UNIVERSITÉ DE STRASBOURG

ÉCOLE DOCTORALE SCIENCES DE LA VIE ET DE LA SANTE

Institut Pluridisciplinaire Hubert Curien CNRS UMR 7178

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Soutenue le **30 Novembre 2023**

Pour obtenir le grade de **Docteur de l'université de Strasbourg**

Discipline / Spécialité : Sciences de la vie / Ecologie-éthologie

Phenotypic and demographic responses to climate and resources variation in a hibernating rodent, the Columbian ground squirrel

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ACKNOWLEDGEMENTS

Pour commencer, je souhaiterai remercier Céline Téplitsky, Michael Kearney, Frédéric Angelier et Sylvie Massemin pour avoir accepté d'évaluer mon travail. Especially Mike, thank you for your help from the begining on the model. Merci également Sylvie pour ton accompagnement sensible et humain depuis le master.

Je ne suis pas toujours très très forte pour exprimer ce que je ressens donc je vais le tourner en blague : Claire et Vincent j'imagine que vous vous en doutez, je ne veux plus jamais entendre parler de vous, j'en peux plus de vos commentaires et de vos retours !!! Bon non, en vrai **merci beaucoup**, pour votre présence, pour votre écoute, pour votre soutien, pour m'avoir permis de commencer en master puis de continuer en thèse, pour votre temps passé à m'aider, pour votre attention pour savoir si je vais bien et pour toutes les autres choses que j'aurai oublié de lister ici (mais pas pour les photos de plongée sur l'île de Pâques pendant que je suis en train de rédiger ma thèse, ça, ça devrait être interdit). J'espère pouvoir continuer à travailler, ou plonger, ou boire un coup, ou profiter de la pluie et de la neige avec vous !

Thank you Steve for your presence all along my Phd. We did not meet a lot in-person, but you were always here to help me and give me the best feedbacks, advices, ideas, or food for thought. Thank you also for your expertise and your incredible investment in the population monitoring, which now allow us to continue studying these (amazing) ground squirrels.

Thank you also Peter, first for being you, and second for all your help in the field. Especially during my PhD and in the context of covid, it would have been impossible to conduct all this work without your help. I will never forget the raclette in Canada with real Swiss cheese at 3 am with jetlag, and thank you, Kathreen, and Anna for always warmly welcoming us.

Thank you Rudy for your welcome in Toronto, and for allowing me to learn a lot from your scientific expertise. Thank you Phoebe for your training in the lab, and for your enormous help on the sample analyses.

Un petit mot pour Christophe et Sylvia (et Mike Rennie et Mélissa à Lakehead !), merci de m'avoir permis de découvrir et de commencer la recherche, le terrain, et les analyses dans des conditions incroyables. Je te l'ai déjà dit mais merci Sylvia pour ta présence et ton énergie, je te souhaite le meilleur pour la suite !

Merci à tous les membres de mon comité de suivi de thèse, Anne Loison, Audrey Bergouignan et Fabrice Bertile de votre écoute et vos conseils pour le bon déroulement de ma thèse. Merci également à Jérome Pansanel pour ton aide pendant mon master et ma thèse pour faire tourner le modèle microclimate.

Merci à toutes les personnes du DEPE, et en particulier les doctorants (+ Hugo) pour tous les moments partagés au labo et ailleurs (quand on était pas confinés chacun chez nous à bronzer au soleil). Nicolas et Benjamin, heureusement que vous étiez là sinon je serai probablement déjà loin dans la montagne. Merci Nico pour ton écoute, nos discussions à regarder par la fenêtre et les fous rires, et tout le reste… merci Benjamin d'être apparu en thèse avec nous et d'être resté (presque) jusqu'au bout, pour ta présence et ton écoute, pour ton humour un peu drôle et pour nous stimuler intellectuellement avec tes questions extrêmement pertinentes. Hugo, si on m'avait dit en master que je serai restée 3 ans en thèse assisse sur le bureau en face de toi j'aurai probablement fait une syncope. J'exagère peut-être un peu, merci d'être là et d'être toi-même, et de me faire quand même (beaucoup) rire. Merci Laura pour tout les moments partagés ensembles, ton écoute et ton énergie : je n'ai jamais vu quelqu'un autant dire oui à chaque fois que je propose un truc. Merci à la (presque) dernière génération de doctorants arrivés, Camille (la plus vieille), Maëlle et Clément pour l'énergie que vous apportez, ainsi que tous les autres doctorants pour les moments passés ensemble.

Finalement, merci à toutes les copines et les copains, les parents, ma sœur adorée, les copines et les copains des parents et de ma sœur préférée et les parents des copines et des copains pour les week-ends et vacances partagés, les repas, les soirées, les pizzas et les fous rires. Merci Ulysse pour les moments partagés en Amérique (et les photos !). Merci Bruno pour m'avoir mis la pression à ta soutenance. Merci Ambre, Mathéo et Agnès pour votre simplicité. Merci Léonard. Et merci Brice, principalement pour ton humour et le matos de spéléo, mais aussi pour les moments partagés et ton soutien.

Et bien sûr merci aux spermophiles pour leur coopération, en particulier Ray la montagne pour nous avoir permis de travailler notre patience.

là

GENERAL THESIS STRUCTURE

LIST OF PUBLICATIONS & COMMUNICATIONS

A. Publications presented in the manuscript

Tamian, A., Saraux, C.*, Scheubel, A., Schull, Q., Sosa, S., Roth, J.D., Pardonnet, S., Filippi, D., Dobson, F.S., Viblanc, V.A.* (2023). Adapted to cope? Behavioral plasticity in response to ambient heat in Columbian ground squirrels. *In prep.*

Tamian, A., Viblanc, V.A.*, Dobson, F.S., Saraux, C.* (2023). Population density and vegetation resources influence demography in a hibernating mammal. *Submitted to Oecologia*.

Tamian, A., Edwards, P. D., Neuhaus, P., Boonstra, R., Neuhaus, A., Emmanuel, P., Pardonnet, S., Palme, R., Filippi, D., Dobson, F. S., Saraux, C.*, Viblanc, V. A.* (2023). Weathering the storm: Decreased activity and glucocorticoid levels in response to inclement weather in breeding Columbian ground squirrels. *Hormones and Behavior, 155*(2023), 105426.

Tamian, A., Viblanc, V. A.*, Dobson, F. S., Neuhaus, P., Hammer, T. L., Nesterova, A. P., Raveh, S., Skibiel, A., Broussard D. Manno, T., Rajamani, N. & Saraux, C.* (2022). Integrating microclimatic variation in phenological responses to climate change: A 28-year study in a hibernating mammal. *Ecosphere*, *13*(5), e4059.

B. Other publications

Karels, T.J., **Tamian, A.**, Saraux, C., Viblanc, V.A. & Boonstra, R. (2023). Arctic ground squirrels choose snow-accumulating habitat to reduce mass loss and survive winter hibernation. *In prep*.

Viblanc, V.A., Pardonnet, S., **Tamian, A.**, McCaw, L., Dobson, F.S., Saraux, C. & Boonstra, R. (2023) Hormonal and oxidative stress responses of male and female Columbian ground squirrels to acute stress. *In prep*.

Dobson, F.S., Saraux, C., **Tamian, A.**, Criscuolo, F., Coltman, D.W., Raveh, S. & Viblanc, V.A. (2023). Comparing lifetime and annual fitness measures for emergence date from hibernation in Columbian ground squirrels. *In prep.*

Tamian, A., Dobson, F.S., Neuhaus, P., Saraux, C., Viblanc, V.A & Oli, M.K. (2023). Determinants of population growth and decline: lessons from a 30-year life table response experiment. *In prep*.

Kemppinen, J.; Lembrechts, J.; Van Meerbeek, K.; Carnicer, J.; Chardon, N.; Kardol, P.; Lenoir, J.; Liu, D.; Maclean, I.; Pergl, J.; Saccone, P.; Senior, R.; Shen, T.; Słowińska, S.; Vandvik, V.; von Oppen, J.; Aalto, J.; Ayalew, B.; Bates, O.; Bertelsmeier, C.; Bertrand, R.; Beugnon, R.; Borderieux, J.; Brůna, J.; Buckley, L.; Bujan, J.; Casanova-Katny, A.; Christiansen, Ditte M.; Collart, F.; De Lombaerde, E.; De Pauw, K.; Depauw, L.; Di Musciano, M.; Díaz Borrego, R.; Díaz-Calafat, J.; Ellis-Soto, D.; Esteban, R.; Fälthammar de Jong, G.; Gallois, E.; Garcia, M.; Gillerot, L.; Greiser, C.; Gril, E.; Haesen, S.; Hampe, A.; Hedwall, P.; Hes, G.; Hespanhol, H.; Hoffrén, R.; Hylander, K.; Jiménez-Alfaro, B.; Jucker, T.; Klinges, D.; Kolstela, J.; Kopecký, M.; Kovács, B.; Maeda, E.; Máliš, F.; Man, M.; Mathiak, C.; Meineri, E.; Naujokaitis-Lewis, I.; Nijs, I.; Normand, S.; Nuñez, M.; Orczewska, A.; Peña-Aguilera, P.; Pincebourde, S.; Plichta, R.; Quick, S.; Renault, D.; Ricci, L.; Rissanen, T.; Segura-Hernández, L.; Selvi, F.; Serra-Diaz, J.; Soifer, L.; Spicher, F.; Svenning, J.; **Tamian, A.**; Thomaes, A.; Thoonen, M.; Trew, B.; Van de Vondel, S.; van den Brink, L.; Vangansbeke, P.; Verdonck, S.; Vitkova, M.; Vives-Ingla, M.; von Schmalensee, L.; Wang, R.; Wild, J.; Williamson, J.; Zellweger, F.; Zhou, X.; Zuza, Emmanuel J.; & De Frenne, P. (2023). Microclimate, an inseparable part of ecology and biogeography. *In revision in Global Ecology and Biogeography.*

Viblanc, V. A., Saraux, C., **Tamian, A.**, Criscuolo, F., Coltman, D. W., Raveh, S., Murie, J.O. & Dobson, F. S. (2022). Measuring fitness and inferring natural selection from long-term field studies: different measures lead to nuanced conclusions. *Behavioral Ecology and Sociobiology, 76*(6), 1-13.

C. Communications

Tamian, A., Saraux, C., Dobson, F. S., Viblanc, V. A. (2023). Density and food influences on demography and population dynamics. *13th International Mammalogical Congress, Invited symposium on data integration for studying mammal populations, 20 July, Anchorage, Alaska, USA.*

Tamian, A., Saraux, C., Dobson, F. S., Viblanc, V. A. (2023). Density and food influences on demography and population dynamics. *16 March, Scientific collaboration with the Centre d'études Biologiques de Chizé, CNRS.*

Tamian, A., Saraux, C., Dobson, F. S., Viblanc, V. A. (2023). Density and food influences on demography and population dynamics. *16 Feb, Scientific seminars of the laboratory, Strasbourg.*

Tamian, A., Edwards, P. D., Boonstra, R., Neuhaus, P., Pardonnet, S., Dobson, F. S., Saraux, C., Viblanc, V. A. (2023). Snowstorm and rainfall effects on ground squirrels. *9 Jan, Scientific team meeting presentation, Strasbourg.*

Tamian, A., Viblanc, V. A., Dobson, F. S., Neuhaus, P., Saraux C. (2022) Integrating microclimatic variation in phenological responses to climate change. *Microclimate Ecology and Biogeography conference, 29 Aug - 1 Sept, Antwerp, Belgium*. Oral presentation.

Tamian, A., Viblanc, V. A., Dobson, F. S., Neuhaus, P., Saraux C. (2022) Phenological changes in relation to microclimates. *15th Ecology & Behaviour Meeting, 21-24 March, Strasbourg, France.* Oral presentation.

Tamian, A., Viblanc, V. A., Dobson, F. S., Neuhaus, P., Saraux C. (2021) Integrating microclimatic variation in phenological responses to climate change. *Graduate conference on behaviour, ecology and evolution, University of Oxford, United Kingdom*. Oral presentation.

Tamian, A., Viblanc, V. A., Dobson, F. S., Neuhaus, P., Saraux C. (2021) Phenological changes in relation to microclimates. *Journées de l'école doctorale, 21-22 Avril, Strasbourg, France.* Oral presentation, price of the second best presentation.

OTHER CONTRIBUTIONS

A. Communication & outreach

- Chair of the organization of the 15th Ecology & Behaviour meeting, an international congress mainly addressed to early-career scientists and hosted almost every year in France. President of the association (SERL) responsible for the 15th edition, which took place in Strasbourg between 21 and 24 March 2022 and gathered around 130 participants (management of the organizing team, administrative management, internal and external communication, funding and grant applications, funding IdEX "Congresses and International Symposia").

- Co-organization of weekly scientific seminars of the laboratory (Department of ecology, physiology, and ethology, IPHC, Strasbourg, between 2020-2023)

- Presentations and discussions around my experience as a scientific woman in middle and high schools in Alsace (between 2020-2023, program co-organized by *Femmes & Sciences* association and *Le jardin des sciences*, University of Strasbourg)

- Invited speaker for a presentation on climate change and phenology, as part of a training on climate change for earth and life science, physics, and chemistry middle and high school teachers (organized by the Maison pour la Science Alsace, 5 May 2023, Strasbourg)

- Invited speaker for a presentation on my PhD thesis to high school girls (organized by the program 'Pour les filles et la Science' from the *Fondation l'Oréal*, 4 February 2023, Strasbourg)

- Participation as a speaker in a project (interviewed in a video) with students from the Master "Scientific communication" of Strasbourg University (January 2022).

B. Teaching & supervision

- Co-supervision of SCHEUBEL Alexandra, 6-month intern from engineering school Agrocampus Ouest, Rennes, France (Jan-Jul 2023).
- Co-supervision of GIOVANNINI Lou, 2-month intern from Ecole Normale Supérieure, Université Paris Sciences & Lettres, France (Jun-Jul 2023).
- Teaching assistant, Animal Biology & Comparative Physiology and Anatomy Courses (2021-2022; University of Strasbourg, 64h).

C. Field & laboratory contributions during my PhD

- Fieldwork as part of the on-going long-term monitoring of Columbian ground squirrels in Sheep River Provincial Park, Alberta, Canada:
    - 2020 & 2021 Sessions cancelled due to Covid-19
    - Oct 2021: Installation of weather stations and climate loggers
    - Apr-Jun 2022: Field season (population monitoring from emergence to the end of lactation)
    - June 2023: Field season (lactation period)
- Laboratory analysis of fecal cortisol metabolites in the University of Toronto Scarborough, in collaboration with Phoebe D. Edwards & Rudy Boonstra (Jul-Aug 2022)

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ABBREVIATIONS

CGS	Columbian Ground Squirrels
DEB	Dynamic Energy Budget
DFA	Dynamic Factor Analysis
ED	Emergence Date
EIA	Enzyme ImmunoAssay
EM	Emergence Mass
FCM	Fecal Cortisol Metabolites
GAM	Generalized Additive Model
GAMM	Generalized Additive Mixed (effects) Model
GLMM	Generalized Linear Mixed (effects) Model
HPA	Hypothalamic-Pituitary-Adrenal (axis)
LM	Linear Model
LMM	Linear Mixed (effects) Model
NDVI	Normalized Difference Vegetation Index
NOAA	National Oceanic and Atmospheric Administration
PCA	Principal Component Analysis
ROS	Reactive Oxygen Species

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GENERAL INTRODUCTION

A. Environmental variations and effects on organisms

Animals constantly face biotic and abiotic constraints from their environment (competition, predation, parasitism, and geology, hydrology, climate, etc.). These constraints act as selective pressures shaping organisms' phenotypic traits and life history strategies (Stearns 1977, 1989). Nonetheless, the environment is not fixed but continuously changing, whether due to intra and inter-annual variability or long-term changes, creating new selection pressures. To face predictable intra-annual (*i.e.* seasonality) and stochastic variation, animals have evolved different strategies and plasticity (Stearns 1976, Yoshimura and Clark 1993, Dayton 2008).

Current long-term changes in climate modify environmental conditions, may limit the scope for adaptive phenotypic plasticity (DeWitt et al. 1998, Merilä and Hendry 2014), and may threaten population viability (Reed et al. 2010). In the context of global change, the frequency of extreme climate (or weather[1]) events is increasing (Stott 2016, Seneviratne et al. 2021, Quante et al. 2021) and has important consequences on animal breeding success and survival (Buckley and Huey 2016, van de Pol et al. 2017, Kucheravy et al. 2021). Extreme climate events, opposed to long-term directional changes in the state of the system (climate change), are punctual, happening stochastically, and also trigger behavioral and physiological reactions from living organisms. To measure organisms' responses to climate change or variability, it is important to consider the spatial extent to which climate or weather conditions are assessed: depending on the size and habitat of animals, microclimate conditions assessed in the local environment surrounding individuals may be more relevant than global climate conditions (Rosenberg et al. 1983, Potter et al. 2013).

Such nuances in the temporal scales (see illustration on Figure 1, parts A.1, A.2, and A.3 in the general introduction) and spatial scales (part A.4 in the general introduction) considered have different effects on animal species (depending on their life histories and phenotypic plasticity) and ultimately on populations.

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[1] **Climate** is defined as the 'long-term' or 'average' weather pattern in a region, or the 'statistical description in terms of the mean and variability of relevant quantities over a period of time ranging from months to thousands or millions of years' (Matthews et al. 2021), whereas the word **weather** is used to describe the conditions of the atmosphere over a short period, with respect to heat or cold, wetness or dryness, calm or storm, clearness or cloudiness (Merriam-Webster Dictionary 2023). Proper definitions of each term are necessary, although climate and weather might be used interchangeably in ecological studies (Shepherd et al. 2015).

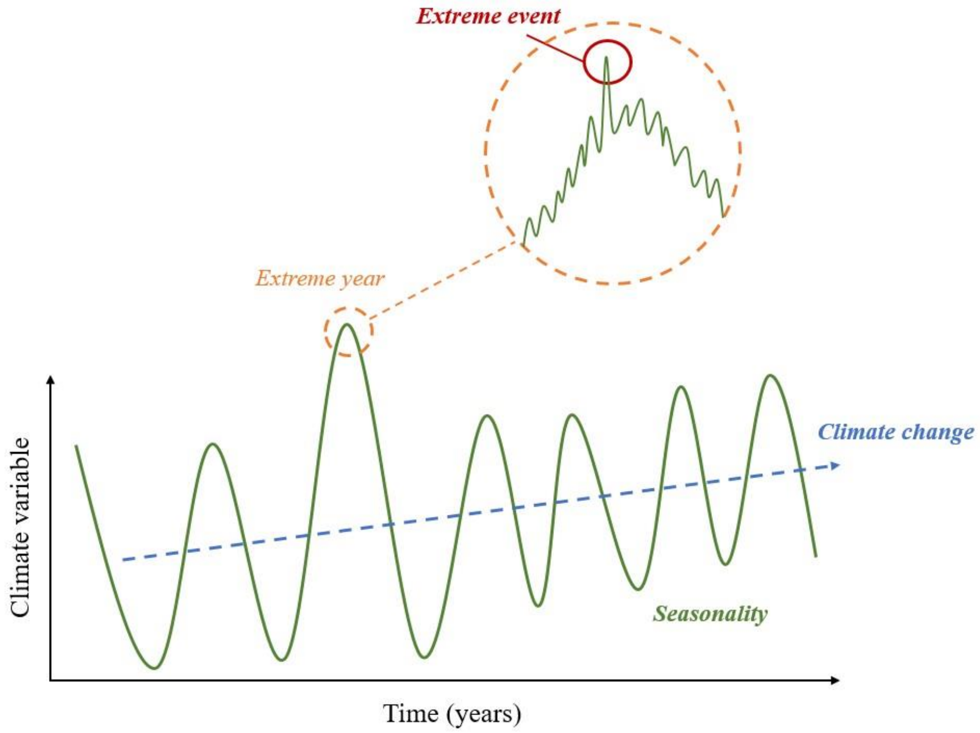


Figure 1. Considerations in climate effects on organisms. *The temporal variation of a climate variable (temperature, precipitation, etc.) is illustrated in terms of seasonality, climate change, and extreme event.*

1. Seasonality and seasonal adaptations of organisms: responding to natural and predictable climate variability in temperate ecosystems

a) Seasons, constraints and adaptations

"Il n'y a plus de saisons !" (French folkoric expression)

Seasons are periods of the year that can be defined mainly in two different ways: from an astronomical perspective according to the natural rotation of Earth around the sun with two equinoxes and two solstices, and from a meteorological perspective according to mean temperature (Trenberth 1983, NOAA 2022). The main four temperate and polar seasons go from colder to warmer seasons (winter and summer), with intermediary periods (spring and autumn). In ecology, seasonality is generally assessed from the perspective of predictable and repeatable changes in the environment, such as changes in photoperiod and/or climate conditions (temperature, precipitations, wind), which ultimately influence primary production, vegetation growth, and food availability for consumers (Battey 2000, Dayton 2008, Lieth 2013, Tonkin et al. 2017, Lisovski et al. 2017). The decrease in the intensity of sunlight reaching the Earth's surface makes winter a period of harsh conditions for most organisms, usually characterized by lower food resources in the environment, and decreased energy availability. On the contrary, warmer seasons are characterized by high primary and secondary production, rapid growth, and reproduction (Lack 1950, Boyce 1979, Conover 1992, Williams et al. 2017a).

Among living organisms, seasonality is an important selective force and animals have adapted to cope with decreased energy availability through various mechanisms, either by moving to avoid winter conditions or by staying and evolving specific traits/strategies to cope with these harsh conditions (Dayton 2008, Varpe 2017, Auteri 2022). Many invertebrate and vertebrate species migrate from one location to another to occupy habitats that are temporarily more resource-rich, and potentially avoid inter and intra-specific competition before returning to their breeding area (Dingle and Drake 2007, Nathan et al. 2008, Milner-Gulland et al. 2011, Dingle 2014, Fudickar et al. 2021). Because increased locomotion usually leads to increased metabolic costs and energy requirements, migration is a trade-off between the costs of staying and the costs of moving (Teitelbaum and Mueller 2019). Species living in seasonal environments that stay during the unproductive period exhibit morphological, physiological, and/or behavioral adaptations to face cold temperatures and resource depletion. These resistance or tolerance adaptations can include fur thickness (Irving et al. 1955, Hart 1956, Prestrud 1991), decreased activity (Chappell and Bartholomew 1981b, Koprowski and Corse 2005), or food caching (Wauters et al. 1997, Morrison et al. 2009, White and Geluso 2012) and storage to maintain energy acquisition during winter (Prestrud 1991, Glanville and Seebacher 2010). Among adaptations of organisms that decrease their activity during winter to minimize energy use, different kinds of dormancy exist, including diapause (suspension of growth and development, generally concerning arthropods, Tauber et al. 1986, Koštál 2006) and hibernation, in a wide range of taxa (Vegis 1964, Mousseau and Roff 1989, Nedergaard et al. 1997, Varpe 2012, Geiser 2013).

Hibernators are heterothermic animal species (*i.e.* endotherms with temporal changes in core body temperature), alternating between torpor bouts and arousals (euthermic phases) on a seasonal basis (Lyman and Chatfield 1955, Geiser 2013). During torpors, metabolism, body temperature, heart rate, respiration, water loss, and other physiological functions are reduced (Heldmaier et al. 2004, Geiser 2013). Hibernation is a strategy that allows drastic decreases of energy expenditure during seasons of resource depletion (decrease to around 5% of normal basal metabolic rate, energy expenditure reduced to <15% of a normothermic animal, Geiser and Ruf 1995, Geiser 2013), and is common in many mammals (monotremes, marsupials, and placentals) and in one bird species (the common poorwill, *Phalaenoptilus nuttallii*, Jaeger 1948, Woods 2002, Geiser 2013). However efficient, even hibernation requires notable amounts of energy for endotherms, both to meet the body's minimum metabolic requirements (basal metabolic rate), and to rewarm the body to normothermia between hypothermic states using internal heat production (Heldmaier et al. 2004). Thus, in order for hibernating animals to survive over several months, energy is stored either externally in food cashes or dens, or internally as fat stores following an hyperphagic phase towards the end of the active season (Lyman 1954, Wall 1990, Humphries et al. 2003). Consequently, energy storage, growth, and reproduction are highly constrained temporally to non-hibernating periods, *i.e.* summers (Varpe 2017). These constraints in energy regulation, a key factor in animal life histories (Brown et al. 2004), have numerous consequences on hibernating species, and on the strategies used.

b) Evolutionary challenges of hibernation

(1) Phenology and environmental cues

Strongly seasonal hibernators, in opposition to opportunistic heterotherms, are dependent on seasonal changes during their life cycle (Boyce 1979, Geiser 2013, Williams et al. 2017a). The compression of somatic and reproductive investments to short active periods (Varpe 2017) may have important fitness consequences, especially if environmental conditions deteriorate. Thus, the timing of animal life cycles – **known as phenology** – typically matches environmental seasonality and food availability during active seasons (Pigeon et al. 2016, Mohr et al. 2020). Phenology is a highly studied life history trait in hibernating species (Iwasa and Levin 1995, Forrest and Miller-Rushing 2010).

However, less research effort has been allocated to understand the diverse factors (or cues) influencing the phenology of hibernation and reproduction events, and the endogenous mechanisms that allow individuals to stay synchronized (Williams et al. 2014a). Dark hibernaculum conditions do not allow hibernators to rely directly on photoperiod cues to terminate hibernation and activate reproductive maturation (through the hypothalamic-pituitary-gonadal axis), meaning that they either rely on a circannual clock (Michener 1984) and/or other environmental cues (Barnes and York 1990). Some species of obligate and relatively long-lived hibernators possess endogenous circannual rhythms that regulate behaviour and physiological functions (golden-mantled ground squirrels, *Callospermophilus lateralis*, yellow-bellied marmots, *Marmota flaviventris*, other species of ground squirrels and chipmunks, Pengelley and Fisher 1963, Heller and Poulson 1970, Ward and Armitage 1981, MacCannell and Staples 2021). Yet, to allow individuals to maintain synchrony with the environment, circannual rhythms must be entrained by external environmental cues, *e.g.* photoperiod, temperature (Bradshaw and Holzapfel 2007, Golombek and Rosenstein 2010).

Regardless, phenological adjustments to the beginning of the active and productive season allow individuals to maximize their breeding opportunities (*e.g.* earlier emergence in male ground squirrels compared to other individuals, Michener 1983, Pra et al. 2022) without emerging too early during harsh climatic conditions and poor resource availability (Barnes 1996, Zervanos et al. 2010). In addition, breeding early in the season, or being able to synchronize breeding with peak resource availability, correlates with higher breeding success, giving more time for the juveniles to accumulate reserves before next hibernation (Williams et al. 2014b) or allowing optimization of energy access for both parents and offspring (Ejsmond et al. 2021). However, different chronologies of events during the productive season exist among species and, depending on the type of strategy employed for energetically sustaining reproduction during the reproduction of seasonal species, different factors may influence individual survival and breeding success.

(2) Reproductive strategies in hibernating species

In general, small hibernating mammals are relatively long-lived for their body mass and exhibit slow life histories (Turbill et al. 2011). Indeed, they spend long periods with low metabolic activities and interrupted growth, which limits the speed of their development. Short active and reproductive periods induce fast reproductive cycles, with most of the species being altricial (Geiser 2013). However, among seasonal species, two main types of breeding strategies that rely on different access to energy during reproduction can be found: income versus capital breeders. Whereas capital breeders produce offspring from stored resources (Figure 2, in red), income breeders produce offspring from concurrent food intake (Figure 2, in blue, Jönsson 1997, Varpe 2017).

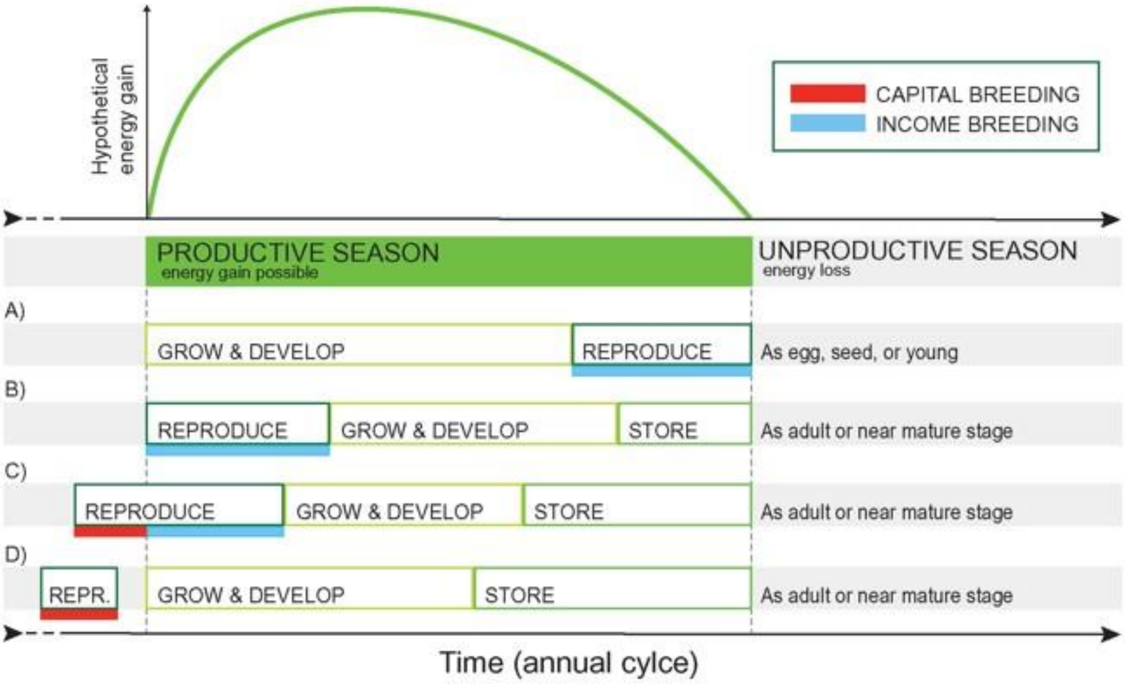


***Figure 2. Presentation of the continuum between capital (red) and income (blue) breeders**. The different strategies are presented (from A to D) with their activity during productive and unproductive seasons (from Varpe 2017).*

Capital breeding, compared to income breeding, induces some energetic costs for accumulating and storing capital but can allow individuals to cope with unpredictable changes in food conditions or risky foraging conditions (Jönsson 1997, Houston et al. 2007, Stephens et al. 2014). Income breeders are constrained temporally to the season with available resources. However, reproductive tactics among seasonal species are now more perceived as a continuum between capital and income breeding and the allocation of capital towards reproduction is a plastic trait that may be linked with reproductive phenology and fecundity (Williams et al. 2017c). Indeed, the breeding success of several seasonal species usually known as income breeders depends on individual body mass or condition at the start of the breeding season, showing carryover effects from the preceding active season (Broussard et al. 2005, Wheatley et al. 2008, Harrison et al. 2011, Rubach et al. 2016). In addition, the capital stored by hibernating species may depend on hibernation conditions (*e.g.* quality, length, Broussard et al. 2005, Culina et al. 2019).

c) Hibernation trade-offs

As presented above and in the literature, hibernation is usually perceived only as an evolutionary response to the seasonality of environmental conditions and resource availability. However, it needs to be highlighted that there are some costs of hibernation, and that other environmental factors may act as selective forces shaping the evolution of hibernation.

First, whereas metabolic depression during hibernation allows for substantial energy savings, the physiological adjustments associated with metabolic depression may carry costs of their own. For instance, rewarming to euthermia in between torpors has been suggested to expose sensitive tissues to increased oxidative damage during reperfusion (Carey et al. 2000, Duffy and Staples 2022). Changes in cellular lipid composition may also render hibernators more prone to oxidative stress, though the amount of oxidative stress in hibernating species appears to be surprisingly well controlled through physiological (*e.g.* enzymatic) or behavioral adaptations (Frank et al. 1998, Carey et al. 2000, Orr et al. 2009, Wei et al. 2018). Other costs associated with hibernation might include reduced immunocompetence (Burton and Reichman 1999, Prendergast et al. 2002), and neuronal tissue damage (Millesi et al. 2001, Clemens et al. 2009). Arousals to normal body temperature are thought to allow individuals to recover from these physiological costs, and hibernation expression can be seen as a trade-off between energy consumption and the depth and duration of torpor bouts (illustrated in Figure 3, Humphries et al. 2003, Boyles et al. 2020). Additionally, while hibernating animals are inactive in their burrow, they are potentially missing reproductive opportunities (*e.g.* mating for males, Michener 1983, Pra et al. 2022).

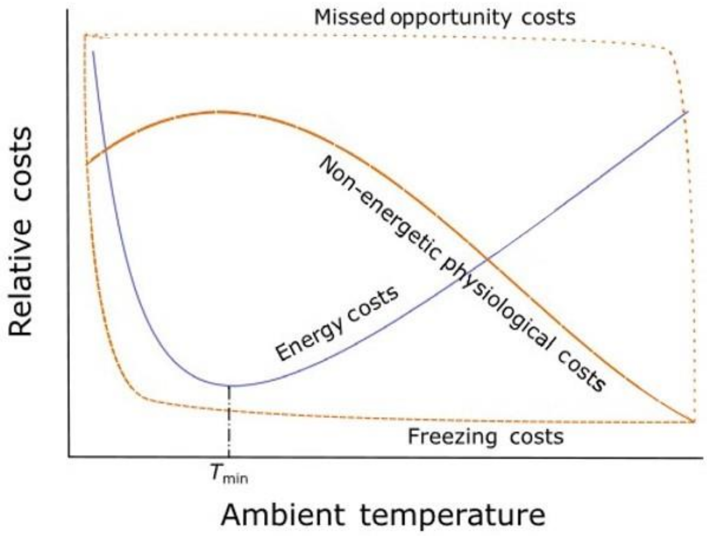


Figure 3. Different relative costs and benefits during hibernation as a function of ambient temperature (proxy of hibernation expression). *Whereas energy costs are minimal at minimum ambient temperature, non-energetic physiological costs are maximal at low temperature, and decrease with increasing temperature. Freezing and energy costs increase drastically at low temperature, and the costs of missing opportunities are constant during hibernation (adapted from Boyles et al. 2020).*

Finally, hibernation can be seen as an adaptive strategy to decrease predation risk (from outside activity – but can still occasionally happen during hibernation) and increase annual survival of individuals, by staying protected in their hibernacula (Bieber et al. 2018, Constant et al. 2020, Ruf and Bieber 2023, Allison et al. 2023). To sum up, numerous factors modulate the expression of hibernation and hibernation phenology in seasonal vertebrates, such as food limitation, predation avoidance, reproductive strategies, physiological costs, or thermal constraints. Seasonality exhibits cyclic patterns in environmental variations, which are usually regular and predictable. However, in the context of current global climate changes, habitats, living conditions, and environmental cues may be modified inducing long-term phenological, reproductive, and population consequences.

2. Long-term changes in relation to climate change

a) Climate change generalities and reports

From the glossary from the Sixth Assessment Report of the IPCC (Intergovernmental Panel on Climate Change), **climate change** is defined as ‘a change in the state of the climate that can be identified (*e.g.* by using statistical tests) by changes in the mean and/or the variability of its properties and that persists for an extended period, typically decades or longer’ (Matthews et al. 2021). A distinction can be made between climate change attributable to human activities, such as contemporary climate change, and natural climate variability (UNFCCC 1992). Current anthropogenic climate change, known as “global warming”, is reflected by a global increase in the Earth’s surface temperature of 1.1°C in 2011-2020 compared to 1850-1900 (and 1.59°C when considering data over land). Other global consequences reported on ecosystems include sea level increase (0.2m between 1901 and 2018), retreat of glaciers, decrease in Arctic and Antarctic sea ice, decreased Northern Hemisphere spring snow cover, and acidification of the surface open ocean (IPCC 2023). Additionally, the scientific interest towards climate change has increased significantly over the last 20 years (Figure 4), and the global effects of climate change on ecosystem functioning and species responses are starting to be well known and reported in a wide diversity of taxa (Martay et al. 2017, Bowler et al. 2017, Radchuk et al. 2019, Schleuning et al. 2020).

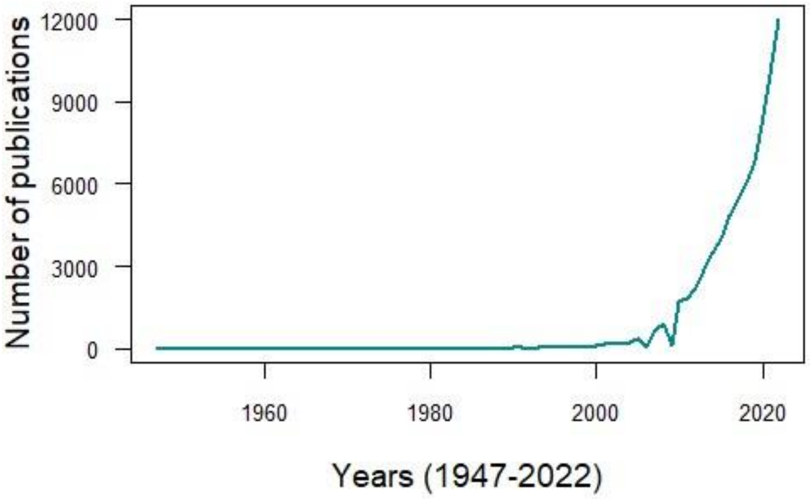


Figure 4. Yearly number of publications with "climate change" in the title between 1947 and 2022. *Since the beginning of the 21st century, the general and scientific interest toward climate change increased significantly (all publications from PubMed).*

However, it is important to highlight that changes are not directional in all ecosystems, and trends cannot be summarized globally. For example, high latitude and altitude ecosystems are more sensitive to climate change (Root et al. 2003, Bhattarai et al. 2022, IPCC 2023, Ramalho et al. 2023). Similarly, species responses to climate change are not similar in all populations (Taper et al. 1995, Marcel et al. 2003, McCain et al. 2016, Bennett et al. 2019) and the level of analysis (individual, population, species, taxonomic group, community, and ecosystem) might condition the direction of observed responses, and influence our conclusions. Among long-term consequences of climate change on populations, spatial and temporal shifts are observed: habitat modifications lead to changes in species distribution and/or to changes in species phenology (Walther et al. 2002a, Parmesan 2006).

b) Long-term effects of climate change on individuals and populations

(1) Spatiotemporal modifications of organisms' presence

With abiotic environmental changes and modifications in thermal regimes, species geographic ranges are generally shifting to more favorable conditions higher in altitude (see example in the European Alps on Figure 5.B), in latitude, and deeper in the oceans (Perry et al.

2005, Dulvy et al. 2008, Thomas 2010, Chen et al. 2011) even though some variation exist (Lenoir and Svenning 2015, Rubenstein et al. 2023). However, topographic variation such as coastlines (Burrows et al. 2014) or mountains (Sekercioglu et al. 2008) may act as natural barriers of range movements, and especially limit species that are already at the "edges", *i.e.* in Alpine or polar ecosystems (see Humphries et al. 2002 for an example). Furthermore, rapid changes may lead species towards ecological traps ("*when organisms make poor habitat choices based on cues that correlated formerly with habitat quality*", Schlaepfer et al. 2002), if rapid changes in the environment creates a mismatch between cues and resource condition, reducing fitness and potentially threatening population viability (Schlaepfer et al. 2002, Hale et al. 2016).

As environmental seasonal cues are changing, numerous species living in seasonal environments also exhibit temporal shifts in their life cycle, with a global trend toward earlier spring activities despite different responses among taxa and species (Figure 5.A, Parmesan 2006, Kearney et al. 2010, Thackeray et al. 2016, Cohen et al. 2018, Chmura et al. 2019, Inouye 2022).

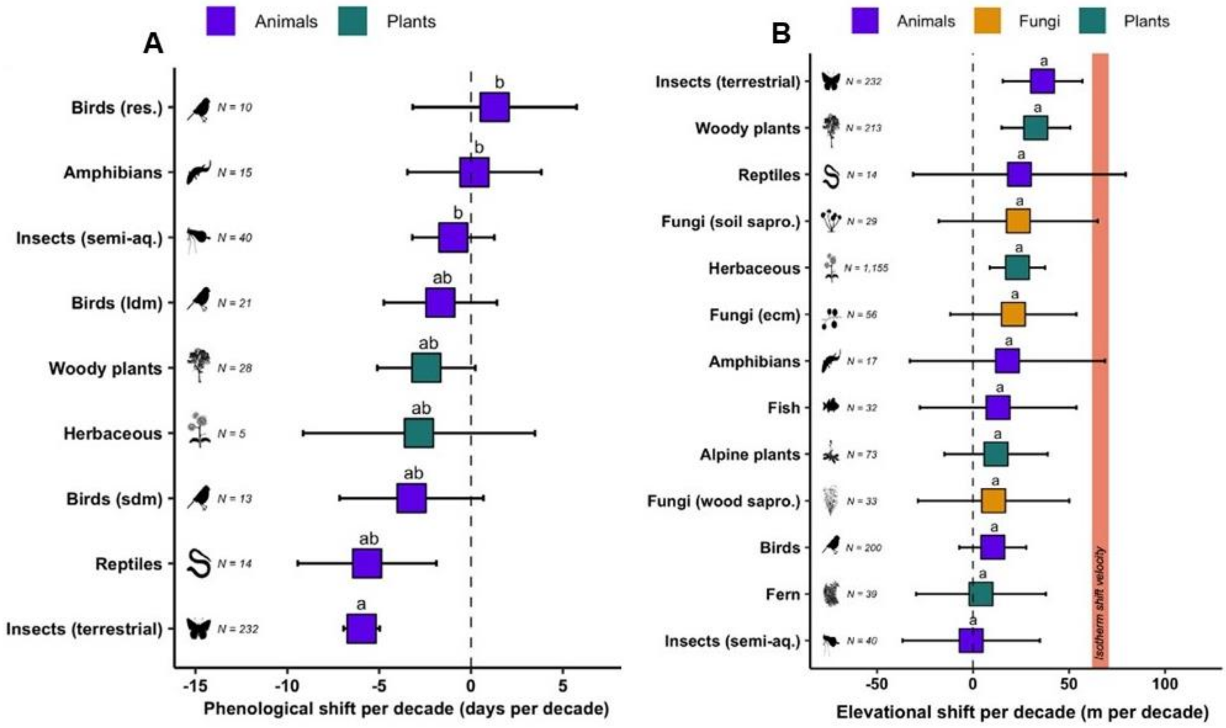


Figure 5. Example of phenological and elevational shifts in different taxa in the European Alps. *(A) Animals (blue) and plants (green) phenological shifts, in terms of advanced (< 0) or delayed (> 0) days per decade. (B) Animals (blue), fungi (orange), and plants (green) elevational shifts of the maximum abundance, in terms of higher m per decade. Values*

correspond to estimated marginal mean with error bars as 95% confidence intervals, and letters to significant differences from post-hoc comparisons. The period of time studied varied but was always more than 10 years and comprised groups with more than five species. The vertical red line corresponds to the velocity of isotherm shift (between +62 and +71 m/decade during the period 1970 – 2019). ldm = long-distance migrants, sdm = short-distance migrants, res. = resident, semi-aq. = semi-aquatic, ecm = ectomycorrhizal, sapro. = saprophytic (adapted from Vitasse et al. 2021).

Because the strength of phenological shifts differs greatly among species, temporal changes may lead to a situation where the phenologies of different trophic levels are mismatched (*e.g.* between herbivores and vegetation growth, between a predator and its prey), a well-known negative consequence of climate change on animal fitness (Visser et al. 2004, Visser and Both 2005, Durant et al. 2007, Plard et al. 2014, Radchuk et al. 2019). The observed spatial and temporal shifts are now well documented and correlated to changes in climate patterns (Amélineau et al. 2021, Schano et al. 2021). What remains unclear, however, is what the environmental cues are that act on species phenologies (Inouye et al. 2000, Williams et al. 2014a, Bonamour et al. 2019), how strongly these cues change, and what coping mechanisms organisms have when facing environmental shifts.

(2) Plasticity of responses or micro-evolution?

Several studies report phenotypic adjustments of individuals and populations to environmental changes (Charmantier et al. 2008a, McGaughran et al. 2021), but it may be challenging to disentangle phenotypic plasticity from micro-evolutionary responses (Teplitsky et al. 2008). Phenotypic plasticity is defined as "the ability of individual genotypes to produce different phenotypes when exposed to different environmental conditions" (Pigliucci et al. 2006), whereas micro-evolution corresponds to changes in allele frequencies in a population, occurring over short evolutionary time scales compared to macro-evolution (Fusco and Minelli 2010, Hautmann 2020). Plasticity or evolutionary responses in ecology are usually inferred from the variability of phenotypic expression under different environmental conditions, using experiments such as translocations of individuals or common garden experiments (de Villemereuil et al. 2016, Lane et al. 2019), or through molecular genetic or statistical analyses ("animal model" analyses, Kruuk 2004, Wilson et al. 2010). Whereas evidences for genetic adaptation to climate change are relatively scarce (Merilä and Hendry 2014, Bonnet et al. 2017), individual responses may also be a combination of both plasticity and micro-evolution (Moiron et al. 2023). Finally, epigenetic (*i.e.* stable heritable traits that cannot be explained by changes in DNA sequence, Deans and Maggert 2015) effects may contribute to climate change adaptations without depending on DNA-encoded genetic changes (McGuigan et al. 2021).

The remaining questions around phenotypic plasticity are whether individual responses will be sufficient or if they are already at the limits of their reaction norms (Nussey et al. 2007, Radchuk et al. 2019). Species with longer generation times might be limited in their genetic adaptations when facing rapid changes. This may be true for hibernating species that generally have a slower pace of life (Turbill et al. 2011). Observed responses of hibernators to climate changes are already identified and discussed, but the extent/way to/in which individuals are able to cope with these changes still need to be explored.

(3) Known challenges for hibernating species

By being highly constrained temporally, hibernators are more sensitive to resource availability and environmental changes happening during the growing season that can affect either their behavioral activity or their foraging conditions (Goldberg and Conway 2021, Wells et al. 2022). Thus, phenological shifts for example, may have important fitness consequences, either positive by lengthening or negative by shortening the active period of animals (Ozgul et al. 2010, Lane et al. 2012, Williams et al. 2014a). Indeed, if the length of the growing season increases, individuals (adults and juveniles) have more time to reproduce and/or accumulate reserves for the subsequent hibernation, leading to increased fitness. However, although hibernators may maintain some level of performance over a wide range of body temperatures, repeatedly high temperatures during the active period may also challenge their thermoregulation, activity, and metabolism, ultimately affecting their survival (Boyles et al. 2011, Levesque et al. 2016). Such limits imposed by thermoregulatory constraints may lead to long-term behavioral adaptations, such as the shift of diurnal activity to nocturnal activity, to avoid warmer hours of the day and associated water loss (Levy et al. 2019).

In addition, as freezing and energy costs during hibernation significantly increase with low temperatures (Figure 2, Boyles et al. 2020), changes in climatic conditions during hibernation may also strongly impact hibernator energetics. It is important to highlight that energetic constraints may differ between large (bears) and small hibernators (ground squirrels, bats), the latter losing respectively more mass during hibernation and having lower storage abilities (Nedergaard et al. 1997, Geiser 2004). The effect of climate change on hibernation strategies, and the challenges faced by hibernators have recently been explored and reviewed (Cordes et al. 2020, Findlay-Robinson et al. 2023, Chmura et al. 2023), but long-term studies are still needed to further document the detailed consequences of climate change on hibernator fitness and population dynamics.

Finally, one other consequence of human-caused climate change is the increase in climate variability and weather extremes in most regions across the globe (IPCC 2023). Thus, in addition to natural seasonal changes in environmental conditions and human-induced long-term climate change, stochastic events are increasing, also affecting animal physiology and behaviour.

3. Unpredictable and punctual extreme events as part of weather stochasticity

a) Climatological and biological definitions of extreme events

Extreme weather events are part of climate variability, and are defined as weather variables occurring “above (or below) a threshold value near the upper (or lower) end of the range of observed values of the variable” (Matthews et al. 2021). Usual examples of extreme events are heat waves, droughts, or heavy rainfalls. However, the last IPCC report states: “by definition, the characteristics of what is called extreme weather may vary from place to place in an absolute sense” (IPCC 2023). Thus, from a more biological perspective, weather events might be defined as ‘extremes’ or not depending on their effect on organisms, at different spatial and temporal scales. The punctual extreme event definition includes changes with subsequent recovery, or with a regime shift (Figure 6.a, van de Pol et al. 2017). Several mechanisms may induce changes in the frequency of extreme weather events: in the case of extremely high temperatures, a shifted mean in temperature distribution toward higher temperatures, increased variability in temperature distribution, or changes in the symmetry of temperature distribution may happen (van de Pol et al. 2017). The frequency of extreme weather and climate events is expected to increase with climate change, along with important ecological consequences that can be measured at different biological levels: on individuals, populations, or ecosystem functioning (Figure 6.b, IPCC 2023).

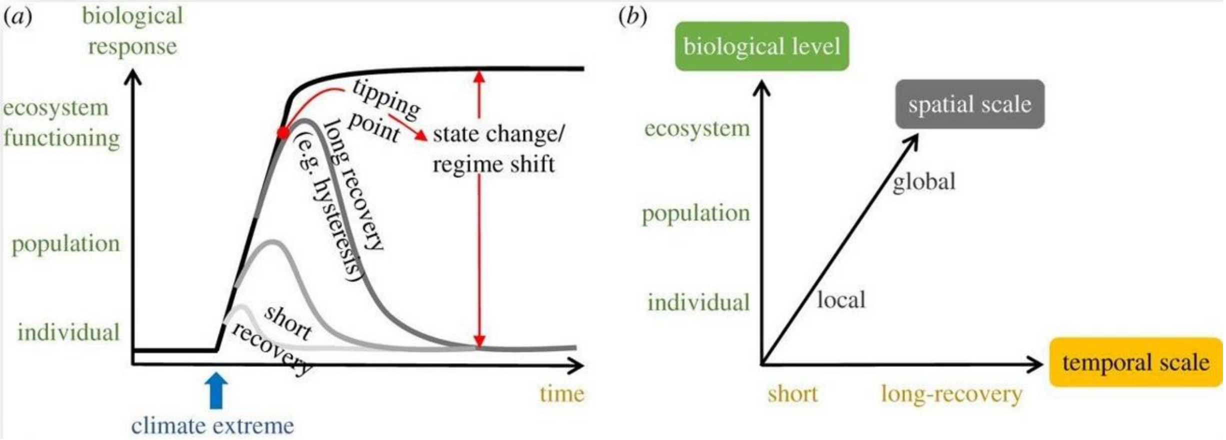


Figure 6. Magnitude of climate extreme effects and scales of assessment*. (a) Amplitude of biological responses following an extreme event and recovery time, from short recovery to long recovery or regime shift and changes in the state of the system. (b) Different biological levels, spatial and temporal scales that determine the impact of extreme events (from Van de Pol et al. 2017).*

b) Documented effects of extreme events on individuals, populations, and species

(1) Physiological and behavioral reactions

Individual, population, and species responses to extreme events depend on their duration, amplitude, and type of event considered (*e.g.* heat wave, storms), but also on their flexibility and ability of organisms to cope with these events. For instance, animals may – to an extent – buffer critically low or high temperatures with behavioral thermoregulation, and find conditions that are more suitable in microenvironments (*e.g.* move to shaded areas, change body posture, Kearney et al. 2009, Barton et al. 2014, Zhou et al. 2022, Allison and Conway 2022). However, buffering temperature extremes via habitat selection strongly depends on species initial range, life history and ecology, dispersal ability, and habitats availability with favorable microclimate (*e.g.* burrow, tree, ground, rocks etc.). Additionally, behavioral adjustments may induce modifications in animals' time-budget, and alter foraging and energy acquisition (Beale et al. 2018, Funghi et al. 2019, Weimerskirch and Prudor 2019, Osborne et al. 2020).

Animals may also buffer high temperatures physiologically by heat exchange (*e.g.* vasodilation, countercurrents, Johansen 1962, Schmidt-Nielsen 1981, Mota-Rojas et al. 2021) and/or evaporative mechanisms, such as sweating and panting (Dmi'el and Robertshaw 1983, Robertshaw 2006) inducing water losses and challenging hydration (Stillman 2019, McFarland et al. 2020, Brischoux et al. 2020). More generally, when facing a perturbation (conditions beyond those normally experienced, *e.g.* extreme event such as heat wave), vertebrates respond physiologically, for instance by activating their hypothalamic-pituitary-adrenal axis (HPA, Boonstra 2004, Wingfield et al. 2011). This endocrine response is adaptive and enables them (us) to face acute stressful events (Angelier and Wingfield 2013, McEwen 2019). In the case of prolonged or repeated stressful events, however, baseline hormonal stress might reach high levels without recovering after a prolonged period of time, having deleterious effects on other individual functions and survival (Sapolsky et al. 2000, Wingfield and Kitaysky 2002a, McEwen and Wingfield 2003, Romero 2004, Wingfield 2005, Landys et al. 2006, Romero et al. 2009).

