasing, isolating foals from group, lunging at and biting at foals. MZNP has a high density of CMZ but, due to high grass availability (Lea et al., 2016), intense resource competition is unlikely. As the aggressive display was perpetrated by a bachelor group, adoption avoidance is also unlikely as bachelors are unlikely to sire offspring. Exploitation is an unlikely explanation for a grazing species. Social pathology is a potential explanation as infanticide behaviour has been recorded in other species without successful male turnover (Watts, 1989), however, this explanation is usually implicated where animals are living in unnatural or stressful conditions. Therefore, sexual selection is the most likely cause of the behaviour we observed. Bachelor males increase their mating opportunities if they can successfully challenge, and displace, the resident stallion. In plains zebra, larger groups of females are more likely to experience a stallion turnover (Vitet et al., 2021). In feral horses, females are more likely to foal in a subsequent year if they have lost a foal (Cameron et al., 2003). Stallions have been shown to play an important role in protecting females from harassment by bachelor males (Linklater et al., 1999). This may explain the higher rates of foal-directed aggression in equids.

4.1.2. Infanticidal behaviour in SWR

Documented infanticide in other equids, in wild and captive conditions, describe biting focused to the legs, neck and head of the foal as well as kicks to the flanks and trampling while the foal is on the ground (Pluháček & Bartoš, 2000; Gray, 2009), which was consistent with the injuries seen in SWR. From the five potential hypotheses, we can conclude predation/exploitation is unlikely. For SWR foals, resource competition, sexual selection, adoption avoidance and social pathology are all potential explanations. Infanticide at SWR may have been partially the result of congregation at supplementary feeding sites due to high resource competition and potentially higher general levels of contact between and within groups. All foals described were born to groups that accessed the supplementary feeding sites regularly. Within SWR, adoption avoidance is possible as stallion takeovers during pregnancy occurred in two of the three infanticides. Foals were killed within days of birth. In August 2020, another foal was born to the same single-male group as SWR6 and SWR19, this foal also died. The body was unable to be retrieved. Infanticide is a potential explanation. Importantly, groups in SWR may commit infanticide even when they do not use the feeding sites. A foal was born to a group of 6 individuals, 5 females and 1 male, which were not recorded to be using feeding sites. The group experienced a turnover of males three months prior to birth, the foal disappeared shortly after birth. As we did not witness infanticides take place in SWR, we cannot distinguish whether the perpetrator was the new stallion, a bachelor group or a group female. We also cannot distinguish whether the behaviour was purposive or accidental. However, due to the similarity of injuries seen in infanticide attempts in other equids, we would argue that these injuries were the result of infanticide.

4.2. Implications for CMZ biology and management

It is disputed whether infanticide plays an important role in the population dynamics of wild equids (Gray et al., 2012). Long term studies of wild plains zebra found no evidence that infanticides take place and foal survival was not impacted by stallion turnover (Vitet et al., 2021). However, in many free-ranging populations, it is extremely difficult to track foals unless intensive behavioural observations are conducted. Vitet and colleagues (2021) used hormone assays to detect non-pregnancy, mid-pregnancy and late pregnancy in mares to know whether a foal had been born even if it died before resighting or had been lost by abortion. Although they could not identify an explanatory variable for resighting rates in their dataset, they note that offspring survival from turnover was impacted when rates were assessed from mid-pregnancy onwards (Vitet et al., 2021). Infanticidal attacks may leave foals wounded and vulnerable to predation. If the aggressive action is not directly witnessed or predation occurs before the foal is found, infanticide may be underreported.

In many equid populations, foal mortality is high but the processes driving foal mortality are not well-documented (Grange et al., 2004). Within three years (2018–2020) in SWR, 5 of 24 foals (approx. 21%) were found dead with infanticide consistent injuries. Although populations in this study did not have biased sex ratios, many CMZ populations with male-biased adult sex ratio are associated with low fertility and low population growth rates (Lea et al., 2018). Male-biased sex ratios could exacerbate competition for females, increase turnover events, increase the frequency and intensity of harassment from bachelor groups and increase the risk of male infanticide. We recommend investigation of behavioural differences and infanticide rates in these male-skewed populations and active management of operational sex ratios in at-risk populations such as translocation of excess males to other reserves.