To sum up, behavioral and physiological modifications during extreme events may lead to changes in the animal's energy balance. For instance, decreased foraging resulting in lower energy acquisition, and increased metabolic activity from thermoregulation resulting in increased energy expenditure, could negatively affect the overall energy balance. Furthermore, if animals are not able anymore to buffer elevated temperature, body temperature may reach critical values and threaten survival (Kavanau and Rischer 1972, Vispo and Bakken 1993, Pörtner 2002, Williams et al. 2016a, Levesque et al. 2016, van de Ven et al. 2019). Extreme events may also lead to increased evaporative water loss inducing hydric stress (Ben-Hamo et al. 2012, Klüg-Baerwald and Brigham 2017, Feng et al. 2019, Mohr et al. 2020, Hajek and Knapp 2022), or to lowered locomotion and speed (Dyer et al. 2023), increasing predation risk (illustrated by thermal performance curves with examples in Kingsolver and Buckley 2017). Consequently, extreme events may negatively affect organisms, and induce reduced survival and/or breeding success.

(2) Consequences on demography and population viability

Through these changes in behavior and physiology, or if animals' responses are insufficient, survival and breeding success may decrease, inducing drastic changes in population dynamics and potentially leading to extinctions (Jenouvrier et al. 2015). As an example, extreme population changes (population size increases or decreases) of birds and lepidopterans were shown to correlate with climatically extreme years (Palmer et al. 2017).

Massive mortalities of seabirds were also observed following marine heatwaves (Jones et al. 2018, Piatt et al. 2020, Quintana et al. 2022). On another hand, sudden heavy precipitation might reduce thermal insulation of fur for mammals, or induce flooding which threatens directly survival of animals living at the ground level (Webb and King 1984, Lovegrove 2003).

From an evolutionary perspective, it is still unclear if climatic extremes are more important for selection and microevolution than mean changes and variability in climate (Kingsolver and Buckley 2017), and experimental studies in natural environments are scarce. Punctual extreme events add a layer of complexity to seasonal climate variability and present an additional challenge for species living in these seasonal – and variable – ecosystems.

c) Potential implications for hibernating species

The short activity window of hibernating species during the growing season temporally constrains energy acquisition. However, the exact consequences of extreme weather events on hibernators have not been widely explored (Kucheravy et al. 2021, Wells et al. 2022). Indeed, animals' coping capacity may depend on the duration, intensity, and frequency of these extreme events. Thus, one might differentiate "real" extreme weather events (see extreme event definition, *e.g.* extreme observed temperatures, extreme biological effects such as population crashes) from punctual "natural" weather variations, usually happening in variable environments. Montane hibernators inhibiting these variables environments are potentially adapted to stochastic weather. Thus, the question remains to what extent punctual changes in weather conditions can be considered "extreme" for these species, *i.e.* to what extent they are adapted to cope with such changes. Furthermore, the scale at which we consider environmental climatic conditions (global or local) may influence our conclusions on organism responses and ability to cope with changes.

4. A matter of scale: macroclimates vs microclimates

a) Spatial heterogeneity in temperate mountainous ecosystems

Traditionally, weather conditions have been assessed from global atmospheric data from satellites over large spatial scales, or weather stations at distant locations, not always reflecting the local conditions experienced by animals themselves (Potter et al. 2013). More recently, advances in technology resolution (sensors, models, Zellweger et al. 2019, Maclean et al. 2021, see Kemppinen et al. 2023 in Annex I for a review) and the development of notions such as microhabitats, small niches, or microclimates allowed for more detailed studies on local climate conditions (Vandermeer 1972, Rosenberg et al. 1983, Jorgensen 2004, Kearney and Porter 2017).

Temperate mountain ecosystems exhibit variations in their physical features such as topography, terrain, soil type, sun's orientation. Such variations can induce heterogeneities over small spatial scales, and create various microenvironments (different vegetation structures, presence of rocks, etc.), which make available numerous shelters and buffers for animals (Vispo and Bakken 1993, Kearney et al. 2009a, Buckley et al. 2015, Senior 2020, Wallace et al. 2021). Heterogeneities in microclimate conditions may also be responsible for differences in the quality of patches of habitat with several populations of the same species living in slightly different conditions, and potentially inducing source-sink dynamics (Amarasekare and Nisbet 2001, O'keefe et al. 2009).

Thus, important ecological questions should be examined considering fine-scale conditions and habitats. The consideration of microclimate is even more important for species living in habitats where the local conditions are different from the conditions around weather stations, usually set to record climate variables at a single 2-meter high point. For instance, environmental conditions under the forest canopy (von Arx et al. 2012, Sanczuk et al. 2023) or at a few centimeters high (Bütikofer et al. 2020, Maclean et al. 2021) for small terrestrial animals might differ greatly from those measured by weather stations.

b) Microenvironments: the case of ground-dwelling species

Facing weather variation, animals find refuges and shelters buffering from outside temperatures and precipitation. This is well known regarding terrestrial ectotherms, which are not always able to thermoregulate physiologically (Guillon et al. 2014, Woods et al. 2015, Pincebourde and Suppo 2016), or hibernators, which need to shelter from low temperatures and predators (Kearney 2020). For hibernating species digging burrows in the ground, soil temperature dynamics are importantly linked to snow cover, temperatures being lower without a thick snow pack (Figure 7, Venäläinen et al. 2001, Decker et al. 2003).

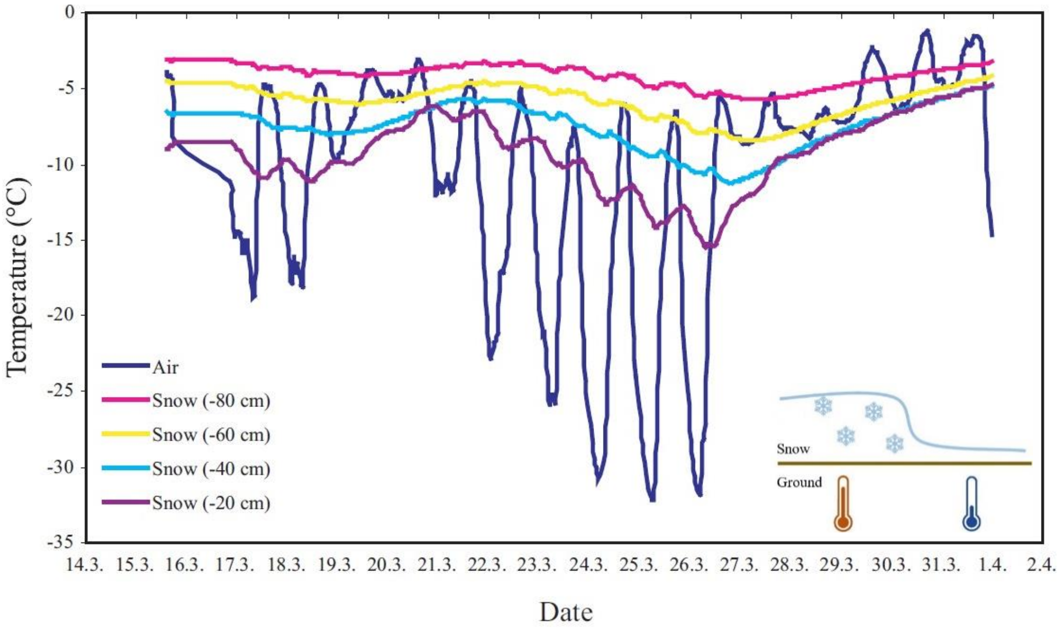


Figure 7. Temperatures measured in the air and at different depths in the snow between March and April*. Temperature at 80cm depth is buffered, being higher and less variable than air temperature (from Venäläinen et al. 2001).*

Thus, in the context of climate change, warmer winters with lower snow cover might induce lowered soil insulation, even though the interaction between climate change, snow, and soil temperature might be complex (Kearney 2020). One hypothesis is that, in the event of punctual extremely cold conditions without snow on the ground, soil temperature may decrease substantially and increase hibernator energy expenditure, challenging overwinter body condition and survival (Vuren and Armitage 1991, Tafani et al. 2013). In addition, spatial variation might induce important local differences in ground thermal regimes, and alter organisms' habitat selection depending on winter characteristics (*e.g.* burrows under trees versus in open areas, Barker and Derocher 2010, Duchesne et al. 2011, Reid et al. 2012). On the ohter hand, the presence of burrows may allow them to thermoregulate behaviourally, in the case of extreme heat events for example (Suggitt et al. 2011).

B. Scope of the thesis

1. General questions & hypotheses

To sum up, environmental variation may affect organisms in many different ways, from long-term directional changes in climate conditions to punctuated extreme events. Phenological and distributional shifts in response to climate change are observed in a wide range of taxa, as well as physiological and behavioral reactions to punctual changes. Whereas hibernators are adapted to cope with environmental seasonal variations, they live in habitat with high spatial heterogeneity and their temporally restricted active period raises other underexplored challenges. Energy acquisition and storage during the active period, crucial for both somatic maintenance and reproduction, may be strongly related to local climate and resource conditions. Proximate physiological and behavioral mechanisms through which animals, especially seasonally-adapted hibernators, respond to local changes in climate and resource conditions, along with their energetic implications, are largely unknown and raise important scientific questions.

This thesis examines how temporally constrained mountain hibernators cope with both punctual and long-term variation in climate and food resources. To this end, it focuses both on phenotypic traits at the level of the individual, including phenology, foraging behavior, mass gain, and the so-called "stress" axis; and on population-level traits (reproduction, survival of specific age classes) to understand how these may be affected by climate and vegetation effects; sometimes markedly contrasted on a local scale (microclimates). I addressed these questions through an application on a hibernating rodent, the Columbian ground squirrel (*Urocitellus columbianus*).

2. Study model

a) Biological & ecological characteristics

Previously classified within the larger genus *Spermophilus*, Columbian ground squirrels (CGS, *Urocitellus columbianus*) are now part of the *Urocitellus* genus or ‘Holartic ground squirrels’, which includes 13 different species and numerous subspecies of small mammalian rodents (Helgen et al. 2009). Ground squirrels are usually medium-sized sciurids (smaller than marmots, genus *Marmota*, and larger that chipmunks, genus *Tamias*) that live underground in burrows.

Columbian ground squirrels inhabit the Rocky Mountains, in North America (Canada and North-western United States). Their range extends from western Alberta and southeastern British Columbia, to western part of Montana, central Idaho, east-central mountainous part of Oregon, and northern and eastern Washington, and from around 200 to 2400 m elevation (Elliott and Flinders 1991, Cassola 2016). Living on open alpine and subalpine meadows, they are a social species organized in matrilineal colonies occupying different burrow systems (Murie and Harris 1978, Festa-Bianchet and Boag 1982, Harris and Murie 1984, Lane et al. 2011, Arnaud et al. 2012). These hibernators store energy as fat reserves prior to winter (Young 1990a, Dobson et al. 1992, example of hibernating patterns on Figure 8) and are known to hibernate individually during several months in a separate burrows (hibernaculum, Young 1990a), rather than hibernating socially.

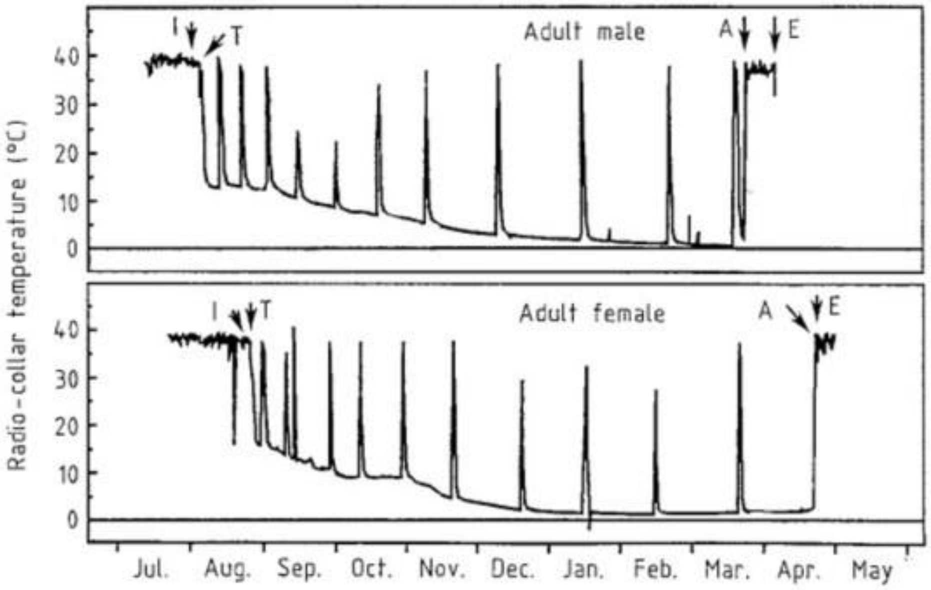


Figure 8. Hibernating patterns of adult male and female Columbian ground squirrels. *Temperature records are from radio-collars deployed in 1986-1987, peaks represent euthermic phases, and (I) date in hibernaculum, (T) date of first torpor, (A) date of last arousal, and (E) date of emergence from hibernaculum (adapted from Young 1990b).*

They enter hibernacula between the end of July and mid-August to hibernate (Figure 9), before emerging in the spring, between the beginning of April and mid-May (depending on their sex and age, Michener 1977, Murie and Harris 1982, Young 1990b, Dobson et al. 1992, Neuhaus 2000). Adult males typically emerge first, followed by adult females and younger individuals. Once they emerge from hibernation and usually within a week, each adult female enters estrus for a single day, and mates with multiple males (Murie and Harris 1982, Murie 1995). Mothers give birth to a litter of 1-7 pups (3 on average, Murie et al. 1980) in an individual nest burrow ca. 24 days later (Murie et al. 1998). Around 27 days following birth, the offspring emerges from the nest burrow around the time of weaning, usually by the end of June/beginning of July (Michener 1977). First successful reproduction typically occurs at the age of 2 or 3, except for some rare exceptions of females mating and weaning a litter as yearlings (one year-old individuals, after their first hibernation, Festa-Bianchet 1981, Zammuto and Millar 1985, Dobson and Murie 1987, Neuhaus et al. 2004, Rubach et al. 2020).

Figure 9. Annual life cycle of Columbian ground squirrels. *The hibernation period is presented in dark blue, and the active period in light blue, which includes emergence from hibernation, reproduction, and re-immergence into hibernation.*

Columbian ground squirrels are diurnal and mostly herbivorous, consuming mainly grass and forbs on meadows, and occasionally some seeds, buds, flowers, other plants, and opportunistically insects (Lambeth and Hironaka 1982, Harestad 1986, Andrusiak and Harestad 1989, Elliott and Flinders 1991). Apart from reproductive activities, they spend most of their daily above-ground time foraging (Betts 1976, MacWhirter 1991). However, being preyed-on by both birds and mammals (eagle, hawk, goshawk, ravens, coyotes, wolfs, badgers, bobcats, martens), they spend a significant amount of time in vigilance, standing at attention and emitting alarm calls in the presence of predators or potential threats (Munro 1929, Miller 1931, Betts 1976, Boag 1990, Elliott and Guetig 1990, Fairbanks and Dobson 2007).

b) What makes them interesting?

Columbian ground squirrels are distributed in North America, in temperate and seasonal ecosystems, and often found in mountainous areas. Thus, they are under constraints of strong seasonal variation, in terms of climate conditions and resource availability. They also live in a spatially heterogeneous environment with the occurrence of unpredictable weather events, making them good models to test whether they can behaviourally and physiologically buffer punctual inclement weather events. Finally, high latitude and altitude ecosystems are known to be especially sensitive to climate change (Root et al. 2003, Bhattarai et al. 2022, IPCC 2023, Ramalho et al. 2023), challenging species' phenotypical and demographical responses.

As hibernators living in a seasonal ecosystem and facing a short growing season, a period during which they have to grow, reproduce, and accumulate reserves for hibernation, CGS are temporally restricted for their activities. This temporal restriction may enhance effects of climate change (both long-term changes, and extreme weather events) on individual survival and breeding success, ultimately affecting population dynamics, depending on individual phenotypical plasticity and ability to cope with environmental changes.

As presented in the introduction in the context of climate change, one important challenge of hibernators is their phenological adjustment to their environment. Previous studies on CGS have shown that they exhibit some plasticity in their hibernation emergence date, as well as moderate heritability of this trait ($h^2 = 0.22 \pm 0.05$ in females and 0.34 ± 0.14 in males, Murie and Harris 1982, Dobson et al. 1992, Neuhaus 2000, Lane et al. 2011, 2019). Moreover, linear long-term trends of adult female emergence date of the same species have been examined, showing a delay in the emergence associated with reduced annual fitness (Figure 10, Lane et al. 2012). However, long-term responses of different populations and different individuals (juveniles, adults, females, males) to climate have not been explored. Indeed, depending on individual sex and age, energetic constraints differ. Additionally, in the heterogeneous environment, even neighboring individuals or populations might live in different microenvironments, and not answer in the same way (*e.g.* phenological differences of ground squirrel population living at different altitudes, Dobson et al. 1992).

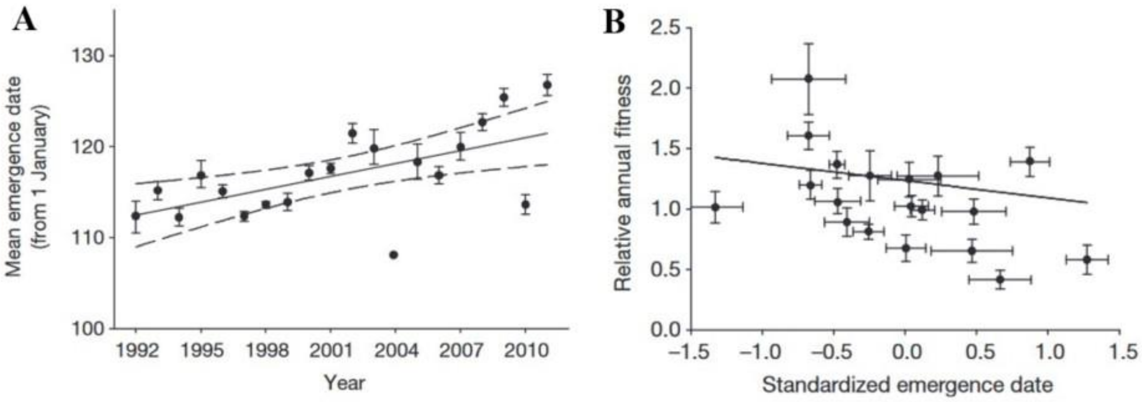


Figure 10. Hibernation emergence date and fitness. *(A) Annual trend in mean emergence date and (B) relationship between emergence date and the relative annual fitness of adult females Columbian ground squirrels (adapted from Lane et al. 2012).*

Additionally, CGS are relatively long-lived (up to 14 years for the oldest female, *personal observations*) and iteroparous, most females raising a litter annually (Murie et al. 1980). In combination with their seasonal life cycle, this allows tests for annual effect, as well as carry-over effects from one active season to another. For example, it has been previously shown that the energy "capital" of females, which influences reproduction, depends on their mass gain during the previous season, demonstrating that Columbian ground squirrels are in the income-to-capital continuum of breeding species (Figure 11, Broussard et al. 2005, Rubach et al. 2016).

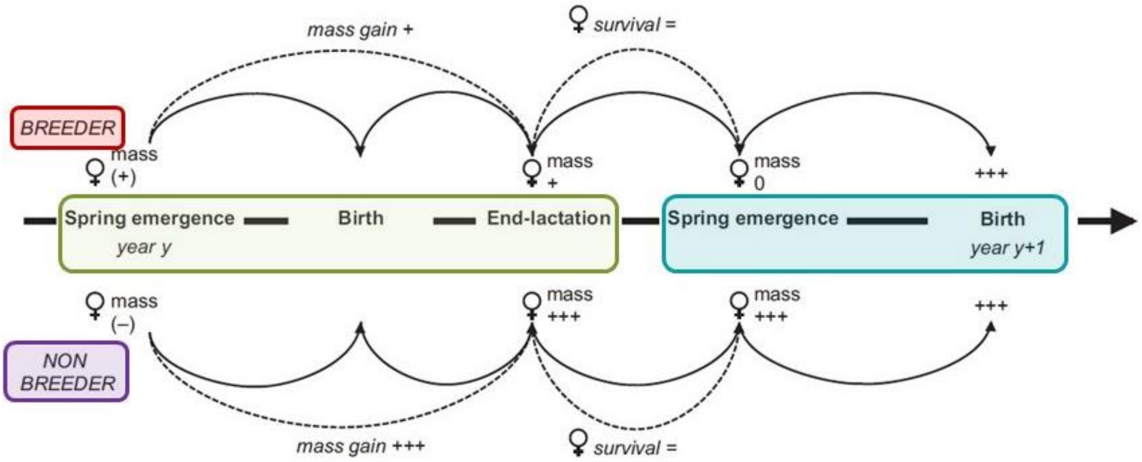


Figure 11. Carry-over effects on female body mass. *A flow diagram of mass changes for breeding (upper part, red) and failed breeding (lower part, purple) female Columbian ground squirrels (3 years and older) between year$_y$ (green) and*

year$_{y+1}$ (blue) is presented. Females that failed during gestation had lower emergence body mass than successful females, but had gained more mass by the time of weaning. However, successful and unsuccessful females have equal chances to survive to the next spring (adapted from Rubach et al. 2016).

Because they are herbivorous, they strongly depend on vegetation growth, quality and availability, which are directly linked to climate. Life history and population dynamics of Columbian ground squirrels were shown to vary with experimental manipulations of food resources (increased or decreased availability, Dobson and Kjelgaard 1985a, 1985b). Most individuals were also shown to be able to forage optimally by selecting food sources maximizing their daily energy intake, which in turn positively affected their fitness (Tyser and Moermond 1983, Ritchie 1988, 1990). However, long-term correlations between food resources and population responses have not been explored yet. Additionally, colony densities may vary spatially and temporally (Boag and Murie 1981, Elliott and Flinders 1991, Dobson and Oli 2001a), but competition for food resources through density-dependence have not been assessed. Indeed, adult males and females are highly territorial, which raises questions about density effects on resource accessibility.

From a methodological perspective, their diurnal activity allows detailed behavioral observations and accurate conclusions about their above-ground time-budget. Finally, only male Columbian ground squirrels disperse, generally as yearlings (Boag and Murie 1981, Dobson 1982, Festa-Bianchet and King 1984, Wiggett et al. 1989, Wiggett and Boag 1992, Neuhaus 2006), which facilitates survival assessment and allows to conclude about interannual variations in demographic rates of other individual classes (females, resident adult males). Thus, the general question on how temporally constrained mountain hibernators cope with variations in climate and food resources was applied to different populations of Columbian ground squirrels that have been monitored for several years, and examined in the different thesis chapters.

3. Thesis structure & predictions

In this thesis, after introducing the reader to both general and specific methodologies used to collect the data, I first examined the question of whether Columbian ground squirrels are behaviourally and physiologically adapted to cope with short periods of inclement weather during their active period (Chapter 1, Figure 12). If animals are indeed well adapted to cope with inclement weather events (snowfalls and rainfalls), one would expect to observe modifications in their above-ground behaviour aimed at avoiding cold and wet conditions outside of their burrow, as well as in their physiology (entrance in reduced metabolic state and reduced solicitation of stored energy reserves), in order to minimize energy loss through decreased insulation and energy expenditure. In contrast, if inclement weather events are stressful, one might expect to observe increased stress hormone levels and decreased body condition following such events as a result. Whereas we can expect montane hibernators to be able to cope with short events of inclement weather, high temperatures may challenge their physiology and ability to thermoregulate, especially in a context of changing climate.

I then tested the relationship between local weather conditions and animal behavior, especially foraging and body condition responses to elevated temperatures (Chapter 2). In the case of prolonged periods of high temperatures, individuals might have difficulties to efficiently thermoregulate, evacuate heat, and avoid water loss and potential hydric stress. The main expected response was behavioral thermoregulation, animals spending more time inside their burrow or in the shade with buffered temperatures, especially during the hottest hours of the day.

Using historical climate and biological data, the effects of long-term changes, inter-annual variations, and spatial heterogeneity in microclimate conditions on ground squirrel hibernation phenology were examined in Chapter 3. An important phenological variable for this hibernating species is the annual date of emergence from hibernation, which determines the phenology of reproduction, itself determining annual fitness. Long-term (20 year) phenological shifts (later emergence date from hibernation) in relation to delayed snowmelt are known to occur in at least in breeding females of one population, but little is known on the consistency of this response over other individual sex and breeding categories and multiple populations considering the heterogeneous nature of their mountain habitat. This spatial heterogeneity in distinct populations, along with carry-over effect from the preceding summer, were evaluated here. On one hand, climate conditions such as snow depth and soil temperature during the hibernation might determine thermal conditions in hibernaculum, inducing higher energy loss and earlier hibernation termination. Snowmelt may act as important cue indicating the beginning of productive season and hibernation emergence. On the other hand, weather conditions during the active period affect foraging conditions and vegetation, determining reserve accumulation and energy storage for hibernation, thus affecting subsequent hibernation emergence date.

Resource availability and access (modulated by intra-specific competition in the colony) being an important determinant of energy acquisition needed for storage and reproduction, the effects of vegetation and population density on body condition, phenology (emergence date), survival, and reproduction were examined in Chapter 4. Inter-annual variability and covariation of these functional traits and demographic rates were tested from long-term trends at the population level. Years with late and low amounts of vegetation should correlate with mean yearly low body condition, low survival, and/or low breeding success. Densely populated years, potentially inducing social stress and increasing competition, should limit individual performance and negatively affect population demography.

Finally, I ended this thesis with a synthesis of the results from the different chapters and an overall comparison and discussion of these results from a more general point perspective. I also examined several limitations inherent to the methodology used, and discussed several challenging perspectives and a way forward for future studies.

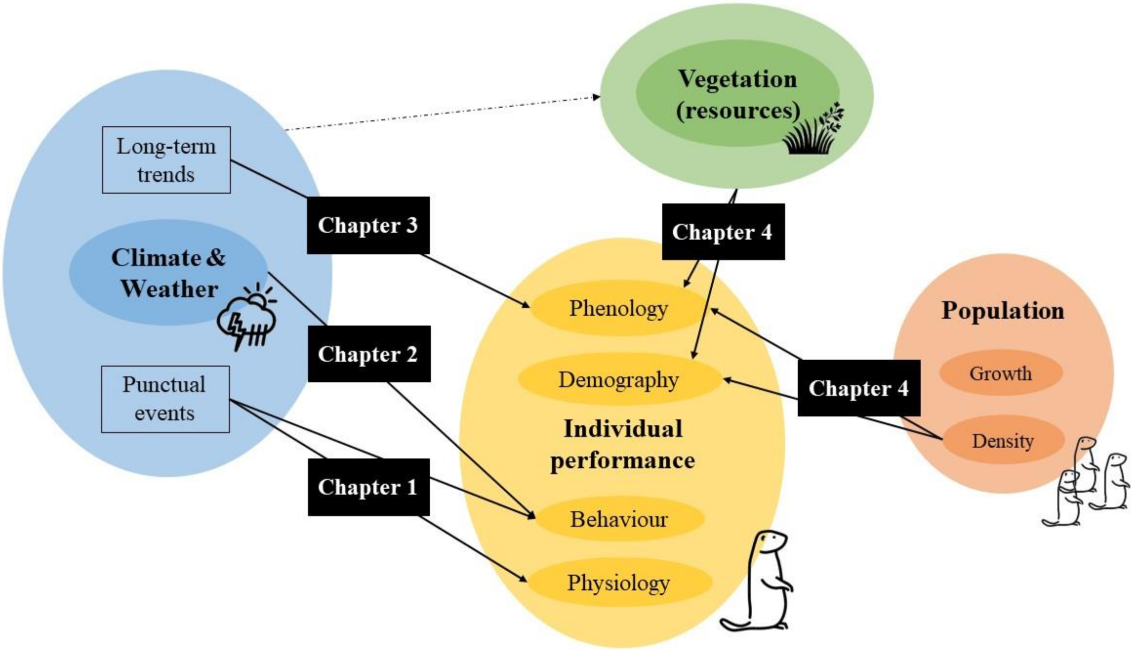


Figure 12. Flow diagram of the thesis questions and different chapters. *First, the effects of punctual events on behavioural and physiological responses (chapter 1). Second, the effect of climate means and extremes on animal behaviour (chapter 2). Third, the long-term effects of climate on one phenological index (chapter 3). Fourth, the effects of both resources and population density on functional traits and demographic rates (chapter 4).*

GENERAL MATERIALS & METHODS

A. Population monitoring

1. Location of the study sites

Columbian ground squirrels were monitored in the Sheep River Provincial Park in Alberta, Canada (50°38’N, 114°39’W), located in the foothills of the Rocky Mountains (Figure 13). This study area includes four different meadows surrounded by mixed forests (primarily lodgepole pine, white spruce, quacking aspen, and birch trees) and composed of vegetation that is representative of grasslands in montane sub-regions (Alberta Parks 2008).

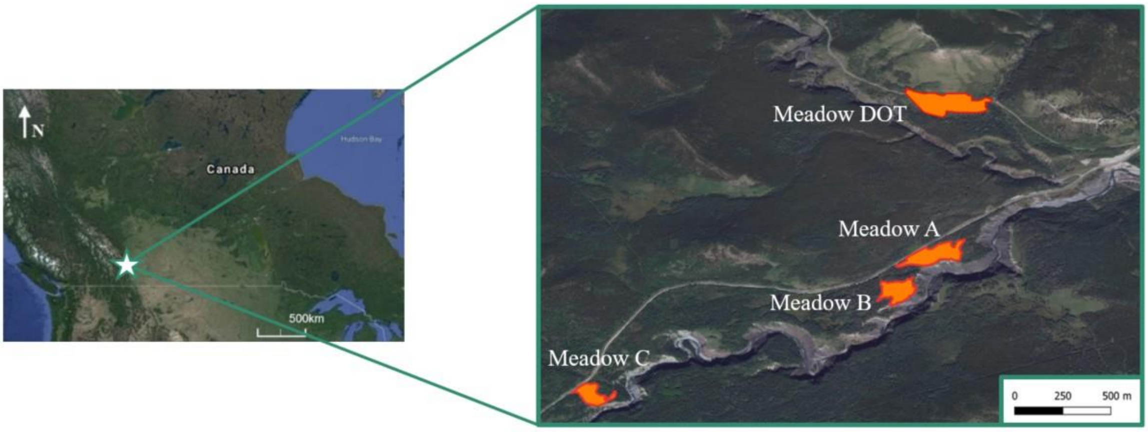


Figure 13. *Location in Canada and map of the different meadows (in orange, A, B, C, and DOT) with monitored Columbian ground squirrel populations (maps assessed from Google, 2022).*

We monitored ground squirrel colonies on: Meadow A (abbreviated *MA*, 3.6 ha, from 1510-1535 m, monitored since 1994), Meadow B (*MB*, 2.6 ha, from 1500-1540 m, monitored since 1992), Meadow C (*MC*, 1.7 ha, from 1540-1560 m, monitored since 1999) and Meadow DOT (*DOT,* 5.7 ha, from 1527-1570 m, monitored since 2001, the different monitoring periods are presented on Figure 14). MA, MB, and MC slopes are southeast facing, whereas DOT slope is southwest facing. When possible, data from all four populations were used to increase sample size and to be able to test microclimate effects over different populations. However, for some biological variables that required more detailed data, studies were performed only on MB.

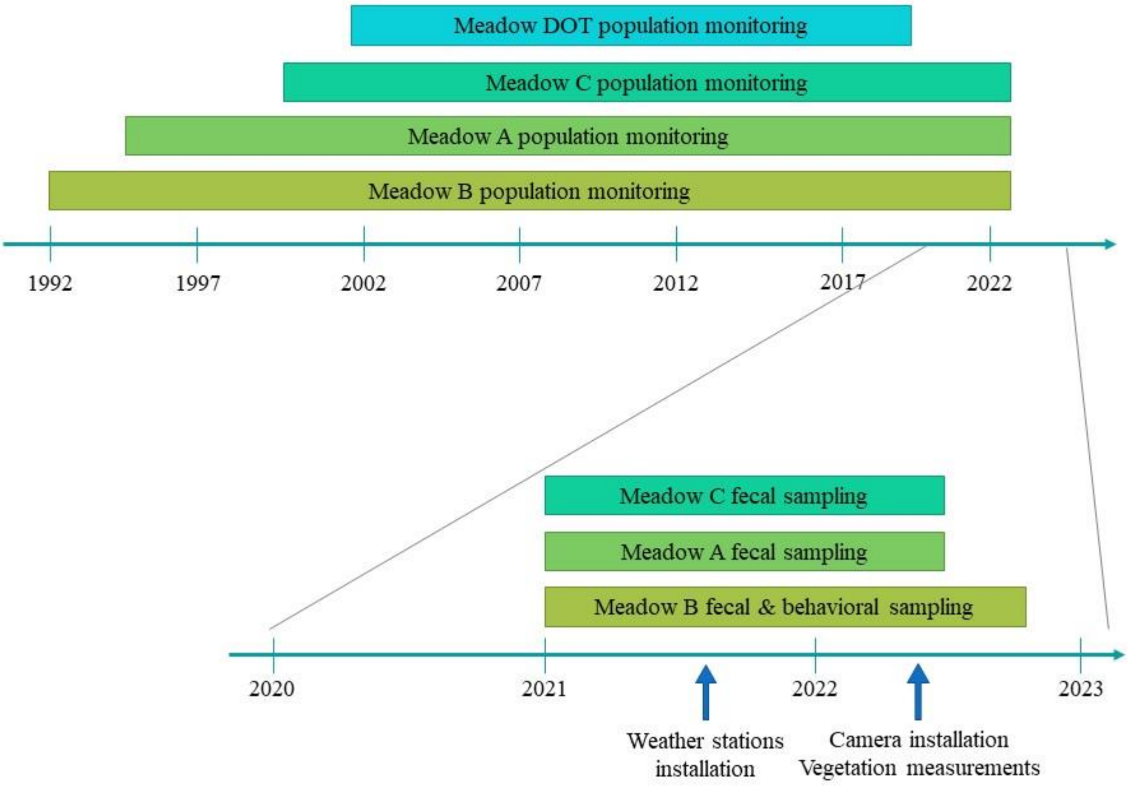


Figure 14. Chronology of population monitoring and data collection. *The duration of the biological monitoring of the different meadows (DOT, MC, MA, and MB, upper part) and the different periods of data collection during my PhD (lower part) are presented. The "population monitoring" includes trapping, marking and monitoring of individuals from the 1st day of emergence (early April) to the end of the reproductive period (juvenile weaning, end of June-early July) to assess mass changes and life histories. The "behavioral sampling" (only on MB) includes daily scan-observations (2-hour periods in the morning) and deployment of light acquisition data-loggers on animals. Fecal sampling (2021 – 2022), behavioral data recording (2019 not shown here, 2022, 2023), and vegetation measurements (2022 – 2023) were conducted only during the active period of animals, whereas weather stations collected data continuously (hourly basis or less) between October 2021 and June 2023.*

2. Generic biological data

Individual data were collected with comparable methods on all four meadows. Individuals were first trapped using live-traps baited with peanut butter (National Live Traps Tomahawk Co., Hazelhurst, WI, USA: 13 × 13 × 40 cm³, Figure 15.A) as juveniles, or when they first appeared in the meadows as immigrant adults, and permanently marked with unique numbered metal ear tags (Model no. 1, National Band & Tag Co., Newport, KY, Figure 15.B).

Sex was determined by visual inspection of genitalia (Murie and Harris 1982). Each year, the meadows were monitored daily from before the first emergences from hibernation to the end of lactation for breeding females. From mid-April to the end of May each year, ground squirrels were captured within a couple of days of emergence from hibernation (usually on the day of emergence or the following morning). The first day of observation or capture (usually the same) of a given squirrel was used as a proxy for hibernation emergence date (ED), typically confirmed by the squirrel's appearance and physical condition (large skin flakes from hibernation lost within few days, large abdominal flaps of skin where fat reserves were accumulated and lost, no defecation upon capture; Murie and Harris 1982). Emergence was recorded as an ordinal date (*i.e.* number of days after 1st January each year), and each ground squirrel was weighed (± 5 g) using a Pesola® spring-slide scale at initial capture (emergence mass, EM, Figure 15.C), and given a unique individual dye mark (Clairol® Hydrience N°52 Black Pearl, Clairol Inc., New York, USA) on its dorsal pelage for visual identification in the field (Figure 15.D). Unfortunately, emergence dates were not collected in 2006 on DOT, in 2003-2006 on MA, in 2002-2003 on MC, in 2020 on MB (see details in Chapter 3 – Supplementary Materials: Table 4), and dates of entry into hibernation were never recorded, as the field seasons ended after the last litter was weaned, roughly a month before immergence into hibernation.

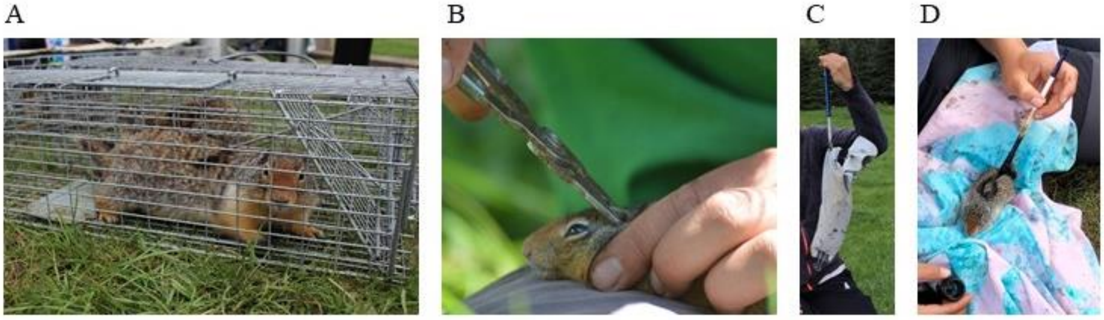


Figure 15. *Trapping (A), definitive marking with ear tags (B), weighing (C), and seasonal marking with hair dye (D) of Columbian ground squirrels.*

In all years, reproductive females were monitored until the first emergence of their offspring from natal nest burrows, around the time of weaning. At that time, females were weighed (± 5 g), litter size was counted, and all pups were captured, sexed, weighed (± 2 g), and ear-tagged. The entire population was trapped each year (confirmed by extensive visual observations from 3-m-high benches and daily trapping until only dye-marked individuals remained in the spring population), we were able to determine the survival of adults, juveniles and yearling females (but not yearling males, due to emigration) from one year (active period) to the next. Trapping masses were taken occasionally along the season, but the effort has been increased in recent years to better monitor mass changes and to study the effects of short-term climate and vegetation changes. Cartesian grids of color-flagged wires on each meadow (10 × 10 m) allowed recording the location of each capture and of each behavioral observation at a ± 1.0 m resolution (see example on MB on Figure 16).

Figure 16. *Example of the Cartesian grid on MB, lines every 10 m and resolution ± 1 m.*

In recent years, additional data was acquired (see lower part on Figure 14): behavioral data including observations and bio-loggers (only on MB in 2019, 2022, and 2023) and fecal samples to measure glucocorticoid hormone levels (on MA, MB, and MC). For the purpose of my PhD and to acquire climate and vegetation data, weather stations were installed on all meadows in 2021, and vegetation measurements were done on MB in 2022 and 2023 (grass height measurements, camera-traps allowing determination of vegetation greening, see details below).

3. Example of population size on Meadow B

As an example, the complete census of Meadow B individuals allows the assessment of the annual population size, and of the annual number of individuals in different age/sex classes (Figure 17). This provides us with an idea of the sample sizes when examining long-term trends in population traits, such as emergence date for example.

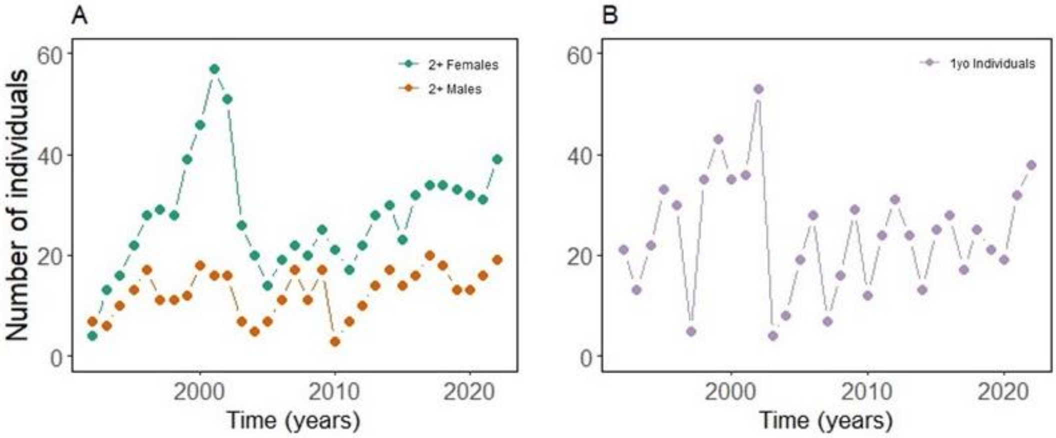


Figure 17. Yearly number of individuals in MB population 1992-2022. *(A) Two years and older females (green) and males (orange) and (B) yearling individuals.*

4. Ethics statement

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures carried out in the field were approved by Auburn University (IACUC protocols) and the University of Calgary. Authorization for conducting research and collecting samples in the Sheep River Provincial Park were obtained from Alberta Environment and Parks and Alberta Fish & Wildlife. On a personal note, I followed the animal experimentation course (certified “concepteur” level) and I was trained by V.A. Viblanc to all field techniques specific to ground squirrels.

B. Behavioural data (MB only)

a) Scan-sampling protocol for behavioral observations

In 2019, 2022, and 2023 (*22 April 2019 – 10 June 2019, 22 April 2022 – 6 June 2022, and 22 April 2023 – 17 June 2023*), behavioral observations were performed daily for two hours in the morning, in the middle of the day, or in the afternoon (*morning*: from 8:07 am for the earliest start until 12:22 pm for the latest end, *midday*: from 12:02 pm for the earliest start until 14:24 pm for the latest end, *afternoon*: from 1:27 pm for the earliest start until 4:42 pm for the latest end) from the top of 3 high observation towers (Figure 18.A, 1 observer per tower, the allocated tower to an observer changing each day).

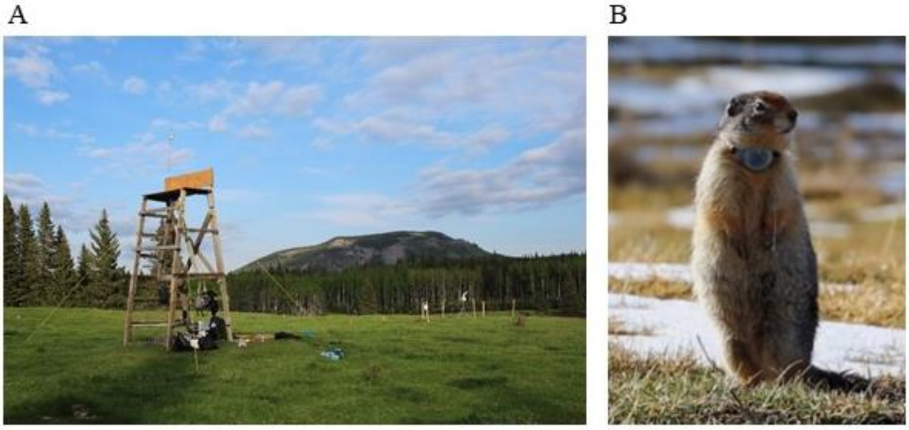
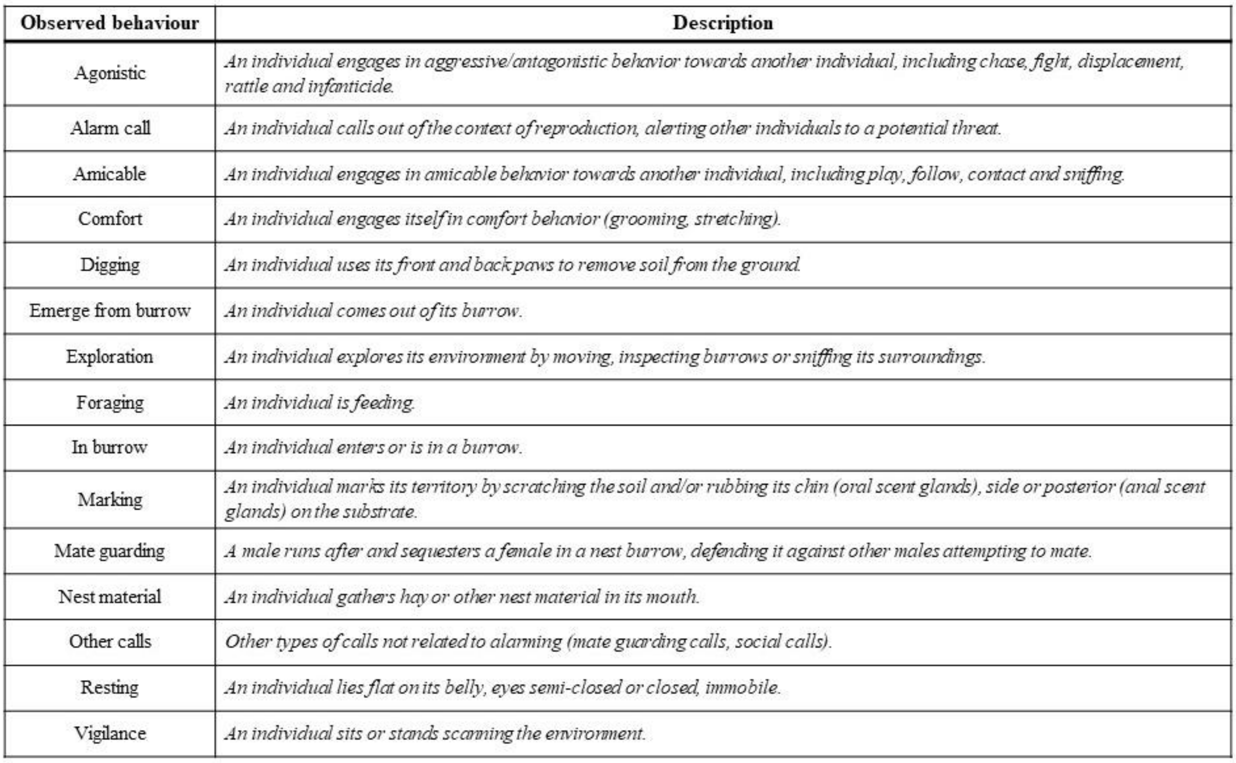


Figure 18. Material used for behavioral sampling. *(A) High observation tower which allows detection and identification of individuals from their back hair dye mark without disturbing their behaviour and (B) a Columbian ground squirrel equipped with a bio-logging collar.*

During these two hours, visual scans of MB were performed every 15 minutes, recording the behaviors of all individuals present (see ethogram on Figure 19) along with their locations within the Cartesian grid. Individuals were identified with binoculars from their back hair dye mark, and data were recorded using CyberTracker application. Foraging was the main behavior of Columbian ground squirrels, which represented 50 ± 0.6 % of their time on average, and between 29% and 72% of their total time budget over the entire active seasons. Daily scans also allowed us to estimate the proportion of individuals that have been seen each day compared to the theoretical number of individuals on the meadow, *i.e.* the number of individuals that already emerged from hibernation and were still alive.



Observed behaviour	Description
Agonistic	*An individual engages in aggressive/antagonistic behavior towards another individual, including chase, fight, displacement, rattle and infanticide.*
Alarm call	*An individual calls out of the context of reproduction, alerting other individuals to a potential threat.*
Amicable	*An individual engages in amicable behavior towards another individual, including play, follow, contact and sniffing.*
Comfort	*An individual engages itself in comfort behavior (grooming, stretching).*
Digging	*An individual uses its front and back paws to remove soil from the ground.*
Emerge from burrow	*An individual comes out of its burrow.*
Exploration	*An individual explores its environment by moving, inspecting burrows or sniffing its surroundings.*
Foraging	*An individual is feeding.*
In burrow	*An individual enters or is in a burrow.*
Marking	*An individual marks its territory by scratching the soil and/or rubbing its chin (oral scent glands), side or posterior (anal scent glands) on the substrate.*
Mate guarding	*A male runs after and sequesters a female in a nest burrow, defending it against other males attempting to mate.*
Nest material	*An individual gathers hay or other nest material in its mouth.*
Other calls	*Other types of calls not related to alarming (mate guarding calls, social calls).*
Resting	*An individual lies flat on its belly, eyes semi-closed or closed, immobile.*
Vigilance	*An individual sits or stands scanning the environment.*

Figure 19. Ethogram of recorded behaviors during scan-samplings.

b) Light collars as biologgers on animals

In 2019, 2022, and 2023 (*20 May 2019 – 9 June 2019*, *13 May 2022 – 21 May 2022*, *10 June 2022 – 16 June 2022*, *23 April 2023 – 7 May 2023*, and *4 Jun 2023 – 14 June 2023*, see deployments on Figure 20) we equipped 123 individuals in total (14 yearlings, 77 older females, and 32 older males) in the MB population with bio-logging collars that were specifically designed for the ground squirrels (Figure 18.B). The collars were radio-collars initially designed to detect contacts (close proximity and social interactions) between individuals. They were also fitted with a light sensor, microcontroller and internal memory chip. For the purpose of my PhD, I used light records to access below versus above-ground activity patterns. The collars continuously recorded ambient light from three red, blue, green (RGB) channels that were summed and combined *in-situ* into a binary variable 1 (light detected) / 0 (no light) every 2 seconds (Mamtag collars, Sextant Technology Ltd., Marton, New Zealand). Thus, we were able to continuously determine if animals were present above ground (light =1) or remained below in their burrow systems (light = 0).

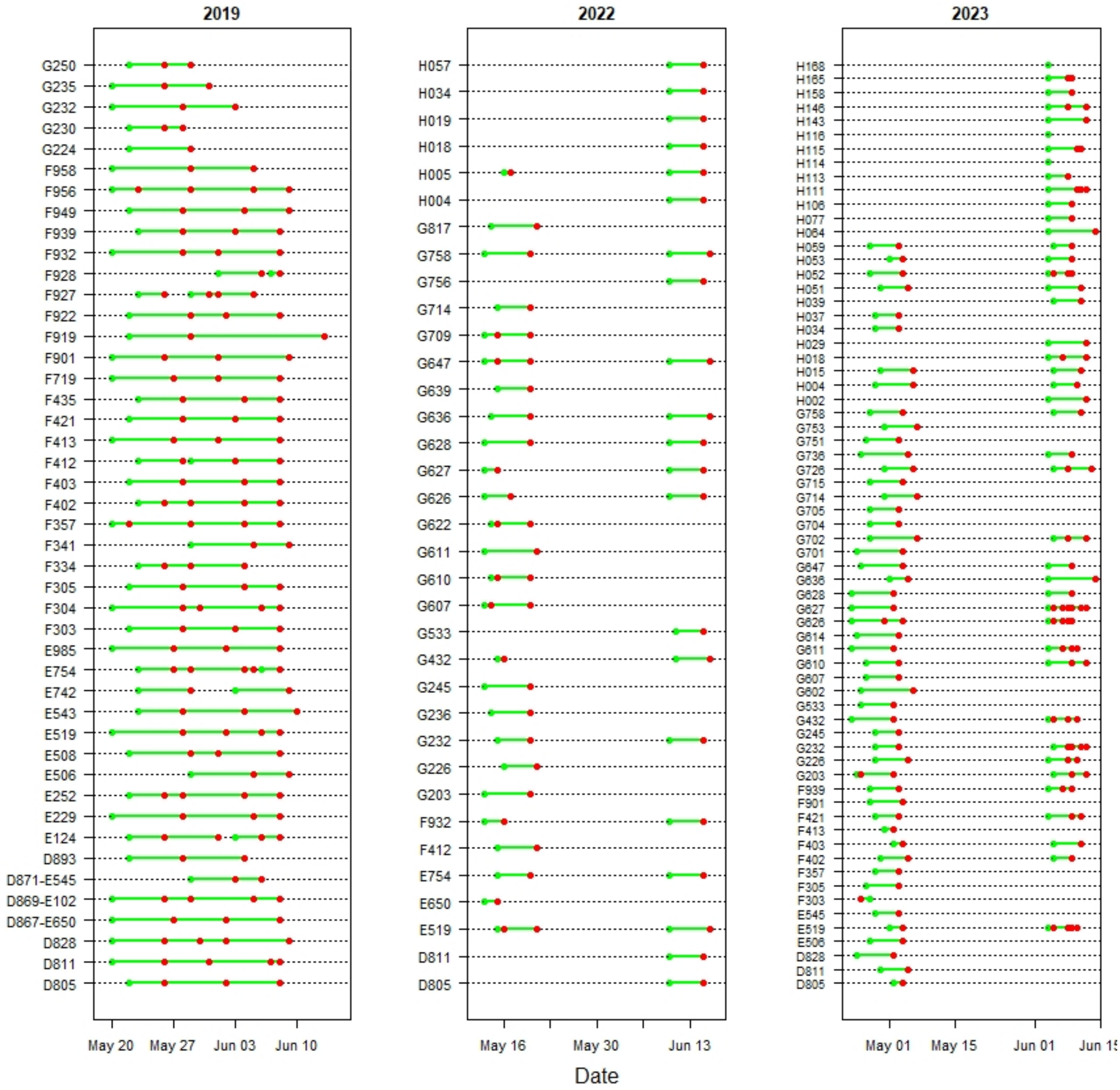


Figure 20. Collar deployments on individuals in 2019, 2022, and 2023 (three panels). *Each green line corresponds to an individual equipped with a collar, along with the dates of beginning (green dot) and end of deployments (final red dot). Intermediary red dots correspond to changes in collar and/or battery.*

From these light data, we were able to determine above-ground activity of the equipped animals during daytime. During this time window, we calculated the hourly proportion of time spent out of the burrow (see Chapter 1 and 2, see example on Figure 21). We also examined the first exit hour everyday relative to sunrise (see Chapter 2). The duration of data logging varied among animals depending on logger-battery life and our success at recapturing the animals. In total between sunrise and sunset, we collected 7 265 469 records in 2019, 3 622 987 records in 2022, and 8 031 495 records in 2023, *i.e.*, 315 332 records on average per day of deployment.

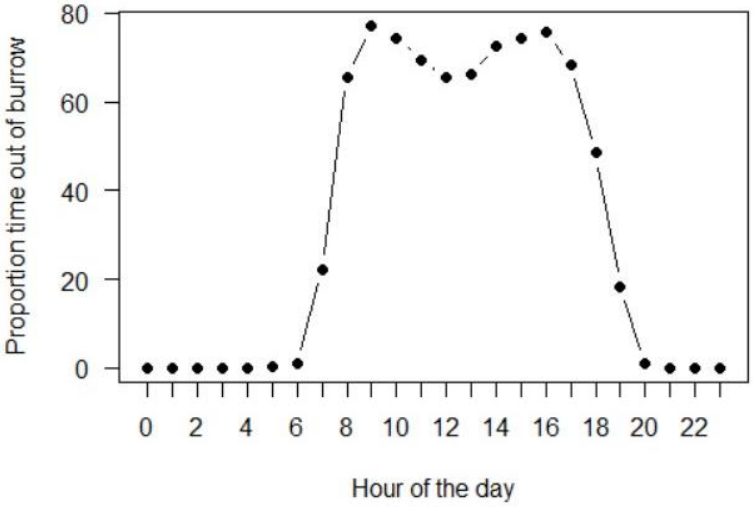


Figure 21. *Mean proportion of time spent out of the burrow calculated per hour, on the entire period of collar deployment and for all individuals (2019, 2022, and 2023).*

C. <u>Physiological data</u>

Enzyme immunoassay for fecal cortisol metabolites

To analyze fecal cortisol metabolite (FCM) levels and assess ground squirrels HPA axis activity, fecal samples were collected in the field from MA, MB, and MC individuals during the active seasons 2021 and 2022. We ensured that traps were clean before being deployed, and fecal samples were collected within minutes of capture of animals. Fecal samples were most often collected either directly into 2 ml sterile vials as the female defecated, or from the floor of the trap. In this latter case, the female was always observed defecating in the trap and the feces collected immediately. We ensured no fecal sample was contaminated by urine upon collection. Contaminated samples were discarded. Fecal samples were stored on ice packs in the field, transferred to a -20 °C freezer within the following hours of sampling and, at the end of the 2022 field season, all samples were transported on dry ice to the University of Toronto and Dr. Rudy Boonstra’s lab. After a 3–week training, I analyzed all samples during summer 2022 with the help of Dr. Pheobe Edwards.

Fecal cortisol metabolites were estimated as previously validated and described for CGS (Bosson et al. 2009). Briefly, lyophilized fecal samples were frozen in liquid nitrogen, and pulverized with a small grinding pestle (see the different steps for sample preparation on Figure 22.1). We weighed 0.050 ± 0.005 g of the sample, recorded the exact weight, and vortexed it in 1 ml of 80 % methanol to extract FCMs (centrifuged 15 min at 2500 *g*). FCM amounts (in ng/g of dried feces) were determined using a 5α-pregnane-3β,11β,21-triol-20-one enzyme immunoassay (EIA, see Figure 22.2), which measures metabolites with a 5α-3β,11β-diol structure (Touma et al. 2003).

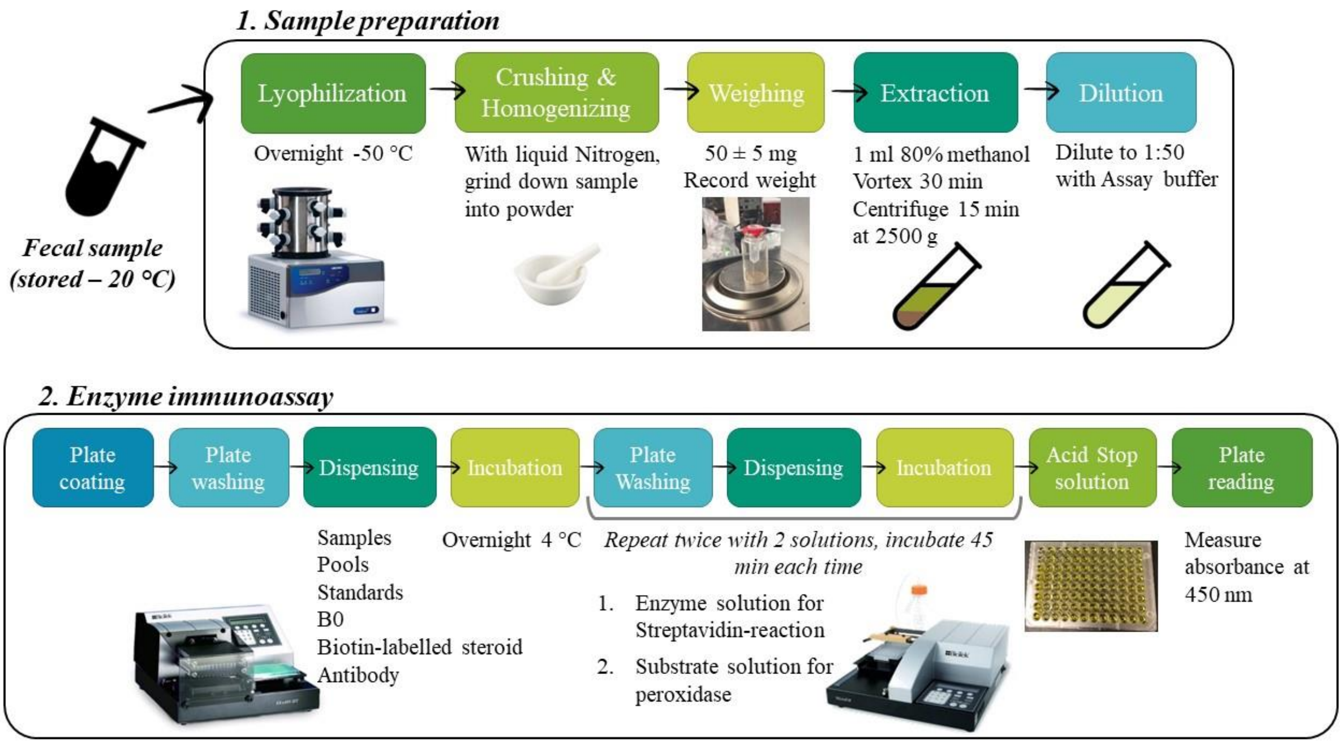


Figure 22. ***Analysis of fecal samples to measure fecal cortisol metabolite levels.*** *(1) Steps of sample preparation and (2) of enzyme immunoassay are presented.*

D. Environmental data

1. Climate & weather data

In previous studies on CGS, the effects of climate had always been assessed using Okotoks weather station (50°43’N, 113°59’W, 1051 m elevation, Lane et al. 2012, Dobson et al. 2016). This weather station was located in an urbanized environment, at around 50 km away from the study site. To consider the spatial heterogeneity in the studies conducted during my PhD, climate and microclimate data were assessed from different available sources, at different spatial and temporal scales. We needed to assess climate and weather conditions on a small spatial scale (*i.e.* in the local physical environment experienced by animals) and in large temporal windows, including in the past. For these reasons, the use of a microclimate model (see presentation below) is optimal but needed to be validated at the location of our study site.

a) Different sources

In the different analyses, we used a variety of climate and weather variables from different sources, either measured directly in the field or modelled from large-scale data. Four standardized weather stations were installed in October 2021 on the four different meadows (Davis, Vantage Pro2), recording air temperature, precipitation, relative humidity, wind speed at 2-m high, and equipped with a sensor recording soil temperature at around 25-cm depth (Figure 23). The weather stations were planned to be installed at the beginning of the active season in 2020 but the access to Canada was not possible before October 2021 due to Covid-19.

Figure 23. *Example of the weather station on MB (on a nice day!) with the temperature sensor in the white shaded and ventilated area, the anemometer, and the rain collector (Davis, Vantage Pro2).*

Unfortunately, the weather stations only record precipitation but not snow depth. Snow depth was thus estimated through the closest accessible weather station located 32 km south of the study site, at a similar elevation (Pekisko weather station, 50°22’N, 114°25’W, 1341 m elevation). Additionally, we accessed past records in precipitations from a weather station situated in Sheep River Provincial Park (Sheep river II weather station, 4km away of the study site, 50°39’N, 114°37’W, 1525 m elevation). The data from these weather stations are available hourly from 2008 and provided by Agriculture and Irrigation, Alberta Climate Information Service, ACIS (https://acis.alberta.ca/weather-data-viewer.jsp, see Figure 24 below for temporal scales).

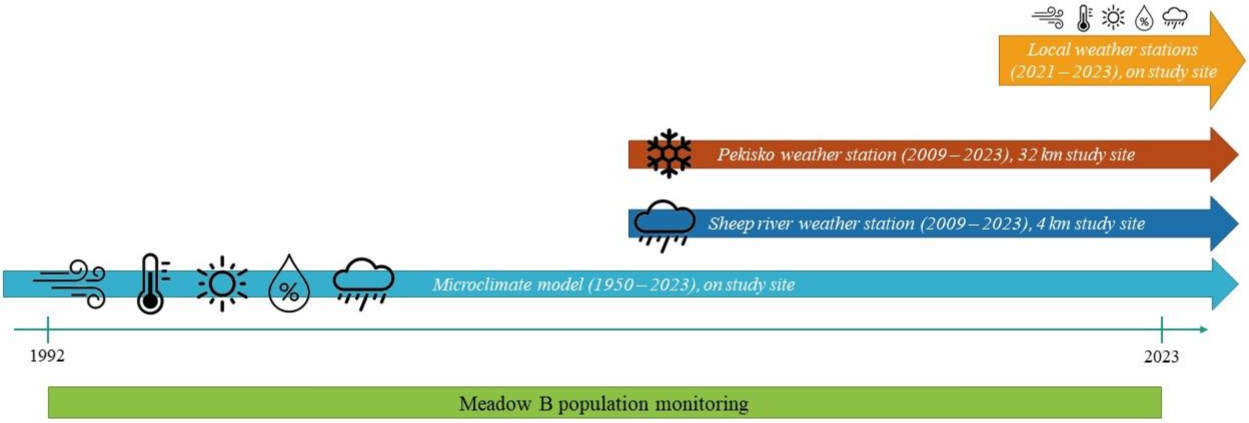


Figure 24. Different climate sources and their temporal availability. *Whereas the longest population monitoring of ground squirrels was on MB from 1992, the local weathers stations (Pekisko, Sheep river, and on the study site) do not cover this entire period. Thus, the use of the microclimate model allows to access small-scale data from long past records.*

Thus, in order to assess environmental conditions of living ground squirrels during the entire population monitoring (longest on MB from 1992, Figure 24), we used modelled climate data from atmospheric records available since the middle of the 20th century. The model, its application, and its validation are presented below.

b) Microclimate model

(1) Presentation & application

NicheMapR is an implementation of a biophysical modelling software in the R programming environment for microclimate and mechanistic niche modelling (https://mrke.github.io/). The microclimate model included in the NicheMapR package (Kearney and Porter 2017, Kearney and Maino 2018, Kearney 2020) includes several functions allowing to downscale global atmospheric climate-forcing data into hourly time series and account for local terrain effects. It applies terrain-specific corrections, including spatial variation in slope (from the Mapzen elevation and Mapzen terrain service, via 'elevatr' package in R; Hollister et al., 2020), soil composition and hydrological properties (acquired from the 'SoilGrids' database in R; Hengl et al. 2017), orientation, and hill shade (see Figure 25). The microclimate model increases the quality and resolution of large-scale weather data, and has been empirically previously validated (Saleeba et al. 2020, Lembrechts and Lenoir 2020, Maclean 2020, Kearney 2020). It includes several modelling functions, but two have global scopes and long-term data availability: *micro_ncep* and *micro_era5* (see below for complete description and comparison).

We initially used the *micro_ncep* function, to calculate hourly microclimate conditions at a fine-scale resolution (5 × 5 m grid), which will be presented in the model validation and in different result sections (Chapter 1 and 3, *micro_ncep* was the only existing function in R when we started the analyses). Because the *micro_era5* function had a greater correlation with local conditions (see below in the validation), we used it in Chapter 2 (last conducted Chapter). Because Columbian ground squirrels live in open grass meadows with adjacent trees, in both functions we estimated climatic variables without specifying shade due to vegetation cover. Whereas precipitations are at a large scale and not downscaled by the microclimate model, we were able to input local recorded precipitations (from 2009 from the Sheep river weather station) in the model to increase its accuracy.

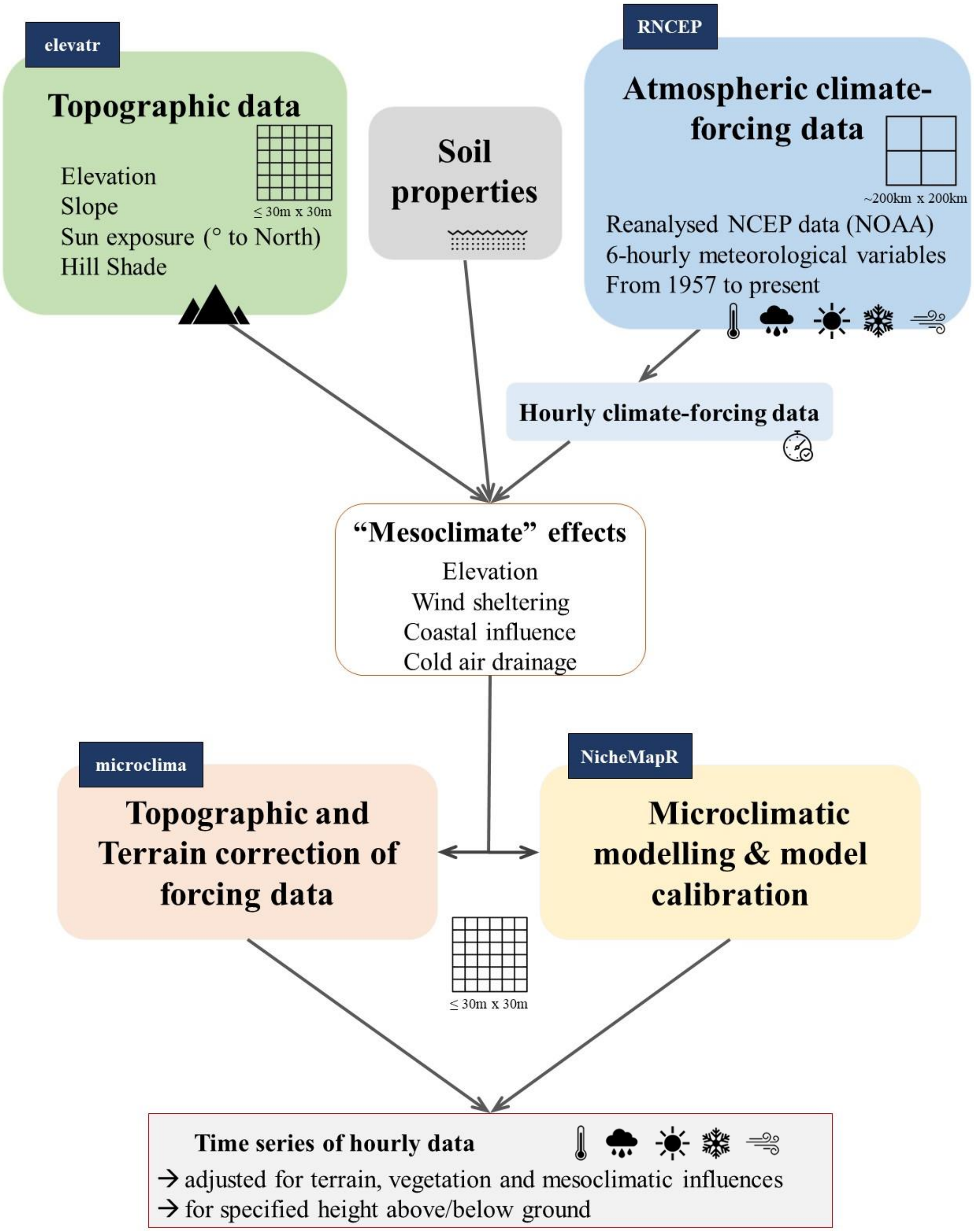


Figure 25. Flow diagram of the microclimate model and example with the micro_ncep function. *The integration of diverse functions from R packages (dark blue boxes) and data sources (colored boxes) allows to downscale atmospheric-climate data.*

(2) Comparison of the modelling functions & in-situ validation

Both *micro_ncep* and *micro_era5* are functions from the NicheMapR package that connect the microclimate model of NicheMapR to different forcing datasets (see Table 1 for comparison details).

Table 1. Comparison between micro_ncep and micro_era5 function characteristics. *The NCEP/NCAR Reanalysis dataset is provided by the NOAA (National Oceanic and Atmospheric Administration, Boulder, Colorado, USA) whereas the ERA5 Reanalysis dataset is provided by the C3S (Copernicus Climate Change Service, European Union).*

Functions	***micro_ncep***	***micro_era5***
Dataset	NCEP/NCAR Reanalysis	ERA5 Reanalysis
Data source	NOAA	C3S
Reference	Kalnay et al. 1996	Hersbach et al. 2020
Packages used	RNCEP, elevatr, microclima	mcera5, elevatr, microclima
Spatial scale	2.5 × 2.5 degree gridded	0.25 × 0.25 degree gridded
Dates available	1957 – 2023	1950 – 2023
Frequency	4-times daily	Hourly

To document potential differences between the uses of the two functions at the location of our study site and determine their accuracy, we compared temporal trends from October 2021 to July 2023 modelled with (1) *micro_ncep* and (2) *micro_era5* against records from the four *in-situ* weather stations (functions applied at the four exact locations of the weather stations on the meadows). Climate variables were averaged by day and over the four locations.

The two modelling functions were similar and highly correlated regarding air temperature (Figure 26 and 27, Pearson correlation test, $r = 0.95$, $p < 0.001$), soil temperature at 25 cm ($r = 0.98$, $p < 0.001$), wind speed ($r = 0.79$, $p < 0.001$), but poorly correlated regarding relative humidity ($r = 0.3$, $p < 0.001$).

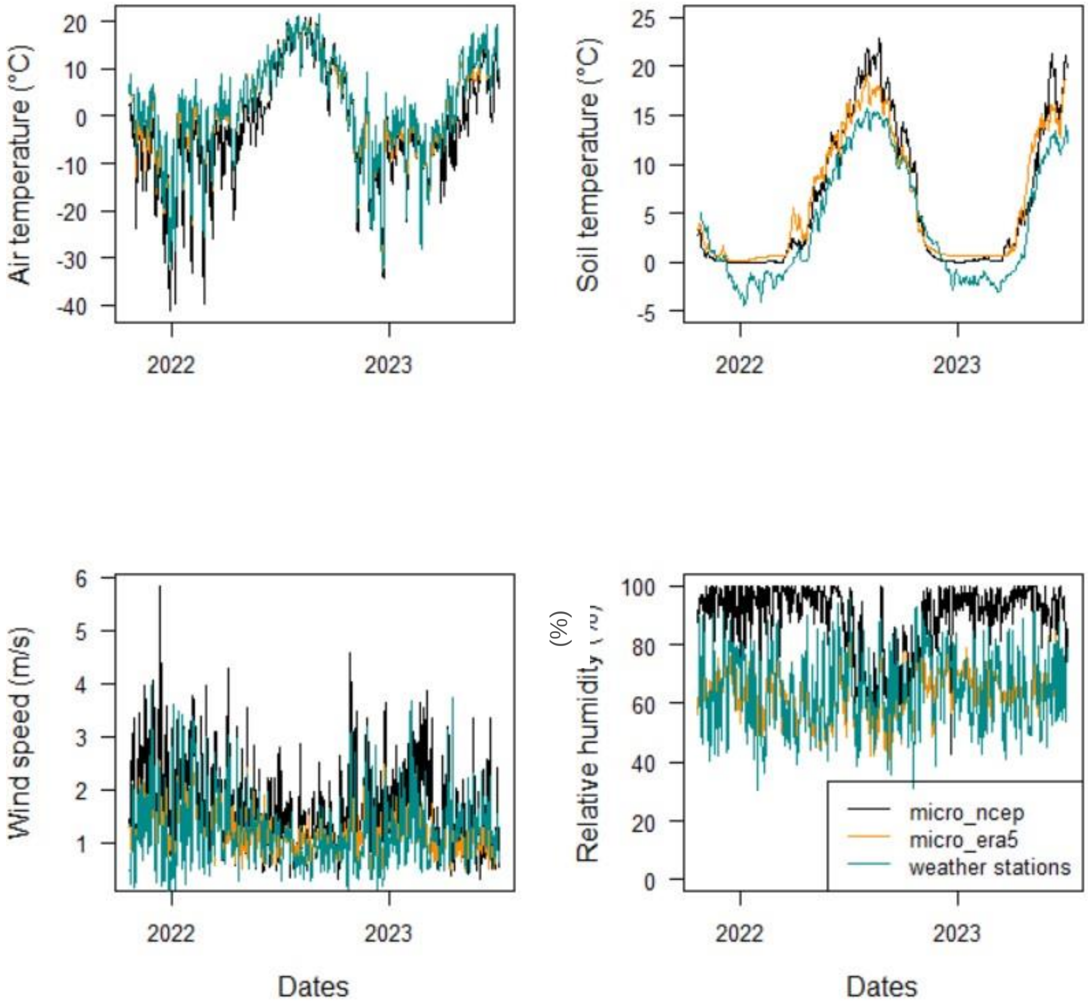


Figure 26. Temporal variations in different climate variables on the study site. *Climate data from the microclimate are presented in black (*micro_ncep *function) and in orange (*micro_era5 *function), and recorded empirical data are presented in blue. Hourly data from 4 different spatial points (location of weather stations on each meadow) were averaged by day and over the 4 locations. The 4 panels correspond to air temperature (in °C), soil temperature at 25-cm depth (in °C), wind speed (in m/s), and relative humidity (%). These variables modelled from the* micro_ncep *function are used in further analyses.*

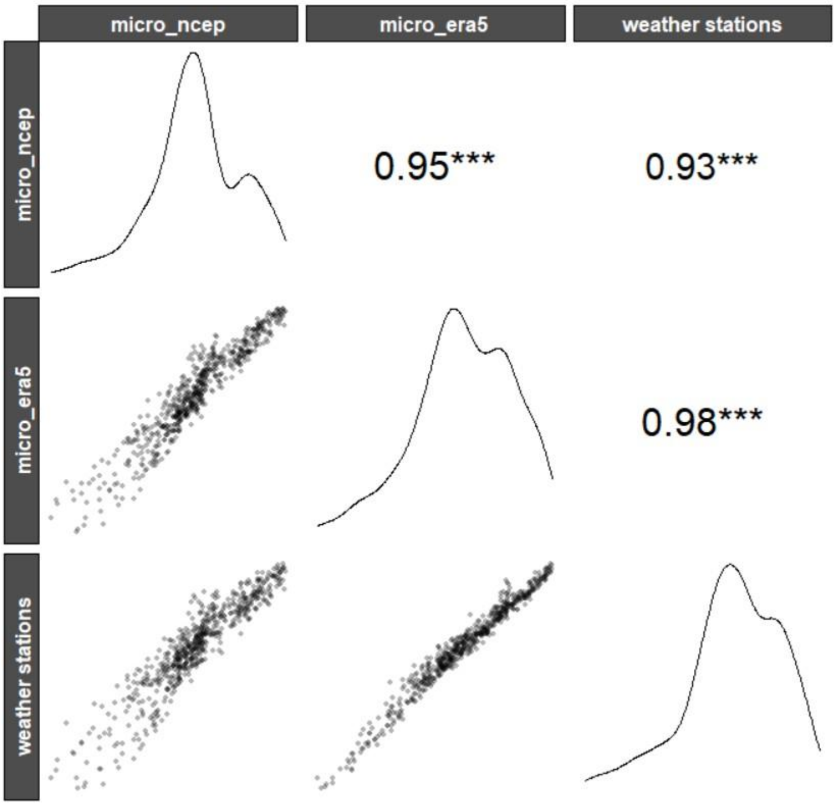
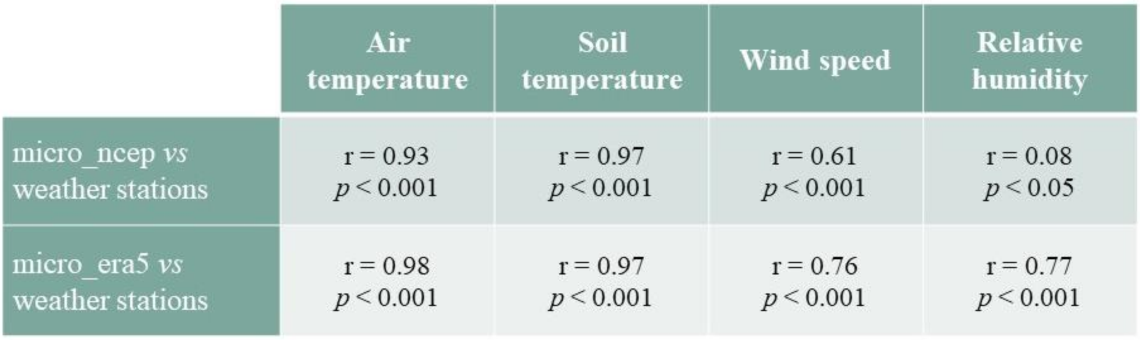


Figure 27. Correlations between modeled (functions **micro_ncep** ***and*** **micro_era5*****) and empirical (weather stations) air temperatures.*** *The Pearson correlation matrix is represented (* = p < 0.05, ** = p < 0.01, *** = p < 0.001).*

However, *micro_era5* correlated more with the empirical records from installed weather stations than *micro_ncep* regarding all four climate variables (see Figure 27 above, Pearson correlation test, statistics in Table 2 below). Unfortunately, only the use of *micro_ncep* was possible when we started some analyses in the context of the thesis, and *micro_era5* was used only in Chapter 2. The results should not change much regarding above and below ground temperatures, but the use of *micro_era5* should greatly improve wind and humidity results.

Table 2. *Correlations between climate variables modelled from the* micro_ncep *and* micro_era5 *functions compared to empirical records from weather stations.*



	Air temperature	**Soil temperature**	**Wind speed**	**Relative humidity**
micro_ncep *vs* weather stations	r = 0.93 $p < 0.001$	r = 0.97 $p < 0.001$	r = 0.61 $p < 0.001$	r = 0.08 $p < 0.05$
micro_era5 *vs* weather stations	r = 0.98 $p < 0.001$	r = 0.97 $p < 0.001$	r = 0.76 $p < 0.001$	r = 0.77 $p < 0.001$

To further validate spatial variation in *micro_ncep*, we used the four previous modelled points (one at each weather station on each meadow) to compare the relative values and differences on each meadow, and calculated the temperature anomaly (difference between the general mean and daily means in empirical data and modelled data). Thus, we can see that in both cases (microclimate model with *micro_ncep* and weather stations), DOT is the coldest meadow compared to other meadows, on which air temperature varies temporally but is generally higher than the mean (Figure 28).

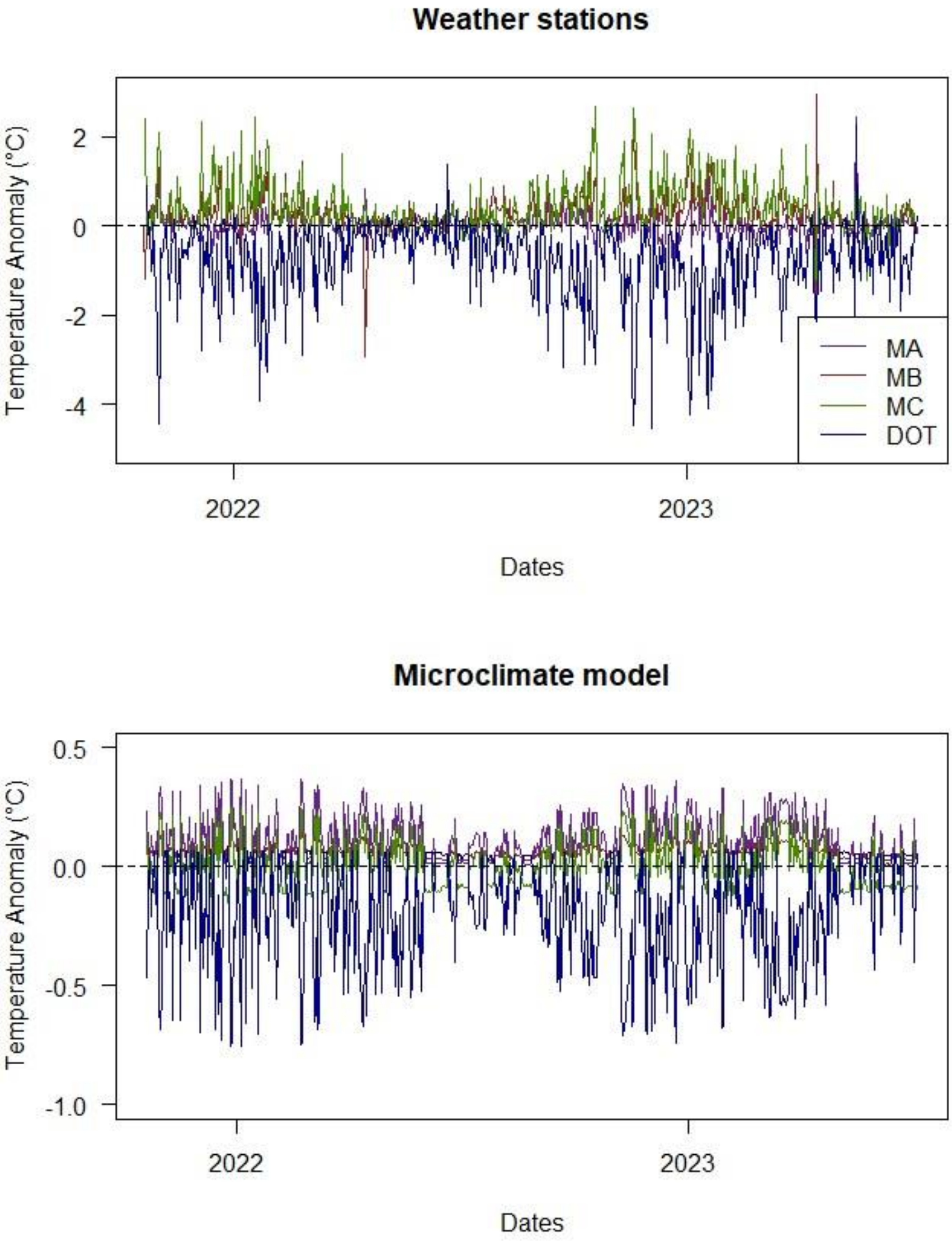


Figure 28. Air temperature anomalies at different locations. *Data from the 4 weather stations (upper panel) and from the microclimate model at the location of the weather stations (lower panel) were centered and compared.*

This validated the use of the microclimate model, in the four locations of the study site, where the microclimate model outputs were highly correlated with empirical data when ran in the place of the weather station. Besides the good predictive ability of the model in other places, this further validated the use of the microclimate model in the context of our studies.

In conclusion: because (1) there was no reasonably close weather station with records of climate data for the entire study period (the closest one being 50 km away at lower altitude in the plains); and (2) weather stations record temperature and precipitation variables, at a standard height of 2 m, which is not representative of what a ground-squirrel might experience a few centimeters above ground, using the microclimate model enabled us to obtain micoclimate data over the entire study period at a high resolution and at the height of the animal (modeled at 10 cm), with relatively good resolution compared to empirical data.

2. Vegetation data

a) NDVI as an index of vegetation growth

To access past records in vegetation, the Normalized Difference Vegetation Index (NDVI) has been extensively used in ecological studies (Pettorelli et al. 2005, 2011, Hurley et al. 2014, Rézouki et al. 2016, Körner et al. 2023). NDVI is derived from the difference between the visible red (RED) and near-infrared (NIR) light reflectance of satellite images. It is calculated as NDVI = (NID-RED) / (NID+RED) and ranges from -1 to 1. NDVI assesses the amount (biomass) of live green vegetation from satellite images: chlorophyll from vegetation absorbs RED whereas mesophyll in the leaves reflects NIR (low values of NDVI correspond to absence or limited vegetation, see Figure 29).

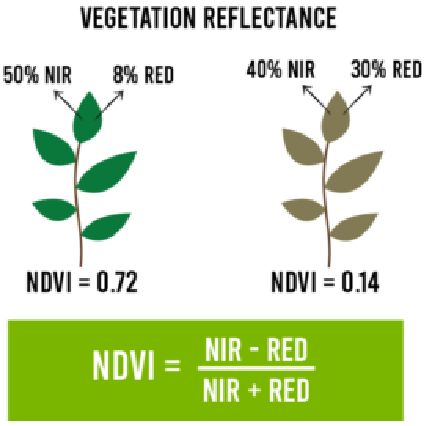


Figure 29. *Example of calculation of the NDVI based on vegetation reflectance of green and brown plants (illustration from https://www.myeasyfarm.com, accessed on 7 January 2023).*

To assess resource effects on ground squirrel demography and life history traits, daily NDVI data for the study site (MB, Lat: 50°38’N, Long: 114°39’W) were downloaded from the Administration-Advanced Very High Resolution Radiometer (AVHRR) dataset of the U.S. National Oceanic and Atmospheric Administration (NOAA National Centers for Environmental Information) for the period spanning 1991-2023 (https://www.ncei.noaa.gov/data/land-normalized-difference-vegetation-index/access/).

We then processed the data to derive indices of vegetation growth. Because some daily NDVI data were missing from the data set (possibly due to cloud cover), we averaged NDVI by week. In each year, vegetation quality and quantity (overall productivity and biomass), and vegetation phenology including timing of greening and vegetation growth speed were indexed from NDVI (Pettorelli et al. 2005). We calculated (1) mean yearly NDVI by averaging weekly values during the active period of the animals (from the first emergences from hibernation ever recorded: week 14, to the approximate period of immergence: week 34), (2) NDVI rate of increase within each year characterizing growth speed and (3) an index of the seasonal phenology (starting week of the increase period of NDVI). The mean yearly NDVI provided us with a measure of yearly vegetation production and overall biomass during the squirrels’ active season, whereas the rate and dates of NDVI increase within each year allowed us to investigate vegetation phenology by determining the rate of spring greening-up and the yearly onset of NDVI increase to identify early vs. late years (Pettorelli et al. 2005). To do so, we smoothed weekly NDVI values (Figure 30.1) and identified the period of highest increase in NDVI through breakpoint analyses of the derivative and considered it as the vegetation-growing period (see example in Figure 30). The start date of this period was considered as the index of phenology, and the mean derivative over this period was considered the growing slope of the year (identified on Figure 30.4). The growing slope was slightly correlated to the index of seasonal phenology, and moderately correlated to the mean NDVI (see correlation matrix on Figure 31).

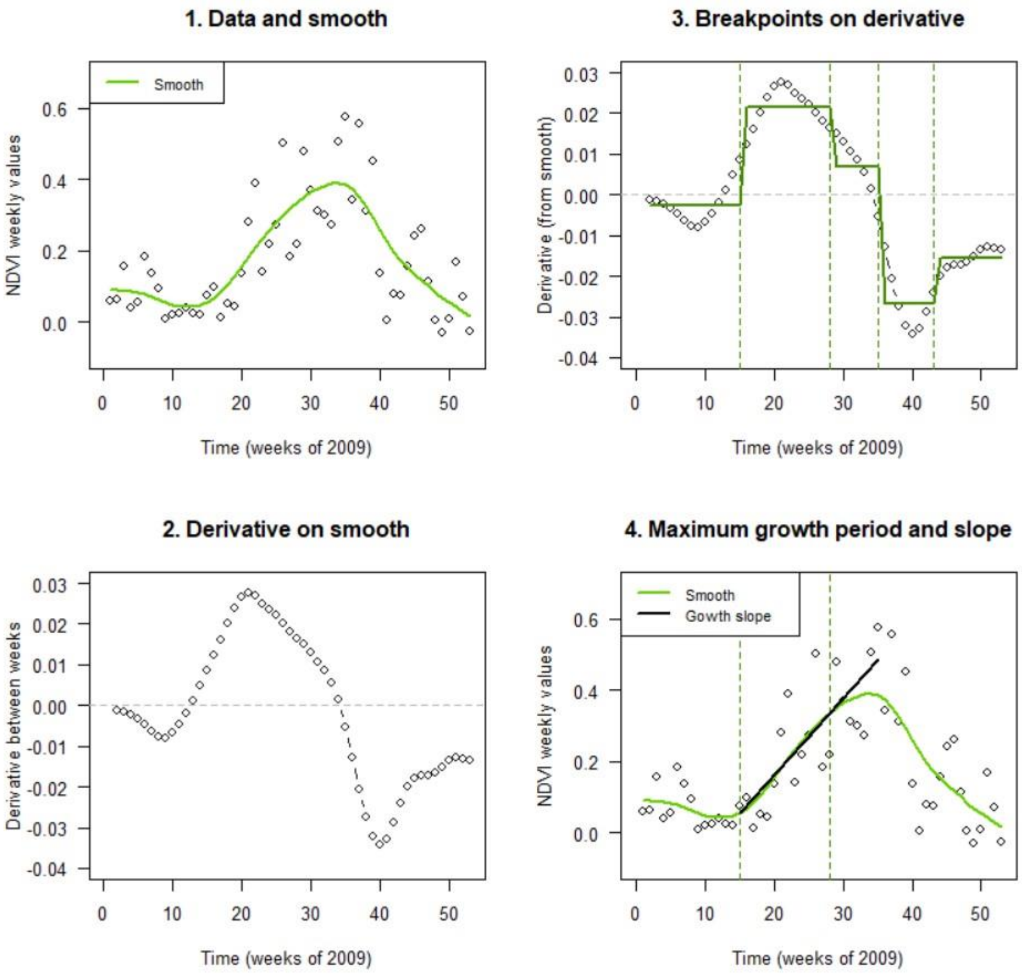


Figure 30. Example of calculation of the vegetation growth slope and seasonal phenology index. *1. Plotted dots are weekly values of NDVI; the plotted green line corresponds to the smooth applied to these values. 2. Plotted dots are derivative values between weeks, calculated on the smooth (positive values correspond to NDVI increase and negative value to NDVI decrease). 3. Plotted dots are derivative values, the vertical dashed lines are breakpoints calculated on the derivative, and the plotted green line show the mean derivative over each segment defined by the breakpoints (shown with vertical dotted lines). 4. The black segment corresponds to the calculated growth slope (from the segment with the highest derivative), and the vertical dashed lines correspond to the beginning and the end of the NDVI increase period (beginning and end of the segment with the highest derivative). The beginning of the NDVI increase period (first dashed line) is considered as the year's phenology index of NDVI (here: week 15 in 2009), while the slope of the black line represents the vegetation growth speed.*

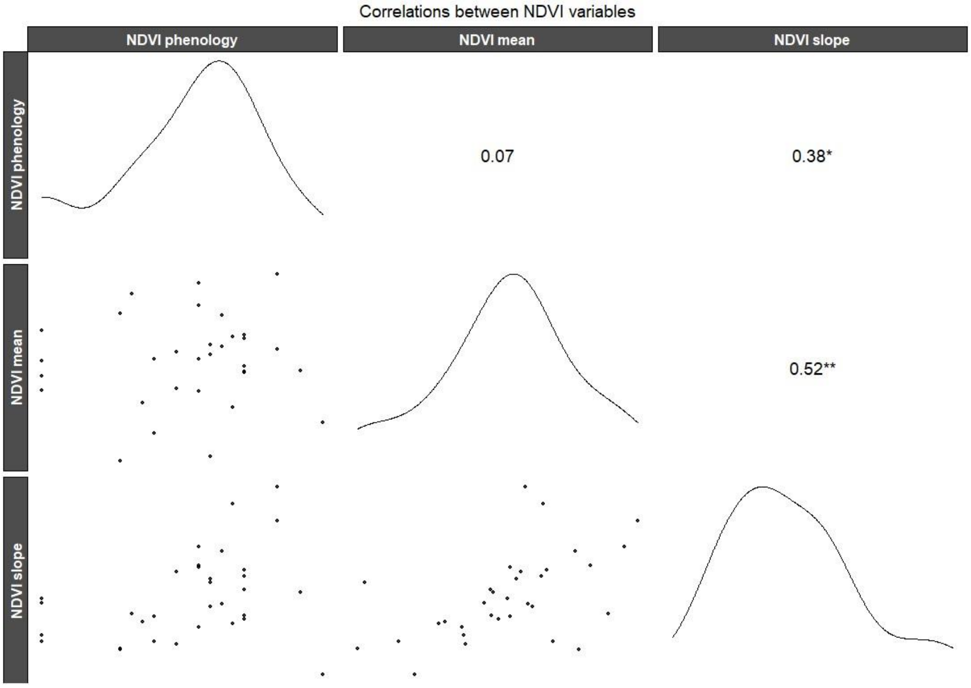


Figure 31. Correlations between yearly NDVI variables used during the analyses. *Pearson correlation matrix between the NDVI phenology index, the mean NDVI, and the vegetation growth slope (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).*

b) In-situ validation

NDVI as a proxy for vegetation growth on the study site was validated against empirical measures obtained in 2022 and 2023. Three camera traps (Reconyx XR6 Ultrafire, Reconyx, LLP, Holmen, Wisconsin, USA, Figure 32.A) were installed in April 2022 and positioned throughout MB. NDVI values were compared to meadow greening calculated from photographs taken from 25 Apr to 28 June 2022 and from 13 Apr to 3 July 2023 (33 photographs per day with pictures above a certain minimum of light retained only, see example of greening on Figure 32.B). Pictures were processed so as to decompose each pixel in red, green, blue values (RGB) and the "greenness" of the pixel was estimated through the ratio of the norm of green over the norm of all colors ($\frac{green}{\sqrt{red^2+green^2+blue^2}}$) allowing to avoid biases due to light differences among pictures. Greenness was then successively averaged per picture and per week.

In addition, 30 wooden poles graduated every centimeter (Figure 32.C) were installed throughout the meadow every 30 m, following linear transects. We compared daily NDVI values to the height of the vegetation (in cm) measured weekly on these poles from 25 Apr to 27 June 2022 and from 19 Apr to 3 July 2023.

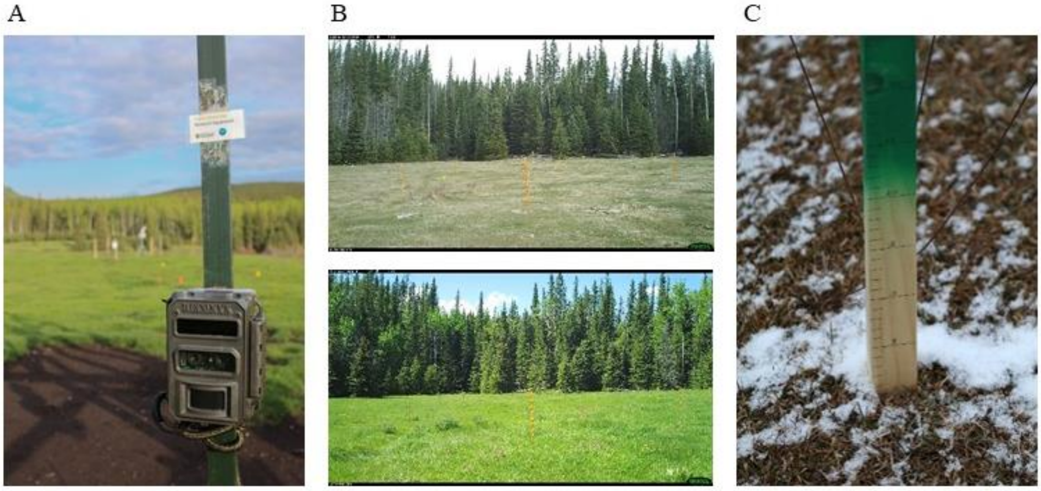


Figure 32. Field installations to validate the use of NDVI on the study site. *(A) Camera trap taking 33 photographs per day, (B) example of greening of the meadow between 26 Apr 2022 and 22 June 2022, and (C) graduated pole installed on the meadow at the beginning of the season in 2022.*

Both vegetation height and greenness measured on MB were found to correlate well with NDVI weekly values (Figure 33, Pearson correlation test between the smooth NDVI values and the green proportion from pictures, in 2022: $r = 0.93$, $p < 0.001$, in 2023: $r = 0.92$, $p < 0.001$, Pearson correlation test between the smooth NDVI values and the vegetation height, in 2022: $r = 1$, $p < 0.001$, in 2023: $r = 0.98$, $p < 0.001$). Thus, as with the microclimate model, the use of NDVI allowed us to obtain vegetation data over the entire study period, and relatively well reflecting the vegetation growth and its interannual variations within the area of our study site. However, due to the resolution of NDVI, spatial differences could not be investigated and NDVI was produced as a single index for all monitored populations.

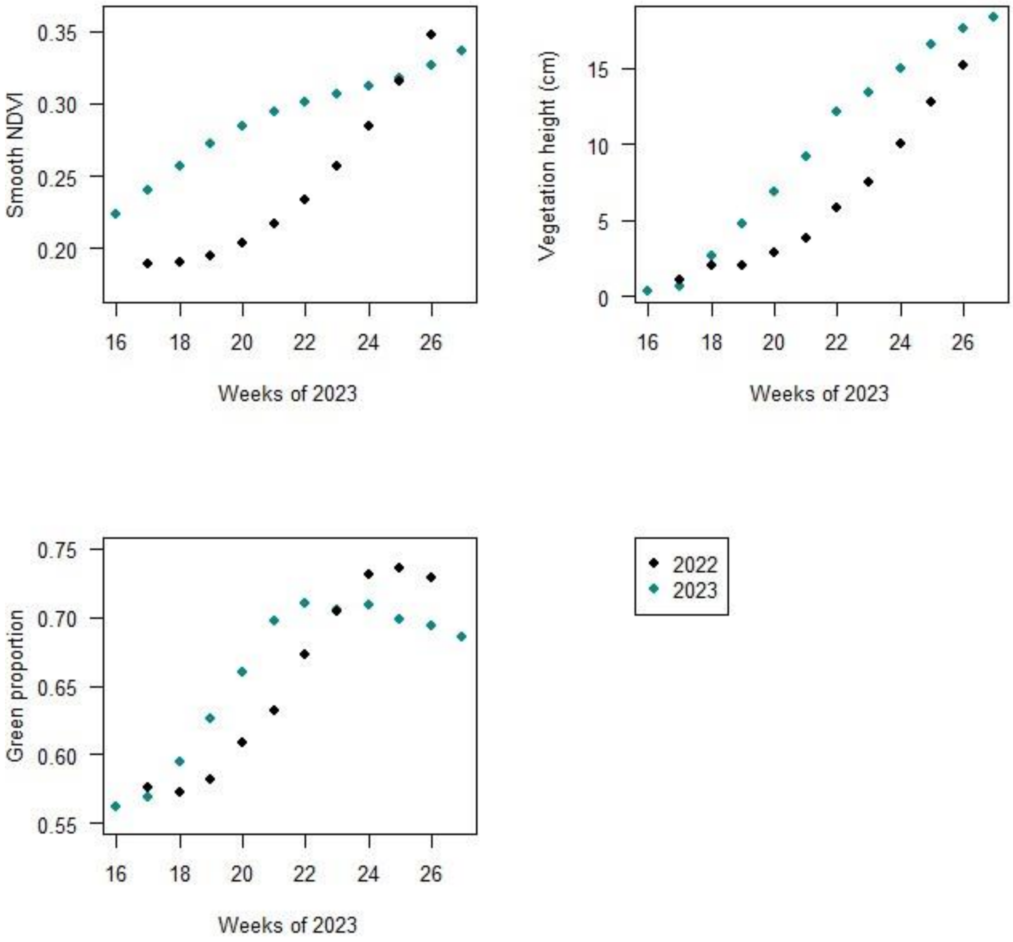


Figure 33. Different measures of vegetation growth in 2022 and 2023. *Vegetation growth between April 25th and June 28th 2022 (in black) and April 13th and July 3rd 2023 (in blue) calculated from (A) smooth based on weekly values of NDVI, (B) vegetation height (cm) measured directly in the field, and (C) the green proportion extracted from pictures of the vegetation taken on the meadow.*

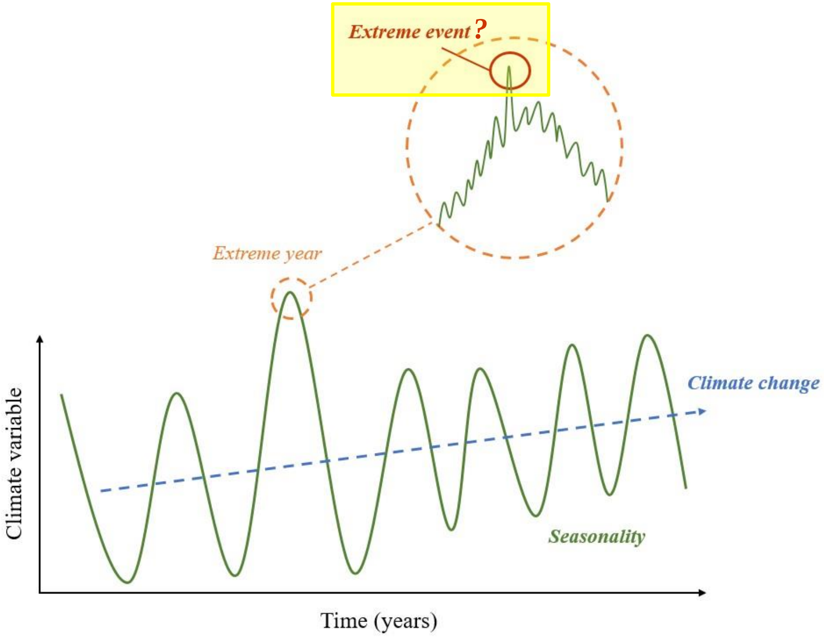
E. Statistical analyses

All statistical analyses were done in R v.4.0.3 (2020-10-10). Although we report statistical tests at a probability error threshold of 5%, results are discussed with regards to biological effect sizes rather than threshold p-values, which is more meaningful and recommended for ecological analyses (Nakagawa and Cuthill 2007, Halsey et al. 2015). Results are presented as means ± SE, along with the number of observations (n). Where appropriate (i.e. for linear models), we ensured that model residuals were normally distributed by visual inspection of density distributions, Q-Q plots, cumulative distribution functions, and P-P plots using the 'fitdistrplus' package in R (Delignette-Muller and Dutang 2015). Whenever interaction effects between independent variables were included in LMs and LMMs, we compared the models with and without the interactions, and chose the final model according to Akaike’s Information Criterion (AIC) (lowest AIC and for ΔAIC < 2, we chose the most parsimonious model, Burnham and Anderson 2004).

On the effects of punctual weather events:

Are they extreme? How do Columbian ground squirrels respond to short periods of inclement weather?