4.3. Conclusion

Incidences of infanticide are rare across the animal kingdom; however, equids display multiple biological characteristics that suggest infanticide would be adaptive. We report an eye-witness account of an aggressive interaction, consistent with behaviours seen in infanticide attempts, between two CMZ foals and a band of bachelor males in MZNP. Furthermore, we document evidence from necropsies of three foals in SWR with injuries consistent with infanticide attempts in other equid species. These findings constitute the first first-hand record for the subspecies CMZ and the first record for the species in the wild. These provide the most direct and comprehensive evidence to date that infanticide takes place within the CMZ subspecies and within Mountain zebra in wild conditions.

Acknowledgements

The work of JAB on this project was funded by the Natural Environment Research Council (NERC) Doctoral Training Partnership (grant NE/L002469/1). Necropsy were performed immediately by Veterinarian Dr Willem Burger with assistance from LV. JAB is also supported through the Chester Zoo Conservation Scholars programme. SS is funded by a Royal Society URF (UF110641). Author contributions: All authors conceived the manuscript; JAB wrote the initial draft of the manuscript. with contributions to the writing and editing from LV, SS. LV recorded group stability, population trends, assisted with three infanticide necropsies documenting and photographing the event. SS recorded the infanticide attempt at Mountain Zebra National Park.

References

Bartoš, L., Dušek, A., Bartošová, J., Pluháček, J. & Putman, R. (2021). How to escape male infanticide: mechanisms for avoiding or terminating pregnancy in mammals. — Mamm. Rev. 51: 143-153.

Berger, J. (1983). Induced abortion and social factors in wild horses. — Nature 303: 59-61.

Cameron, E.Z., Linklater, W.L., Stafford, K.J. & Minot, E.O. (2003). Social grouping and maternal behaviour in feral horses (*Equus caballus*): the influence of males on maternal protectiveness. — Behav. Ecol. Sociobiol. 53: 92-101.

Cameron, E.Z., Setsaas, T.H. & Linklater, W.L. (2009). Social bonds between unrelated females increase reproductive success in feral horses. — Proc. Natl. Acad. Sci. USA 106: 13850-13853.

Chen, J., Weng, Q., Chao, J., Hu, D. & Taya, K. (2008). Reproduction and development of the released Przewalski's horses (*Equus przewalskii*) in Xinjiang, China. — J. Equine Sci. 19: 1-7.

Dorj, U. & Namkhai, B. (2013). Reproduction and mortality of re-introduced Przewalski's horse. — J. Life Sci. 7: 623-629.

Duncan, P. (1982). Foal killing by stallions. — Appl. Anim. Ethol. 8: 567-570.

Ebensperger, L.A. (1998). Strategies and counterstrategies to infanticide in mammals. — Biol. Rev. 73: 321-346.

Feh, C. & Munkhtuya, B. (2008). Male infanticide and paternity analyses in a socially natural herd of Przewalski's horses: sexual selection? — Behav. Process. 78: 335-339.

Grange, S., Duncan, P., Gaillard, J.M., Sinclair, A.R.E., Gogan, P.J.P., Packer, C., Hofer, H. & East, M. (2004). What limits the Serengeti zebra population? — Oecologia 140: 523-532.

Gray, M.E. (2009). An infanticide attempt by a free-roaming feral stallion (*Equus caballus*). — Biol. Lett. 5: 23-25.

Gray, M.E., Cameron, E.Z., Peacock, M.M., Thain, D.S. & Kirchoff, V.S. (2012). Are low infidelity rates in feral horses due to infanticide? — Behav. Ecol. Sociobiol. 66: 529-537.

Hrabar, H. & Kerley, G.I.H. (2013). Conservation goals for the Cape mountain zebra *Equus zebra zebra* — security in numbers? — Oryx 47: 403-409.

Hrdy, S.B. (1979). Infanticide among animals: a review, classification, and examination of the implications for the reproductive strategies of females. — Ethol. Sociobiol. 1: 13-40.