To test for environmental effects on Columbian ground squirrels, I first focused on short periods of inclement weather (see illustration below), and examined behavioral and physiological modifications. In this study, to qualify inclement weather, we compared observed weather events to past records modelled on the study site. These events of inclement weather were characterized by a few days of snowstorms or heavy rainfall during the active season of animals. From these observed events, we described changes in ground squirrels' behavioral activity patterns during the events, and hormonal 'stress' levels or body mass changes after the events compared to controls.



CHAPTER 1

Punctual events, Physiology, and Behaviour

Weathering the storm: Decreased activity and glucocorticoid levels in response to inclement weather in breeding Columbian ground squirrels

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[Article published in *Hormones and Behavior* in 2023]

155(2023), 105426, DOI: https://doi.org/10.1016/j.yhbeh.2023.105426

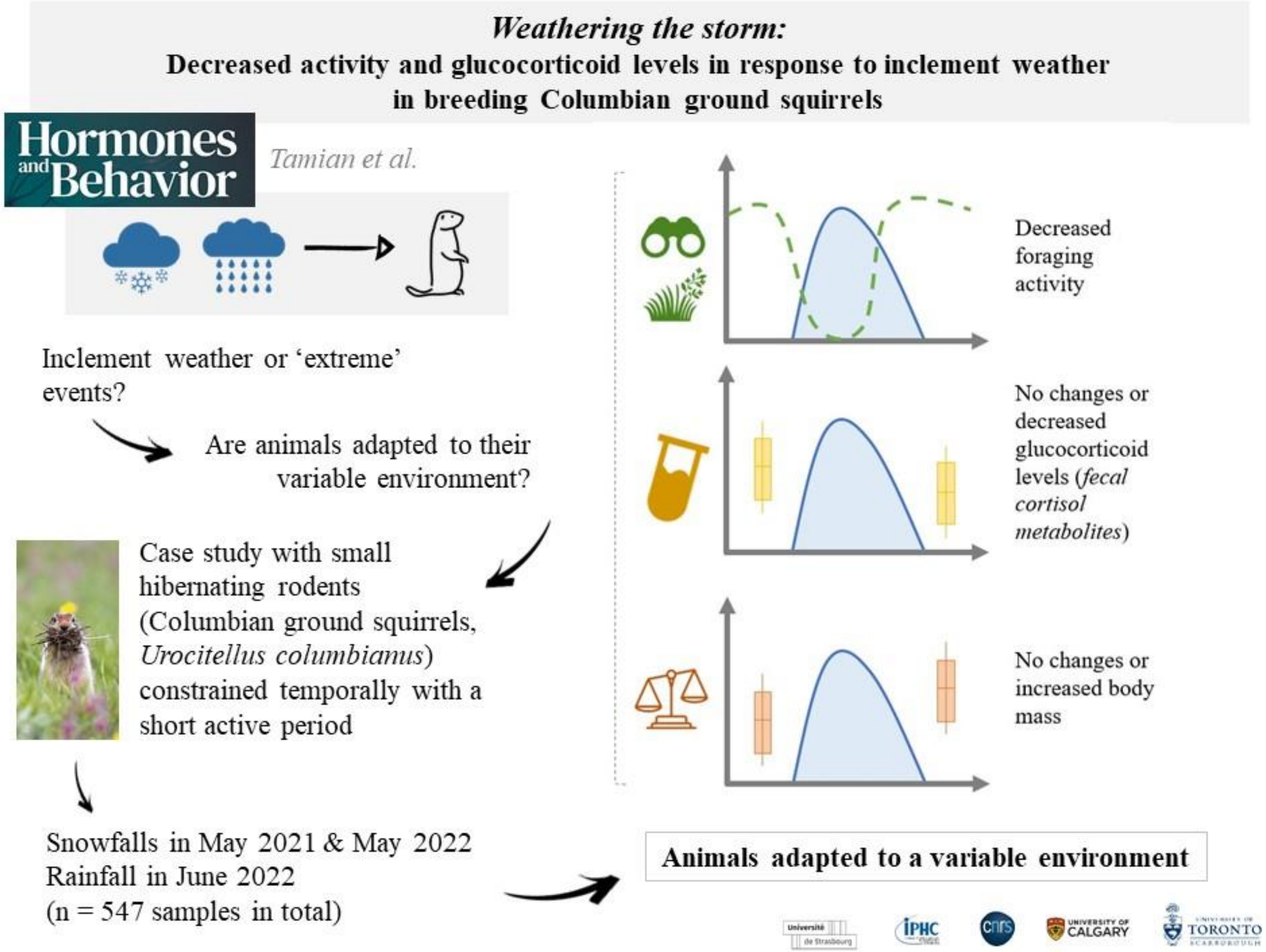
The Method section in this chapter has been slightly modified compared to the published version to avoid repetition.

A. Abstract

Inclement weather can rapidly modify the thermal conditions experienced by animals, inducing changes in their behavior, body condition, and stress physiology, and affecting their survival and breeding success. For animals living in variable environments, the extent to which they have adapted to cope with inclement weather is not established, especially for hibernating species with a short active season that are constrained temporally to breed and store energy for subsequent hibernation. We examined behavioral (foraging activity) and physiological (body mass and fecal cortisol metabolites) responses of Columbian ground squirrels (*Urocitellus columbianus*), small hibernating rodents inhabiting open meadows in Rocky Mountains, to 3 events of inclement weather (two snow storms in May 2021 and May 2022, one heavy rainfall in June 2022). We found that individuals adapted to inclement weather conditions by (1) reducing above-ground activity, including foraging, (2) decreasing the mobilization of stored resources as indicated by a decrease in the activity of the hypothalamo-pituitary-adrenal (HPA) axis and lower fecal cortisol metabolites in the hours/days following periods of inclement weather; and (3) compensating through increased foraging and more local activity when favorable conditions resumed. As a result, body mass and growth did not decrease following short periods of inclement weather. Columbian ground squirrels were well-adapted to short periods of inclement weather, coping *via* modifications of their behavior and the activity of the HPA axis.

Keywords: Behavioural shifts, energy mobilization, extreme climate events, hibernating sciurid, HPA activity, precipitation-modified behavior, weather-induced stress responses

B. Graphical abstract



Weathering the storm:

Decreased activity and glucocorticoid levels in response to inclement weather in breeding Columbian ground squirrels

Tamian et al.

Inclement weather or 'extreme' events?

Are animals adapted to their variable environment?

Case study with small hibernating rodents (Columbian ground squirrels, *Urocitellus columbianus*) constrained temporally with a short active period

Snowfalls in May 2021 & May 2022
Rainfall in June 2022
(n = 547 samples in total)

Decreased foraging activity

No changes or decreased glucocorticoid levels (*fecal cortisol metabolites*)

No changes or increased body mass

Animals adapted to a variable environment

C. Introduction

With global change, the frequency of extreme weather events is increasing along with their effects on organisms, and are predicted to continue increasing in the future (van de Pol et al. 2017, Seneviratne et al. 2021, Quante et al. 2021). Such events can induce marked changes in animal phenology, physiology, and behavior, consequently affecting survival and breeding success (Krause et al. 2016b, van de Pol et al. 2017, Kucheravy et al. 2021). Even for animals adapted to harsh climate conditions, the occurrence of extreme heat, long freezing events, or heavy precipitation can threaten individual survival and reproductive success, and as a result population dynamics (see Frederiksen et al. 2008, Ropert-Coudert et al. 2015, Wingfield et al. 2017 in birds). Energy regulation is a key factor in animal life histories (Brown et al. 2004). Energy acquisition and expenditure can be strongly influenced by weather conditions either *via* effects on foraging resources (modifications of resource availability and acquisition; (Allison and Conway 2022) or *via* effects on animal behavior (*e.g.* foraging behavior) and metabolism (*e.g.* thermoregulation, Levy et al. 2019, Schweiger and Frey 2021).

From a climatology perspective, extreme weather events may be characterized as unusual weather events, contributing to climate variability, but that lie at the extremes of historical records in a given place (*e.g.* heat and cold waves, heavy rainfalls, and snow storms, Matthews et al. 2021). Another perspective defines extreme weather events from a biological point of view, an event being considered as extreme when it significantly impacts individuals, populations, or ecosystem functions (Ummenhofer and Meehl 2017, van de Pol et al. 2017). Thus, from an animal's perspective, the extent to which events of inclement weather constitute extremes depends on their effects on the organism, especially on body condition and stress physiology, and on whether and how animals cope with these environmental changes (Bize et al. 2010, Krause et al. 2016a, Wingfield et al. 2017). Assessing the extent to which various species are able to cope (behaviorally and physiologically) with inclement weather conditions is necessary for discerning the scope of plasticity that they might exhibit in the current context of rapid global change (Ummenhofer and Meehl 2017, Harris et al. 2018).

Assessing behavioral and physiological modifications to inclement weather should be particularly relevant for animals inhabiting mountainous temperate ecosystems where variations in thermal conditions are high, favorable living conditions temporally constrained (*viz.* to spring and summer months), and the occurrence and intensity of snowstorms and rainfalls is projected to increase with ongoing climate change (Seneviratne et al. 2021, Quante et al. 2021). Many species rely strongly on foraging conditions during the spring/summer period to sustain their reproduction. This short temporal window is especially challenging for hibernating species, that not only rely on energy income for reproduction, but also for replenishing fat stores in anticipation of the following hibernation (Humphries et al. 2003b). Thus, resource acquisition and storage during the active period may have strong influences on survival and reproductive success (Murie and Boag 1984, Zervanos et al. 2014, Carrier et al. 2022). Inclement weather that occurs during the short spring/summer period can have direct consequences on the mortality of individuals (*e.g.* flooding, Golet et al. 2013, Viblanc et al., *personal observations*), or indirect consequences through food consumption, reflected in reduced body mass (Lenihan and Vuren 1996, Neuhaus et al. 1999). As an example, Belding's ground squirrels survival, reproduction, and behavior was strongly impacted by a long spring snowstorm inducing sharp mass loss, decreased reproduction, and increased mortality in the population (Morton and Sherman 1978). What remains unclear however, is how animals that live in fluctuating environments react to inclement weather: their hormonal, physiological, and behavioral responses (Wingfield et al. 2011).

When conditions are beyond those normally experienced, *e.g.* stressful weather events, vertebrate behavior and physiology is modified through the hypothalamic-pituitary-adrenal axis (HPA; Boonstra 2004, Wingfield et al. 2011). Several studies showed that the activity of HPA axis increases when conditions become more stressful, which can be measured in glucocorticoids concentrations (hormonal mediators of allostasis that trigger a "stress response", McEwen and Wingfield 2003, Baker et al. 2013, Walker et al. 2015, Romero and Wingfield 2015, Boonstra et al. 2020, King et al. 2023). Even though the stress response allows individuals to quickly respond to unpredictable stressful events and is considered adaptive (Boonstra et al. 2001, Angelier and Wingfield 2013, McEwen 2019), long exposure to stressful stimuli can also have deleterious effects on individuals and affect their long-term survival (Boonstra et al. 1998, Sapolsky et al. 2000, Wingfield and Kitaysky 2002b, Romero 2004).

Glucocorticoids are responsible for energy mobilization and fat and protein catabolism, which can ultimately lead to changes in body condition (Sapolsky et al. 2000, Angelier et al. 2007). In case of inclement weather and limited access to food, nutritional stress usually triggers the HPA axis (Pravosudov and Kitaysky 2006, Jenni-Eiermann et al. 2008) and the response is reflected in increased circulating cortisol concentrations, an important glucocorticoid in mammals (Vijayan et al. 2010, Haase et al. 2016, Zhang and Buck 2022). In contrast, one hypothesis would be that species adapted to unpredictable environments, such as mountainous ones, could save energy by shutting down the HPA axis and decreasing energy mobilization. This can be the case for hibernating species, which already rely on shelters (e.g. burrows, dens) as part of their natural history, and seasonally limit their metabolism. One non-invasive way to assess HPA activity is to examine fecal cortisol metabolites (FCMs, Sheriff et al. 2011, Palme 2019, Gormally and Romero 2020, Zhang and Buck 2022).

We tested the hypothesis that animals inhabiting mountainous ecosystems may have adapted to cope with short periods of inclement weather. For this test, we studied Columbian ground squirrel (*Urocitellus columbianus*), a model species that is regularly subjected to highly variable and unpredictable weather conditions in its montane habitat (Zammuto and Millar 1985). To do so, we examined the effect of inclement weather on activity patterns, foraging behavior, glucocorticoid levels, and body mass of ground squirrels during two late snowfall events in 2021 and 2022 and one rainfall event in 2022. Specifically, we (1) tested whether these weather events were considered as ‘extreme’ from a climatology perspective, (2) described behavioral changes during inclement weather in 2022, and (3) analyzed whether hormonal ‘stress’ levels or body mass changed after weather events compared to controls. Unlike several species showing increased activity of the stress axis during periods of inclement weather (Wingfield et al. 1983, Romero et al. 2000, Bize et al. 2010, Krause et al. 2016a) or food deprivation (Kitaysky et al. 1999, 2007), we predicted that in response to inclement weather, these mountain rodents would adapt by weathering the storm, *i.e.*, saving energy by reducing their overall activity and metabolism.

We expected animals to avoid wet and cold weather conditions by increasing the time spent in sheltered burrow-systems and reducing above-ground foraging activity. We thus expected to observe less individuals foraging during periods of inclement weather, but expected foraging activity to re-increase to higher levels afterwards, perhaps compensating for the inclement periods of lowered resource acquisition. We further expected decreases in metabolic activity during inclement weather to be reflected in overall decreased levels of glucocorticoid levels (Haase et al. 2016) shortly after the period of inclement weather conditions (as measured through fecal glucocorticoid metabolites FCM; Bosson et al. 2009, Sosa et al. 2020), reflecting an overall metabolic state geared towards minimized use of stored energy (for associations between metabolic activity and GCs, see Bonier et al. 2009, Haase et al. 2016). In turn, we predicted such adaptations to result in minimal loss of body mass during periods of detrimental weather.

Columbian ground squirrels are an ideal model for testing the effects of acute inclement weather on animal condition and stress, and the hypothesis of adaptation. These hibernating herbivorous, and diurnally active rodents inhabit open meadows in the Rocky Mountains (Elliott and Flinders 1991) that are honeycombed with communal and individual burrows in which the animals sleep and raise their young, and in which they may take shelter from inclement weather conditions and predators. They hibernate from August to April of the following year (about 70% of the year, Murie and Harris 1982, Dobson et al. 1992). Their active period, during which reproduction and fattening for subsequent hibernation occur, is thus notably short. During the active season, they spend most of their daily time-budget foraging on vegetation, being inactive in their burrows at night. Their daily time-budget varies during the season, with variations according to sex and breeding status (Ritchie 1990, MacWhirter 1991). Columbian ground squirrels rely strongly on current foraging conditions to sustain reproduction, although their stored energy capital at the start of the breeding season is also important (Dobson et al. 1999, Broussard et al. 2005, Rubach et al. 2016).

During their short active period, unfavorable weather conditions might be expected to negatively impact above-ground squirrel activity and thus the time they spend foraging. Rainfall and snow cover can render the animals wet, decreasing fur insulation, and inducing high energy losses (Webb and King 1984, Lovegrove 2003). Additionally, vegetation is not accessible when covered with snow (Korslund and Steen 2006), and prolonged periods of snow cover during the active season (*e.g.* 2 consecutive weeks of snow storm – more than half of the mating season in normal years) are known to negatively affect male survival and female breeding success (Neuhaus et al. 1999). However, for shorter spells of inclement weather, little is known on whether the animals are able to adapt or not.

D. Materials & Methods

1. Data collection

The study was conducted during the 2021 and 2022 active seasons of Columbian ground squirrels from MA, MB, and MC populations. We checked the weather forecast for the Sheep River area (Alberta, Canada) to anticipate possible periods of inclement weather occurring in our populations. We were able to anticipate 2 periods of inclement weather in 2022: one late spring snow storm and one period of summer rainfall. We target-trapped and weighed the animals, and collected fecal samples just before (control) and after (treatment) these events occurred, i.e., typically the next day within 24h (average 6.8 ± (SD) 5.9 h, min = 0.25 h, max = 22.9 h) following the end of the period of weather deterioration (see Figure 34).

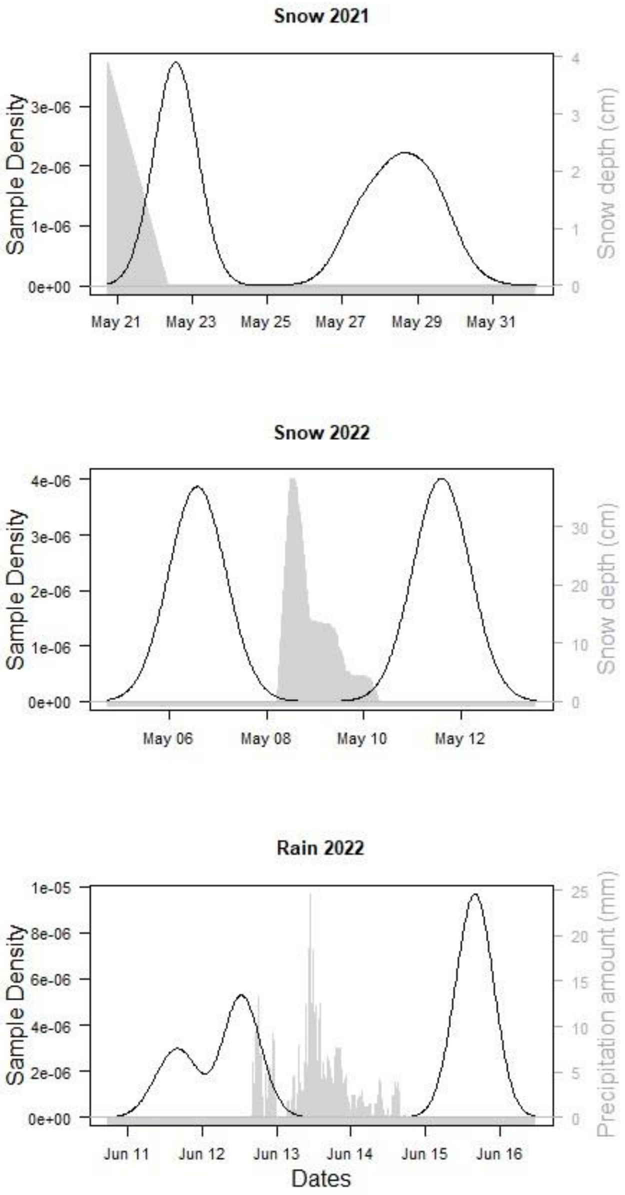
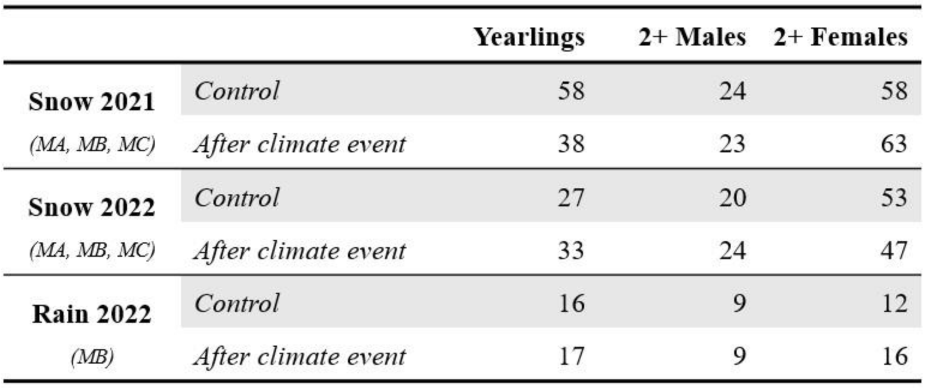


Figure 34. *The density of fecal sampling times and dates compare to snow and rain events are presented for each weather event (grey filled area, snow depth in 2021 and 2022, and rain in 2022).*

In 2021, we were not able to anticipate a sudden snow storm, and thus only acquired fecal samples after the event occurred. Thus, we collected control samples roughly one week after the event had passed. In 2021, animals were weighed and feces were collected just after a snow storm in May (treatment, between 11:00 am and 3:37 pm on 22 May 2021), and around a week after this event (control, between 10:43 am on 27 May 2021 and 10:10 am on 30 May 2021). In 2022, animals were weighed and feces were collected just before a snow storm in May (control, between 10:50 am and 6:55 pm on 6 May 2022), and just after (treatment, between 11:15 am and 7:11 pm on 11 May 2022), before a rainfall in June (control, between 09:54 am on 11 June 2022 and 6:23 pm on 12 June 2022, only on MB) and just after (treatment, between 11:01 am and 7:02 pm on 15 June 2022, only on MB). Given that FCM concentrations reflect circulating glucocorticoid levels *ca.* 7 h before sampling (Bosson et al. 2009), the fecal samples collected thus reflected the activity of the stress axis immediately after (or towards the end of) respective storms. On a few occasions, individuals were not weighed and the sample was not considered in further statistical analyses. Overall, we acquired 547 fecal samples for 272 individuals of known age, but see Table 3 for details of the number of observations per meadow and event.

Table 3. *Number of samples analyzed (fecal samples with known body mass and individual characteristics) for each weather event, condition (control and after), and age/sex category. In total, 547 fecal samples were obtained from 272 different individuals.*



		Yearlings	2+ Males	2+ Females
Snow 2021	*Control*	58	24	58
(MA, MB, MC)	*After climate event*	38	23	63
Snow 2022	*Control*	27	20	53
(MA, MB, MC)	*After climate event*	33	24	47
Rain 2022	*Control*	16	9	12
(MB)	*After climate event*	17	9	16

2. Analysis of fecal cortisol metabolites (FCMs)

Fecal cortisol metabolites were determined by enzyme immunoassay from fecal samples prepared as described in the general materials and methods. All samples were run in duplicate (except 3 samples with one of the measures out of the range, and thus only the other one was kept) on eighteen 96 well plates, showing a high repeatability (R = 0.896, SE = 0.008, CI = [0.878, 0.910], p-value < 0.001, n = 1251 observations, using the *rpt* function from 'rptR' package). Pooled samples with low value (~60 % binding) and high value (~30 % binding) were run on each plate as controls. Mean intra-assay coefficient of variation was 9.7 %, and the mean inter-assay coefficients of variation based on the pools were 12.42 % (low pool) and 13.74 % (high pool).

3. Behavioral sampling & analyses

a) Behavioral observations

As described in the behavioral sampling procedure (general materials and methods), behavioral observations were performed daily on MB. For this study, we selected data from May 4th to 15th 2022 (*i.e.*, 5 days before, 2 days during and 5 days after the snow event). During this period, scans were performed in the morning, from 8:30 am for the earliest start, until 11:40 am for the latest end.

From these observations, we calculated 4 daily behavioral variables: the mean number of observations per scan, the daily percentage of individuals seen above-ground (compared to the actual number of individuals alive and emerged from hibernation on the meadow as of the particular day), the percentage of time spent foraging compared to other behaviors, and the mean total area used by each individual (in m^2). For each individual, area use was estimated from the minimum convex polygon of all observations on a given day (with *mcp* function from 'adehabitatHR' package, with a minimum of five observations per individual).

b) Above-ground activity from loggers

As described in the behavioral sampling procedure (general materials and methods), ground squirrels in MB population were equipped with bio-logging collars fitted with a light sensor, that allowed to determine the hourly proportion of above-ground activity between 8 am and 6 pm. For this study, we selected data from June 10th to June 16th 2022 (*i.e.*, 3 days before, 2 days during and 2 days after the rain event), which leaded to 16 individuals equipped (4 yearlings, 6 older females, and 6 older males). It represented 1,516,780 records, *i.e.*, 13,543 records per day per individual, on average, over a period of 7 days.

4. Characterization of weather events

a) Observed snow & rain events

To quantify snow and rain events, we used several climate sources. Local rainfall in 2022 was recorded at the weather station installed on MB. However, because the weather station did not record snow, we relied on the weather station situated in Pekisko (closest available data) to assess snow in 2021 and 2022. In 2022, to obtain more detailed local data, we combined records from this weather station with the camera traps installed on MB for vegetation assessment (photos taken every 30 min). The cameras faced wooden poles that were graduated every 5 cm. This set up provided us with continuous records of snow depth at various locations on the meadow.

In 2021, it snowed up to 15 cm between the 19-20th of May. Snow remained on the ground until the 22nd of May. In 2022, it snowed during the night between 8th and 9th May up to 38 cm, and the snow melted on the 11th of May. It rained continuously on the 13th and 14th of June 2022 (up to 9.8 mm/h, 110 mm in total over the two days). Because weather events could not be controlled and happened at different biological periods in the season (*i.e.*, during the mating season for earlier events *vs.* during lactation for later events), further analyses on biological measures and samples were conducted separately for each weather event (Snow 2021, Snow 2022, Rain 2022).

b) Extreme events? Assessment of long-term trends

We tested whether the observed events could be characterized as ‘extreme’ from a climatology perspective, compared to the past climate in the study area. To access past and long-term local weather conditions, we used the microclimate model to estimate past snow events on the studied meadow (using the *micro_ncep* modelling function). Local precipitation events from the weather station situated in Sheep River Provincial Park were available starting in 2009. We used this data as input in the model to increase its accuracy, and ran the model from 2009 to 2022. We estimated hourly climate conditions at 4 different points on MB, estimating the snowfalls and snow depth on the ground (in centimeters).

From the modelled snow data and recorded precipitation, we calculated the number of snowfalls and rainfalls (uninterrupted snow/rain events), the duration of snow and rain events (in h), and the mean snow depth of the snow events (in cm) or the total precipitation amount of the rain event (in mm) from the 15th of April each year (average day of first hibernation emergences, typically by older males) until the beginning of August (in the period of immergences into hibernation, Young 1990b, Neuhaus 2000).

5. Data analyses

a) Age & sex considerations

Because the sampled animals differed in terms of sex and reproductive characteristics, we examined separately reproductive males and reproductive females (mature individuals), and one-year-old individuals, usually non-reproductive (immature individuals), resulting in three sex/age categories: ‘Yearlings’, ‘2+ Females’, and ‘2+ Males’.

b) Weather effects on physiology (FCMs & body mass)

For each weather event (Snow 2021, Snow 2022, and Rain 2022), we ran a linear mixed effects model (LMM) with the FCM levels as the dependent variable, and tested for an interaction between the period at which the sample occurred, hereafter called treatment (control *vs* after weather event) and the sex/age category. We included body mass as a covariate in the analyses and controlled for plate, individual, and meadow effects as random terms. Because sampling hour did not significantly affect FCM levels, we did not consider it in the models (LMM, $t = 0.837$, $p = 0.403$, n = 547 observation, N = 272 individuals). The ranges of trapping times compared to the occurrence of weather events are shown Figure 34. We compared the models with and without the interactions, and chose the final model according to Akaike's information criterion (lowest AIC and for ΔAIC < 2, we chose the most parsimonious model; Burnham and Anderson 2004). When the interaction was retained within the best model, we ran LMMs for each sex/age category separately to test for treatment effects on FCM levels.

To test whether there was a difference in body mass between the control period and after the weather event, we ran similar linear mixed effects models (for each of the 3 weather events and testing the interaction of the treatment with the sex/age category, controlling for individual and meadow effects, set as random terms) but with body mass as the response variable.

Because individuals were expected to fatten-up during their short activity period, we further examined whether the weather events impacted their mass gain. During the 2022 active season, we weighed individuals on MB (several individuals of each individual category every week) and calculated the average weekly mass when trapped (yearlings: n = 348 captures and 39 individuals in total, 2+ females: n = 456 captures and 39 individuals, 2+ males: n = 299 captures and 19 individuals). We then compared these patterns to patterns in mean weekly body mass from the long-term monitoring of the same population (same trapping and weighing methods as described previously). This represented 2067 captures of yearlings (n = 664 individuals), 5487 captures of 2+ females (n = 320 individuals), and 1481 captures of 2+ males (n = 186 individuals) spanning 24 years (1994, 1999-2021).

E. Results

1. Long-term trends in weather events

Based on the microclimate model, during the 2009-2022 period, the meadows experienced an average of 7.1 ± 0.4 snowfall events during the active periods of the ground squirrels. There was important interannual variability in the number of events, their duration and the snow depth, with lowest values recorded in 2016 (only a couple of very short events) and an increasing trend since (Figure 35.A, C, G). These snow events usually lasted 12.3 ± 1.5 h and spread from only a few millimeters up to 13 cm (Figure 35.C, H). Compared to all other weather events, the two snow events studied here lasted an average duration, but the amount of snow on the ground was quite different between the two, with a moderate amount in 2021 (4.5

cm *vs* 4.1 cm on average) and a large amount in 2022 (6.8 cm *vs* 4.1 cm on average, above the 90th percentile) (Figure 35.H).

From the weather data in the provincial park that were recorded from 2009 to 2022, there were on average 15.1 ± 0.4 rainfall events (13 to 17) during the active period of ground squirrels (Figure 35.B). The number, duration and amount of rain events were also characterized by an important interannual variability, without displaying longer-term trends. These rainfalls usually lasted 2.8 ± 0.1 days and ranged from only a few millimeters up to 255 mm in total (Figure 35.E, I). Compared to all past rainfalls, the event in June 2022 was only slightly longer than the average (3 days *vs* 2.8 days on average, Figure 35.F) and relatively extreme (110 mm *vs* 18 mm on average, Figure 35.J).

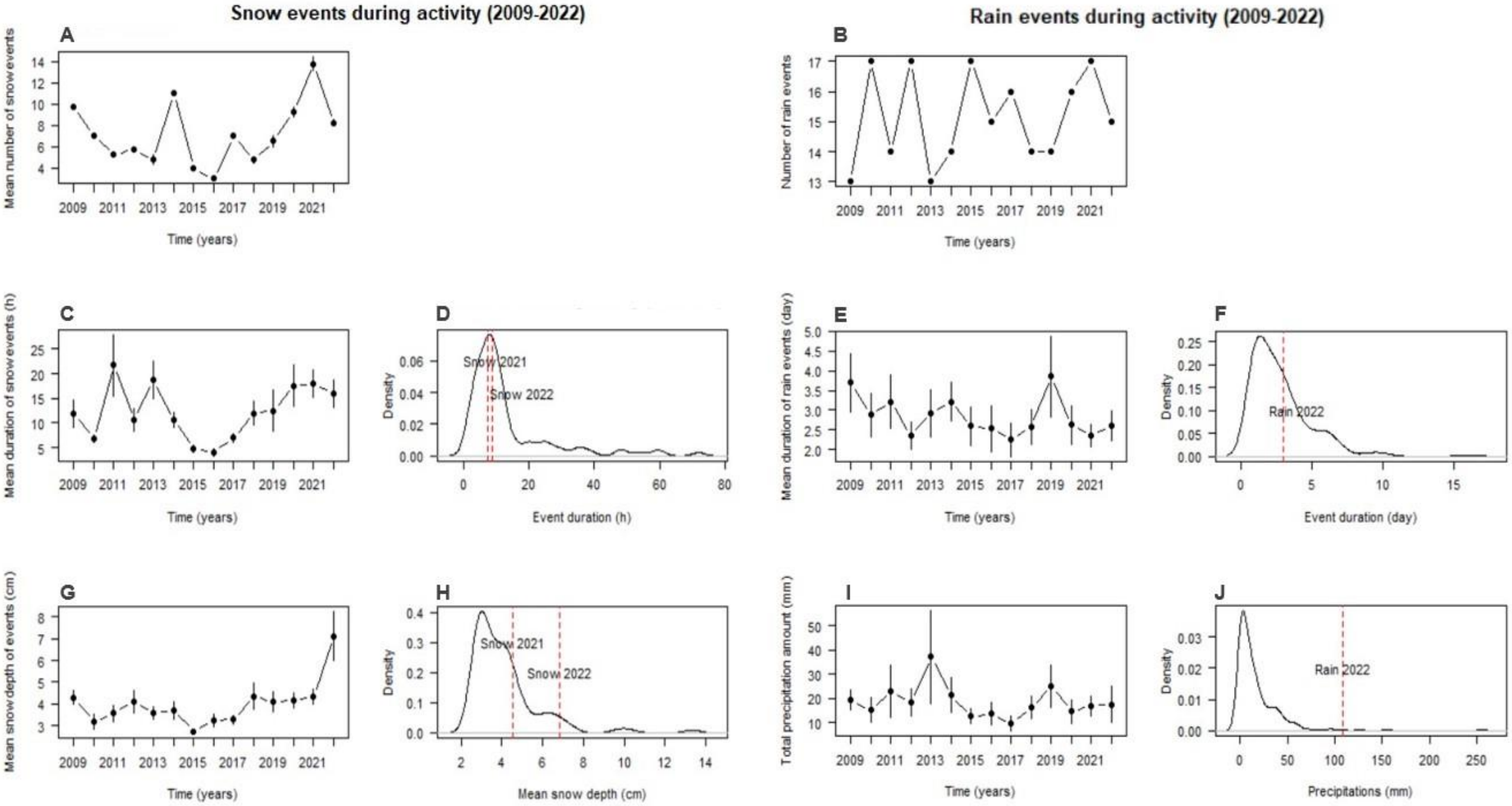


***Figure 35.Weather events characterization**: long-term trends in climate regarding past snow (days with snow on the ground) and rain (continuous rainfall) events from 2009 to2022: (A) and (B) yearly number of snow and rain events, (C & E) yearly mean duration of snow and rain events, (D) duration of 2021 and 2022 snow events (in hours), (F) duration of 2022 rain event (in days), (G) mean yearly snow depth of events, (H) snow depth of 2021 and 2022 snow events (in cm), (I) mean yearly total precipitation of events, (J) total precipitation of 2022 rain event (in mm).*

2. Behavioral responses to weather event

During the May 2022 snow event, the total number of observations per scan and the percentage of individuals seen above ground dropped drastically after the snowfall (when the meadow was snow-covered), on May 9th (from 49 observations par scan on average to 2 observations per scan, and from >92 % of individuals seen on average to 7 %) (Figure 36.A, B). The percentage of time spent foraging by those active above ground was <20 % on May 9th, coincident with the low number of observations (Figure 36.C). In comparison, before the snow event (between May 4th and 8th), yearlings spent 63 ± 1 % of their time foraging, 2+ females 56 ± 2 %, and 2+ males 32 ± 2 %. However, after snow had melted (between May 11th and 15th), males foraged 88 % more than they did before, but females and yearlings did not (LMs, males: $p < 0.001$, $t = 11.44$, females: $p = 0.82$, $t = 0.236$, yearlings: $p = 0.103$, $t = 1.871$, $n = 9$ observation sessions for all). Finally, we were not able to calculate the area used by individuals on May 9th because of too few observations of animals being active. However, older males and females covered a 53 % and 31 % larger area before the snowfall (males: 858 ± 127 m^2, females: 278 ± 35 m^2) than after (LMMs, males: 403 ± 54 m^2, $p = 0.001$, $t = -3.273$, $n = 91$ observations, females: 192 ± 24 m^2, $p = 0.056$, $t = -1.922$, $n = 202$ observations, Figure 36.D). The area used by yearlings did not seem to change before and after the snowfall (209 ± 34 m^2 before and 197 ± 30 m^2 after, $p = 0.776$, $t = -0.286$, $n = 135$ observations).

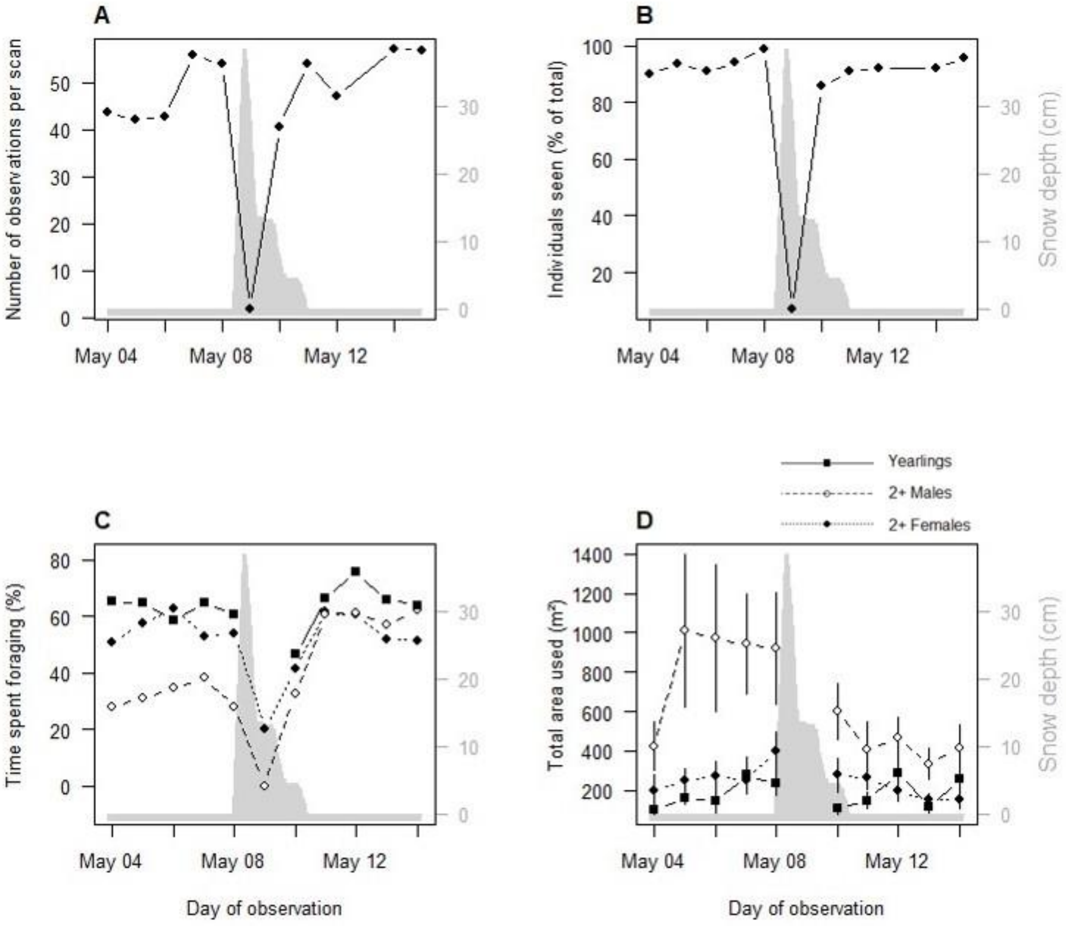


Figure 36. From behavioral observations during the snow event in 2022, we calculated the number of observations per scan (A), the percentage of individuals seen compared to the theoretical number of individuals alive and having already emerged from hibernation (B), the percentage of time spent foraging (C), and the total area used by individual (D). The last two were calculated independently for each sex/age category (yearlings in black squares, older females in black circles, older males in white circles). The grey filled area in the background corresponds to the snow depth on the meadow during the weather event.

During the rainfall in June 2022, the above-ground activity during the day (8am-6pm) decreased significantly for all sex/age categories (LMs, yearlings: $p < 0.001$, $t = -583.2$, females: $p < 0.001$, $t = -474.1$, males: $p < 0.001$, $t = -805.7$, Figure 37). Indeed, between June 10th-12th, yearlings, 2+ females, and 2+ males spent 82 ± 0.09%, 67 ± 0.09%, and 88 ± 0.06% of their time outside of their burrow respectively, compared to 11 ± 0.07%, 14 ± 0.07%, and 11 ± 0.07% during the rainfall (June 13th and 14th). After the rainfall, the above-ground activity increased to similar levels to 75%, 67%, and 82% of their time outside of their burrow respectively.

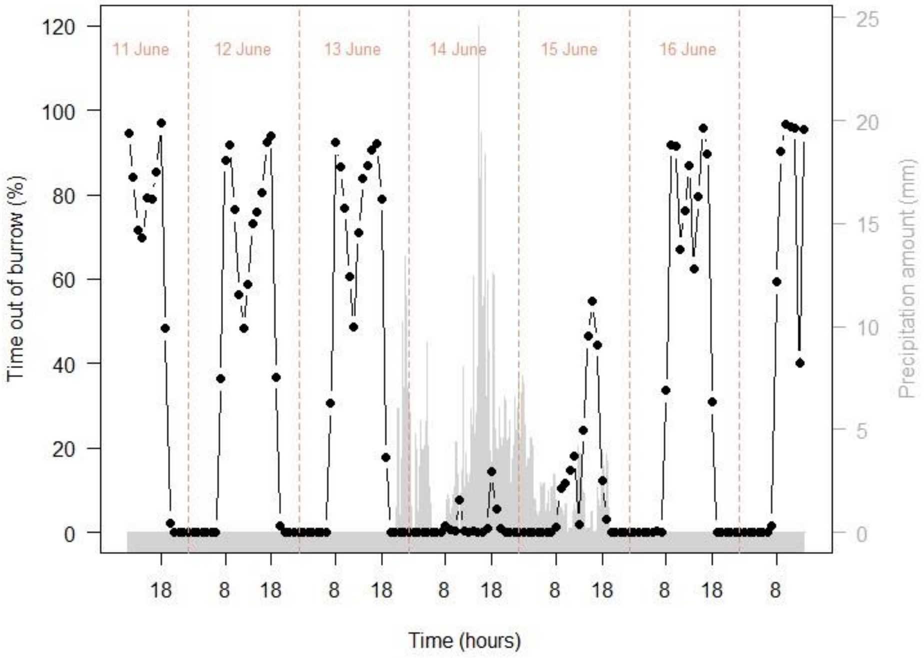


Figure 37. *Hourly percentage of time spent out of the burrow, or diurnal above-ground activity of all individuals during the period of the rain event in 2022. The above-ground activity decreased during hours and days with rain (13th and 14th of June 2022, strong precipitation presented in grey in background).*

3. Physiological responses to weather events

When testing for snowfall and rainfall effects on fecal cortisol metabolites and on body mass, the interaction between treatment (control/after the snow event) and sex/age category was always retained in the best models (LMMs, $3.5 < \Delta AIC < 8.7$), except for FCM levels in 2021 ($\Delta AIC = 0.2$, see sample sizes in Table 3).

For the snow event of 2021, there was no difference in FCM levels between the control (a week after the weather event, Figure 38.A in red) and the treatment (just after the event, Figure 38.A in blue, $p = 0.067$, $t = -1.845$, $n = 264$ observations). When examining changes in mass, weather effects differed depending on the sex/age category (LMM, $\Delta AIC = 6.9$). Whereas yearlings and older males tended to be 5% and 8% heavier a week after the snow event respectively, than just after the event (yearlings: 28 ± 6g heavier, $p < 0.001$, $t = 4.890$, $n = 96$ observations, 2+ males: 30 ± 13g heavier, $p = 0.036$, $t = 2.314$, $n = 47$ observations), the body mass of older females did not change (0.4% difference, -13 ± 11g, $p = 0.249$, $t = -1.161$, $n =$

121 observations, Figure 38.B). Because the control was sampled 1 week after the treatment, conclusions cannot be drawn easily, since changes in mass could simply reflect the typical mass gain of individuals at this time of the season (see typical mass gain between week 20 and 21 on the blue line, Figure 39).

For the snow event in 2022, the weather effects on FCM levels and body mass depended on the sex/age category (LMMs, FCM: ΔAIC = 8, mass: ΔAIC = 3.9). All individuals had lower FCM levels after the snowfall than before, but the effect was stronger in adult males: 48 % lower FCM levels after the event for older males *versus* 43 % and 34 % lower FCM levels for older females and yearlings, respectively (yearlings: -162 ± 40 ng/g, $p < 0.001$, t = -4.075, n = 60 observations, 2+ females: -163 ± 33 ng/g, $p < 0.001$, t = -5.003, n = 100 observations, 2+ males: -426 ± 138 ng/g, p = 0.004, t = -3.093, n = 44 observations, Figure 38.C). Whereas the body mass of adult males did not change (p = 0.856, t = 0.184, n = 44 observations, Figure 38.D), yearlings and older females had 3 % higher body mass after the event than before (yearlings: 14 ± 4 g, p = 0.002, t = 3.620, n = 60 observations, 2+ females: 17 ± 6 g, p = 0.008, t = 2.809, n = 100 observations).

For the rain event in 2022 on MB individuals, weather effects on FCM levels and body mass again depended on the sex/age category (LMMs, FCM: ΔAIC = 3.5, mass: ΔAIC = 8.7). Only yearlings had statistically significant lower FCM levels after the rainfall than before (27 % lower, -155 ± 60 ng/g, p = 0.017, t = -2.606, n = 33 observations, Figure 38.E), but older females had lower FCM levels that approached significance (2+ females: 17 % lower, -48 ± 22 ng/g, p = 0.077, t = -2.196, n = 28 observations, 2+ males: p = 0.972, t = -0.036, n = 18 observations). In terms of body mass, there were no significant changes before and after the weather event for any sex/age category (yearlings: 33 ± 21 g, p = 0.136, t = 1.532, n = 33 observations, 2+ females: -28 ± 22 g, p = 0.220, t = -1.255, n = 28 observations, 2+ males: -24 ± 13g, p = 0.090, t = -1.808, n = 18 observations, Figure 38.F).

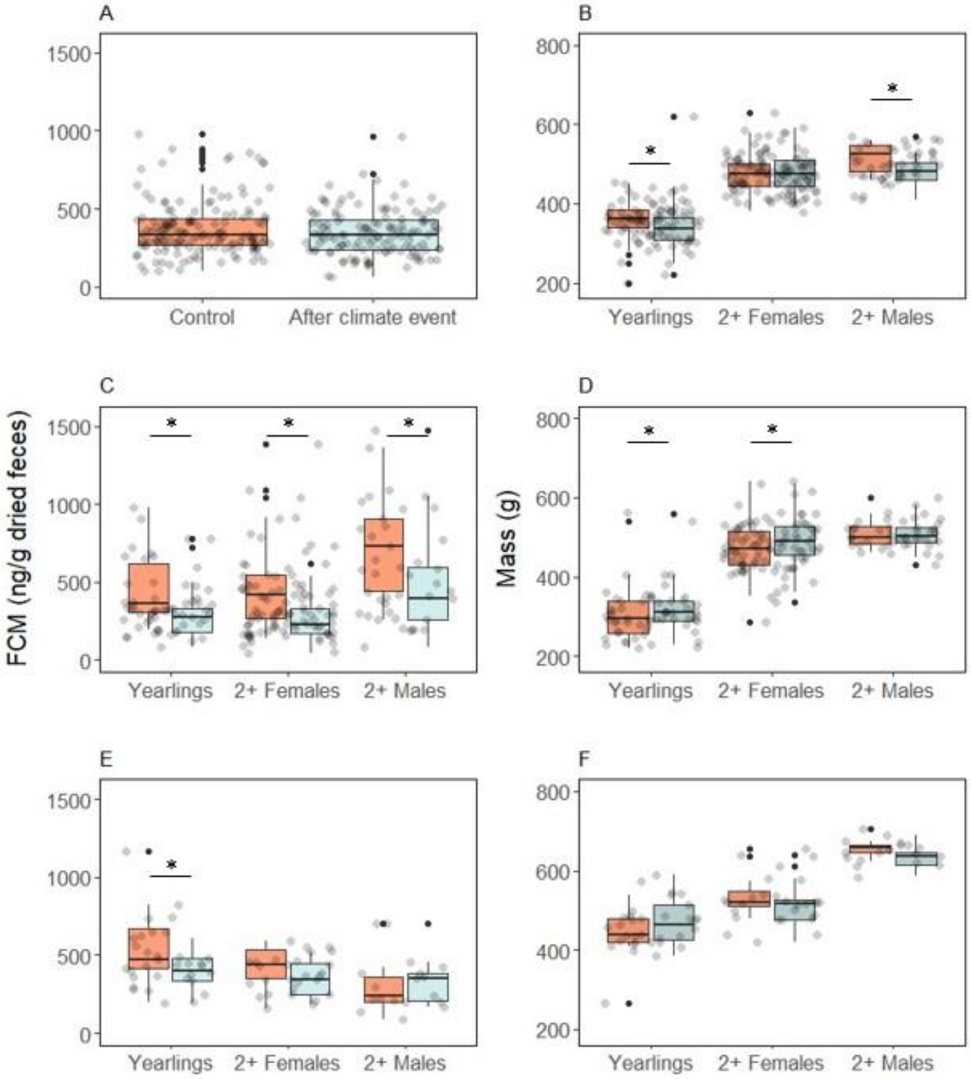


Figure 38. *Fecal cortisol metabolite concentrations (FCM, left) and mass (right) comparisons between the control (orange) and after the climatic event (light blue), for each climatic event (A and B: snow in 2021, C and D: snow in 2022; E and F: rain in 2022) and for each sex/age category (yearlings, older females, older males).*

Finally, the trend in body mass through the active season did not seem to differ greatly in 2022 compared to previous years of monitoring (n = 23 years, Figure 39), for any of the sex/age categories. During the 2022 snow event, all individuals gained mass at a similar rate as in past records (first interval of dashed lines, Figure 39). During the period of rain in 2022, although older females seemed to always lose mass, the mass of older males stayed constant compared to other years (second interval of dashed lines, Figure 39). Finally, only yearling individuals seemed to experience a decreased body mass after the rain in 2022 compared to a slight increase in other years, but this trend was not statistically significant when testing for weather effects on yearling mass (see above).

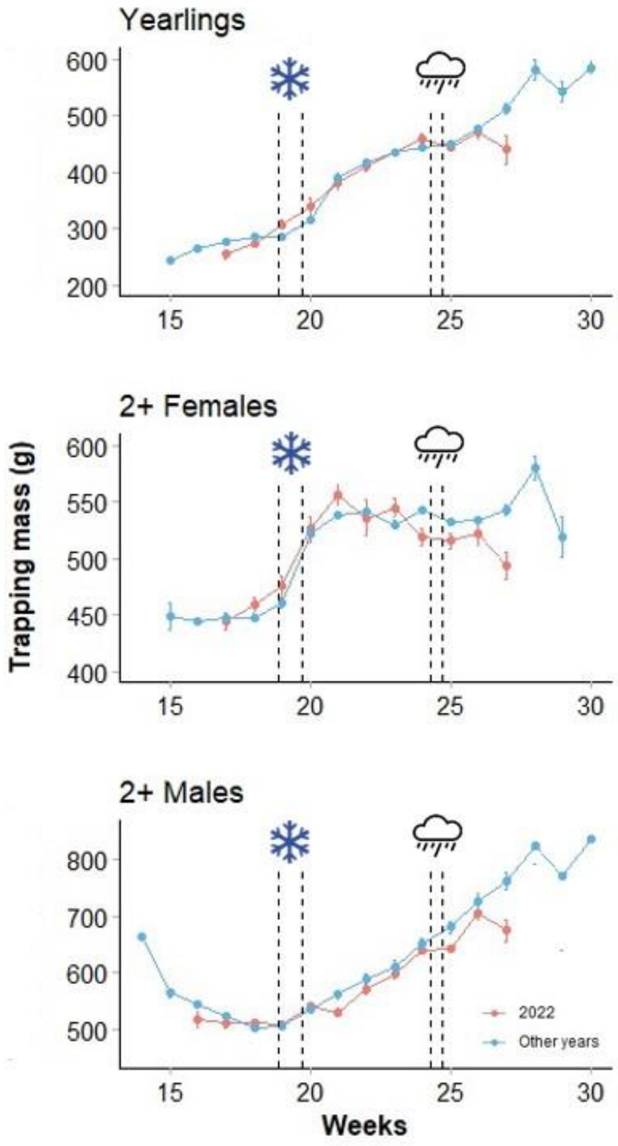


Figure 39. *Comparison between theoretical (blue, calculated from other years) and observed (red, in 2022) weekly body mass (given in weeks since 1 January; week 15 = beginning of April, week 30 = end of July) during the active season of ground squirrels for each individual category (yearlings, older females, older males). The mean body mass (in g) along with standard errors is plotted for each week during active season. The vertical dashed lines represent the occurrence and duration of 2022 weather events (snow and rain).*

F. Discussion

We examined the effects of periods of inclement weather on the behavior, mass change, and physiology of Columbian ground squirrels, a species inhabiting mountain regions with pronounced weather variation (Zammuto and Millar 1985). Consistent with our predictions, we found that behavioral activity and foraging decreased during events of inclement weather. Following these events, fecal cortisol metabolites decreased in most individuals, or did not change, likely reflecting an overall decrease in metabolic activity and metabolic rate (Haase et al. 2016), and lower use of stored energy resources (*i.e.* carbohydrates and fat). Thus, inclement weather did not strongly impact the body mass of individuals, despite decreased foraging activity during the weather events. Taken together, these results confirm the hypothesis that these mountain rodents have evolved to cope with short periods of inclement weather both through behavioral and metabolic adaptations.

For small mammals, high precipitation and humidity in the environment, combined with low temperatures, can impose substantial thermoregulatory energy losses by decreasing body insulation through wet fur (Webb and King 1984, Lovegrove 2003, McCafferty et al. 2017). A way for animals to minimize such costs is to modify behavior (Williams et al. 2016b, Levy et al. 2019). Indeed, during snowfalls and rainfalls, we observed that ground squirrels spent less time foraging above-ground, but rather increased the time spent in their burrows. The few observations we made of ground squirrels briefly emerging from their burrows during the rainy/snowy periods showed that their fur was dry (AT, CS, VAV, *personal observations*), confirming that burrows were indeed efficient shelters against the elements.

Due to reduced foraging, energy intake during inclement weather periods was necessarily reduced as well. In our study, the costs of missed foraging opportunities might have been somewhat higher for males than females. We found that older males, but not females, tended to compensate and forage more just after the snow melt in 2022. Similarly, the activity of older males, which exhibited a large activity range prior to the snowfall during the mating season, appeared to be restricted to smaller areas during and after the snow melt, spending more time foraging and potentially avoiding areas with remaining snow (Stokes et al. 2001, Korslund and Steen 2006). In ground squirrels, the mating season is a period of intense territoriality and aggression for males (Murie and Harris 1978, 1988, Manno and Dobson 2008), which do not gain, but at best maintain their body mass through daily foraging activities (Raveh et al. 2010; see Michener and Locklear 1990 for similar results on Richardson's ground squirrels). Thus, it would make sense that males have more at stake than females in terms of energy acquisition during this period, and that the costs of missed foraging opportunities may rapidly accumulate. For instance, during a prolonged period of snow cover (2 weeks) during the mating season in 1996, Neuhaus et al. (1999) found male survival and mass changes to be more strongly impacted than female survival. Yet, if there was some immediate cost to decreased foraging activity in the present study, this was not clear, at least in terms of changes in body mass. Individuals did not exhibit decreased body mass after inclement weather, and some of them even gained mass over the few days (yearlings and older females in 2022 snow event). Nor did individuals appear to gain less mass than usual when compared with past records of body mass gain at similar periods of the year, and when compared with results in the earlier study (males lost up to 96 g of mass between 30 April and 14 May 1996, Neuhaus et al. 1999).

Our hypothesis to explain the lack of effects of detrimental weather conditions on body mass is that the animals have generally adapted to cope with the fluctuating environments characteristic of their mountain habitat, and geared behavioral and physiological responses towards saving energy in times of hardship. Whereas changes in weather conditions have been found to induce increased HPA activity in various species (Wingfield and Kitaysky 2002b, Bize et al. 2010, Krause et al. 2016a), we found the opposite: individuals emerging from their burrows following a period of inclement weather had lower (or similar) fecal cortisol metabolite levels as compared to before the weather event. Because FCM levels reflect circulating cortisol levels some 7 h before (Bosson et al. 2009), the glucocorticoid levels measured in our study would have coincided with the physiological status of the animals shortly after, rather than during, the respective periods of inclement weather. These results suggest a general hypo-responsiveness of the HPA axis which may have persisted shortly after storms had passed, quite possibly related to a general hypometabolic response aimed at saving energy during periods of inclement weather (see Haase et al. 2016 for cortisol – metabolic rate relationships in mammals). Whereas it would have been interesting to know whether such a hypometabolic response was even more pronounced during the inclement weather events, this was unfortunately impossible since ground squirrels were mostly sheltered below ground and could not be trapped.

To conclude, we were able to combine behavioral and physiological examinations to assess the effects of inclement weather on a species well-adapted to variable and unpredictable environments. Whereas our results suggest that these mountain rodents have adapted to life in a stochastic climatic environment, the question remains whether such events will increase in terms of frequency or duration in the future with climate change (*i.e.* real 'extreme weather events' opposed to inclement weather, van de Pol et al. 2017, McPhillips et al. 2018, Seneviratne et al. 2021). Past research (Neuhaus et al. 1999) shows that even these highly adapted animals may suffer in terms of reproduction or survival if periods of inclement weather last for more than a few days. If the amplitude of observed events in our study was high (high snow depth and high precipitation amount), their duration was average compared to past climate records. This corroborates the idea that animals have adapted to 'short' detrimental weather events without direct consequences on their stress levels or body condition, but consequences could well be different in the case of more frequent and prolonged storms (Morton and Sherman 1978, Harris et al. 2018). If the frequency of weather events increases with global change, individuals might not be able to compensate, with negative consequences on survival and population viability (Neuhaus et al. 1999).

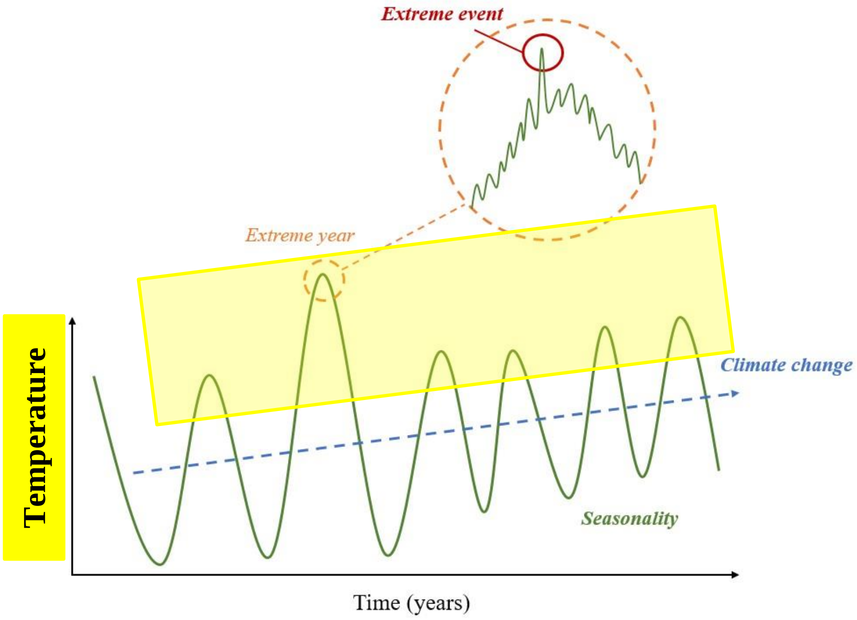
G. Acknowledgements

We are grateful to Edward A. Johnson and Steven Vamosi (Directors, Biogeosciences Institute, University of Calgary), Judy Mappin-Buchannan and Adrienne Cunnings (Managers, Kananaskis Field Stations), and Kathreen Ruckstuhl (faculty member responsible for the R.B. Miller Field Station) for housing and facilities during fieldwork. We are grateful to Alberta Parks, and Alberta Environment, Fish & Wildlife for granting us access to the study sites and support with the long-term research. We are particularly grateful to Edward A. Johnson and Kathreen Ruckstuhl for their continued support and friendship throughout the years. We are specifically indebted to Jan O. Murie for initiating the long-term research on Columbian ground squirrels and for his continued advice and friendship, and to David A. Boag for his earlier work on the species. We wish to thank two anonymous reviewers for insightful comments on a previous version of the paper. This research was supported by an Initiative d'Excellence (IDEX attractivité) research grant to C.S. a CNRS Projet International de Coopération Scientifique grant (PICS-07143) to V.A.V, a research grant from the Fondation Fyssen to V.A.V, a USA National Science Foundation grant (DEB-0089473) to F.S.D., and a fellowship grant from the Institute of Advanced Studies of the University of Strasbourg to F.S.D. and V.A.V. A.T. was supported by a PhD scholarship from the Ministère de l'Enseignement Supérieur, de la Recherche et de l'Innovation. F.S.D. thanks the Région Grand Est and the Eurométropole de Strasbourg for the award of a Gutenberg Excellence Chair.

From punctual inclement weather to seasonal thermal conditions:

How do thermal conditions vary? How do Columbian ground squirrels respond to hot conditions?

Columbian ground squirrels are able to cope with short periods of inclement weather, such as snowstorms or heavy rainfalls. However, it remains unclear how they behaviorally and physiologically respond to high temperatures during their active period. Indeed, whereas they are highly adapted (physiologically, anatomically, and behaviorally) to low temperatures, the extent to which they are able to cope with ambient temperatures potentially above their critical thermoneutral zone, e.g. when facing extreme heat, and how this affects their body condition is less clear.



CHAPTER 2

Heat and Behaviour

Adapted to cope? Behavioral plasticity in response to ambient heat in Columbian ground squirrels

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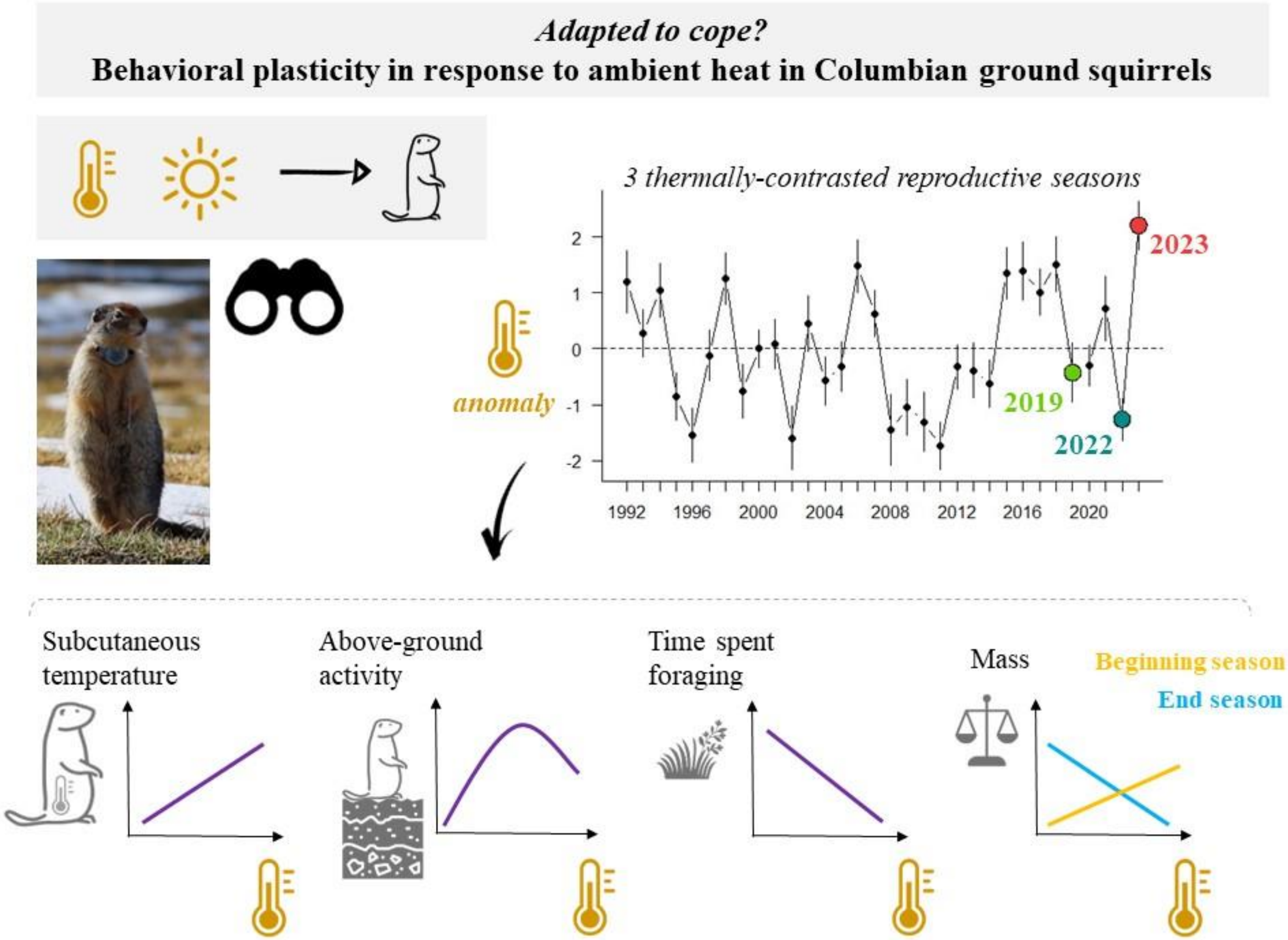
[Article in preparation]

A. Graphical abstract



B. Introduction

With global increases in heat waves (Stott 2016, van de Pol et al. 2017, Stillman 2019, Seneviratne et al. 2021), heat-associated mortality events are becoming increasingly documented across animal populations (*e.g.*, birds; McKechnie and Wolf 2009, McKechnie et al. 2012, insects; Rocha et al. 2017, corals; Estaque et al. 2023, and mammals, including humans; Stillman 2019). This has probed more and more studies to focus on the thermal limits that animals are able to tolerate, and the extent to which they are able to cope with increasing temperatures via morphological, behavioral or physiological plasticity (Kearney et al. 2009b, Boyles et al. 2011, Huey et al. 2012, Sunday et al. 2014, Khaliq et al. 2014, Buckley et al. 2015, Gunderson and Stillman 2015, McKechnie and Wolf 2019).

In contrast to ectotherms, whose body temperature (T_b) mostly conforms to that of their environment, endotherms are defined by their ability to produce (or dissipate) metabolic heat to maintain their core body temperature within a narrow temperature range (Schmidt-Nielsen 1997). Mitchell et al. (2018) described four different zones of mammalian thermoregulation: the thermoneutral zone (TNZ) with minimal changes in animal metabolic rate (Scholander et al. 1950), the prescriptive zone (above or below the TNZ) where sustainable increased metabolic rate and evaporative heat loss are required to regulate T_b, the tolerance zone where malfunction can occur outside of the prescriptive zone but life is not yet threatened, and the survival zone beyond the tolerance zone where survival is threatened. In response to higher temperatures than those normally experienced, mammals may thermoregulate either physiologically by reaching the prescriptive zone and increasing their evaporative heat (and water) loss (Tattersall et al. 2012, Dowd et al. 2015, Williams et al. 2016a), and/or behaviorally by modifying their activity to avoid hot conditions (Fuller et al. 2021). Behavioral modifications may include temporal changes (*i.e.*, switch from diurnal to nocturnal activity, decrease in time spent in high temperatures, Levy et al. 2019, Schweiger and Frey 2021, Zhou et al. 2022) or spatial changes at different scales (*i.e.*, cache use, habitat range changes, Aublet et al. 2009, Fuller et al. 2016, Osborne et al. 2020).

Thermoregulatory behaviors can be seen as adaptations to avoid critical temperatures, sublethal costs of heat exposure (*e.g.*, increased oxidative stress; Jacobs et al. 2020, 2021, declines in motor and cognitive performance; Danner et al. 2021, Soravia et al. 2021, declines in reproductive performance; Hansen 2009), or ultimately death (see above). However, the benefits of thermoregulation importantly trade-off with energy lost (via thermoregulation-related energy expenditure) or not acquired due to changes in patterns of activity (Cunningham et al. 2021). For example, animals that modify their behavior and time budget to thermoregulate might spend less time foraging, and acquire less energy during periods of elevated temperatures (Carrascal et al. 2001, du Plessis et al. 2012, Mason et al. 2017, van de Ven et al. 2019). Negative impacts of high temperatures on animal foraging behavior, and complex relationships with body condition (Webb 1979, Bronikowski and Altmann 1996, Edwards et al. 2015, Funghi et al. 2019, van de Ven et al. 2019, Allison and Conway 2022), have hence been documented in several taxa. Yet, the consequences of such trade-offs for individual fitness remain unclear.

Among endotherms, the extent to which adaptive thermoregulation, *viz.* any thermoregulatory adaptation arising from phenotypic plasticity within generations or genetic change across generations (Angilletta et al. 2010), will be sufficient to cope with increasingly extreme warm weather events is also unclear, though there is growing evidence that endotherms show remarkable variability in their ability to tolerate high ambient and body temperatures (Boyles et al. 2011, Khaliq et al. 2014, McKechnie and Wolf 2019). Of particular interest are the concepts that species inhabiting regions of more variable climates (such as regions of high latitude or altitude) should have broader thermal tolerances (climate variability hypothesis; Stevens 1989, Khaliq et al. 2014), and that co-adaptation between thermoregulation and thermal sensitivity, *i.e.* the reaction norm of a given performance across a range of T_b, should shape endothermic responses such that heterotherms should be able to maintain some level of performance / activity over a broader range of T_b than strict homeotherms (Angilletta et al. 2010, Boyles et al. 2011).

In this context, seasonally hibernating species offer an interesting and powerful model to study adaptive thermoregulatory responses to extreme temperatures in endotherms. Most seasonal hibernators live in strongly variable climatic environments, and are highly specialized to respond to seasonal temperature decreases both via behavioral and physiological adaptations. For instance, despite relying on internal heat production as all endotherms, hibernators show drastic fluctuations in their core (and peripheral) body temperatures, with some species such as arctic ground squirrels, *Urocitellus parryii*, championing extremes ranging from some 38°C during normothermia (Long et al. 2005) to as low as -2.9°C during hibernation (Barnes 1989). What is less known, however, is how hibernators respond to high temperatures potentially driving them out of their thermoneutral zone (Levesque et al. 2016, 2023). Yet, even high altitude environments with strong seasonality are expected to be increasingly exposed to unpredictable and extreme heat events (Seneviratne et al. 2021, IPCC 2023), challenging these cold-adapted species. Whereas high plasticity in thermoregulatory behaviors of small hibernating mammals are known, the relationships between their thermal environment, their behavior, and their body condition have not always been clearly identified (Vispo and Bakken 1993, Sharpe and Van Horne 1999, Long et al. 2005, Williams et al. 2016b).

In this study, we tested the hypothesis that Columbian ground squirrels (*Urocitellus columbianus*), small hibernating mammals inhabiting strongly seasonal habitats in the Rocky Mountains, were able to adapt to periods of high ambient temperatures through behavioral plasticity. Columbian ground squirrels do not have access to free-standing water but access water through food consumption (Karasov 1983), so that facing high temperatures is not just about thermoregulation but also water loss through sudation. We focused on animals during their short 4-mo. spring-summer active season and tested the extent to which: (1) T_b was related to ambient air temperature, and (2) above-ground activity, foraging time budget, and mass gain changed with air temperature. Columbian ground squirrels live in open meadows surrounded by shaded forests, and honeycombed by burrow systems in which they hibernate over winter, and use during the spring/summer periods to sleep and shelter from sudden storms (Tamian et al. 2023). In contrast, little is known on how ground squirrels use burrows to behaviorally thermoregulate in warm conditions, *i.e.*, is there a critical threshold in above-ground ambient temperatures constraining them to disappear underground? An early study on ground squirrel activity suggests that these animals may favor the coolest hours of the day for activity (Elliott and Flinders 1980). However, this study equated ground squirrel activity to the presence of animals in live-traps, and may suffer from potential biases due to the selective trapping of 'trap-shy' or 'trap-happy' animals (Elliott and Flinders 1980). In addition, behavioral thermoregulation does not necessarily imply decreasing overall activity in warmer conditions than usual, but could also be achieved by conducting activities in areas where temperature is lower. For instance, by shifting activities to shaded areas (forest edges surrounding the meadow).

Here, using a mix of observational, bio-logging and trapping (mass) data, we focused on behavioral and body mass response to high temperatures. We predicted that animals would adapt to high temperatures by (1) decreasing the proportion of time spent above ground under hot conditions, (2) shifting their activity to cooler hours of the day (Elliott and Flinders 1980, Levy et al. 2019), and (3) shifting their activity centers to cooler (shaded) areas of their habitat. However, we expected patterns to differ among individuals, depending on differences in energetic and water requirements linked to sex and breeding status. For instance, we expected lactating females requiring large amounts of energy to sustain lactation and exporting large amounts of water in milk to be more vulnerable (Zhao et al. 2020) and thus more dependent on vegetation for water and energy income. We further also expected decreased foraging activity under hot conditions to lead to decreased mass gain over the season, a potential cost to behavioral thermoregulation in this seasonal mammal, highly dependent on energy storage over summer to sustain hibernation. Working over 3 contrasted breeding seasons (2019, 2022, 2023, including a relatively hot reproductive season in 2023, see Results) was expected to provide initial insights on within and among season variation in behavioral responses.

C. Materials & Methods

1. Data collection

Columbian ground squirrels were monitored on MB as presented in the general materials and methods, and we recorded individual behavior in 3 recent years: 2019, 2022, and 2023. Behavioral samplings included direct scan observations and above-ground activity data obtained from light sensors on bio-logging collars (as presented in the general materials and methods in the “Behavioural data” section), from which we calculated the proportion of time spent above-ground, the time budget of animals, the proportion of time spent foraging, and their location on the meadow. This represented around 110 days of observation and 61 days of collar deployment over the three years (see details on Figure 20). In addition, in 2023, 9 adult females were equipped with subcutaneous thermal PIT tags (passive integrated transponders; Biomark® BioTherm 13 Pit Tag, Boise, ID USA) which, in addition to individual identification, transmitted instantaneous subcutaneous temperature when read at capture. For 46 captures of these females (between 24 May and 29 June), we recorded the subcutaneous temperature as soon as the individual was caught in our trapping bags (between 10 and 73 seconds after trap closing).

2. Defining yearly thermal conditions compared to long-term trends

Local air temperatures at 10 cm above ground (average height of ground squirrels) were modelled hourly (1992-2023) over four different locations dispersed over the study site using the *micro_era5* function, and averaged over the four locations. We then compared estimated air temperatures during years of study (2019, 2022, and 2023) to past records (from 1992). To do so, we only selected the reproductive season (beginning of April to the end of June) and only day hours during which animals were theoretically likely to be active (sunlight, based on the hours of sunrise and sunset at the location from "suncalc" R package). We then (1) averaged hourly diurnal temperatures into daily measures, (2) estimated the average seasonal pattern, *i.e.* average daily temperature at any given date within a year, and (3) estimated the anomaly by centering the daily temperatures by the average seasonal temperature of the day. This allowed for comparisons among years and within years, and further allowed removing seasonal increases in temperature, thereby preventing relationships with temperature from being driven by seasonal patterns. Anomalies translate into how hot (positive anomaly values) or cold (negative anomaly values) a day is compared to what is usually recorded at that time of the year. We then tested the relationships between temperature anomalies, behavioral and body mass data.

3. Data analyses

a) Age, sex and reproductive categories

Because we expected behavioral responses to heat to vary depending on physiological constraints (*e.g.* lactation), we grouped individuals by their sex, age, and breeding status into four categories: yearlings (males and females of one year-old), 2+ males (two year-old and more), 2+ breeding females (two year-old and more females which successfully weaned a litter), 2+ non-breeding females (two year-old and more females which did not wean a litter).

b) Relationships between squirrel subcutaneous temperature and ambient temperature

Using the data from the 9 adult females that were equipped with subcutaneous thermal PIT tags in 2023, we tested how subcutaneous temperatures varied with ambient air temperatures at that time (hourly data obtained from the microclimate model ran at 10 cm-high). Because the duration between trap closing and the temperature measurement could not be standardized (done as soon as the individual was in the bag, 10-73 seconds), we ran a linear mixed model of the body temperature as a function of ambient temperature with the lag from trapping closing to measurement as a covariate, and the individual (ID) as a random factor.

c) Plasticity in above-ground activity patterns in relation to ambient temperature

We tested the plasticity of ground squirrels in their above-ground activity patterns in relation to ambient temperatures in several steps using different parameters: i) the daily proportion of time spent above ground, ii) the hours of individual emergence for the day and immergence for the night, iii) the hourly proportion of time spent above ground all inferred from collar light data. We then focused on iv) the percentage of time spent above ground foraging and v) the spatial use of the meadow; both estimated from our scan observations. While these different analyses all had their specificities (especially different time scales, etc. see below), they were all performed according to similar rules: i) the effect of temperature was tested using both linear and quadratic terms to test for potential optima in temperature, ii) the effects of temperature were tested in interaction with the individual sex/age/breeding status category, iii) year (and individual identity when run at the individual scale) were included as random effects, iv) model selection was based on AIC starting from the full model $y \sim T_{amb}$ x Category + T_{amb}^2 x Category. Further, all analyses were done using both absolute temperatures and temperature anomalies. Results were usually quite similar and, for clarity purposes, results on absolute temperatures are presented in Supplementary Materials: Figures 50 to 53.

Collar light data were recorded every 2 seconds and aggregated at different time scales. The daily proportion of time spent above ground was studied at the individual level (thus including ID as a random term) using two different measurements: using all daylight hours (from sunrise to sunset, “suncalc” package) or focusing only on hours between emergence and immergence of the squirrel. Models used for the 2 daily proportions of time spent above ground as well as the hourly proportion were Generalized Linear Mixed Models (GLMM) with a binomial distribution (number of recorded above-ground occurrences = success, number of below-ground occurrences = failures).

Emergence and immergence times were estimated from collar light data as the absolute time difference in hours between sunrise and the first (or the last and sunset) complete minute recorded above ground. Because data were collected every 2 sec, this meant that 30 successive above-ground occurrences had to be recorded for an emergence (or immergence) to be considered valid. This avoided potential errors in the light sensor to affect our results. Unfortunately, in 2019 and 4 (out of 35) deployments of 2022, data were only recorded during the day (and not night) preventing us to estimate emergence and immergence. Analyses on emergence and immergence were thus conducted on a subsample of collar data (2022 and 2023). Linear mixed models were then used to test for the effect of ambient temperatures on emergence and immergence hours with year and ID as random terms.

To estimate the effect of temperature on global daily activity patterns and spatial usage of Columbian ground squirrels, we compared cold, mild and warm days. These were defined according to the 1st and 3rd quartiles of temperature anomalies (or absolute temperatures). Cold days were days of temperature 2.5 °C below seasonal average (or days < 11 °C), warm days displayed temperatures 2.8 °C above seasonal average (or days >17 °C). Differences in daily activity patterns (mean proportion of time spent above ground per hour within a day) and space usage (kernel density maps) were then described for the three different air temperature scenarios.

Finally, because observations were performed by 15-min scans 2-hours a day, we decided to estimate time budget (low sample sizes, as well as for mass changes, see below) weekly rather than per day. To do so, for each of the three years of study and for each individual category, we selected the day of first emergence from hibernation as the start of the season (J0). From this day, we calculated the following weeks until the end of the monitoring period (week 1, week 2, etc.). Then, for each week relative to the year, we calculated the mean foraging activity (from time budget, centered by the mean foraging activity of the week and category over the 3 years of study). We performed similar weekly anomaly calculations for the mean air temperature. We further calculated the mass change (gain or loss, presented as Δmass) every two weeks (because of too few trappings per week) and its anomaly during study years compared to all the years with available trapping data (1994, 1999-2023) for each category separately (see illustration in Supplementary Materials: Figure 54). Patterns of foraging and mass changes were not uniform along the season, and these variables (anomalies) allowed correction for seasonal changes. Thus, we ran a linear mixed effects model with the anomaly of weekly percentage of time spent foraging as a response variable and the anomaly of averaged air temperature during the week as an explanatory variable, and year as random variable.

d) Effects on body mass

Changes in behavior may influence ground squirrels’ energy intake and affect their body mass. We thus tested for an effect of the proportion of time spent foraging on Δmass. Additionally, we tested for a direct link between Δmass and air temperature. In both cases, we ran a linear mixed effects model with the Δmass anomaly as the response variable, year as random factor and either the anomaly of proportion of time spent foraging or the air temperature anomaly in interaction with the sex/age/breeding category as the explanatory variables. Because the link between mass changes and air temperature changes might be different between the beginning and the end of the reproductive season, we tested for an interactive effect of the week in the model.

D. Results

1. Characterizing thermal contrasts between our study years

Compared to the past 30 years, 2019, 2022 and 2023 were highly contrasted in terms of thermal conditions (Figure 40). 2023 was the warmest year on record (17 % higher than average), 2022 one of the coolest (10 % lower than average), while 2019 was intermediate (3 % lower than average) (Figure 40, 2019: 12.82 ± 0.52 °C and -0.43 °C difference on average, 2022: 11.98 ± 0.59 °C and -1.26°C difference on average, 2023: 15.46 ± 0.57 °C and +2.21 °C difference on average).

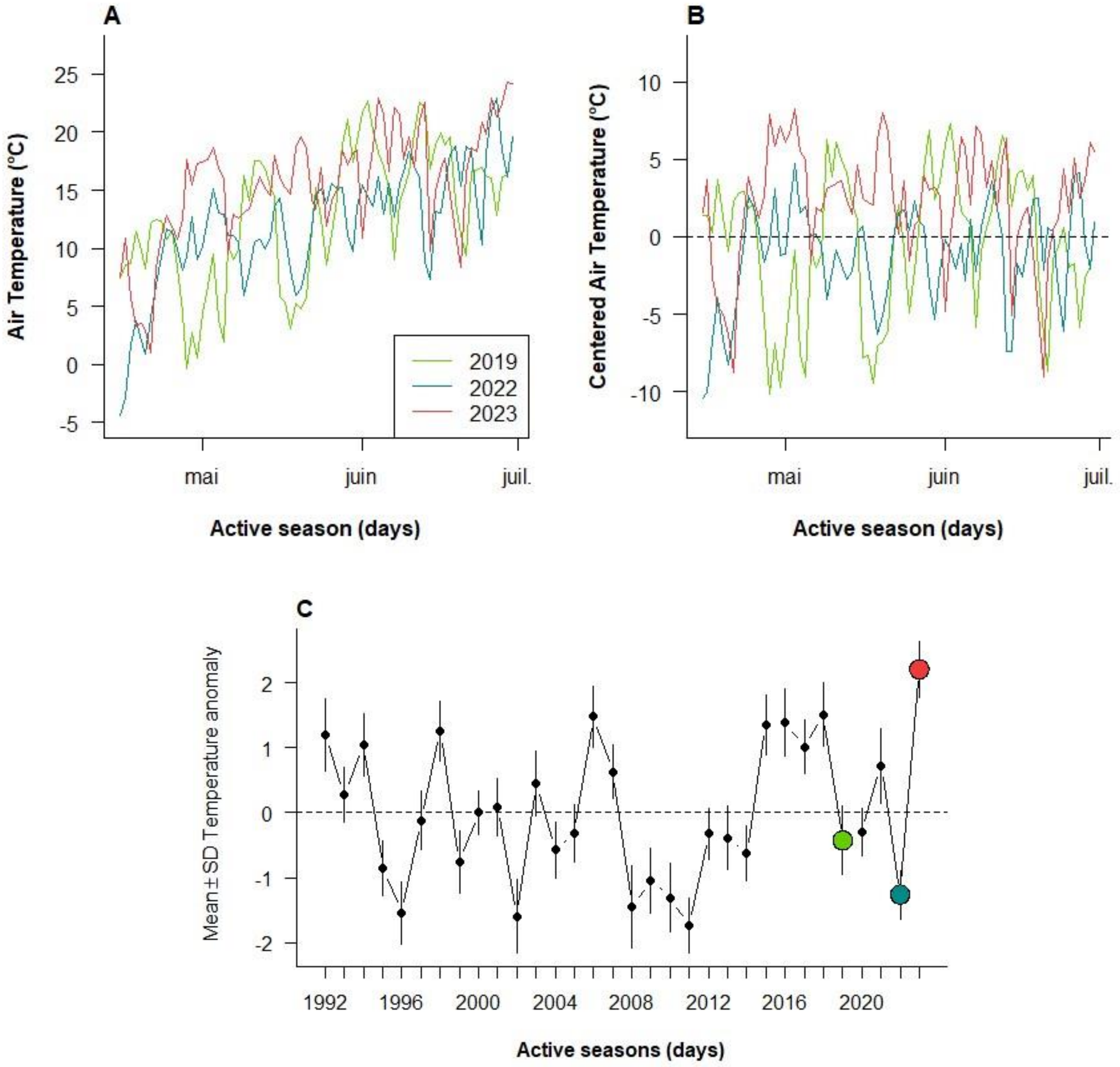


Figure 40. *Air temperatures during sunlight hours modelled from the microclimate model with the* micro_era5 *function. (A) Mean daily air temperatures during 2019 (green), 2022 (blue), and 2023 (red) reproductive seasons; (B) daily anomalies of air temperatures during the different seasons (centered by the mean daily value over the last 30 years 1992-2023), and (C) mean annual anomalies of daily temperatures averaged over the reproductive season (15th April – 30th June).*

2. ***Relationship between ambient temperature and animal body temperature T_b***

Using the data from the 9 adult females that were equipped with subcutaneous thermal PIT tags in 2023, we found a linear significant effect of ambient air temperature on subcutaneous body temperature at capture, the animals' T_b increasing by 0.10 °C (± 0.02) for a 1 °C increase in ambient temperature (LMM, t = 4.597, p < 0.001, n = 46 obervations, Figure 41).

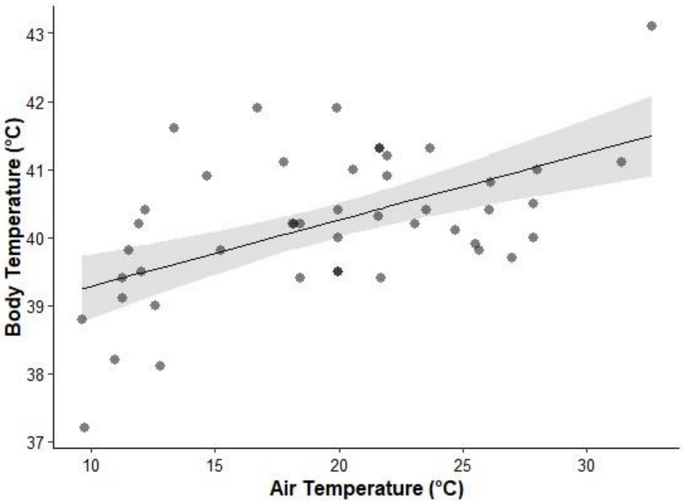


Figure 41. *Relationship between air temperatures and body temperatures from thermal PIT tags of 9 ground squirrel adult females (°C).*

3. Plasticity in above-ground activity patterns in relation to ambient temperature

Above-ground activity patterns were obtained from light-loggers as presented in the general materials and methods.

a) Plasticity in daily amounts of above-ground activity

Considering daily activity from sunrise to sunset hours, Columbian ground squirrels spent an average of 57 ± 1 % of the day above-ground. This proportion varied quadratically with daily air temperature (absolute or anomaly), according to individual age/sex/breeding status category (Figure 42.A; and see Supplementary Materials: Figure 50), increasing initially then decreasing again for warmer temperatures (GLMM binomial, best model retaining linear and quadratic effects of temperature and their interaction with individual category, n = 1043, N = 95). While all four individual categories increased their time spent above ground when temperature went from below to above average (36 to 59% point increase), only yearlings seemed strongly affected by warm temperatures (25% point decrease in time spent above ground between the maximum reached at 2°C above average and the hottest days 8 °C warmer than average vs. 3, 4 and 5% point decrease in breeding females, adult males and non-breeding females respectively).

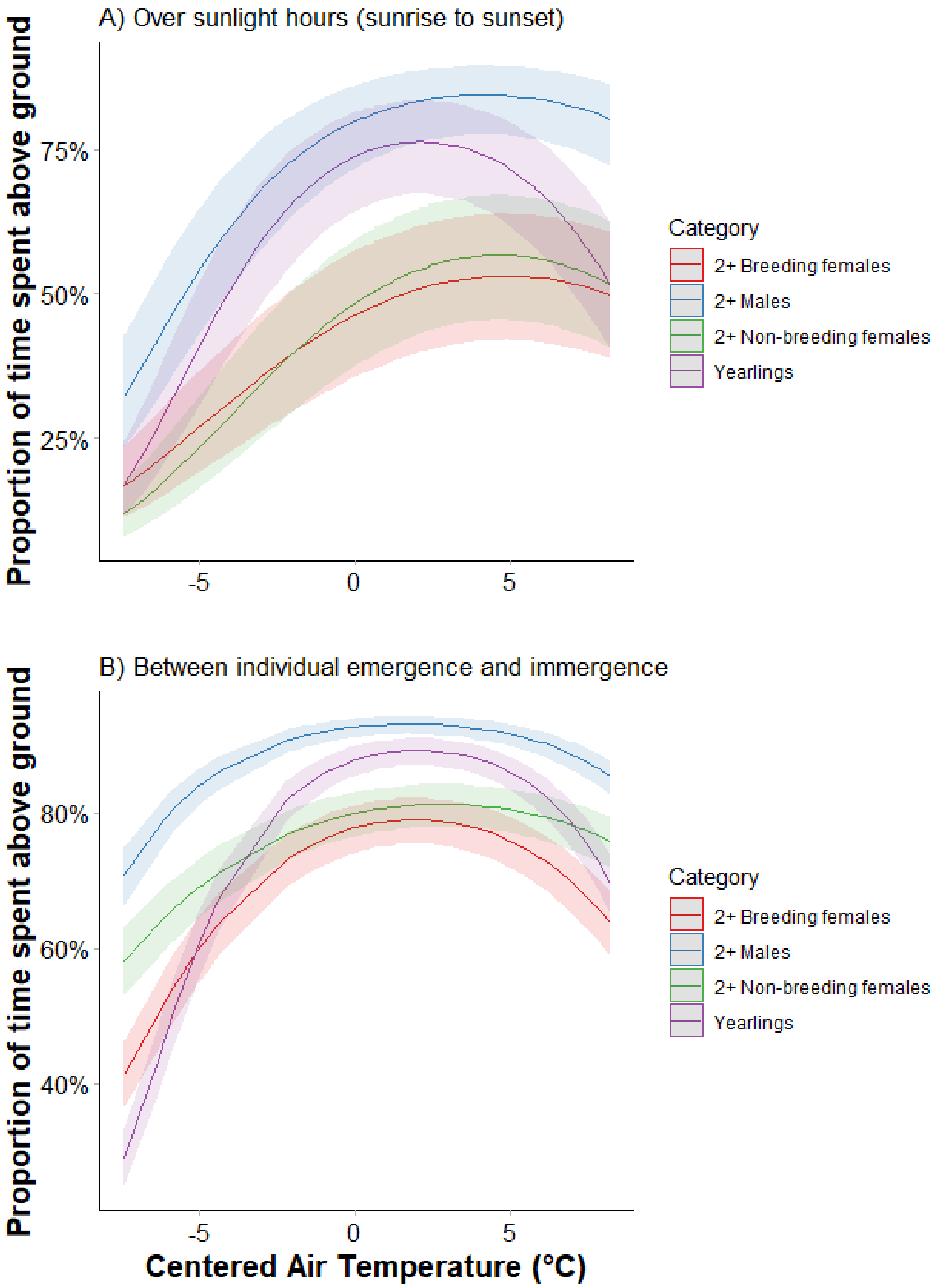


Figure 42. *Proportion of time spent above ground from collar light data according to daily temperature anomalies, as predicted by the best model (GLMM binomial including a linear and quadratic effect of daily temperature anomalies both in interaction with individual age/sex/breeding status categories), (A) based on daylight hours (sunrise and sunset estimated every day) and (B) based on daily activity (emergence and immergence estimated per individual every day).*

Further focusing on the hours of animal activity, *i.e.* after morning emergences from burrows and before evening immergences, on average, individuals spent 80 ± 0 % of their time above-ground. Again, this proportion was highly influenced by air temperature (linear and quadratic effects), individual category and their interaction (GLMM binomial, n = 653 observations, N = 90 individuals, Figure 42.B). The proportion of time spent outside increased with daily air temperature (absolute or anomaly), reaching a maximum at anomalies of 1.8 °C to 2.6 °C above average depending on individual categories (Figure 42.B; or absolute temperatures of 15.2 °C to 17.0 °C, see Supplementary Materials: Figure 50), then decreasing again for warmer temperatures.

b) Plasticity in the timing of daily activities

Columbian ground squirrels were only recorded above-ground during daylight hours (after sunrise and before sunset). They emerged from their burrows some 2.5 ± 0.1 hours after sunrise, non-breeding females first (2.0 h) followed by adult males (2.4 h), adult breeding females (2.8 h) and yearlings (3.5 h). They immerged for the night some 2.8 ± 0.0 hours before sunset, adult males and breeding females first (2.9 h), followed by non-breeding females (2.6 h) and finally yearlings (2.1 h). However, these patterns were modulated by daily average temperatures.

Regardless of their age/sex/breeding status category, individuals (1) emerged earlier (relative to sunrise) when the anomaly in temperature the previous day was above average (LMM: linear effect est. = -0.105 ± 0.028, t = -3.758, $p < 0.001$, quadratic effect: est. = -0.012 ± 0.004, t = -2.987, p = 0.003, n = 464, Figure 43.A). This effect however was not significant when considering absolute temperatures (LMM: best model retaining only the category as explanative variable, n = 464, see Supplementary Materials: Figure 51); and (2) immerged later (relative to sunset) in days when the anomaly in temperature was higher (LMM: est. = -0.078 ± 0.016, t = -4.967, $p < 0.001$, n = 471, N = 67 individuals; Figure 43.B). In this latter case, a similar pattern was found for absolute temperatures, though the effect was stronger in adult males and breeding females, than in non-breeding females and yearlings (LMM: interaction category*temperature retained in the best model, n = 471, N = 67 individuals, see Supplementary Materials: Figure 51).

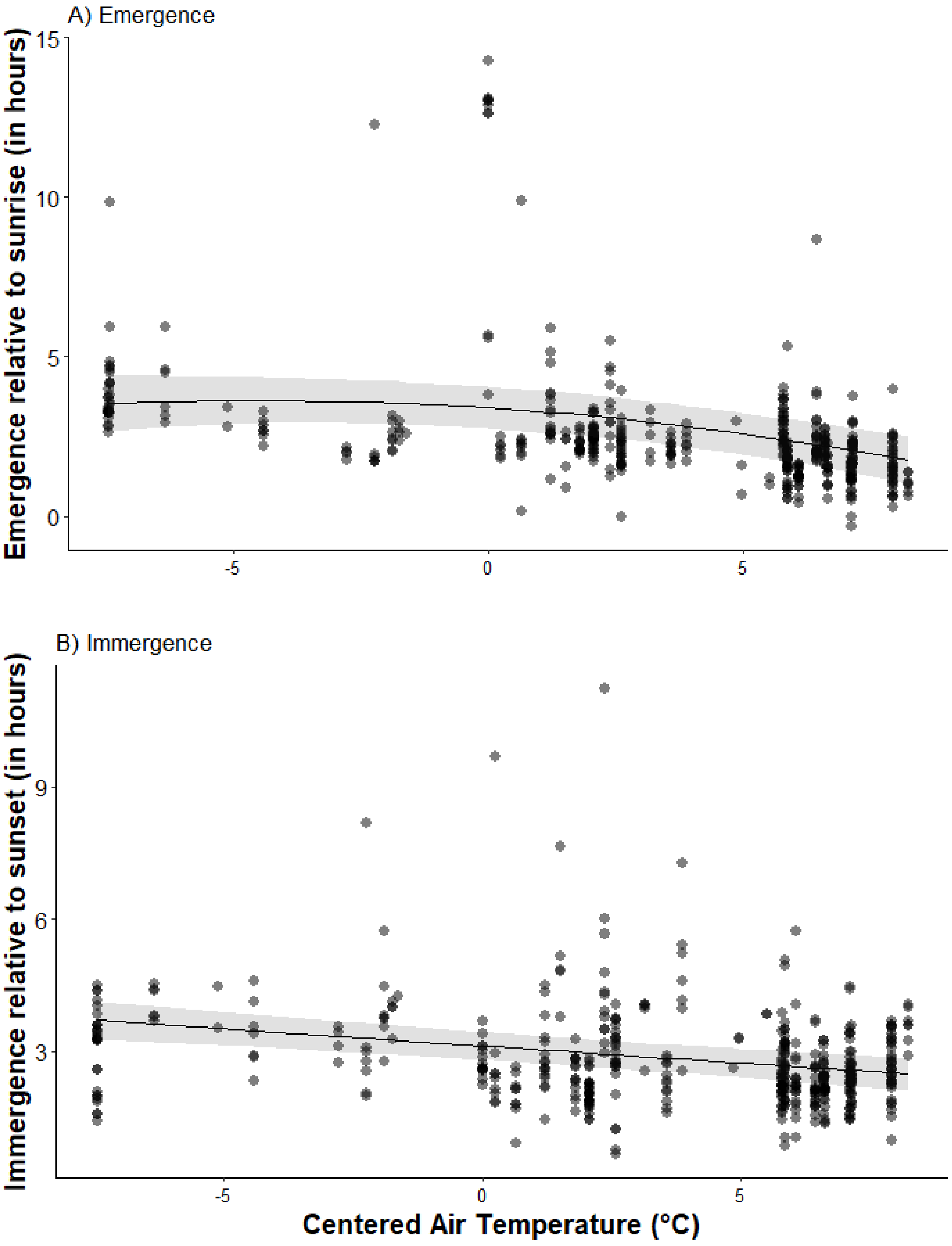


Figure 43. Daily start and end of activities from collar light data according to daily temperature anomalies. *(A) Emergence of burrow (in hours relative to sunrise) and (B) immergence into burrow (in hours relative to sunset). The black lines represent the prediction of the best linear mixed model (including individual sex/age/breeding status category as well as temperature anomaly as a linear and quadratic effect for emergence but only as a linear effect for immergence).*

At a daily scale, average activity patterns differed between individual categories and varied with mean daily temperature (absolute or anomaly). During colder days (*i.e.* more than

2.5 °C less than average, or < 10 °C), individuals (and especially yearlings) spent less time above-ground and focused above-ground activity to the warmest hours of the day (early afternoon, see Figure 44 and Supplementary Materials: Figure 52). Differences between mild and warm days were less pronounced, individuals spending slightly more time above-ground during the warmest period of the day in mild days than in warm ones.

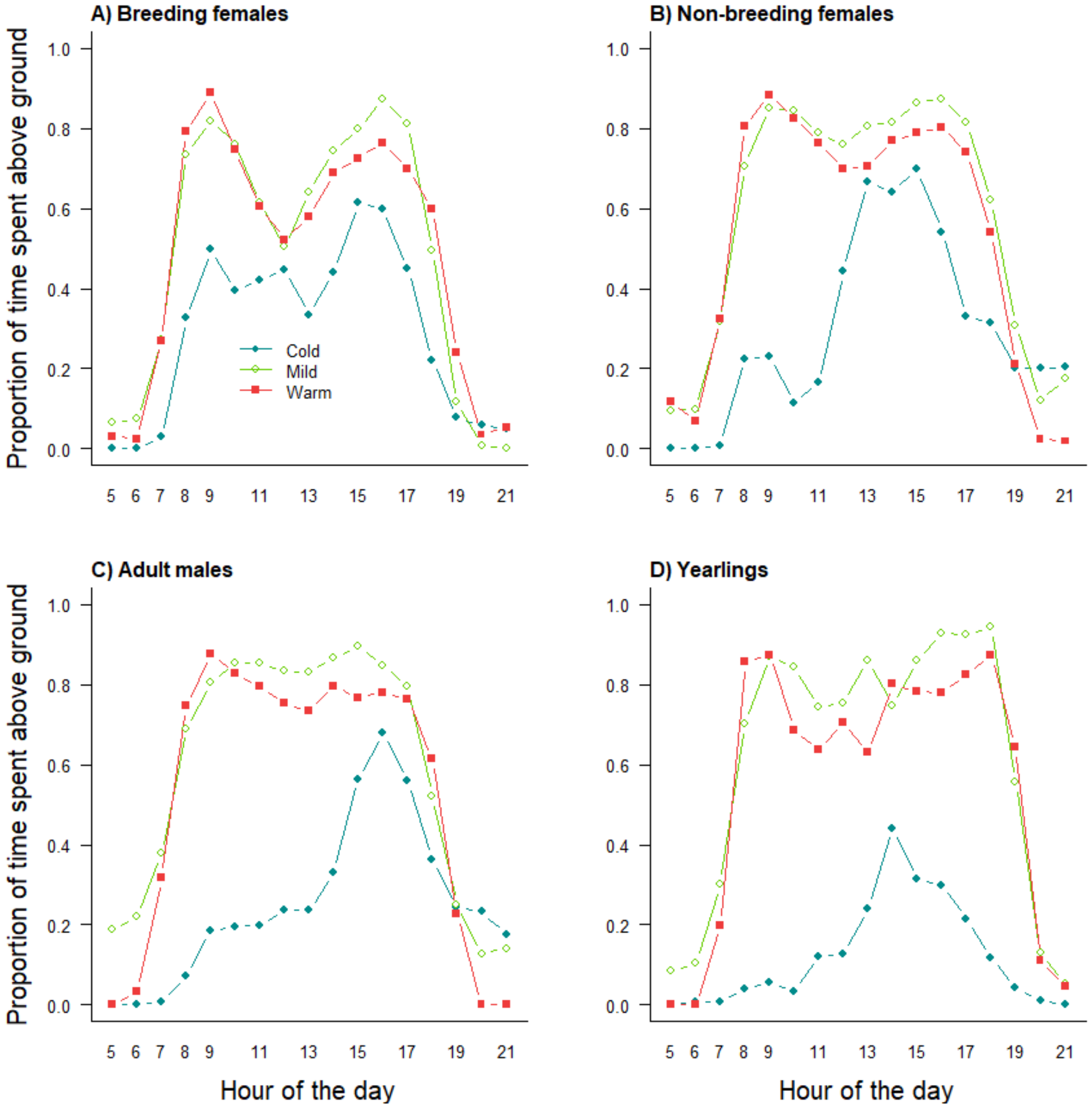


Figure 44. Daily above-ground activity patterns*. Hourly proportion of time spent above ground according to hours of the day for cold (in blue), mild (in green) and warm (in red) days in (A) breeding females, (B) non-breeding females, (C) adult males and (D) yearlings. Days were grouped in cold, mild and warm days based on the quartile of daily temperature anomalies (cold days < 25% quartile = -2.5 < mild days < 2.8 =75% quartile < warm days).*

At an hourly scale, the proportion of time spent above ground also varied quadratically with temperature, and differently according to individual sex/age/breeding status category (best model including a linear and quadratic effect of daily temperature anomalies both in interaction with individual age/sex/breeding status categories, n = 6295, N = 95 individuals). Breeding females and yearlings spent less time above ground as hourly ambient temperature increased (clear optimum temperature around 22 °C for breeding females and 25 °C for yearlings, Figure 45).

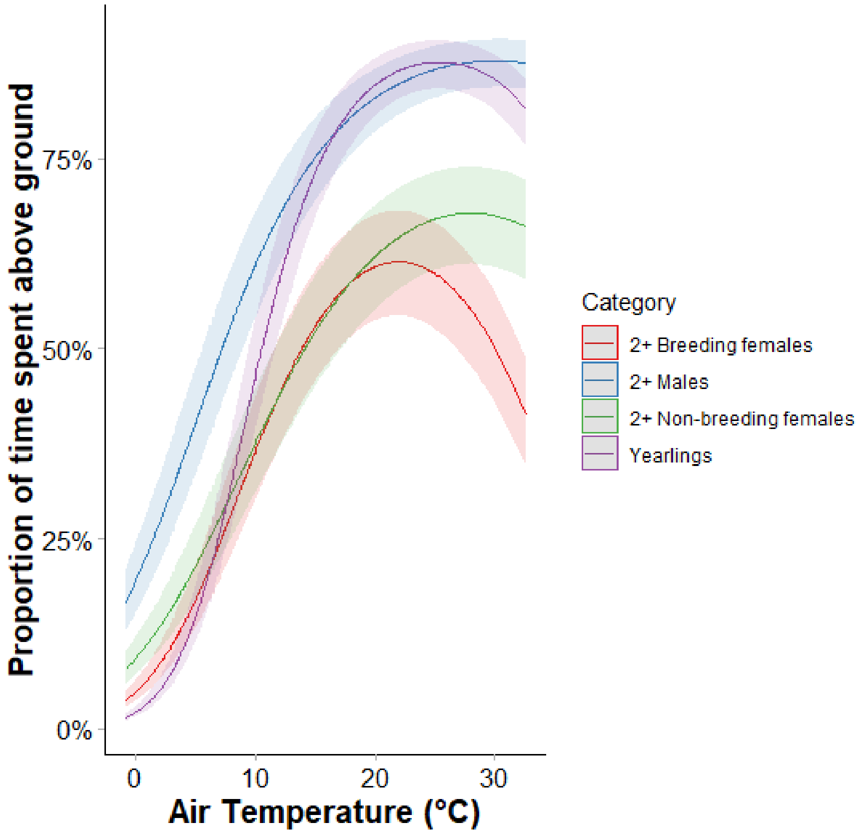


Figure 45. *Proportion of time spent above ground from collar light data according to hourly temperature, as predicted by the best model (GLMM binomial including a linear and quadratic effect of daily temperature anomalies both in interaction with individual age/sex/breeding status categories).*

4. Plasticity in foraging behavior in relation to ambient temperature

a) <u>Behavioral time budgets in relation to ambient temperature</u>

The relationship between the percentage of time spent foraging and daily temperature anomaly varied according to individual sex/age/breeding status categories (interaction in the best model; ΔAIC = 3.83). Yearlings and adult males (2+) tended to forage less when air temperature was higher (LMMs, yearlings: est. = -1.6 ± 0.4 % foraging, t = -3.667, p = 0.002, n = 22 weeks, 2+ males: est. = -1.1 ± 0.5 %, t = -2.258, p = 0.034, n = 24) but these effects were not statistically significant regarding breeding and non-breeding adult females (breeding 2+ females: est. = -0.2 ± 0.6, t = -0.295, p = 0.774, n = 21, non-breeding 2+ females: est. = -0.5 ± 0.5, t = -0.823, p = 0.421, n = 21, Figure 46).