Joubert, E. (1972). The social organisation and associated behaviour in the Hartmann *zebra Equus zebra hartmannae*. — Madoqua 1: 17-56.

Lea, J.M.D., Kerley, G.I., Hrabar, H., Barry, T.J. & Shultz, S. (2016). Recognition and management of ecological refugees: a case study of the Cape mountain zebra. — Biol. Conserv. 203: 207-215.

Lea, J.M.D., Walker, S.L., Kerley, G.I.H.I., Jackson, J., Matevich, S.C. & Shultz, S. (2018). Non-invasive physiological markers demonstrate link between habitat quality, adult sex ratio and poor population growth rate in a vulnerable species, the Cape mountain zebra. — Funct. Ecol. 32: 300-312.

Linklater, W.L., Cameron, E.Z., Minot, E.O. & Stafford, K.J. (1999). Stallion harassment and the mating system of horses. — Anim. Behav. 58: 295-306.

Lloyd, P.H. & Rasa, O.A.E. (1989). Status, reproductive success and fitness in Cape mountain zebra (*Equus zebra zebra*). — Behav. Ecol. Sociobiol. 25: 411-420.

Lukas, D. & Huchard, E. (2014). The evolution of infanticide by males in mammalian societies. — Science 346: 841-844.

Lukas, D. & Huchard, E. (2019). The evolution of infanticide by females in mammals. — Phil. Trans. Roy. Soc. B: Biol. Sci. 374: 20180075.

Opie, C., Atkinson, Q.D., Dunbar, R.I.M. & Shultz, S. (2013). Male infanticide leads to social monogamy in primates. — Proc. Natl. Acad. Sci. USA 110: 13328-13332.

Packer, C. & Pusey, A.E. (1982). Cooperation and competition within coalitions of male lions: kin selection or game theory? — Nature 296: 740-742.

Penzhorn, B.L. (1984). A long-term study of social organisation and behaviour of Cape mountain zebras *Equus zebra zebra*. — Z. Tierpsychol. 64: 97-146.

Penzhorn, B.L. (1985). Reproductive characteristics of a free-ranging population of Cape mountain zebra (*Equus zebra zebra*). — J. Reproduct. Fertil. 73: 51-57.

Pluháček, J. & Bartoš, L. (2000). Male infanticide in captive plains zebra, *Equus burchelli*. — Anim. Behav. 59: 689-694.

Pluháček, J., Bartoš, L. & Čulík, L. (2006). High-ranking mares of captive plains zebra *Equus burchelli* have greater reproductive success than low-ranking ones. — Appl. Anim. Behav. Sci. 99: 315-329.

Ransom, J.I. & Kaczensky, P. (2016). Behaviour of horses, zebras and asses. — In: Wild equids: ecology, management, and conservation (Ransom, J.I. & Kaczensky, P., eds). John Hopkins University Press, Baltimore, MD, p. 33-34.

Rubenstein, D.I. (1981). Behavioural ecology of island feral horses. — Equine Vet. J. 13: 27-34.

Van Schaik, C.P. & Kappeler, P.M. (1997). Infanticide risk and the evolution of male-female association in primates. — Proc. Roy. Soc. Lond. B: Biol. Sci. 264: 1687-1694.

Van Schaik, C.P. & van Noordwijk, M.A. (2010). Infanticide. — In: Encyclopedia of animal behavior (Breed, M.D. & Moore, J., eds). Elsevier, Oxford, p. 138-143.

Vitet, C., Duncan, P., Ganswindt, A., Mabika, C. & Chamaillé-Jammes, S. (2021). Do infanticides occur in harem-forming equids? A test with long-term sociodemographic data in wild plains zebras. — Anim. Behav. 177: 9-18.

Watts, D.P. (1989). Infanticide in mountain gorillas: new cases and a reconsideration of the evidence. — Ethology 81: 1-18.

Zharkikh, T.L. (1999). The cases of infanticide in the Przewalski horses at Askania-Nova. — Vestn. Zool. 80-82.