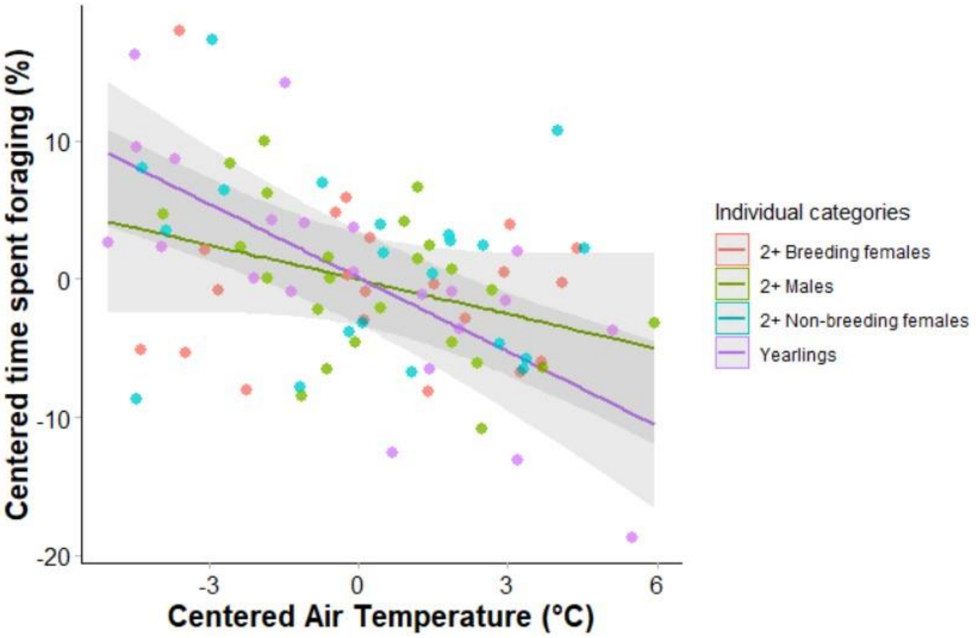


Figure 46. *Relationship between the centered air temperature and the centered time spent foraging (% of the total time-budget by week, n = 88) for each individual category (2+ Males in green, 2+ Breeding females in red, 2+ Non-breeding females in blue, and Yearlings in purple). Significant effects are plotted as the slope (solid line) along with the 95% confidence interval (grey shaded area).*

b) Space use in relation to ambient temperature

Individual ground squirrels’ preferred foraging areas varied depending on ambient temperature. Foraging densities were higher towards the center of the meadow (yellow parts and close lines on Figure 47), where little shade is present, during colder days (less than 2.5 °C below average), whereas they were higher towards the meadow edges (western and northern), where trees (and shade) are present during warmer days (more than 2.8 °C above average). Similarly, foraging density on the lower (south-eastern) part of the meadow, exposed to sun, also decreased with increasing temperatures (both anomalies and absolute temperatures, Supplementary materials: Figure 53).

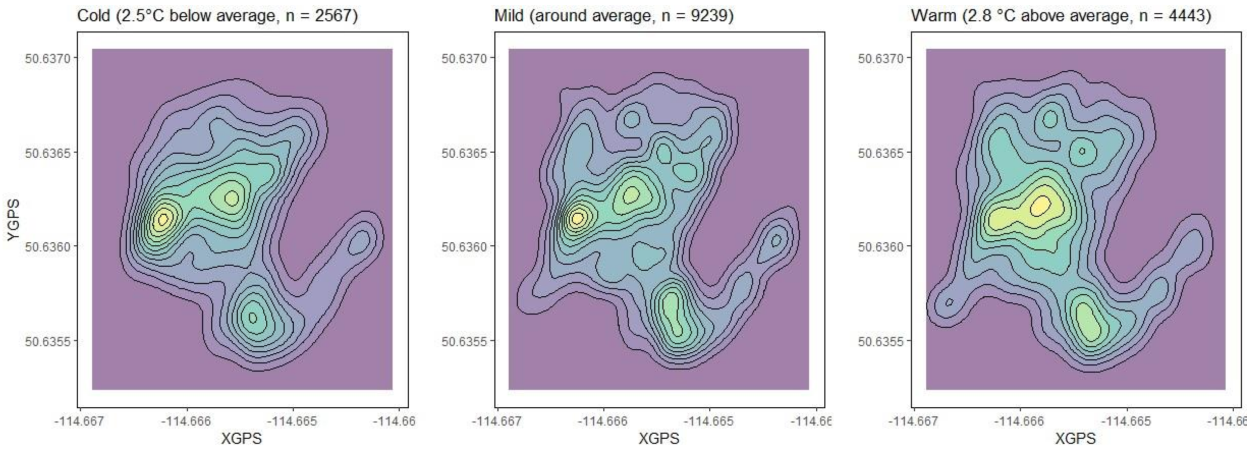


Figure 47. *Densities of foraging areas on the meadow for different thermal conditions: cold (air temperature less than 2.5°C below average, 25% quantile), mild (air temperature between 2.5 °C below and 2.8°C above average, 75% quantile), and warm (air temperature more than 2.8 above average °C). Higher densities are presented in lighter colors (yellow, light blue/green) and lower densities toward purple.*

5. Mass dynamics

a) Relationship with foraging

Ground squirrels did not tend to gain more mass when they foraged more than usual (no interaction with age/sex/breeding category in the best model, LMM, est. = +0.02 ± 0.77 g/2 weeks, t = 0.028, p = 0.978, n = 48, Figure 48).

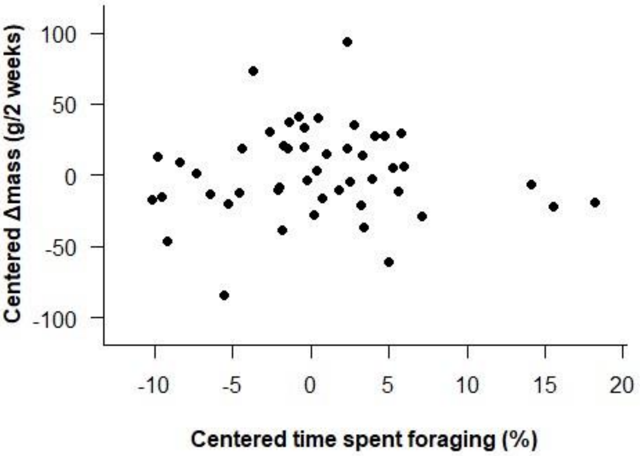


Figure 48. *Relationship between the time spent foraging (% of the total time-budget) and centered Δmass (g) calculated over two weeks, all individual categories and studied years included.*

b) <u>Relationship with air temperature</u>

Ground squirrels' mass gain was higher with more elevated air temperatures, but this effect diminished and reversed through time in the seasons (the best model retained no interaction with age/sex/breeding category, and statistically significant interaction between air temperature and weeks, est. = -1.9 ± 0.9, t = -2.031, p = 0.048, n = 48): centered Δmass increased with air temperature during the first weeks (LMM, est. = +13.3 ± 5.6 g/2 weeks per 1°C increase in air temperature, t = 2.375, p = 0.0222, n = 48) and decreased with air temperature during the last weeks (see dynamics on Figure 49).

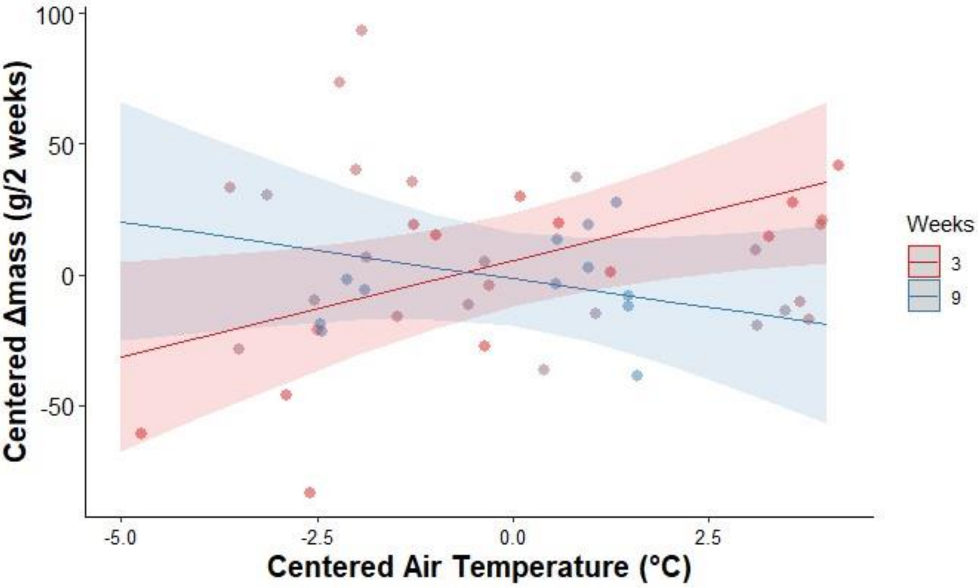


Figure 49. *Relationship between centered air temperature and centered Δmass (g) calculated over two weeks, all individual categories and studied years included. Slopes are plotted for the first two weeks of data ("Week 3", red line) and the last two weeks of data ("Week 9", blue line) are plotted along with the with the 95% confidence interval (shaded area).*

E. Discussion

By comparing average levels of behavior and seasonal mass gain within and among three contrasted years, we found that under warm conditions, ground-squirrels: (1) reduced above-ground activity, specifically foraging behavior, after a certain threshold in ambient temperature was met; (2) shifted activity patterns to the early and late hours of the day; and (3) concentrated their activity to meadow outskirts, close to the tree line, where more shade was likely available. We also found that individual mass gain during the season decreased at high temperatures, but changes in mass were not clearly related to foraging activity. Together, these results support the hypothesis that Columbian ground squirrels adapted to ambient temperatures and minimized thermoregulatory costs through behavioral plasticity.

The 2019, 2022 and 2023 study seasons were markedly different in terms of climate conditions at our study site. Over our 30-year long term study, climate anomalies calculated in reference to the long-term average indicated that 2022 was among the coldest years (on average 1.3 °C below average, 10 %), 2019 intermediate (0.4 °C below average, 3 %), and 2023 was the hottest year on record (2.2 °C above average, 17 %), with an extended period above normal average (51 days from 23 April to 13 June when temperature was maintained above average but for three 1-day period). The microclimate patterns observed at our study site were consistent with the El Niño-Southern Oscillation (ENSO), the breeding period of 2019 being characterized by a weak El Niño episode, 2022 by a moderate La Niña episode, and 2023 by a moderate to strong El Niño episode (ONI index, NOAA). These climate conditions provided ample contrast and variation to test for temperature effects on behavior.

As predicted by the co-adaptation hypothesis (Angilletta et al. 2010, Boyles et al. 2011), Columbian ground squirrels (strongly heterothermic endotherms) were able to maintain above-ground activity over a broad range of ambient (and likely body) temperatures, ranging from -0.9 °C to 32.6 °C. We found that subcutaneous T_b at capture was directly related to ambient temperature in our species. An increase in peripheral temperature associated with an increase in ambient temperature implies a decrease in the body core-skin temperature gradient with decreased conductivity and heat loss capacity (Lovegrove et al. 1991), and an increase in core temperature if physiological or behavioral thermoregulatory adaptations do not occur. Whereas this may be advantageous at low ambient temperatures, decreased heat loss can also become problematic in warm conditions. Consistently, ground squirrels appeared to show high plasticity in patterns of activity in relation to ambient temperature, which varied seasonally and differed between categories of individuals. The daily proportion of time spent above-ground showed a quadratic relation with ambient temperature, above-ground daily activity initially increasing with ambient temperature anomalies before decreasing again. This likely reflected conditions where animals used behavioral thermoregulation above and below critical temperatures defining their thermoneutral optimum.

Negative temperature anomalies were associated with days colder than average, and may have been days of relatively poor weather (rain, snow) known to negatively affect above-ground behavior in ground squirrels (Tamian et al. 2023). Cold days may have been prohibitively costly in terms of thermoregulation, at a time when the animals are active and body temperature is regulated around the point of normothermia. For instance, closely related Arctic ground squirrel (*Urocitellus parryii*) are known to have a lower critical temperature of 18°C (when not hibernating), below which metabolic thermoregulatory costs increase rapidly (Chappell 1981), and indeed proportionally decrease their above-ground activity with standard operative temperatures ranging from 17 °C to -2 °C. Although we did not measure standard operative temperatures in our study (which integrates radiant and convective thermal inputs with wind speed; Gagge 1940, Bakken 1976), it is interesting that patterns or activity are remarkably similar in our species, decreasing proportionally below average daily ambient temperatures of 14.3 °C to 17.4 °C, depending on categories of individuals. Similar behavioral patterns of daily activity in relation to low temperatures have also been observed in Thirteen-lined ground squirrels, *Ictidomys tridecemlineatus* (Vispo and Bakken 1993).

The energy costs of thermoregulation at lower ambient temperatures are further illustrated by patterns of foraging and body mass gain in our species: in weeks of lower ambient temperature than average, the proportion of foraging time-budget relative to other behaviors increased above the long-term average as well. In other words, when weeks were colder, animals spent less time above ground but devoted more of that time foraging, and this effect was more pronounced in yearlings than adults (see Figure 44). Yearlings have higher surface to volume ratios than adults (weighing on average 42 % less than adults, *viz.* 256 g *vs.* 445 g, at emergence from hibernation in our long-term data set), so that heat loss to the environment would have been greater in the former. Thus, it is not surprising that the proportion of time spent above ground *decreased* more rapidly – but the amount of time spent foraging *increased* more rapidly – in yearlings than adults. Similarly, mass gain over a couple of weeks was lower than average during periods colder than average, and this was especially true early on in the season. Finally, it is interesting to note that during cold days, the pattern of above-ground activity was highest between 1 pm and 5 pm (Figure 44), consistent with the idea that animals targeted the warmest hours of the day to conduct their daily activities.

Columbian ground squirrels also showed remarkable behavioral plasticity at high ambient temperatures. Under warmer conditions than average, the animals (1) spent less time above-ground; (2) increased their total foraging area as foraging activity shifted from the center to the edge of the meadow; and (3) shifted behavioral activities to earlier and later hours of the day. Shuttling between burrows and the surface periodically, foraging on the edge of the meadow where surrounding trees provided shaded areas (AT, CS, VAV; *personal observations*), and shifting patterns of activity to earlier and/or later hours of the day, were behavioral strategies probably allowing animals to benefit from microclimates of lower temperature and avoid the costs of thermoregulation above their upper critical temperature due to direct sun exposure. Shuttling strategies in response to high temperatures have previously been reported in other ground squirrel species, including at lower (Thirteen-lined ground squirrels; Vispo & Bakken 1993, antelope ground squirrels, *Ammospermophilus leucurus;* Chappell and Bartholomew 1981, Cape ground squirrels, *Xerus inauris*; Bennett et al. 1984) and higher (Arctic ground squirrel; Long et al. 2005) latitudes. On the other hand, subcutaneous temperatures of >42 °C recorded in our data indicate that individuals may have tolerated transient hyperthermia, perhaps in order to maximize the time spent-above ground or given locations and to maximize daily energy intake, consistent with optimal foraging modelling in this species (see Belovsky 1986).

Interestingly, responses to high temperatures differed between individual categories. Notably, breeding females and yearlings appeared to reduce their hourly above-ground activity more rapidly than males and non-breeding females at high temperature anomalies (warmer hours than average). Besides surface/volume likely explaining the marked decrease in above-ground activity for yearlings at high temperature anomalies (heat transfers being faster than adults), breeding females are constrained by lactation and the necessity to export both energy and water in the form of milk. Water stress, despite being importantly related to temperature, has generally received less focus than heat stress in ground squirrels, perhaps because water loss through sweat is thought to be low in these sciurid rodents (*e.g*. Chappell and Bartholomew 1981). Yet, sweat glands are known to occur on the volar surface of the foot in Columbian ground squirrels (Kivett 1978), and may contribute to increased evaporative water loss at high temperature. This might explain why we did not detect an association between mass gain and foraging time, if mass changes were primarily related to changes in body water content, animals losing more body water with higher temperatures. Our current data indicate that water content of their forage ranges from 56 to 68 %, on average over the season, suggesting that water may play a more important role in body mass variations than previously thought, especially in warm weather conditions. In addition, whereas foraging behavior during periods of above-ground activity decreased relative to normal when temperature anomalies were high for adult males and yearlings, this was not the case for females. A decrease in physical activity in favor of more "static" behaviors such as vigilance (generally standing or laying on four legs), comfort (self-grooming), or resting (no movement and eye closing) would reduce heat production (see Suplementary Materials: Figure 55). Yet, for breeding females that strongly rely on daily energy income to sustain reproduction (Broussard et al. 2005), such decreases may be prohibitive, explaining why they are not observed reducing their foraging activity during these periods. Why similar patterns are not observed in non-breeding females, though, is unclear, and warrants further research into putative constraints requiring foraging activity to be maintained.

To conclude, our results suggest that Columbian ground squirrels minimized the costs of thermoregulation both to low and critically high temperatures, through high behavioral plasticity. Whereas patterns of activity shifted both spatially and temporally, a proper understanding of how these animals optimally exploit the thermal heterogeneity of their habitat will require further studies of microclimatic conditions considering tree shade and vegetation height, under varying climatic conditions. In addition, heat and water challenges being importantly linked to forage resources, it seems necessary to integrate temporal and spatial variation in vegetation water content and nutritional quality to completely understand how these variables factor into behavioral decisions regarding individual energetics and reproduction. Finally, as the occurrence of extreme events, such as heat waves, are predicted to increase in the future, one important question is whether these animals will compensate for climate change by gradually shifting to crepuscular or nocturnal activity as suggested for some rodent species by biophysical models (Levy et al. 2019; see Briscoe et al. 2023 for a review on mechanistic forecasting).

F. Supplementary Materials

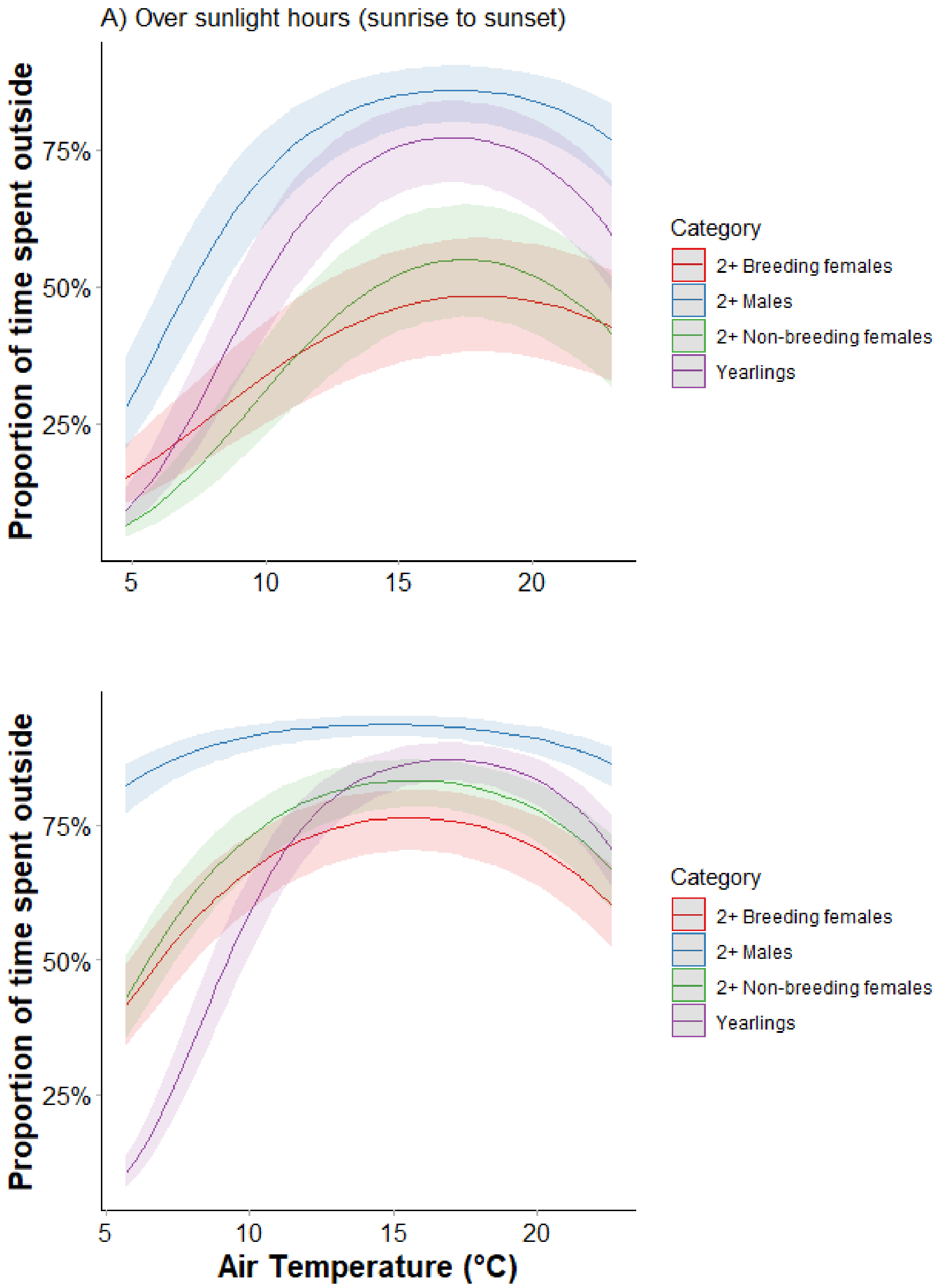


Figure 50. *Proportion of time spent above ground from collar light data according to daily absolute temperature, as predicted by the best model (GLMM binomial including a linear and quadratic effect of daily temperature both in interaction with individual age/sex/breeding status categories). (A) based on daylight hours (sunrise and sunset estimated every day) and (B) based on daily activity (emergence and immergence estimated per individual every day).*

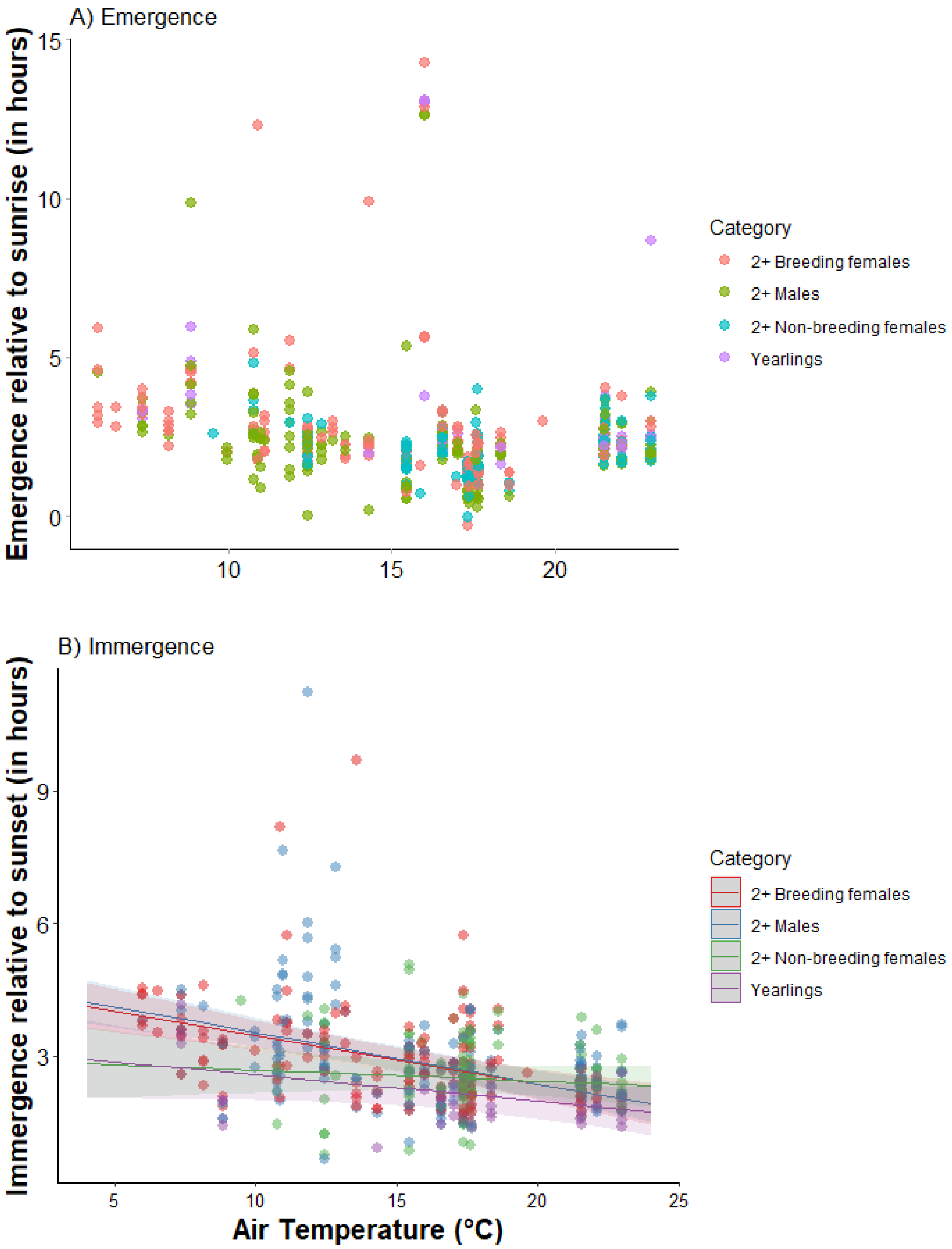


Figure 51. Daily start and end of activities from collar light data according to daily absolute temperature. *(A) Emergence of burrow (in hours relative to sunrise) and (B) Immergence into burrow (in hours relative to sunset). The best linear mixed model for emergence only included the individual age/sex/breeding status category, while for immergence it included individual age/sex/breeding status category in interaction with temperature.*

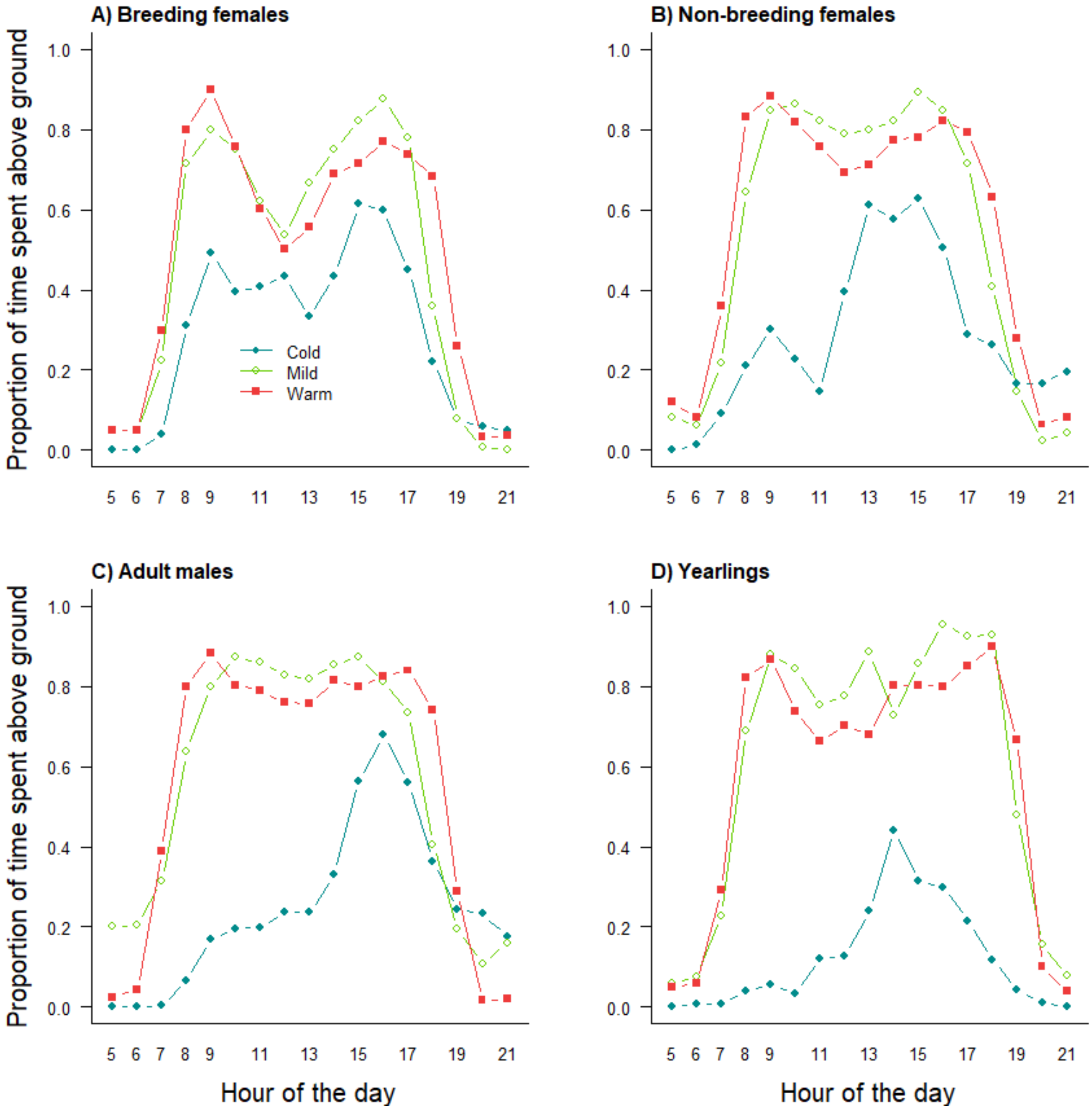


Figure 52. Daily above-ground activity patterns. *Hourly proportion of time spent above ground according to hours of the day for cold (in blue), mild (in green) and warm (in red) days in (A) breeding females, (B) non-breeding females, (C) adult males and (D) yearlings. Days were grouped in cold, mild and warm days based on the quartile of daily absolute temperatures (cold days < 25% quartile = 11 °C < mild days < 17 °C =75% quartile < warm days).*

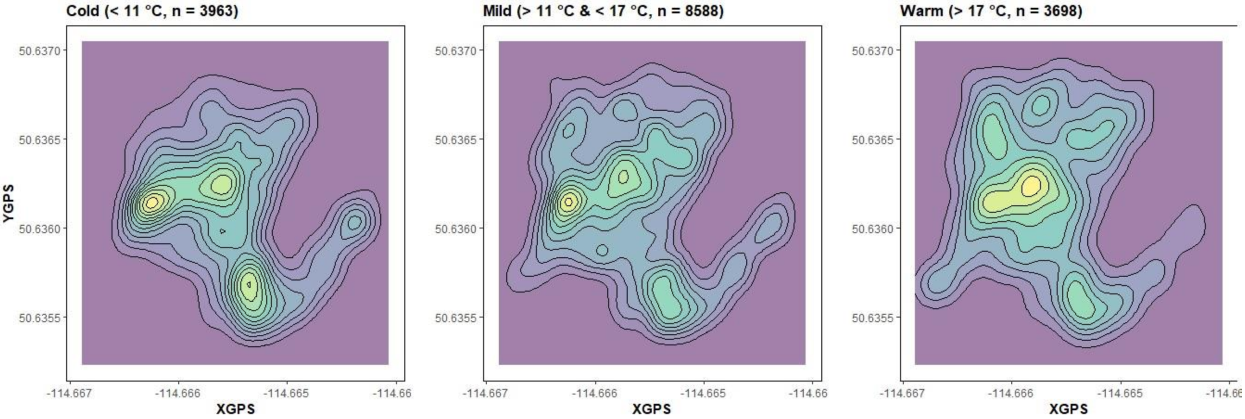


Figure 53. *Densities of foraging areas on the meadow for different thermal conditions: cold, mild, and warm (cold < 25% quartile = 11 °C < mild < 17 °C =75% quartile < warm). Higher densities are presented in lighter colors (yellow, light blue/green) and lower densities toward purple.*

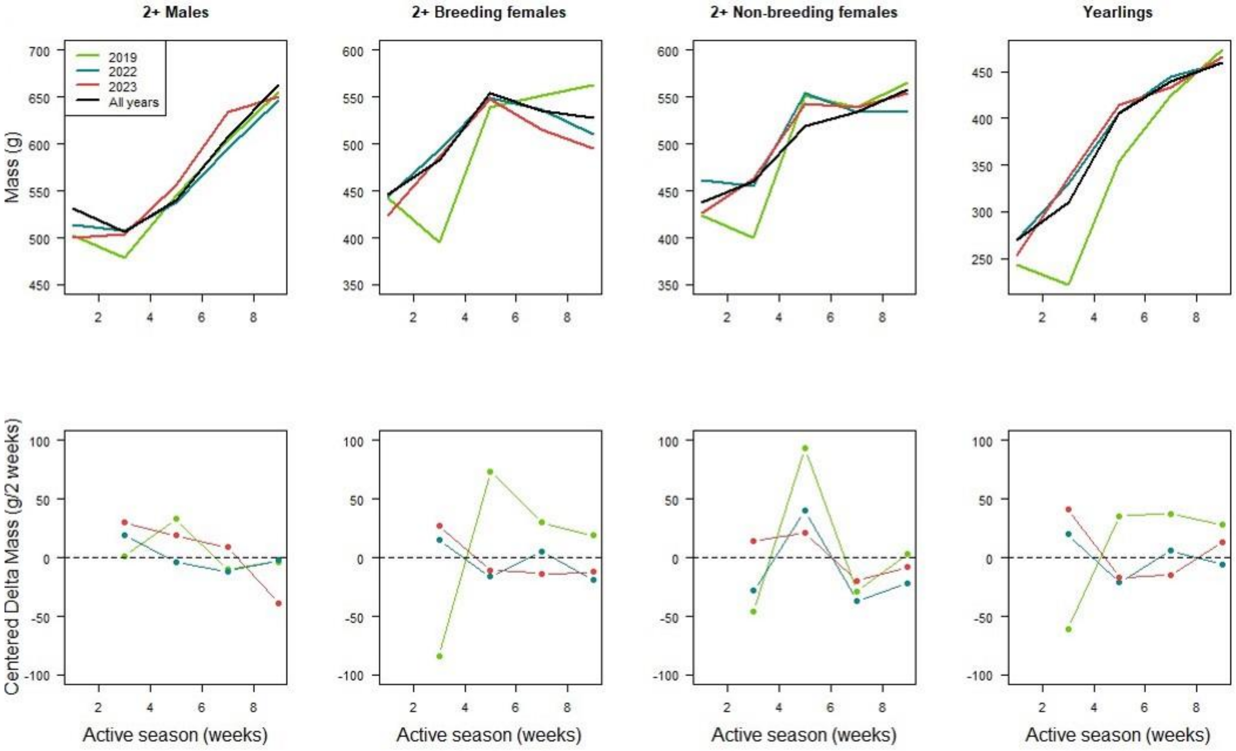


Figure 54. *Body mass dynamics (upper part) and centered Δmass (lower part) through the season for each individual category. Trapping masses are averaged and Δmass are calculated over two weeks for sufficient sample sizes. The different studied years are presented (2019 in green, 2022 in blue, and 2023 in red) and the mean general Δmass over all the years was subtracted to each year to obtain centered values (mean mass as the black line on the upper part).*

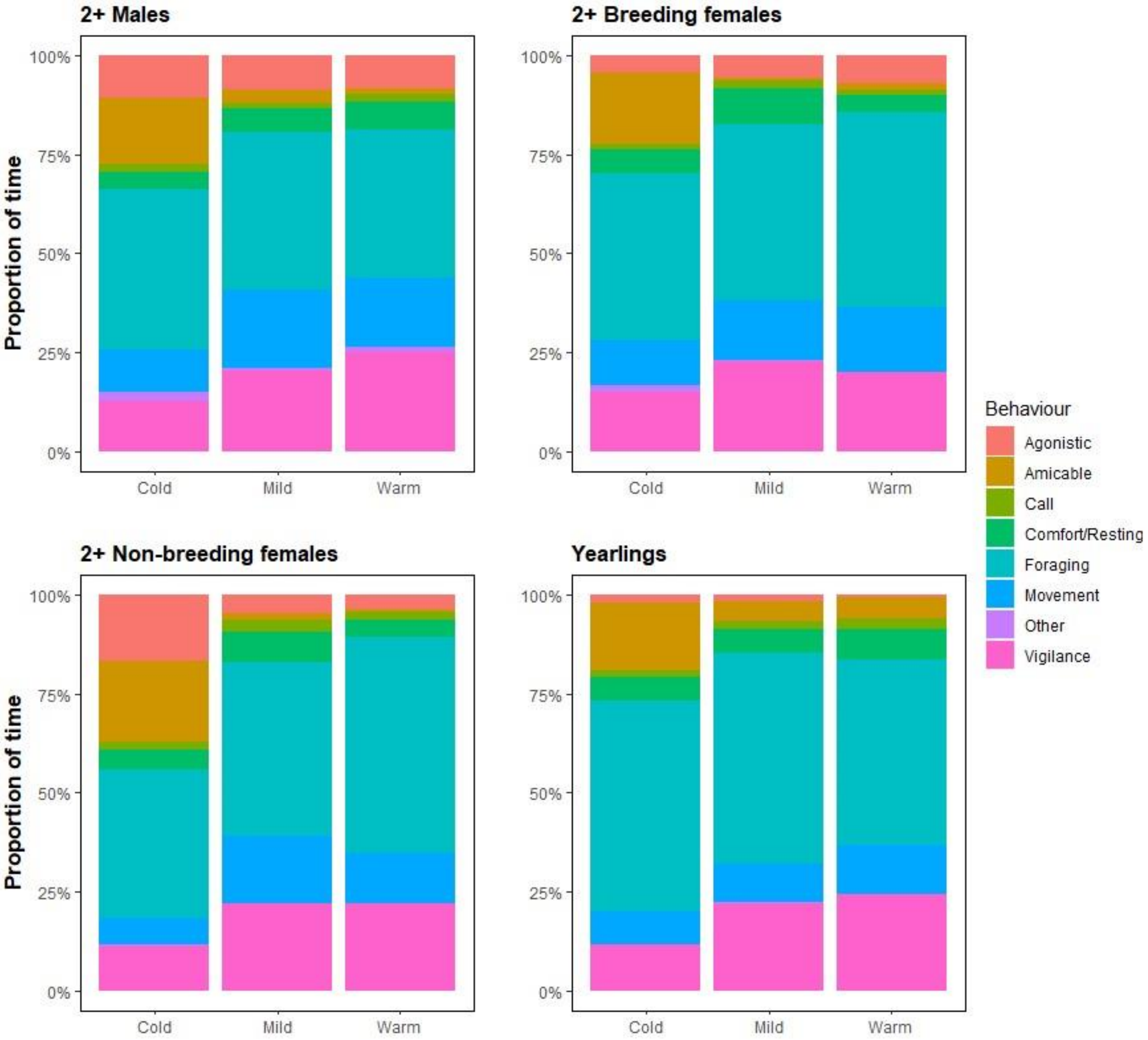
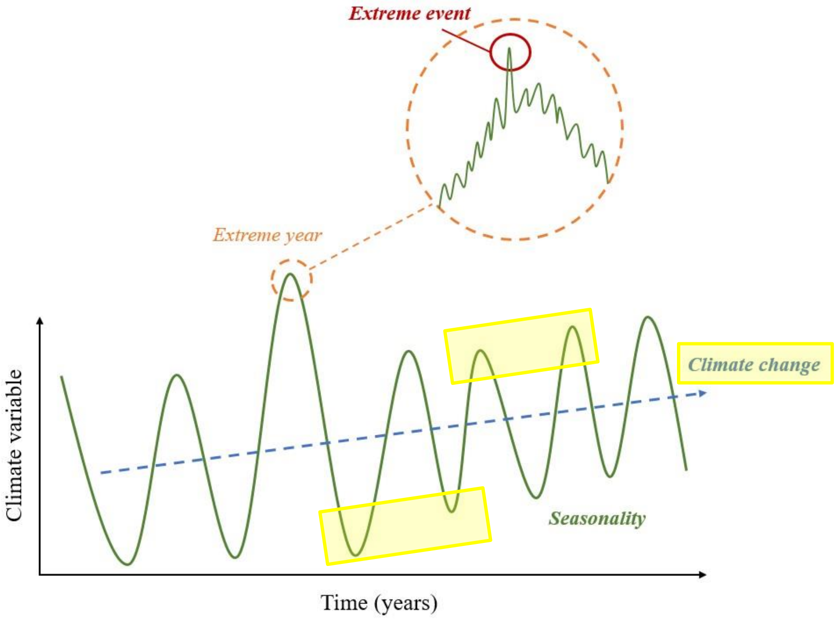


Figure 55. *Behavioural time-budgets for each sex/age/breeding category (adult males, adult breeding females, adult non-breeding females, and yearlings) and thermal conditions (Cold: quantile < 25%, Mild: 25% > quantile < 75%, Warm: quantile > 75% of centered air temperatures). Compared to the ethogram in the general materials and methods, some behaviors have been grouped (alarm calls and other calls into "calls", comfort and resting, digging, emerge, exploration, marking, nest material in "movement", and mate guarding into "other") to identify more "static" versus "dynamics" behaviors.*

From seasonal thermal conditions to interannual variations and long-term trends in climate:

How do climate conditions vary temporally and spatially? How do Columbian ground squirrels' phenology respond to these variations?

Columbian ground squirrels respond to changes in daily air temperature during their breeding season, and the modification of their behavior and physiological conditions may affect their entire annual life cycle. Thus, the question remains of how their phenology, an important life-history trait for hibernating species, varies with long-term climate trends considered both during the active season and during hibernation.



Indeed, for now we examined only the energetic constraints during the active season (foraging, mass gain) but winter conditions are also energetically challenging for animals, and are generally less studied (Williams et al. 2015). Additionally, in the next chapters the time scale will switch from only a few years to long-term trends and interannual comparisons.

CHAPTER 3

Long-term trends and phenology

Integrating microclimatic variation in phenological responses to climate change: a 28-year study in a hibernating mammal.

Running head: Phenology and microclimate

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[Article published in *Ecosphere* in 2022]

13(5), e4059, DOI: https://doi.org/10.1002/ecs2.4059

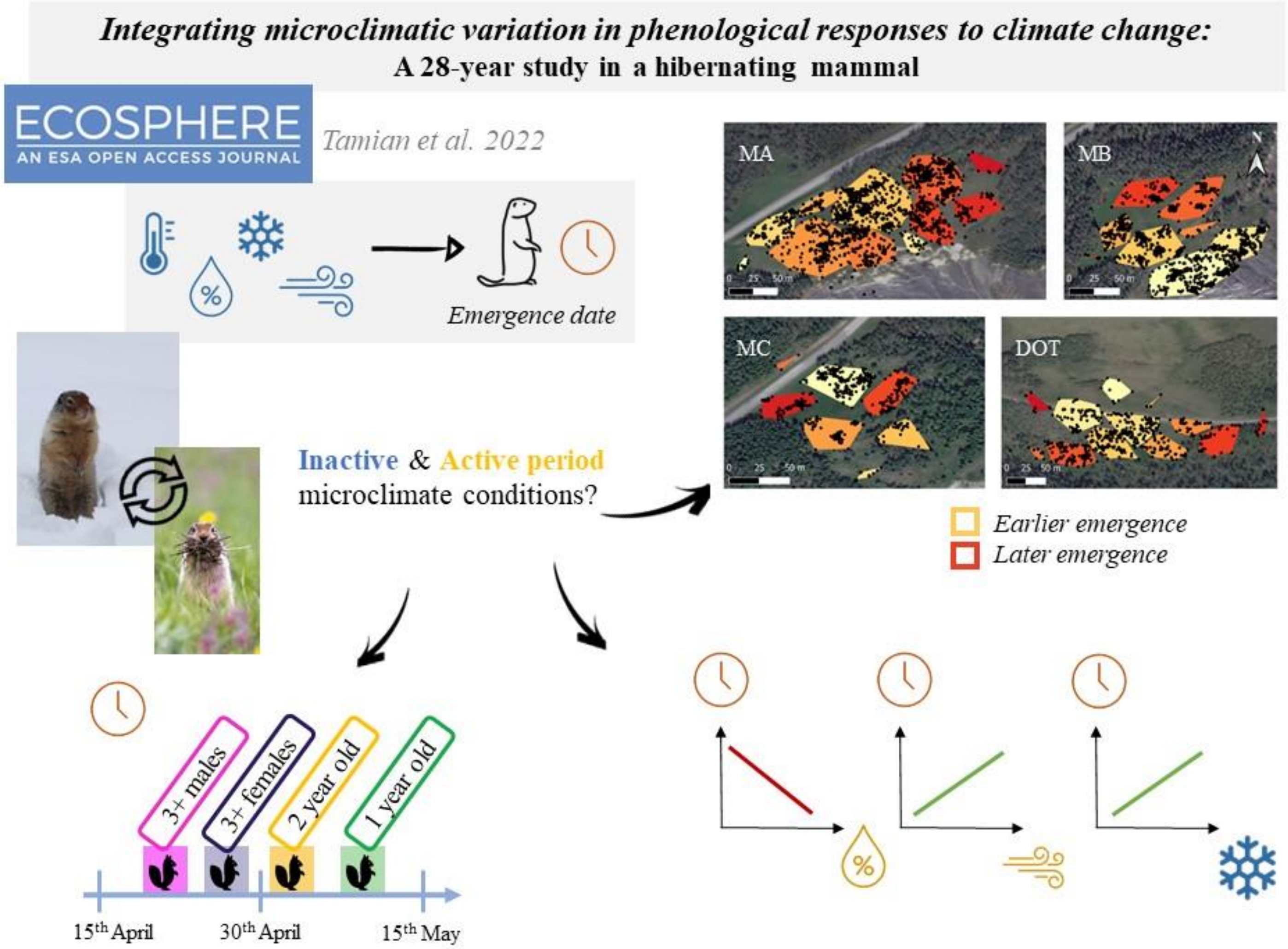
The Method section in this chapter has been slightly modified compared to the published version to avoid repetition.

A. Abstract

Phenological shifts associated with directional changes in climate, resulting in earlier spring activities, have been documented in several animal species. However, the extent to which species respond to overall climate change versus local climate variation is rarely studied. In addition, climate data are usually averaged over large spatial scales, even though local heterogeneity in habitats may be high, and species might be more susceptible to changes in local rather than global climate conditions. In this study, we examined the effects of spatio-temporal climate variation and climate change on the phenology of a hibernating mountain rodent, the Columbian ground squirrel (*Urocitellus columbianus*). Over 28 years of research (1992-2019), we studied the relationship between the microclimatic conditions experienced by adult and juvenile ground squirrels from four neighboring meadows, and their dates of emergence from hibernation. We used a microclimate model to calculate microclimate variables (local snow depth, soil temperature, air temperature, wind speed, and humidity) at an hourly scale, a 5-m spatial resolution, and at animal height on the study sites over 28 years. Emergence dates varied with age and sex, among years, as well as among and within meadows, with some areas averaging earlier emergence from hibernation than others (up to 10 days of difference). While emergence dates tended to be delayed throughout the study period, long-term temporal changes and interannual variability in emergence dates differed among meadows and depended on individual age and sex. Dates of hibernation emergence were correlated with local climate variables considered either during hibernation or during the preceding summer. Ground squirrels emerged earlier in years or at locations when/where snow melted earlier (years: all individuals excluding 2-year-old males, locations: yearlings and older females), and when the previous summer was less windy (≥3-year-old individuals) and more humid (2-year-old males). Two-year-old male ground squirrels also emerged later in locations where snow depth during winter was higher. Using a microclimate model provided more realistic predictions of phenological responses to climate, highlighting its potential for research on animal responses to abiotic change.

Keywords: Phenology, climate variability, hibernation, exogenous effects, microclimate model, spatial variability, snow cover.

B. Graphical abstract



C. Introduction

The extent to which animals are able to adapt to current climate change (e.g. earlier springs in the temperate zones) and increasing fluctuations in extreme events (e.g. storms, droughts, late snow falls) is of major concern in terms of biodiversity loss and ecosystem function (Walther et al. 2002b, Martay et al. 2017, Pires et al. 2018). In particular, variations in winter and spring onsets are occurring globally in seasonal ecosystems around the world (Menzel and Fabian 1999, Kunkel et al. 2004, Kreyling and Henry 2011, Pachauri et al. 2014a, 2014b), affecting the annual timing, known as phenology, of life cycle events (e.g. reproduction) in many animal species (Sparks and Yates 1997, Charmantier et al. 2008b, Cohen et al. 2018). Phenological shifts are generally consistent with the direction of climate change in most animal species, such as earlier spring activities following earlier spring onsets (Parmesan and Yohe 2003, Radchuk et al. 2019). The strength of phenological shifts, however, varies greatly within and among species, between trophic levels, and between geographical locations, from little or no changes to marked changes in seasonal timing (Visser and Holleman 2001, Strode 2003, Winder and Schindler 2004, Parmesan 2007, Thackeray et al. 2016, Chmura et al. 2019, Radchuk et al. 2019, see Parmesan and Yohe 2003, Root et al. 2003 for meta-analyses).

Because global climate change is altering phenological cycles (Menzel 2002, Cleland et al. 2007, Chmura et al. 2019), and because phenological shifts have marked consequences for individual reproduction (e.g. match-mismatch of reproduction and peak resource availability; Visser and Both 2005, Durant et al. 2007) and population dynamics (Ozgul et al. 2010), it is critical to understand how species phenology responds to fluctuations in climate. The need to distinguish phenological responses to long-term climate change from responses to short-term climate variability complicates this understanding. While climate variability usually considers year-to-year variation in climate, climate change only refers to those variations that persist for longer periods, usually greater than a decade (World Meteorological Organization 2015). Another layer of complexity is added by the temporal and spatial scales over which inferences are drawn. Numerous studies rely on satellite or weather station data, often collected several kilometers or tens of kilometers from studied populations (Graae et al. 2012, Potter et al. 2013). However, individuals are often more affected by local climate conditions, which are shaped by spatial heterogeneity in habitat topography, soil composition, or sun exposure (Suggitt et al. 2011, Maclean et al. 2017, Zellweger et al. 2019). This is especially true in rugged areas, such as mountain habitats, where heterogeneity is high over relatively small spatial scales (Huggett

1995, Nagy and Grabherr 2009, Gultepe 2015). Local contrasts in slope angles, sun exposure, soil composition, and vegetation cover can cause climate to vary, through variations in solar radiation, wind exposure or humidity, from scales of 1-10 kilometers (*i.e.*, mesoclimates) to scales of 0-100 meters (*i.e.*, microclimates) (Barry 1992). Due to a lack of climate data at a fine resolution (<1 km), few studies have examined phenological responses to microclimatic conditions (James et al. 1984, Suggitt et al. 2011, Potter et al. 2013, Varner and Dearing 2014, Paruchuri et al. 2019).

In this study, we examined the relationship between the phenology of hibernation and climate in a mountain hibernator, the Columbian ground squirrel (*Urocitellus columbianus*). We focused on hibernation emergence dates (ED) for individual ground squirrels from four neighboring meadows monitored over a period ranging from 18-28 years, depending on the meadow. Our objectives were to (1) examine spatial (among and within the four meadows) and temporal (over the years) variation of ground squirrel ED and to (2) determine how this variation might be explained by local and temporal variation in microclimatic conditions (scale of tens of meters, as experienced by the animals). Columbian ground squirrels are colonial ground-dwelling sciurid rodents inhabiting burrow systems in subalpine meadows of the northern regions of the Rocky Mountains. Columbian ground squirrels are an interesting model system to investigate changes in phenology in relation to microclimates. These relatively long-lived animals (the oldest squirrel in our data set lived up to 14-yo.) hibernate for ~8 months of the year, and are only active for a short summer period during which reproduction occurs (Murie and Harris 1982, Young 1990b, Dobson et al. 1992). Because of this short active season, phenological shifts may be important in determining animal reproduction or ability to fatten for winter. A long-term delay adult female ED, correlating with lower spring temperature and delayed snowmelt, has already been reported (9.4 days over a 20-year period) in one of our four populations (Lane et al. 2012). While this study suggested phenological plasticity in ground squirrel ED, likely due to climate change (see also Dobson et al. 2016), it only considered adult females from a single population and used wide-scale climate variables to test for their effects on ground squirrel ED (the weather data came from a meteorological station located in a prairie habitat, some 55km from the study site). Yet, although some emigration occurs (Wiggett et al. 1989), adult ground squirrels are largely highly philopatric and have a relatively small ranges over their lifetimes (e.g. female range rarely exceeds 1 km^2 and male range around 4.2km^2; Murie and Harris 1978, Festa-Bianchet and Boag 1982, Harris and Murie 1984, Arnaud et al. 2012). Thus, these animals are more likely to respond to very local climate conditions than to wide-scale climate variations. In addition, previous studies have shown that adult males emerge from hibernation first, followed by adult females, and lastly yearlings (Murie and Harris 1982, Dobson et al. 1992). However, data was lacking on whether different age or sex-categories show similar or different phenological plasticity to local climate, both within and between neighboring populations (resolution of ~300m).

To study the relationship between emergence date and climate, we performed a three-step analysis. First, we analyzed how variable ED was both within and among four different meadows over a 28-year period. We analyzed variation in ED in relation to individual (sex and age) and environmental characteristics (year, hibernation location) that were expected to affect hibernation emergence. Second, although our four study sites are located in the same valley, they present observable variability in slope, topography, and exposure to sun both within and among sites. Thus, we expected local climate variations to occur, affecting variation in ED both among meadows and within different parts of a given meadow. In the absence of local weather stations, we used a microclimate model (see Kearney et al. 2020, Saleeba et al. 2020, Lembrechts and Lenoir 2020, Maclean 2020, Kearney 2020) to predict climate since 1992 on the four study sites, at a 5-m resolution, and at the height of the animal (10 cm above ground, or 1-m below ground, roughly the depth of a hibernaculum; Young 1990a). We then analyzed climate variation and tested whether we could detect a directional change in local climate patterns over the past 28 years (viz. climate change). Finally, we tested whether variation in ED could be explained by the local climate conditions encountered by the animals. Because emergence from hibernation is strongly related to the energetic state of individuals at the end of the hibernation (Norquay and Willis 2014, Williams et al. 2014a, Fietz et al. 2020), there are two theoretical ways that climate could affect ground squirrel emergence dates: 1) by modifying individual energy expenditure during hibernation (Humphries et al. 2002, Pretzlaff and Dausmann 2012, Geiser 2013), and 2) through carry-over effects from one active season to another (Norris 2005, Saino et al. 2017, Moore and Martin 2019). On one hand, the energy available and stored by individuals during the active period should affect their ability to fast during hibernation (as Columbian ground squirrels are “fat-storing” hibernators, Murie and Boag 1984, Humphries et al. 2003). On the other hand, harsh climate conditions during winter could also affect ground squirrel energy expenditure, and thus influence individual ability to fast and hibernate (Davis 1976, Humphries et al. 2004, Fietz et al. 2020).

As the environment is multifactorial by nature, our analyses included several climate variables that could affect either energy storage before hibernation or energy expenditure during hibernation (as recommended by McGuire et al. 2021). Specifically, we expected areas or years with earlier snowmelt and higher underground temperature to be associated with earlier ED (see Lane et al. 2012). The presence and depth of the snow cover directly impacts soil insulation and ground temperature, potential external cues for hibernating individuals. In addition, we expected carry-over effects of climate on ED to occur from one year to the next. During hibernation, ground squirrels rely almost exclusively on body fat (Shaw 1926). Overwinter survival depends on fat reserves accumulated during the previous season (e.g. Murie and Boag 1984), and low fat deposition is likely to affect ED the subsequent year. We did not measure body mass before hibernation in our study, but we reasonably expected foraging conditions (length and quality of forage) during the previous summer to directly reflect the amount of fat accumulated and burned during winter (Dobson et al. 1992, Neuhaus 2000). Thus, we expected unfavorable forage conditions – e.g. low rainfall (reflected by humidity) to be associated with low primary productivity (Dobson and Kjelgaard 1985b, Neuhaus et al. 1999, Dobson et al. 2016), and strong winds to be associated with more time spent by animals in vigilance rather than foraging (Fairbanks and Dobson 2007). Through the decrease of fur thermal insulation (McCafferty et al. 2017), humidity might also negatively affect the time foraging, which will have detrimental effects on the accumulation of fat stores and ultimately on hibernation duration. This may cause individuals to emerge earlier due to the early depletion of overwintering fat stores, or conversely to delay emergence by more pronounced hypometabolism and a longer torpor period until resource availability is high again (i.e. avoiding early arousals when resource availability is low). Juveniles, which are less able to mitigate the survival costs of low-fat energy stores during hibernation, might experience this more than adults (Murie and Boag 1984). Alternately, more favorable conditions during the previous summer could also allow for longer hibernation periods and later ED in juveniles, allowing them to avoid the period of high territorial aggression displayed by reproducing adults early in the season (Murie and Harris 1978, 2011). Taken together, the joint investigation of summer and winter condition effects on ED in different neighboring populations was expected to provide us with a comprehensive understanding of how these mountain-adapted rodents deal with local heterogeneity in weather conditions, and whether different populations respond differently to long-term changes in climate patterns.

D. Materials & Methods

1. Emergence date sampling

Emergence dates were recorded on the four different meadows as described in general materials and methods. At the time when study was conducted, available monitoring data were continuing until 2019 only.

a) Age classification

Because male Columbian ground squirrels emigrate, and new individuals occasionally leave and arrive on meadows, some individuals on the Meadows A, B, C, and DOT were not known from birth (19% of the cases overall, 455/2436). For these individuals, recorded age resulted from estimates of physical characteristics mainly distinguishing between yearlings, 2-year-old, and older individuals. To establish meaningful age bins in further analyses we conducted a preliminary analysis investigating how emergence date varied according to age using individuals of known age (n = 4681 observations, N = 1981 individuals). We ran a linear mixed effects model (LMM) of emergence date (dependent variable) as a function of age (independent categorical variable), specifying individual identity as a random factor in the model to account for repeated measures of individuals among years. Tukey HSD tests were applied for post-hoc comparisons. Emergence date of one-year-old individuals (ordinal date ± SE = 126.5 ± 0.3 days) was significantly later than all other age categories (Tukey HSD, $p < 0.001$). For 2-year-old individuals, emergence ordinal date (121.7 ± 0.3 days) was significantly earlier than yearlings ($p < 0.001$) but significantly later than older age categories (116.7 ± 0.2 days; $p < 0.001$). No other significant differences were found among older age categories (all $p > 0.05$). Thus, individuals aged 3 and older were combined in a unique age category (≥3-year-old) for subsequent analyses allowing us to use all existing ED data (n = 5873 observations in total, N = 2436 individuals). Sample sizes per meadow, year and age and sex classes are provided in Supplementary Materials: Table 4).

b) Spatial clustering

Hibernacula are usually used by the animals during a few days after emergence until collective burrows were re-excavated for the active season. First capture locations on the

Cartesian grid should thus provide a reasonable approximation of the area where individuals hibernated. Recorded Cartesian coordinates were converted to GPS coordinates using a database of GPS and Cartesian coordinates collected in 2018-2019. We estimated the error associated with the conversion of Cartesian to GPS coordinates at a median of 5.5 ± (SD) 3.4m (n = 225 coordinates). Coordinates were collected for 74% of the emergences, 4351/5873. The missing emergence sites were before 1994 on MA, 1999 on MB, 2006 on MC, and 2012 on DOT. Emergence locations were also not recorded in 2000-2007 on MA. Thus, sample sizes varied among years.

To evaluate spatial heterogeneity in emergence dates within a meadow, we investigated the spatial autocorrelation of emergence dates. Specifically, we examined whether correlation between emergence dates at two locations depended on the distance between them. To do so, we used variograms (Matheron 1963) that measured the value of the spatial variance at different distance intervals. For any two data points, we measured their spatial distance and estimated the variance in ED. The variance was then averaged over bins of distance intervals. Empirical variograms were based on all emergence locations, but with pairs of points considered only if points were in the same year and within the same meadow. We modelled variograms using a least square method (Rivoirard et al. 2008). The empirical variogram of emergence date exhibited spatial structure (i.e., increasing variance when the distance between sampling points increased before levelling off; see Supplementary Materials: Figure 63), meaning that the emergence dates were not random, such that spatially close locations had closer emergence dates than more distant locations. Based on the modelled variogram, we found that variance in emergence date stabilized at a range of about 40 m. Thus, hibernation emergence locations were grouped into spatially relevant clusters according to their GPS coordinates to understand whether individuals on some parts of the meadow emerged earlier than others.

We built clusters by grouping emergence locations according to an iterative algorithm (adapted from Woillez et al. 2007, see also Saraux et al. 2014). The algorithm started with each location as its own cluster. Each iteration of the algorithm then: (1) calculated the distances between the center of gravity of all clusters on the meadow; (2) merged the two closest clusters if the shortest distance among all cluster distances was below a threshold distance of 40 m (according to above variogram results). The process was reiterated until none of the distances between clusters were lower than 40 m. In total, we defined eleven, eight, seven, and twelve clusters for Meadows A, B, C, and DOT respectively (named differently depending on the meadow), which corresponded to hotspots of ground squirrel emergences in the habitat (compared to zones where ground squirrels were rarely seen emerging). Emergence locations and built clusters were imported into QGIS (3.10.12 long-term released version, QGIS.org, QGIS Geographic Information System, 2021) and presented on a Google satellite map © 2021 (see Figure 56).

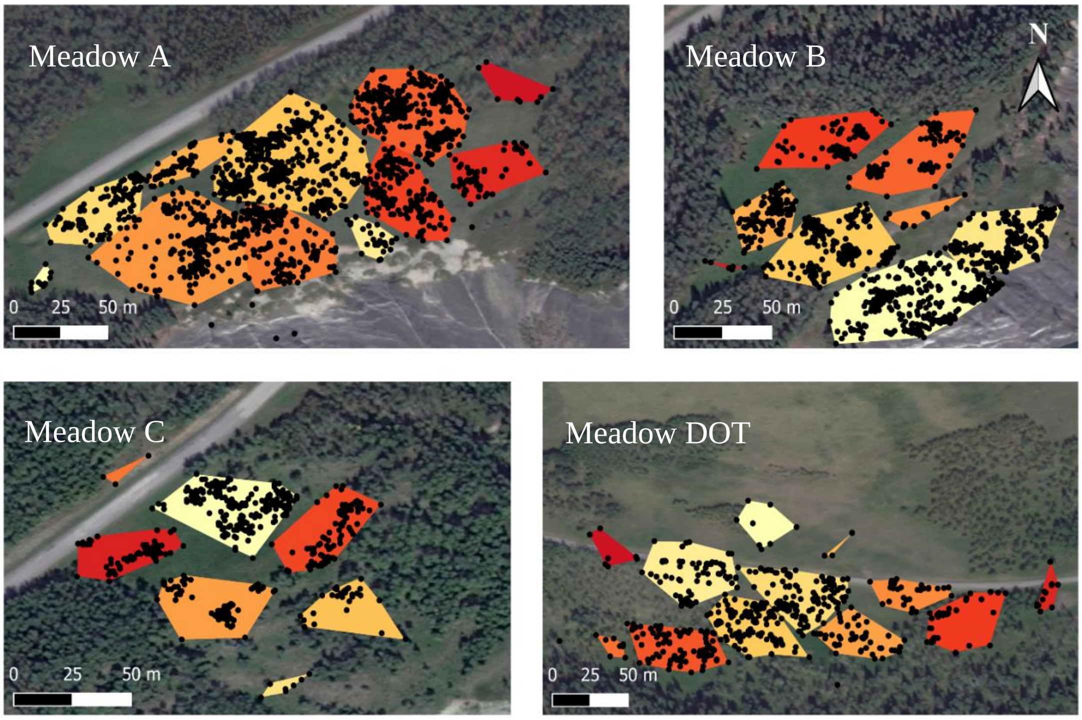


Figure 56. Emergence locations (black dots) grouped into spatial clusters on each meadow. *Clusters are represented by minimum convex polygons and colored according to their mean emergence date. Lighter colors correspond to earlier mean emergence date of the cluster (yellow and light orange) and darker colors correspond to later mean emergence date of the cluster (dark orange and red). Mean ordinal emergence date on clusters ranged from 118 to 127 on MA, from 118 to 130 on MB, from 120 to 124 on MC and from 114 to 124 on DOT.*

2. Microclimate data

We calculated hourly microclimatic conditions using the microclimate model (*micro_ncep* function), at a fine-scale resolution (5 x 5 m grid) for each meadow during the entire monitoring period (from 1992-2019). The model estimated snow depths (cm) on the ground as well as temperatures (°C), wind speed (m/s), and relative humidity (%) at 10 cm above ground (roughly the height of a ground squirrel). In addition, we estimated temperature at various soil depths below ground (0, 2.5, 5, 25, 50, 75, 100, 125, 150, and 200 cm). Hibernating burrows are usually around 70 cm in depth and rarely exceed 1 m (Young 1990a). Soil temperatures at various depths were highly correlated (see Supplementary Materials: Figure 64). Thus, we used 1-meter-deep temperatures as representative of hibernaculum conditions.

To estimate spatial and temporal variation of climate, we averaged the model-generated time series at two different spatial scales for each meadow: i) over the entire meadow and ii) separately within each cluster. Hourly data were averaged over 24-hour periods. Based on the average ordinal day of first hibernation emergence (older males) across all years (15 April ± 2 days) and previous studies showing that immergence occurs close to the beginning of August (Young 1990b, Neuhaus 2000), we further divided the year in two phases: the active season (15 April – 1 August), and the inactive (hibernation) season for the remainder of the year. This allowed us to test for possible effects of climate conditions during the winter and the previous summer on individual emergence dates. Besides air temperature (°C), soil temperature at 1m depth (°C), relative humidity (%), wind speed (m/s), and snow depth (cm), we considered two additional climate variables: first day of the calendar year without snow cover on the ground (over the entire meadow or within each given spatial cluster, in ordinal date), and number of days with snow falls during the active period for investigation of late snowstorms on ED.

3. Data analyses

a) Quantifying variance in emergence date

(1) Emergence date variance partitioning

To identify how much variance in ground squirrel emergence dates (ordinal days) was explained by endogenous (sex, age category, individual ID), spatial (meadow, emergence cluster within the meadow), and temporal (year) factors, we used a linear mixed effects model (LMM) with all variables specified as random terms (with *1|variable*):

$$\textit{Emergence date} \sim (1|sex) + (1|age_{category}) + (1|\ ID) + (1|meadow/cluster) + (1|year)\ \mathbf{[1]}$$

This model allowed us to partition variance components between the different random terms; (1|meadow/cluster) representing both the effect of the meadow, and the effect of the cluster nested within the meadow.

(2) Effects of sex and age on emergence date

Because sex and different age (especially sexually mature versus immature individuals) classes face different reproductive constraints, we tested the effects of individual endogenous characteristics (sex and age specified as independent variables) on emergence date, while controlling for spatial and temporal effects (set as random terms):

$$Emergence\ date \sim sex + age_{category} + sex{*}age_{category} + (1|ID) + (1|meadow/cluster) + (1|year)$$ **[2]**

b) Spatio-temporal variation in emergence date

(1) Spatial variation in emergence date

Because of differences in slope and exposure at different meadows, we expected emergence dates to vary spatially. To test for spatial variation in emergence date, we assessed among and within meadow differences using separate LMMs. Differences were tested by specifying emergence date as the dependent variable, meadow (or cluster) as an independent variable, and year and the sex-age category (determined based on sex and age results) as random terms:

$$Emergence\ date \sim meadow_{[MA\ vs.\ MB\ vs.\ MC\ vs.\ DOT]} + (1|year) + (1|\ sex\text{-}age_{category}) + (1|ID)$$ **[3]**

$$Emergence\ date \sim cluster + (1|year) + (1|sex\text{-}age_{category}) + (1|ID)$$ **[4]** (one LMM per meadow)

(2) Temporal variation in emergence date

Individuals might be phenotypically plastic, and time their ED to interannual variations, including directional changes in climate variables. We differentiate between interannual variation in ED and long-term directional changes in ED. Because emergence locations were not always known (especially in the early years, see above), we did not include spatial clusters in the following models [5] and [6], enabling us to study potential changes in time over a larger data set (e.g. starting in 1992 instead of 1999).

(i) Interannual variation in EDs

To explore interannual variation in hibernation emergence dates, we built generalized additive mixed models (GAMMs) with emergence date as the response variable, and year as the independent variable. This facilitated identification of changes between years, and detection of nonlinear temporal variation. Because temporal trends could differ among meadows or according to the individual sex-age category, we considered interactions (specified with "by =") between the continuous variable (year) and these factors by constructing separate GAMMs, keeping individual ID as a random variable:

$$\textit{Emergence date} \sim s(\textit{year}, \textit{by} = \textit{meadow}) + s(\textit{year}, \textit{by} = \textit{sex-age}_{\textit{category}}) + \textit{sex-age}_{\textit{category}} + \textit{meadow} + (1|\textit{ID})\ \mathbf{[5]}$$

(ii) Long-term directional changes in ED

In a context of climate change, most phenological studies showed advanced spring season (Walther et al. 2002b, Cleland et al. 2007, Dingemanse and Kalkman 2008). Yet, in Columbian ground squirrels, females older than 3 displayed a delay in emergence over a 20-year period (Lane et al. 2012). To test whether this long-term directional pattern was consistent over the 4 populations and over other sex/age categories, we ran the previous model (model **[5]**) as a LMM, and assessed interactions between continuous (year) and categorical (meadow or sex-age$_{\text{category}}$) variables:

$$\textit{Emergence date} \sim \textit{year} + \textit{sex-age}_{\textit{category}} + \textit{meadow} + \textit{year}*\textit{meadow} + \textit{year}*\textit{sex-age}_{\textit{category}} + (1|\textit{ID})\ \mathbf{[6]}$$

c) Spatio-temporal variation in climate

(1) Spatial variation in climate variables

To investigate microclimates, we assessed correlations among the 11 previously defined climate variables (air temperature during the active and inactive period, soil temperature at 1m depth during the active and inactive period, relative humidity during the active and inactive period, wind speed during the active and inactive period, snow depth during inactive period, first day of calendar year without snow and number of days with snow during the active period), using a Principal Component Analysis (PCA) on data averaged by year and by cluster on each meadow. The objective was to identify principal variations related to spatial variables (clusters and meadows) given the large amount of climate data at a micro-scale within the meadows. Spatial differences in climate were analyzed from resulting components (PCs), through LMs at two different scales:

PCs ~ meadow **[7]**
PCs ~ cluster **[8]**

Using our results (see below), only climate variables expected to affect emergence date were kept for subsequent analyses. These included (1) snow depth ($depth_{snow}$) and soil temperature ($temp_{soil}$) at a 1-meter depth during hibernation (inactive phase), and (2) air temperature ($temp_{air}$), relative humidity and wind speed during the previous summer (active phase), in addition to the ordinal date of spring snowmelt ($day_{snowmelt}$) and the number of days with snow during the active period ($days_{snow}$).

(2) Temporal variation in climate variables

Because interannual variability in climate often result from different processes: trend in climate (e.g., due to climate change), cyclic effects (e.g., El-Niño) and random year-to-year variation, we decided to use a 2-step approach. First, we focused on short-term variation using GAMs. Then we looked for directional trend in ED (as expected as a result of climate change) using LMs.

(i) Climate variation

To explore how climate varied, we ran GAMs for each climate variable as a function of year. This allowed identification of changes between years, and detection of nonlinear temporal variation. Because the interannual variability of climate could differ among meadows (or among clusters), we also considered interactions of years with these factors in the following GAMs:

Climate variable ~ s(year, by = meadow) + meadow **[9]**
Climate variable ~ s(year, by = cluster) + cluster **[10]** *(one GAM per meadow)*

(ii) Climate change

To test for long-term directional climate changes over 28 years, we ran the previous GAMs **[9]** & **[10]** as LMs **[11]** & **[12]**, with each climate variable as a dependent variable and the year, meadow (or cluster) and their interaction as independent variables:

*Climate variable ~ year*meadow + meadow* **[11]**
*Climate variable ~ year*cluster + cluster* **[12]** *(one LM per meadow)*

d) Climate – emergence date relationships

We tested how climate might affect emergence date through two processes: energy expenditure and carry-over effects of energy stored before hibernation. Because Columbian ground squirrels start breeding at 2-years-old, the emergence date of ≥ 2-year-old individuals may vary with climate differently between males and females. Thus, we considered five sex-age categories for these analyses: yearlings, 2-year-old females, 2-year-old males, ≥3-year-old females, and ≥3-year-old males.

(1) Mean relationships between climate & emergence date

To test for overall relationships between microclimates and hibernation emergence dates, emergence dates were averaged by year, meadow, and sex-age category. We built separate LMMs for the different age-sex categories, with emergence date as the dependent variable and climate variables (averaged by meadow and year) as independent variables:

Emergence date ~ depthsnow_winter + tempsoil_winter + tempair_active n-1 + humidityactive n-1 + windactive n-1 + daysnowmelt + dayssnow_active + (1|Year) + (1|Meadow) **[13] (one LMM per age-sex category)**

Individual responses in emergence date might differ due to different constraints between spatial heterogeneity (related to spatial living locations) and temporal heterogeneity (plasticity that matches interannual variation). To distinguish between temporal *vs.* spatial effects of climate on hibernation emergence dates, we ran two further analyses, building separate models for different sex-age categories.

(2) Year-centered approach: are spatial differences in ED related to spatial variation in climate?

To investigate spatial effects of climate variables on emergence date, we removed all temporal variation by centering emergence date and climate variables by year and sex-age category (mean of the years and category subtracted to each observation). During the active season, ground squirrels might visit most locations on the meadow in a single day. Because spatial clusters were defined from hibernation emergence locations and not the total active range of individuals, we included in the model only climate variables considered during hibernation (inactive phase). For each individual emergence date, we associated the climatic conditions of the given winter averaged over the spatial cluster in which the individual emerged. Thus, the linear mixed model was:

$$Emergence\ date \sim depth_{snow\_winter} + temp_{soil\_winter} + day_{snowmelt} + days_{snow\_active} + (1|cluster) + (1|ID) \quad \textbf{[14]}$$

(3) Spatial-centered approach: are interannual differences in ED related to interannual variation in climate?

To investigate temporal effects of climate variables on emergence date, we centered emergence date and all climate variables (including those considered during the active phase) by cluster and sex-age category. This way, spatial variations in climate were entirely removed from the data, and we tested whether yearly differences in climate could explain differences in emergence date among years.

$$Emergence\ date \sim depth_{snow\_winter} + temp_{soil\_winter} + temp_{air\_activen-1} + humidity_{activen-1} + wind_{activen-1} + day_{snowmelt} + days_{snow\_active} + (1|year) + (1|ID) \quad \textbf{[15]}$$

e) Statistical specifications

When the assumptions of a normal distribution of residuals were not met, we applied a Box-Cox transformation (from MASS R package, Box and Cox 1964) to the data (monotonous transformation), or removed extreme values from the data (i.e., emergence dates later than the 22nd of May for which we likely missed the actual emergence date, representing 2.7% (116/4351) of observations). Results with and without transformation or extreme values yielded similar results, and we present results obtained from complete and untransformed data sets for clarity (exceptions are specified in the results). Post-hoc multiple comparisons were run to compare all factor levels of categorical variables, using the Least Square Means (LSM) test, to account for multiple testing (Lenth 2016). GAM(M)s were run using the '*gam*' and '*gamm'* function in R ('mgcv' package, Wood 2017). Principal Component Analysis was run using the '*PCA'* function, and components accounting for a meaningful amount of variance (*i.e.* from 75%, O'Rourke and Hatcher 2013) were then considered in the models **[7]** and **[8]** ('FactoMineR' package, Lê et al. 2008). Whenever interaction effects between independent variables were included in LMs and LMMs, we compared the models with and without the interactions, and chose the final model according to Akaike's Information Criterion (AIC) (lowest AIC and for ΔAIC < 2, we chose the most parsimonious model, Burnham and Anderson 2004). However, we also analyzed meadows separately to assess difference in trends between populations. The results on the link between climate and emergence date are presented from the full models (i.e. with all tested variables included). Multicollinearity was checked and when variables were collinear, the one with the highest variance inflation factor (VIF) was removed, meaning that only variables with variance inflation factors below three remained in the models (Zuur et al. 2010). Finally, to allow comparisons of effect sizes in the last models **[13], [14],** and **[15]**, we standardized (by subtracting the mean and dividing by the standard deviation) all explanatory climate factors.

E. Results

1. Quantifying variance in emergence date

a) Emergence date variance partitioning

Across all individuals, meadows, and years, mean emergence date from hibernation was 2 May ± 4 days (ordinal date 122 ± 4) and ranged from 6 April to the 30 May (ordinal date 96-150). The model **[1]** with age, sex, meadow, cluster within meadow, year, and individual as random effects explained 65.7% of the total variance in emergence date (LMM, n = 4351 data points). Variables contributing most were age (26.3%), year (16.1%), and individual identity (14.6%). Within-meadow cluster, sex, and meadow explained fairly trivial amounts of variation, 3.9%, 3.1%, and 1.7% of the variance in ED, respectively.

b) Effects of sex and age on emergence date

Age, sex, and their interaction, were all retained in the best model explaining emergence date (LMM **[2]**, n = 4351, N = 1950 individuals, 26 years). We found differences in emergence date between males and females only in individuals ≥3 years old, with males emerging around 7.3 ± 0.4 days earlier than females (post-hoc LSM, z = -18.3, p < 0.001). Post-hoc comparisons revealed significant differences in emergence dates between four categories: yearlings, 2-year-old individuals, ≥3-year-old females and ≥3-year-old males (LSM, all p < 0.001). On average, older males (≥3-year-old) emerged first around 21 April (111.2 ± 0.2 days), followed by older females (≥3-year-old) around 28 April (118.5 ± 0.2 days), 2-year-old individuals around 2 May (121.8 ± 0.3 days), and finally one-year old individuals around 6 May (125.5 ± 0.2 days).

2. Spatio-temporal variation in emergence date

a) Spatial variation in emergence date

Analyses on spatial differences in hibernation emergence dates showed that individuals on DOT emerged 2.2 ± 0.5 days and 1.8 ± 0.4 days earlier than individuals on MC and MB, respectively. MC and MB individuals emerged 3.3 ± 0.4 days and 3.6 ± 0.3 days earlier than MA individuals (LMM **[3]**, post-hoc LSM: all p < 0.001, n = 5873, N = 2436 individuals, 28 years). Similarly, within-meadow differences in emergence dates were found between different clusters (see cluster colors on Figure 56). As an example, on MB, individuals in “bottom-west” cluster emerged between 1.9 ± 0.5 and 8.8 ± 2.5 days earlier than individuals from other clusters (LMM **[4]**, post-hoc LSM: p < 0.001, n = 1315, N = 579 individuals, 21 years). In contrast, individuals from the “top” cluster emerged between 4.6 ± 0.9 and 8.1 ± 0.8 days after individuals from other clusters (post-hoc LSM: p < 0.001, n = 1315, N = 579 individuals, 21 years, see Figure 56 and Supplementary Materials: Table 5).

b) Temporal variation in emergence date

(1) Interannual variability in ED

Emergence date exhibited marked interannual variations over the course of the study on all meadows and for each age-sex category (GAMMs **[5]**; $2.09 < edf < 8.73$, $3.43 < F < 31.61$, $p < 0.001$, Figure 57), except for older males (≥3-year-old) on MB and MC ($p = 0.158$ and $p = 0.652$, respectively). Cyclic patterns over time were observed with a period varying from 6-10 years, depending on the period or the meadow considered. Older males (≥3-year-old) showed less variation in emergence date over time on MA than on DOT. Emergence date of yearlings and older females on MA was especially delayed between 1995-97 as compared to 1999, as well as in 2011 compared to 2014-17. On MB, yearlings' and ≥3-year-old females' emergence date exhibited shorter fluctuations at the start of the monitoring period (1992-2002) than in recent years (2005-2019). Emergence date appeared to be delayed from 2005 to 2009, then advanced from 2009-2016, and delayed again from 2016-2019. On DOT, emergence date for yearlings and older individuals showed lower variation than on other meadows, with fluctuations of smaller amplitude. MC individuals exhibited the greatest variation during the most recent years, emergence dates being advanced between 2013 and 2015 and delayed between 2016 and 2018. In general, the emergence date of 2-year-old individuals showed slightly lower fluctuations and a more stable pattern. On all meadows, individual emergence dates appeared to be earlier in 2015 and 2016 when compared with other years. Whereas the emergence date of MA and MB individuals was later in 2010 than other years, it was earlier on MC and DOT.

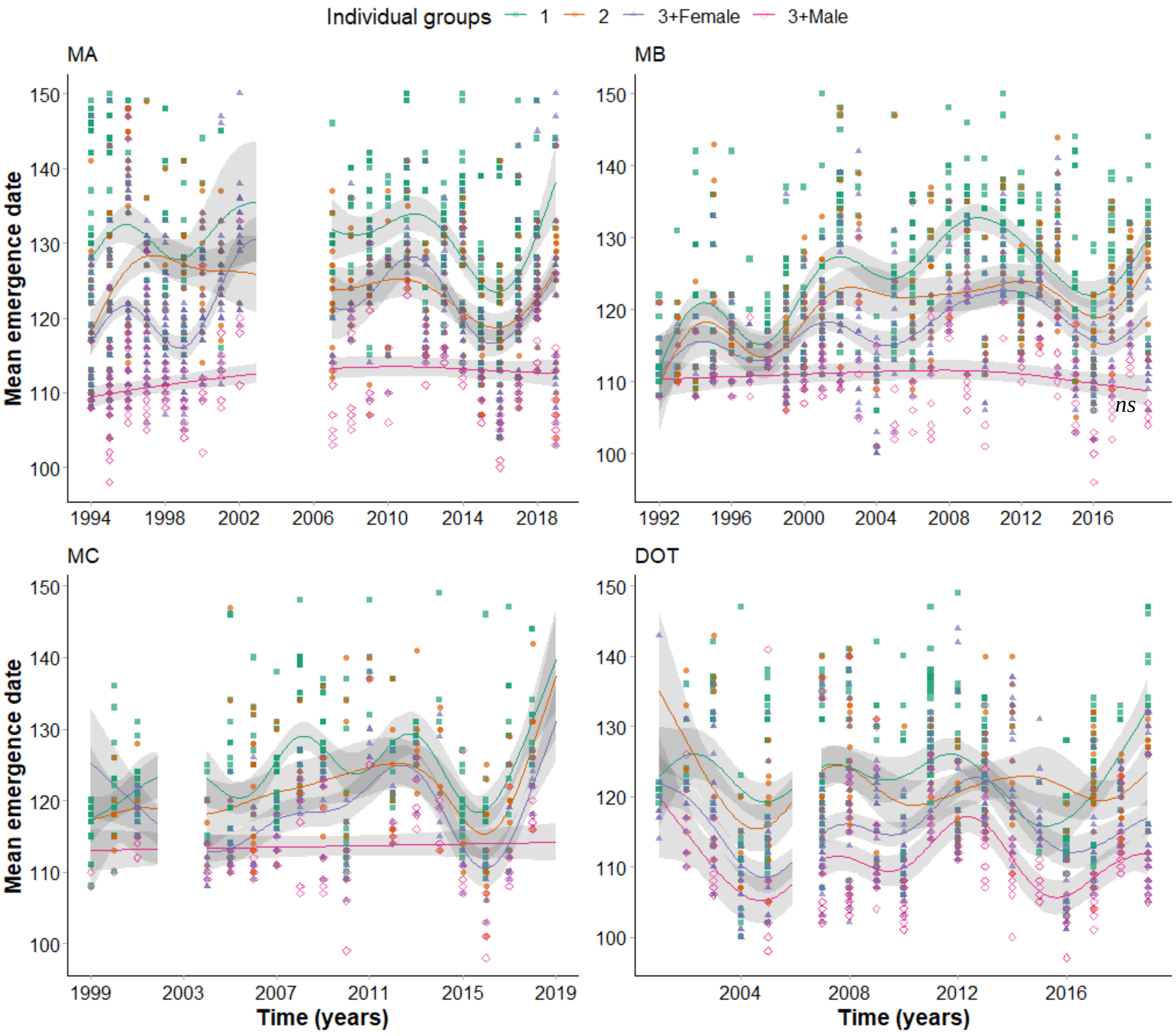


Figure 57. Interannual variability in emergence date. *Temporal patterns of emergence dates (in ordinal day) on each meadow (MA, MB, MC, DOT) and for different individual categories. Yearlings (green squares, 1), 2-year-old individuals (orange circles, 2), older females (blue triangles, 3+ Female), and older males (pink diamonds, 3+ Male) are represented. Plotted lines correspond to significant temporal trends and their 95% confidence interval according to GAMs* ***[5]****, excepting the temporal pattern of older males on MB and MC which is non-significant (ns).*

(2) Long-term directional changes in ED

When testing for long-term directional linear changes in emergence dates, the interaction between year and meadow was retained in the best model (LMM **[6]**, ΔAIC = 35, n = 5873, N = 2436 individuals). On DOT, emergence dates of individuals did not significantly change in a directional manner through time for any of the sex-age categories (LMM, interaction year*sex-age not retained, ΔAIC = 5, t = 1.094, p = 0.274, n = 1303 observations, N = 503 individuals, see Supplementary Materials: Figure 65). On MC, regardless of sex and age (interaction year*sex-age not retained, ΔAIC = 2), emergence from hibernation was significantly delayed over time, averaging a 4-day later emergence over an 18-year period (LMM, +0.22 ± 0.06 day/year, t = 3.769, p = 0.001, n = 704 observations, N = 385 individuals). On other meadows, temporal trends differed according to the sex-age category (ΔAIC = 17 and 22 for MA and MB, respectively). On MA, over a 22-year period, emergence was advanced by 2 days for 2-year-olds (LM, -0.11 ± 0.05 day/year, t = -2.290, p = 0.023, n = 399 observations, N = 399 individuals), delayed by 3 days for ≥3-year-old individuals (LMMs, ≥3-year-old Females: +0.14 ± 0.04 day/year, t = 3.254, p = 0.001, n = 767 observations, N = 290 individuals; ≥3-year-old Males: +0.13 ± 0.04 day/year, t = 3.058, p = 0.003, n = 368 observations, N = 155 individuals) and stable in yearlings (LM: p = 0.053, t = -1.936, n = 625 observations, N = 625 individuals). On MB, the hibernation emergence of yearlings, 2-year-old individuals and ≥3-year-old females was delayed by 11, 7 and 5 days, respectively over 28 years (LMs; yearlings: +0.40 ± 0.04 day/year, t = 9.110, p < 0.001, n = 631 observations, N = 631 individuals; 2-year-old: +0.26 ± 0.06 day/year, t = 4.678, p < 0.001, n = 318 observations, N = 318 individuals; LMM: ≥3-year-old Females: +0.17 ± 0.05 day/year, t = 3.455, p < 0.001, n = 531 observations, N = 164 individuals), whereas the emergence date of ≥3-year-old males did not significantly change through time (LMM; p = 0.379, n = 227, N = 83).

3. Spatio-temporal variation in climate

a) Spatial variation in climate variables

The three most influential components of the PCA explained 78.3% of the variance in climate variables and each generated an eigenvalue higher than one. The first component (PC1), explaining 39.4% of the variance, primarily reflected air and soil temperature during the summer (active period), soil temperature during the winter (inactive period), and negatively correlated to wind speed and humidity during the summer (see Figure 58 and Supplementary Materials: Table 6). Winter air temperature and humidity were positively correlated with the second component (PC2, explaining 24.5% of the variance), whereas snow depth during winter negatively loaded on PC2. The third component (PC3, explaining 14.4% of the variance), was mainly related to wind speed during the winter. The three principal components varied depending on the meadow (LMs **[7]**, p < 0.001), and depending on the cluster (LMs **[8]**, p < 0.001, see Supplementary Materials: Figure 66).

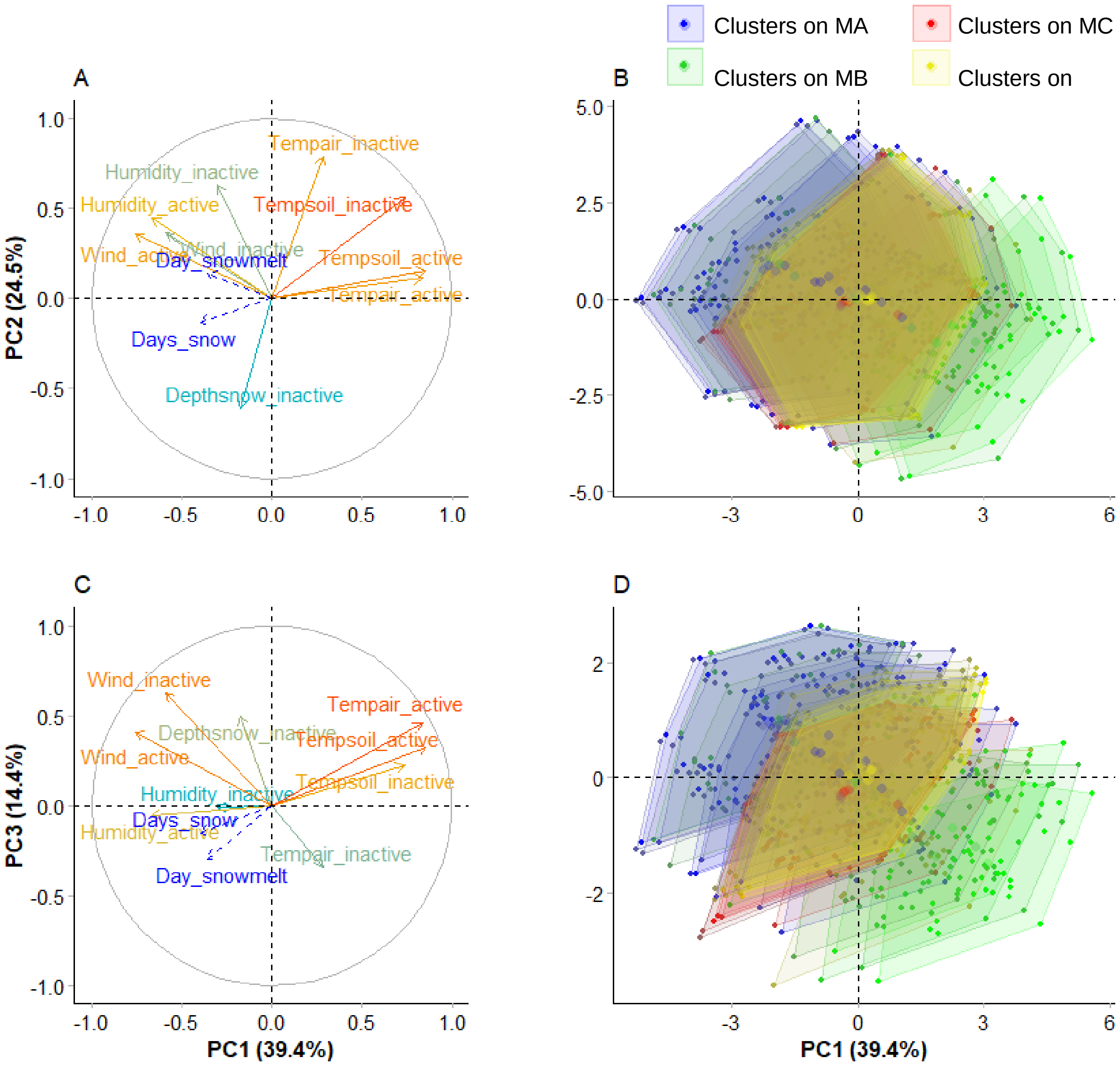


Figure 58. ***Spatial variation of climate conditions****. Left side: Climate variables, considered either during the active or inactive period, included in the Principal Component Analysis along dimensions 1 and 2 (A) and dimensions 1 and 3 (C). The number of days with snowfalls during the active period (Days_snow) and the ordinal day of snowmelt (Day_snowmelt) were not used to estimate the PCA but projected on it. Right side: Annual mean values of climate conditions for each cluster of the four meadows. Clusters are represented by minimum convex polygons colored by meadows (MA in shades of blue, MB in shades of green, MC in shades of red and DOT in shades of yellow), with their center of gravity represented by larger dots.*

As presented on Figure 58.B, D, clusters on MA were between 9.9% and 29.2% windier during the inactive period, between 16.3% and 26.4% windier during active periods, and between 0.6% and 2.7% more humid during the active period than clusters from other meadows. Conversely, soil temperatures of clusters on MB were higher, during both winter (between 1.7% and 4.5%) and summer (between 2.4% and 6.6%), and the air during summer was between 1.4% and 4.3% warmer than on other meadows. Despite those general patterns, within-meadow variability was also quite high among clusters, especially on MB, which displayed both very positive and negative PCs clusters (Figure 58.B, D and Supplementary Materials: Figure 66).

b) Temporal variation in climate variables

(1) Climate variation

As predicted, all climate variables exhibited temporal variability (GAMs **[9]** and **[10]**; $6.51 < \text{edf} < 8.78$, $2.79 < F < 15.33$, all $p < 0.01$, Figure 59). Interannual variation in these variables was similar among meadows (no year*meadow interactions retained, N = 28 years), although the absolute values in wind speed and relative humidity varied across meadows. This confirmed previous results that MA was the windiest and most humid meadow, while MB was the least humid and windy, MC and DOT being intermediate. Summer air temperature exhibited particularly high interannual variation, increasing and decreasing from 1 to 4°C from one year to the next (e. g. between 1997 and 1998), fluctuating on average between 7-13°C. During 2006-2007, air temperature was particularly high, and much warmer than the next four years. Conversely, the relative humidity during summer was particularly low in 2006-2007 compared to the next four years, staying relatively stable over the rest of the study period. Wind speed during the active period showed regular cycles oscillating between 0.8-1.2 m/s every five years. During the inactive period, soil temperature varied between 2-5 °C within approximately five-year periods. Winter snow depth showed interannual variation as well, with especially low values in 2018 and 2019. Similarly, the ordinal day of snowmelt varied between mid-February and 10 April, depending on the year. Number of days with snow during the active period contrasted between years of no late snowstorms (1992-1995, 1997-1998, 2004-2006, 2012, 2015-2016) and years of late snow falls (between 2000-2003, and between 2008-2013).

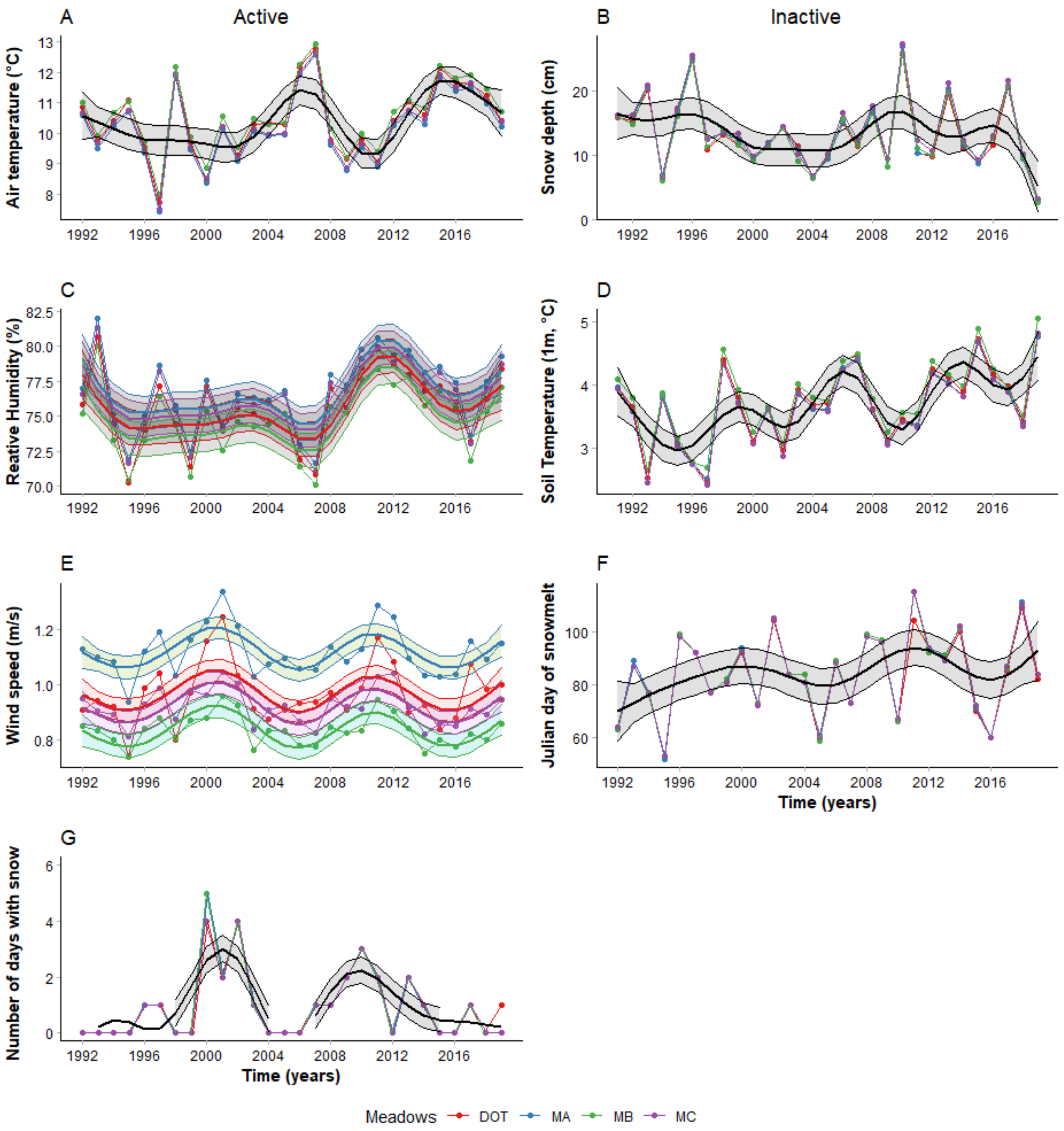


***Figure 59. Climate interannual variability**. Annual mean values of climate variables averaged by meadow. The lines correspond to temporal patterns of climate variables estimated by the GAM **[9]** on the four meadows (MA in blue, MB in green, MC in purple, and DOT in red) between 1992 and 2019. Climate time series were divided into two periods: inactive from the 1st of august to the 15th of April (right column) and then active the rest of the year (left column) and averaged by year. Where no difference was found between meadows (all variables except wind and humidity), a single prediction line was plotted for all locations (in black) with its 95% confidence interval (shaded area).*

(2) Climate change

Temporal trends in climate did not differ between meadows (LMs: no year*meadow interactions retained, N = 28 years, see Supplementary Materials: Figure 67). Over 28 years, air temperature increased by 1.40°C during the active season (LM **[11]**, Active: 0.05 ± 0.01°C/year, t =3.510, p < 0.001). Winter (inactive period) soil temperature at a 1-m depth increased at a slower rate of 0.03 ± 0.01°C/year (i.e. 0.84°C over 28 years, LM, t = 5.642, p < 0.001). Conversely, the snow depth during the inactive period (winter) decreased by -0.13 ± 0.06 cm per year, or 3.64 cm over the 28 years of study. During the active period, relative humidity increased by 0.08 ± 0.03 % points per year (LM, t = 2.818, p = 0.006). Moreover, the first ordinal day of complete snowmelt increased through time (delay of 13 days within 28 years, i.e. 0.45 ± 0.18 day/year, LM, t = 2.509, p = 0.014). The only climatic variable that appeared to remain relatively invariant over the study period were wind speed and the number of snow days during the active period (LMs, wind: t = 0.070, p = 0.944, nbdays: t = 0.207, p = 0.836). In summary, the climate tended to be warmer, more humid and with a smaller snow cover during winter. Yet, this snow cover disappeared later in the spring.

4. Climate – emergence date relationships

a) Mean relationships between climate & emergence date

Among the different climate variables considered during winter (inactive phase) or the previous summer (active phase), only the air temperature during previous summer had a VIF greater than three (between 5.244 and 5.460 according to the sex-age category considered) and was removed from the model (probably due to its high correlation with soil temperature; see Supplementary Materials: Figure 68). The ordinal day of snowmelt and wind during summer were positively related with annual mean emergence date (Figure 60). Emergence dates were delayed with a delay in the first day of snow melt for all individual categories, apart from 2-year-old individuals (LMMs **[13]**, yearlings: t = 2.211, p = 0.036, n = 84 observations, 2-year-old females: t = 1.713, p = 0.099, n = 82 observations, 2-year-old males: t = 0.424, p = 0.676, n = 77 observations, ≥3-year-old females: t = 2.994, p = 0.007, n 83 = observations, ≥3-year-old males: t = 3.958, p < 0.001, n = 83 observations). Ground squirrels therefore emerged earlier when (or/and where) snow melted earlier; emergence date being delayed by approximately 0.25 day for a day of delayed snowmelt. Similarly, the summers with higher wind speed correlated with later emergences, but only for older females (≥3-year-old, LMM, t = 2.230, p = 0.037, n = 83 observations). Indeed, older females emerged around 13 days later per 1 m/s increase in wind speed.

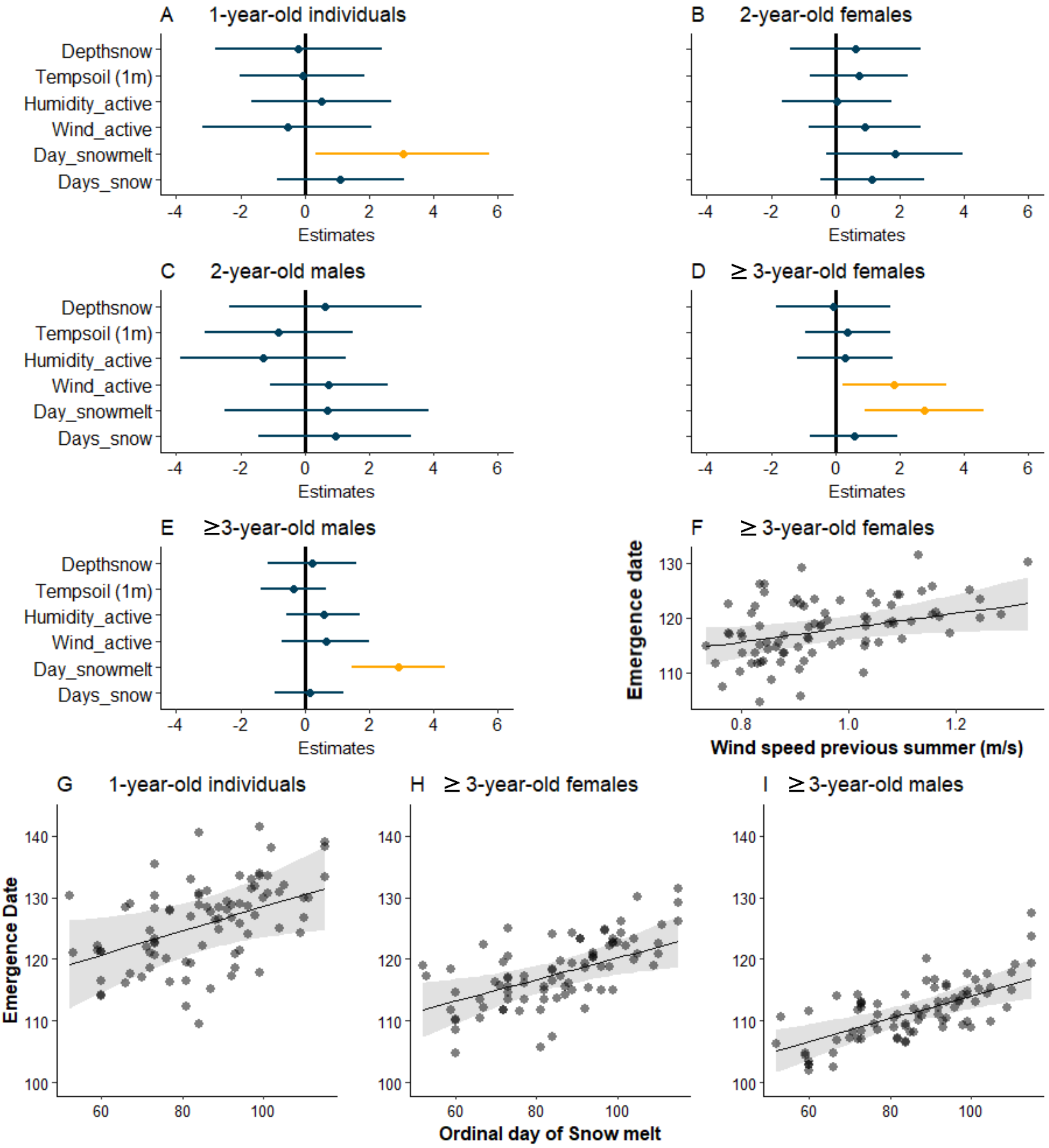


*Figure 60. **Climate effects on hibernation emergence date of different individual categories**. From (A) to (E): Estimates and 95% confidence intervals from the full LMM **[13]** explaining annual mean emergence dates per meadow are presented. Significant effects are presented in orange while non-significant ones are blue. (F) Annual mean emergence date as a*

function of the wind speed during the previous active period for ≥3-year-old females and model predictions (predicted line and 95% CI around). From (G) to (I): Annual mean emergence date as a function of the ordinal day of snow melt and model predictions for one-year-old individuals (G), ≥3-year-old females (H) and ≥3-year-old males (I).

b) Year-centered approach: are spatial differences in ED related to spatial variation in climate?

Once centered per year, no strong correlations were highlighted between climate variables, and we included all four of them in the model (VIFs < 1.308, see Supplementary Materials: Figure 69). Day of snowmelt was related positively to emergence date, but only in yearlings and older (≥3-year-old) females; individuals emerged later when they emerged from locations where snow melted later (Figure 61; ED around 0.2 day later for a day of delayed snowmelt, ED and climate variables centered by year, LMMs **[14]**, yearlings: t = 2.253, p = 0.024, n = 1450 observations, N = 1450 individuals, ≥3-year-old females: t = 2.389, p = 0.017, n = 1392 observations, N = 559 individuals). Males of 2-year-old and ≥3-year-old seemed to emerge around 2 days later in locations where the snow depth was 1cm higher during hibernation (LMMs, 2-year-old males: t = 2.303, p = 0.023, n = 280 observations, N = 280 individuals, ≥3-year-old males: t = 2.085, p = 0.039, n = 693 observations, N = 300 individuals, effect only marginally significant after transforming the data, Box-Cox transformation, p = 0.058). Soil temperature during winter tended to have an effect on the emergence date of yearlings and older males, with individuals emerging earlier from warmer hibernacula (Figure 61, LMMs, yearlings: t = -1.625, p = 0.105, n = 1450 observations, N = 1450 individuals, ≥3-year-old males: t = -1.498, p = 0.137, n = 693 observations, N = 300 individuals).

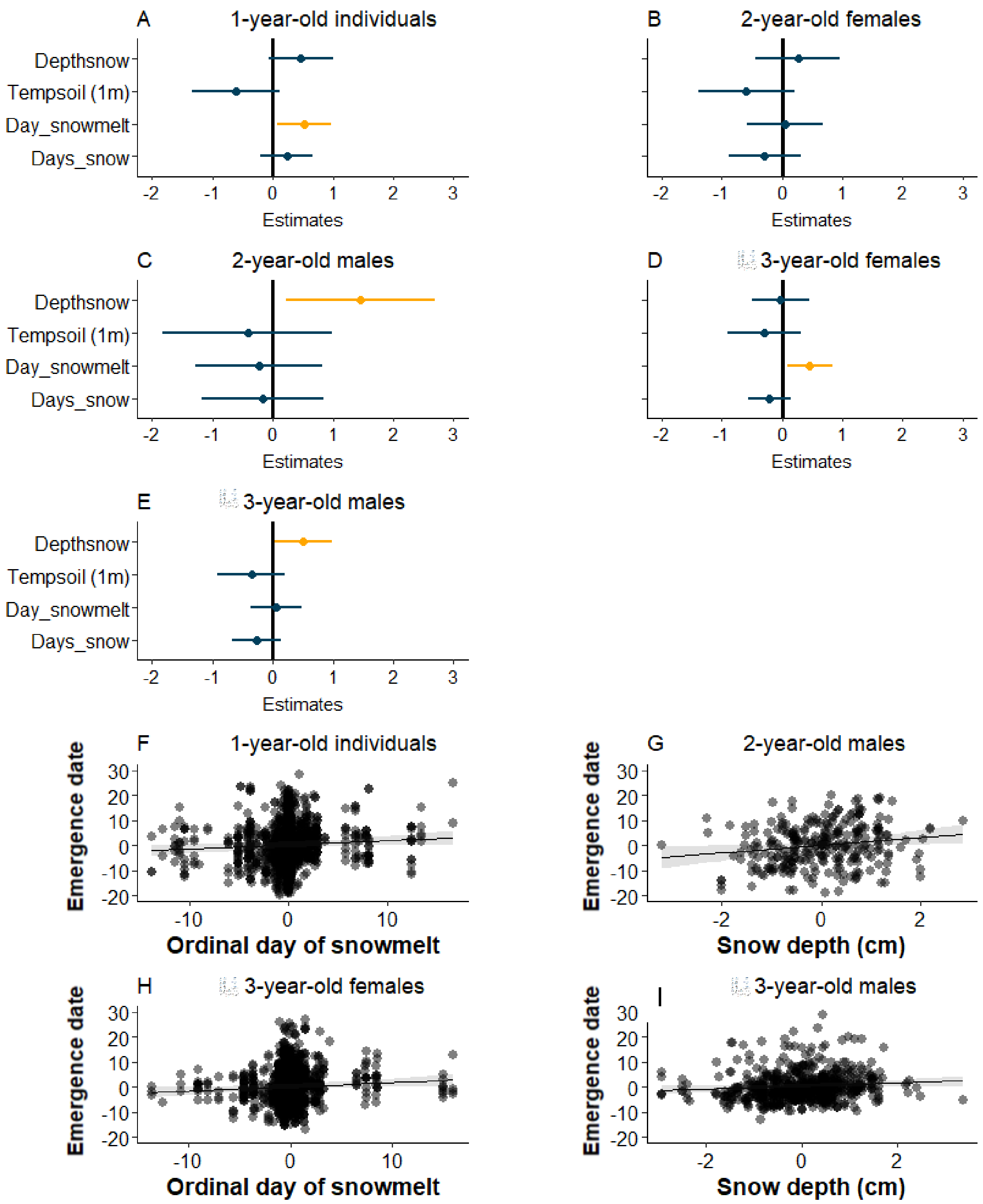


Figure 61. Spatial climate effects on centered emergence dates of different individual categories. *From (A) to (E): Estimates and 95% confidence intervals from the full LMMs* **[14]** *are presented with significant effects in orange. From (F) to (I): Spatial effect of climate on emergence date (year-centered observations to remove temporal variance). Model predictions are indicated along with their 95% confidence intervals (shaded area) on top of observations. Significant spatial effect of the ordinal day of snowmelt on emergence date of (F) yearlings and (H) older females (≥3-year-old and more). Significant spatial effect of the winter snow depth on the emergence date of (G) 2-year-old males and (I) ≥3-year-old males.*

c) Spatial-centered approach: are interannual differences in ED related to interannual variation in climate?

When conducting the analysis, air temperature during the previous summer had a VIF greater than three (between 4.154 and 5.249) and was removed from the model (probably due to its high positive correlation with soil temperature, see Supplementary Materials: Figure 70). Regarding temporal effects of climate on emergence date, the ordinal day of snow melt was positively correlated to emergence date for all individuals excepting 2-year-old males (emergence dates and climate variables centered by cluster, LMMs **[15]**, yearlings: $t = 3.015$, $p = 0.003$, $n = 1450$ observations, $N = 1450$ individuals; 2-year-old females: $t = 2.821$, $p = 0.006$, $n = 525$ observations, $N = 525$ individuals; 2-year-old males: $t = 1.275$, $p = 0.211$, $n = 280$ observations, $N = 280$ individuals; ≥3-year-old females: $t = 3.246$, $p = 0.002$, $n = 1392$ observations, $N = 559$ individuals; ≥3-year-old males: $t = 3.610$, $p < 0.001$, $n = 693$ observations, $N = 300$ individuals). Thus, individuals emerged later in years when snow melted later (around 0.1 day later for a day of delayed of snowmelt, see Figure 62). Similarly, active periods with high wind speed correlated positively with delayed emergence dates of older individuals the next year (between 13 and 17 days delay per m/s increase, LMMs, ≥3-year-old females: $t = 3.357$, $p = 0.001$, $n = 1392$ observations, $N = 559$ individuals; ≥3-year-old males: $t = 2.803$, $p = 0.006$, $n = 693$ observations, $N = 300$ individuals). Earlier emergence date of 2-year-old males correlated with higher summer humidity (LMMs, 2-year-old males: $t = -2.197$, $p = 0.036$, $n = 280$ observations, $N = 280$ individuals), although this effect was only marginally significant after transforming the data (Box-Cox transformation, $p = 0.054$). Depth of snow during winter may have a positive effect on the ED of older individuals, and the emergence date of 2-year-old males seems to be negatively associated with the hibernation soil temperature (ED around 3 days earlier per degree increase of soil temperature at one-meter depth).

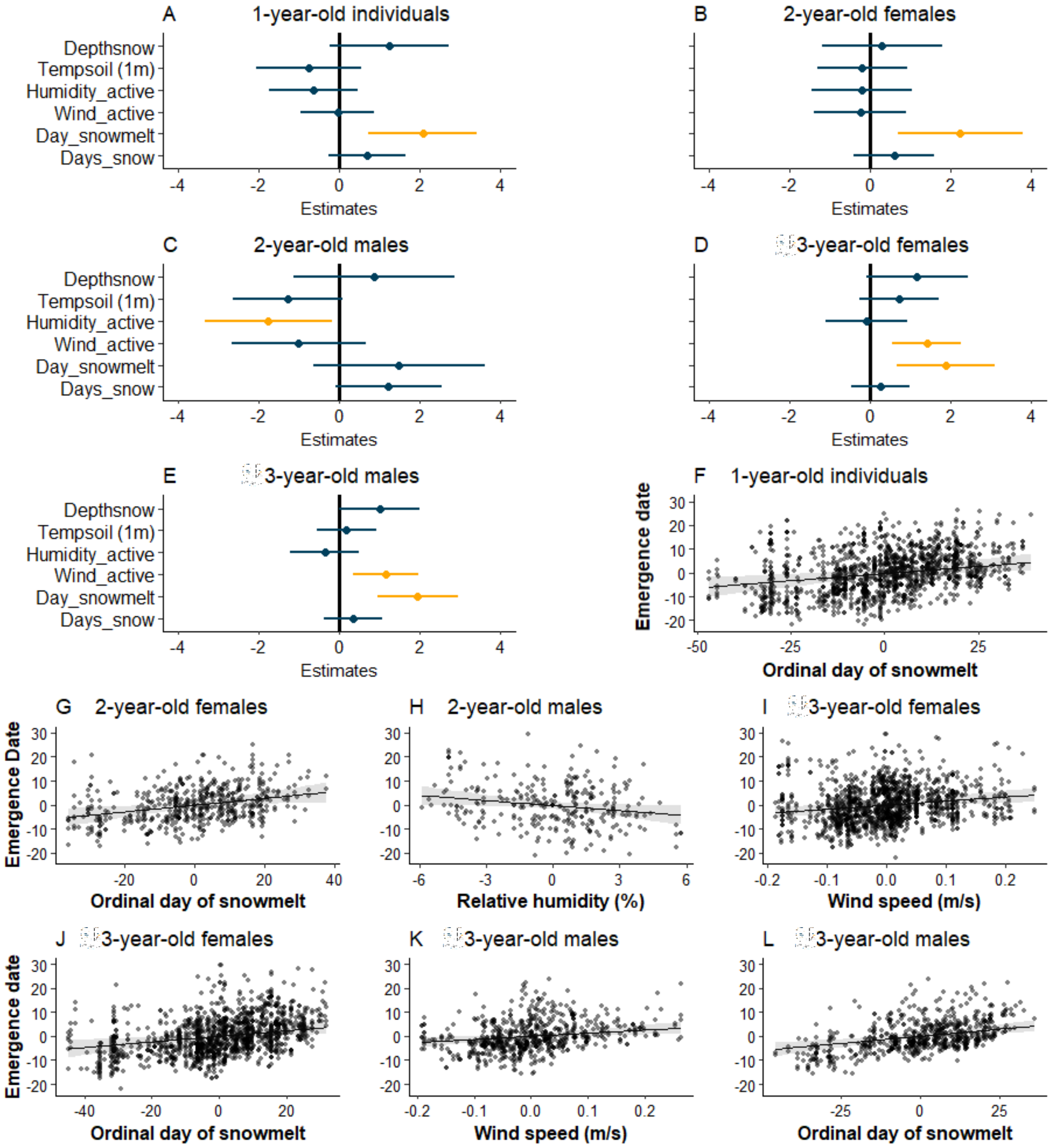


***Figure 62*. Temporal climate effects on centered emergence dates of different individual categories.** *From (A) to (E): Estimates and 95% confidence intervals from the full LMMs* **[15]** *are presented with significant effects in orange. From (F) to (L): Temporal effect of climate on emergence date (all cluster-centered to remove the spatial variance). Model predictions are indicated along with their 95% confidence intervals (shaded area) on top of observations. Significant temporal effects of the ordinal day of snowmelt on the ED of (F) yearlings, (G) 2-year-old females, (J) ≥3-year-old females, and (L) ≥3-year-old males. Significant temporal effects of (H) relative humidity during preceding summer on the ED of 2-year-old males, and of wind speed on the ED of ≥3-year-old (I) females and (K) males.*

F. Discussion

Consistent with previous studies (Murie and Harris 1982, Dobson et al. 1992), ground squirrel age and sex accounted for a substantial amount of variation (29.4%) in ED. Older males emerged first from hibernation, followed by older females, 2-year-old individuals, and finally yearlings. Individual identity had a significant effect in the overall pattern of emergence date. This is consistent with previous knowledge on the heritability of emergence date of Columbian ground squirrels ($h^2 = 0.22$ in females and 0.34 in males, Lane et al. 2011). The magnitude of the individual effect (14.6% of variance) was similar to the temporal variance in ED (16.1% of variance), in spite of the strong individual plasticity in ED from year to year (Lane et al. 2012). These results confirm the importance of including among-individuals heterogeneity in understanding population dynamics (Hamel et al. 2018).

ED varied over space and time (21.7% of variance), suggesting that this trait is plastic and capable of responding to climate variation (see also Dobson and Kjelgaard 1985b, Dobson 1988, Lane et al. 2019). Also, separating the temporal and spatial effects of climate on the emergence, either by centering by cluster or by year, enabled us to compare different mechanisms. Indeed, philopatric individuals (such as adult Columbian ground squirrels) are adapted to the environmental conditions in which they live. Oppositely, interannual variations in climate are more stochastics and might trigger plastic responses of animals. Whereas the years effects (16.1% of variance) are consistent with previous observations that ED was delayed through time in some populations (Neuhaus et al. 1999, Lane et al. 2012), our results importantly show that part of the variance (5.6%) in ED was also explained by spatial variation both within and among meadows. Such spatial heterogeneities were unlikely explained by variations in altitude (Dobson et al. 1992), since the four study populations are less than 70-m apart in terms of elevation. Interestingly, the variation in ED among individuals from different locations was even higher within than among meadows, ground squirrels from some parts of the meadows emerging earlier than others. This suggests that local heterogeneities occur at a micro-scale (~40 m; see Results) rather than meso-scale (1-10 km). These results add to the growing literature showing that several animal and plant species are more sensitive to microscale than wider environmental variations (Lampei et al. 2019, Lundblad and Conway 2021, Wang and Hou 2021). Concurrently, spatial microclimate differences were found between clusters, suggesting a potential link between ED and local habitat conditions.

1. Emergence date relationship to climate

Our analyses of microclimate effects on ED focused on two distinct temporal phases: the climate conditions during the previous summer (active period) and those during hibernation (inactive period). First, our results showed the importance of carry-over effects of the period preceding hibernation on ground squirrel emergence dates the subsequent year. In particular, we found that delayed emergence occurred in older individuals when the preceding summer was windier and associated with lower humidity (see Supplementary Materials: Figure 70 for temporal correlations between climate variables). Carry-over climatic effects on animal phenology have mainly been documented in birds (Ockendon et al. 2013, Finch et al. 2014), fish (Wilson et al. 2021), and insects (Dingemanse and Kalkman 2008, McCauley et al. 2018). Yet, carry-over effects are widespread, including in mammals, and are thought to be primarily mediated through macronutrient supply (reviewed by Harrison et al. 2011). Climate directly affects food availability/quality or/and foraging patterns (Levy et al. 2016), which may have direct consequences on individual mass gain before hibernation. In particular, strong wind conditions may have decreased the time spent foraging for ground squirrels (Fairbanks and Dobson 2007), while low humidity associated with decreased precipitation may have resulted in reduced primary productivity and inadequate forage quantity/quality, also affecting the accumulation of fat stores (Dobson and Kjelgaard 1985b, Neuhaus et al. 1999, Dobson et al. 2016). In turn, lower fat stores might have resulted in longer hibernation to avoid emerging when resources were scarce, explaining tardier emergence dates. Whereas our study suggests climatic carry-over effects on phenology, such effects are largely understudied, and yet likely widespread. For instance carry-over effects of food availability on the relationship between temperature and hibernation emergence were shown in edible dormice (Fietz et al. 2020).

Similarly, climate conditions during hibernation had marked effects on the timing of emergence of ground squirrels, either by affecting the quality of hibernation and animal energy expenditure, or acting as cues indicating the end of the winter period. Years with later snow melt resulted in later emergences for one-year-old individuals, 2-year-old females, and ≥3-year-old individuals. Similarly, spatial clusters where snow melted later resulted in yearlings and ≥3-year-old females emerging later than in other areas. This was true both at the spatial and temporal scales, and for almost all individual categories, suggesting the necessity for snow to melt and uncover growing vegetation resources before ground squirrels emerge. In addition, a melting and wet snow pack makes it hard for the animals to easily move around the meadow

(VAV, FSD, PN; *personal observations*) and likely increases the costs of thermoregulation, increasing overall energy expenditure at a time where their remaining fat stores are likely depleted. Local climate may thus act as a strong cue on the timing of ground squirrel emergence, though the question of the immediate mechanism that stimulates emergence remains for future research.

We found a positive spatial correlation between snow depth and emergence of 2-year-old and ≥3-year-old males, suggesting that males of 2-year-old and older emerged later in locations where snow depth was higher during the inactive period. Higher snowpack or delayed snowmelt during the inactive period may also insulate and stabilize soil temperatures and hibernation conditions, decreasing fat reserve consumption, and thus allowing a longer hibernation or emergence in better condition (the latter important for reproduction; Rubach et al., 2016). Snowpack thickness is an important variable influencing hibernating species, such as alpine marmots (Canale et al. 2016), hoary marmots (Patil et al. 2013), or northern Idaho ground squirrels (Goldberg and Conway 2021). A negative correlation between the soil temperature during inactive period (at one-meter depth) and the emergence date of yearlings and ≥3-year-old males also seemed to apply. As for northern Idaho ground squirrels (Goldberg and Conway 2021), this result possibly indicates that unfavorable hibernating conditions lead to earlier emergence. For example, elevated soil temperatures prevent animals from lowering their body temperature sufficiently for efficient energy conservation during hibernation. In short, fat-storing and hibernating species might be sensitive to local environmental conditions and especially elevated temperatures, which are directly influencing energy expenditure during this period of fast.

Climatic variables correlating with ED differed between sex-age categories. Explanations for such diverging responses may be both ecological and physiological. Young (1990a) reported the existence of long post-hibernation euthermic phases only in adult males, remaining several days underground before emerging despite favorable aboveground conditions, possibly to allow for gonadal maturation (Williams et al. 2017b). Physiological and phenological differences in sex and age categories might therefore reflect distinct constraints between reproductively mature individuals and non-mature individuals. Yearlings and 2-year-olds that emerge later in the spring likely do so when resources become more abundant and perhaps to avoid predator exposure or the social aggression related to reproduction (Murie and Harris 1982, Constant et al. 2020). By contrast, mature individuals may optimize their emergence date according to the trade-off between energy resources (remaining fat stores, food availability) and the advantages of an early reproduction (Neuhaus 2000). Furthermore, reproductive strategies might differ between males and females, with males taking advantage of earlier hibernation emergence to seek more breeding opportunities (Richardson's ground squirrels, Michener, 1983) and establish their territories (Manno and Dobson 2008). Thus, environmental influence on a population might differ importantly between individuals with different life history constraints (*e.g.* age classes, Pardo et al. 2013, Bonamour et al. 2020).

2. Long-term changes in climate in relationship to emergence date

Previous studies have reported shifts in animal phenology linked to changes in climate, species especially responding to temperature (more so at high latitudes) and precipitations (Cohen et al. 2018). In the context of climate change, advancements in spring phenology have been found in the vast majority of cases (Parmesan and Yohe 2003, but see Radchuk et al. 2019). However, in studying long-term effects of climate change on animal phenology, mammals are poorly represented (Parmesan and Yohe 2003, but see Radchuk et al. 2019). Among those studied, *Marmotini* species appear to respond differently to directional changes in climate. The phenology of Uinta ground squirrels did not change over time, despite a locally changing climate (Falvo et al. 2019). Yellow-bellied marmot have been found to emerge earlier in response to warming air temperatures (Inouye et al. 2000), while Columbian ground squirrels had been shown to display a trend toward later emergence, related to late spring snowstorms (Lane et al. 2012).

Interestingly, our results are only partially consistent with previous findings on Columbian ground squirrels. We found evidence for delayed snowmelt (13 days) over the 28 years of study despite a general warming trend (air temperature increased by 1.40°C during the active season), and a positive effect of the first day without snow on emergence date (except for 2-year-old individuals). Despite such a climatic trend, different phenological responses were observed in Columbian ground squirrels of different age categories and meadows. As an example, on MC and MB, all individuals (except older males on MB) delayed emergences by 4 to 11 days over the 18 to 28 year period. Yet, no changes in emergences were observed on DOT for any age category, and long-term temporal trends in ED differed substantially depending on individual age and sex on MA. Two-year-old individuals advanced their emergence by 2 days over 22 years, whereas older individuals delayed their emergence by 3 days in the same period, and no change in ED was found for yearlings. Differences in ED trends between closely located meadows may be related to the spatial heterogeneity of field, slope, or sun and wind exposure, with different effects of microclimates. Similar results were found in a recent study on the North American deer mouse (*Peromyscus maniculatus*), where temperature and photoperiod were positive cues of breeding phenology, the relationship varying among regions (McLean and Guralnick 2021). Whether variation in ground squirrel phenology is linked to behavioral plasticity versus long-term adaptation remains to be seen. The small spatial scales tested here, and the diverging responses among nearby populations might suggest phenological plasticity, as recently suggested (Lane et al. 2019).

Many species responses to climate are likely to occur at micro-scale, rather than meso- or macro-scale (e.g. sockeye salmon; *Onchorhynchus nerka*; Martins et al. 2012). As highlighted here, the relationships between phenology and climate variables are likely to differ between neighboring populations of the same species, in response to contrasting microclimate conditions, even over small spatial scales. Thus, studies focusing on climate-phenology relationships in animals would benefit by integrating fine local scale information on habitat.

3. Conclusion and further perspectives

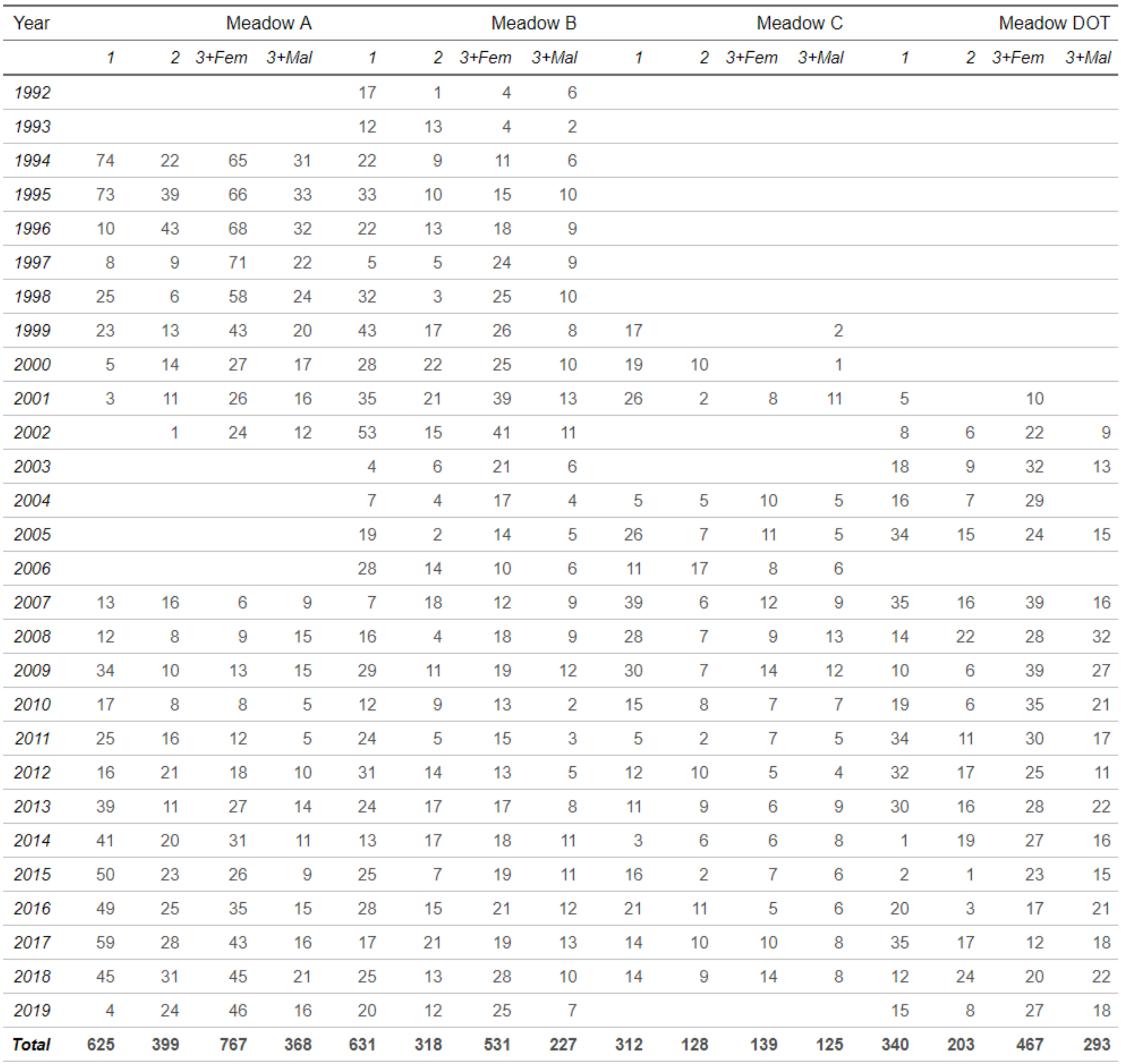
Taken together, our results point to important differences in microclimate effects on animal phenology in neighboring populations. Of course, phenology may be linked to individual fitness, and thereby the dynamics of the populations. Thus, we might also expect variation in these variables over small spatial scales. Integrating microclimatic variation in the study of climate change is likely to provide more accurate predictions of a variety of animal responses to a changing world.

G. Acknowledgements

We are grateful to Edward A. Johnson (Director of the Biogeosciences Institute, University of Calgary), Judy Mappin-Buchannan and Adrienne Cunnings (Managers, Kananaskis Field Stations), and Kathreen Ruckstuhl (faculty member responsible for the R.B. Miller Field Station) for housing and facilities during fieldwork. The fieldwork was aided by many volunteers and students over the years, and we thank them for their excellent efforts. We are specifically indebted to Jan O. Murie for initiating the long-term research on Columbian ground squirrels, and to David A. Boag for his earlier work on the species. Jan O. Murie provided insightful comments on the manuscript, and we are grateful for his continued advice, help and friendship over the years. This research was supported by a USA National Science Foundation grant (DEB-0089473) and a fellowship grant from the Institute of Advanced Studies of the University of Strasbourg to F.S.D., a Natural Sciences and Engineering Research Council of Canada grant to J.O. Murie, a CNRS Projet International de Coopération Scientifique grant (PICS-07143), an AXA Postdoctoral Research Fellowship, a Fyssen Foundation Postdoctoral Fellowship, and a Fyssen Foundation research grant to V.A.V., and an Initiative d’Excellence (IDEX attractivité) research grant to C. Saraux. F.S.D. thanks the Région Grand Est and the Eurométropole de Strasbourg for the award of a Gutenberg Excellence Chair during the time of writing. A.T. was supported by a PhD scholarship from the Ministère de l’Enseignement Supérieur, de la Recherche et de l’Innovation.

H. Supplementary Materials

Table 4. Table of emergence date observations per year on the four different meadows. *The number of observations is presented for each individual (sex-age) category: 1-year-old individuals (1), 2-year-old individuals (2), ≥3-year-old females (3+Fem), and ≥3-year-old males (3+Mal).*



Year	Meadow A				Meadow B				Meadow C				Meadow DOT			
	1	2	3+Fem	3+Mal	1	2	3+Fem	3+Mal	1	2	3+Fem	3+Mal	1	2	3+Fem	3+Mal
1992					17	1	4	6								
1993					12	13	4	2								
1994	74	22	65	31	22	9	11	6								
1995	73	39	66	33	33	10	15	10								
1996	10	43	68	32	22	13	18	9								
1997	8	9	71	22	5	5	24	9								
1998	25	6	58	24	32	3	25	10								
1999	23	13	43	20	43	17	26	8	17			2				
2000	5	14	27	17	28	22	25	10	19	10		1				
2001	3	11	26	16	35	21	39	13	26	2	8	11	5		10	
2002		1	24	12	53	15	41	11					8	6	22	9
2003					4	6	21	6					18	9	32	13
2004					7	4	17	4	5	5	10	5	16	7	29	
2005					19	2	14	5	26	7	11	5	34	15	24	15
2006					28	14	10	6	11	17	8	6				
2007	13	16	6	9	7	18	12	9	39	6	12	9	35	16	39	16
2008	12	8	9	15	16	4	18	9	28	7	9	13	14	22	28	32
2009	34	10	13	15	29	11	19	12	30	7	14	12	10	6	39	27
2010	17	8	8	5	12	9	13	2	15	8	7	7	19	6	35	21
2011	25	16	12	5	24	5	15	3	5	2	7	5	34	11	30	17
2012	16	21	18	10	31	14	13	5	12	10	5	4	32	17	25	11
2013	39	11	27	14	24	17	17	8	11	9	6	9	30	16	28	22
2014	41	20	31	11	13	17	18	11	3	6	6	8	1	19	27	16
2015	50	23	26	9	25	7	19	11	16	2	7	6	2	1	23	15
2016	49	25	35	15	28	15	21	12	21	11	5	6	20	3	17	21
2017	59	28	43	16	17	21	19	13	14	10	10	8	35	17	12	18
2018	45	31	45	21	25	13	28	10	14	9	14	8	12	24	20	22
2019	4	24	46	16	20	12	25	7					15	8	27	18
Total	**625**	**399**	**767**	**368**	**631**	**318**	**531**	**227**	**312**	**128**	**139**	**125**	**340**	**203**	**467**	**293**

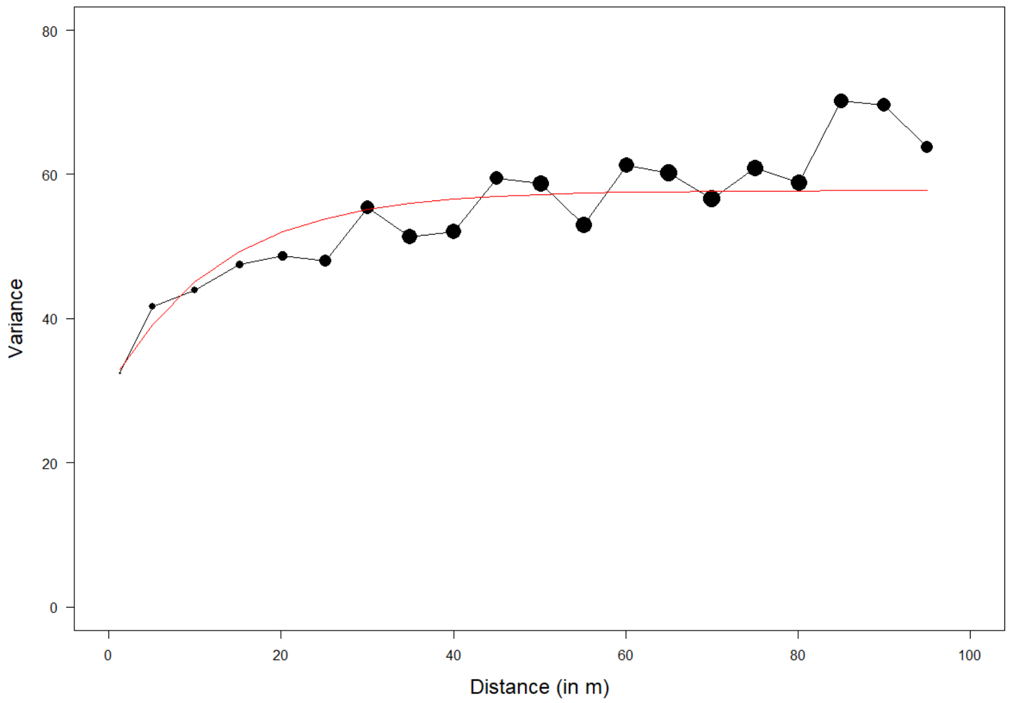


Figure 63. Variance of emergence date as a function of the distance between emergence locations, all meadow and individual categories considered. *The black dots and line represent the empirical variogram, the size of the point representing the number of pairs of points considered in each distance bin. The red line represents the modelled variogram. The variance stabilized around 40 meters distance between two emergence locations.*

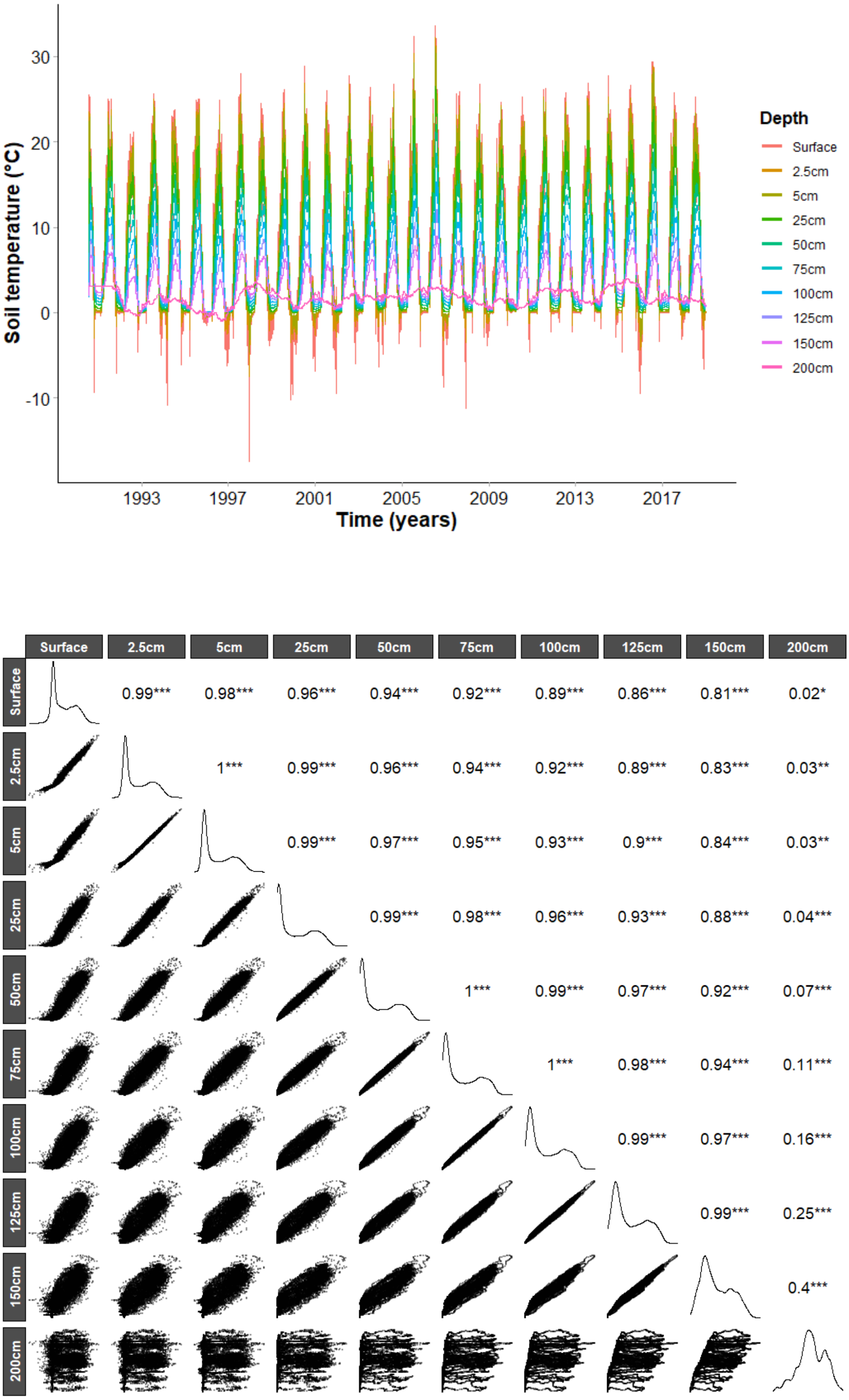
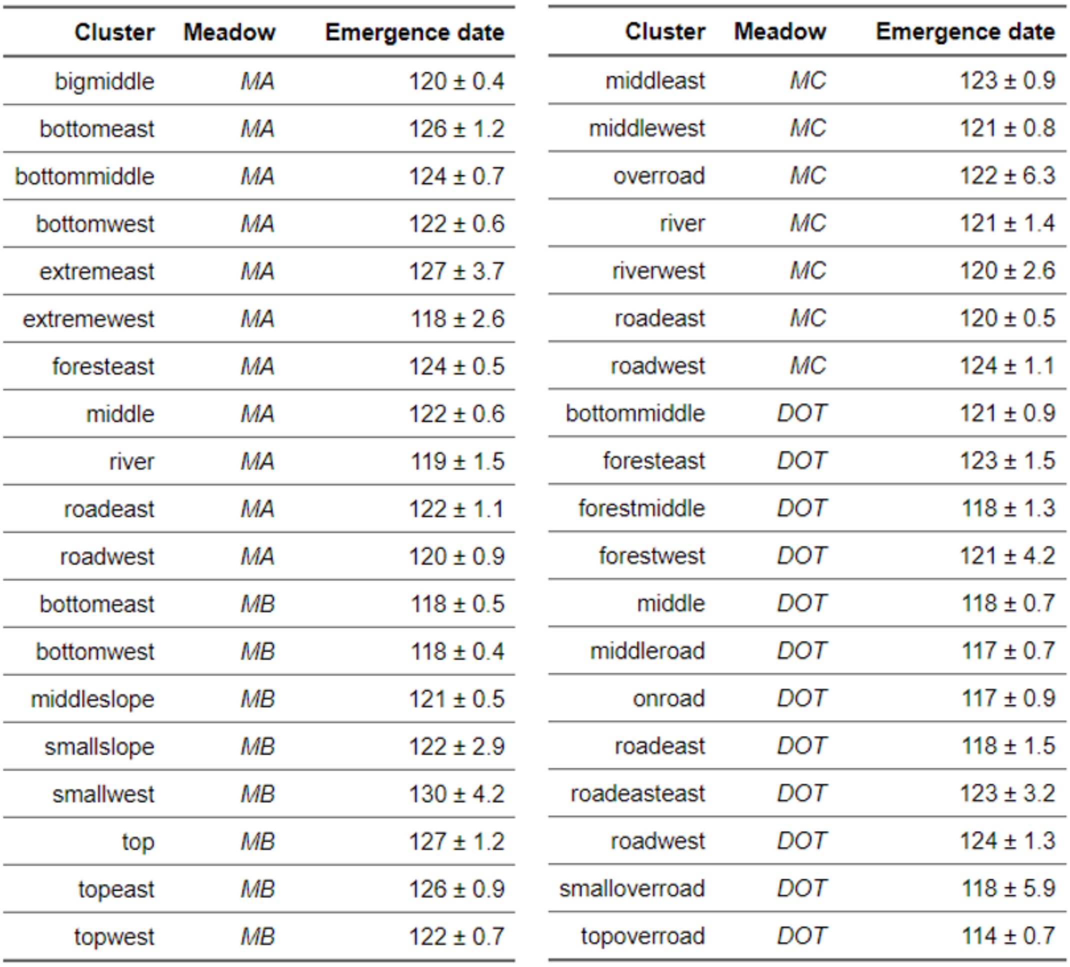


Figure 64. Correlations between soil temperatures at different depths. *Upper part: Daily mean of soil temperatures at various depth (different colors) over the 28 years of study. Lower part: Pearson correlation matrix between soil temperature at depths between surface and two-meter depth (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).*

Table 5. Summary table of spatial variation in emergence date. *The mean emergence date (in ordinal day) of each cluster within each meadow (MA, MB, MC, DOT) is presented along with standard errors.*



Cluster	Meadow	Emergence date
bigmiddle	*MA*	120 ± 0.4
bottomeast	*MA*	126 ± 1.2
bottommiddle	*MA*	124 ± 0.7
bottomwest	*MA*	122 ± 0.6
extremeast	*MA*	127 ± 3.7
extremewest	*MA*	118 ± 2.6
foresteast	*MA*	124 ± 0.5
middle	*MA*	122 ± 0.6
river	*MA*	119 ± 1.5
roadeast	*MA*	122 ± 1.1
roadwest	*MA*	120 ± 0.9
bottomeast	*MB*	118 ± 0.5
bottomwest	*MB*	118 ± 0.4
middleslope	*MB*	121 ± 0.5
smallslope	*MB*	122 ± 2.9
smallwest	*MB*	130 ± 4.2
top	*MB*	127 ± 1.2
topeast	*MB*	126 ± 0.9
topwest	*MB*	122 ± 0.7
middleast	*MC*	123 ± 0.9
middlewest	*MC*	121 ± 0.8
overroad	*MC*	122 ± 6.3
river	*MC*	121 ± 1.4
riverwest	*MC*	120 ± 2.6
roadeast	*MC*	120 ± 0.5
roadwest	*MC*	124 ± 1.1
bottommiddle	*DOT*	121 ± 0.9
foresteast	*DOT*	123 ± 1.5
forestmiddle	*DOT*	118 ± 1.3
forestwest	*DOT*	121 ± 4.2
middle	*DOT*	118 ± 0.7
middleroad	*DOT*	117 ± 0.7
onroad	*DOT*	117 ± 0.9
roadeast	*DOT*	118 ± 1.5
roadeasteast	*DOT*	123 ± 3.2
roadwest	*DOT*	124 ± 1.3
smalloverroad	*DOT*	118 ± 5.9
topoverroad	*DOT*	114 ± 0.7

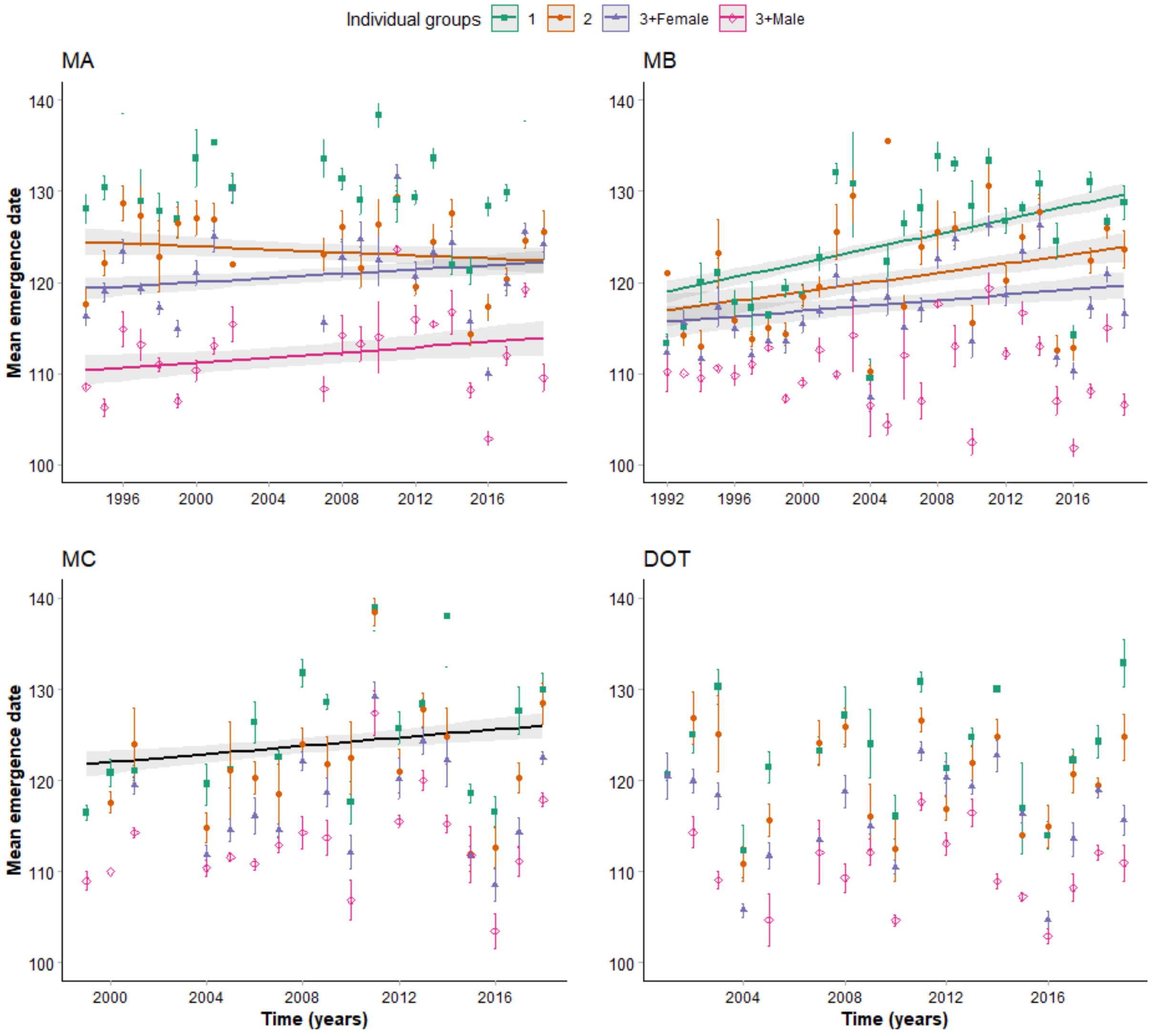
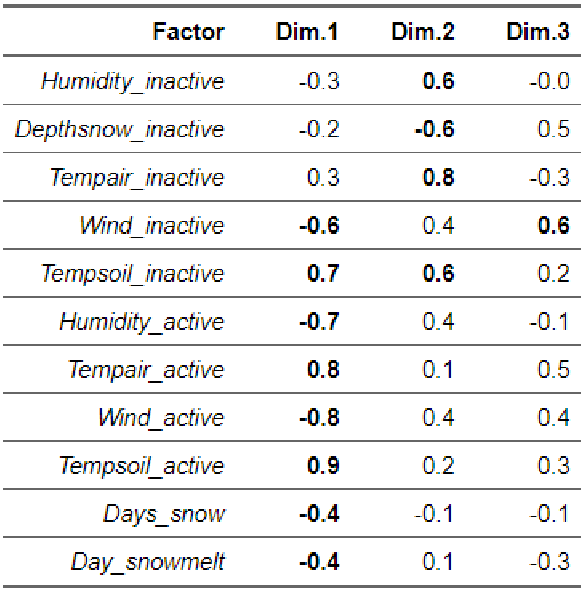


__Figure 65. Long-term directional changes in emergence date.__ Linear temporal trends of annual mean emergence dates (in ordinal day) on the four different meadows (MA, MB, MC, DOT) and for each individual category. Points represent annual means ± standard error in emergence dates of yearlings (green squares), 2-year-old individuals (orange circles), older females (blue triangles), and older males (pink diamonds). Plotted lines correspond to significant temporal trends and their 95% confidence interval.

Table 6. *Table of the contribution of the 11 climate variables to the principal component analysis performed on annual time series of climate variables per cluster. Loadings of climate factors on the three first components of the principal component analysis (cumulative explained variance: 78.3%).*



Factor	**Dim.1**	**Dim.2**	**Dim.3**
Humidity_inactive	-0.3	**0.6**	-0.0
Depthsnow_inactive	-0.2	**-0.6**	0.5
Tempair_inactive	0.3	**0.8**	-0.3
Wind_inactive	**-0.6**	0.4	**0.6**
Tempsoil_inactive	**0.7**	**0.6**	0.2
Humidity_active	**-0.7**	0.4	-0.1
Tempair_active	**0.8**	0.1	0.5
Wind_active	**-0.8**	0.4	0.4
Tempsoil_active	**0.9**	0.2	0.3
Days_snow	**-0.4**	-0.1	-0.1
Day_snowmelt	**-0.4**	0.1	-0.3

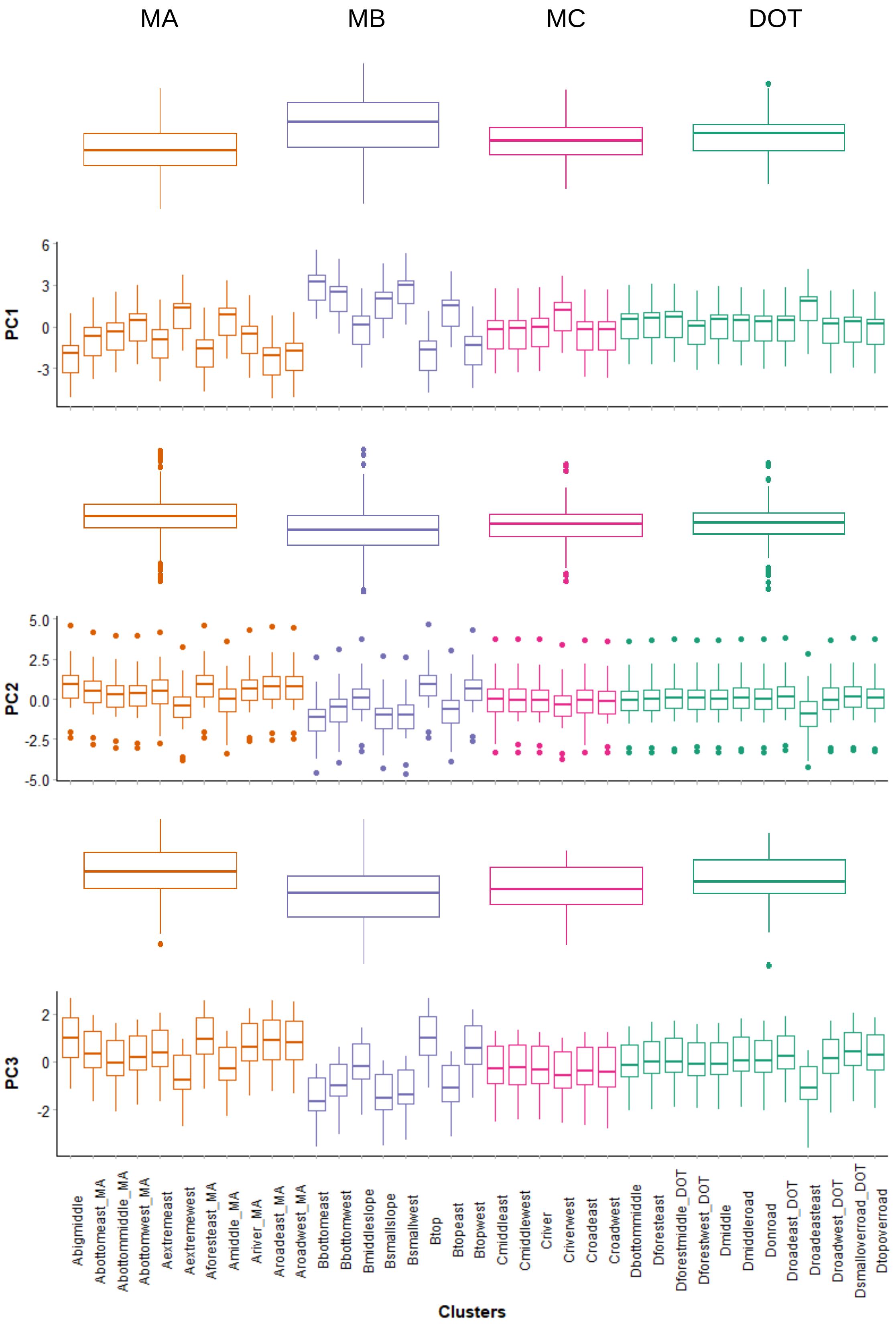


Figure 66. Spatial variability of climate. *Principal components, built from climate variables, compared between meadows (highest boxplots of each panel) and clusters of the different meadows (lowest boxplots of each panels).*

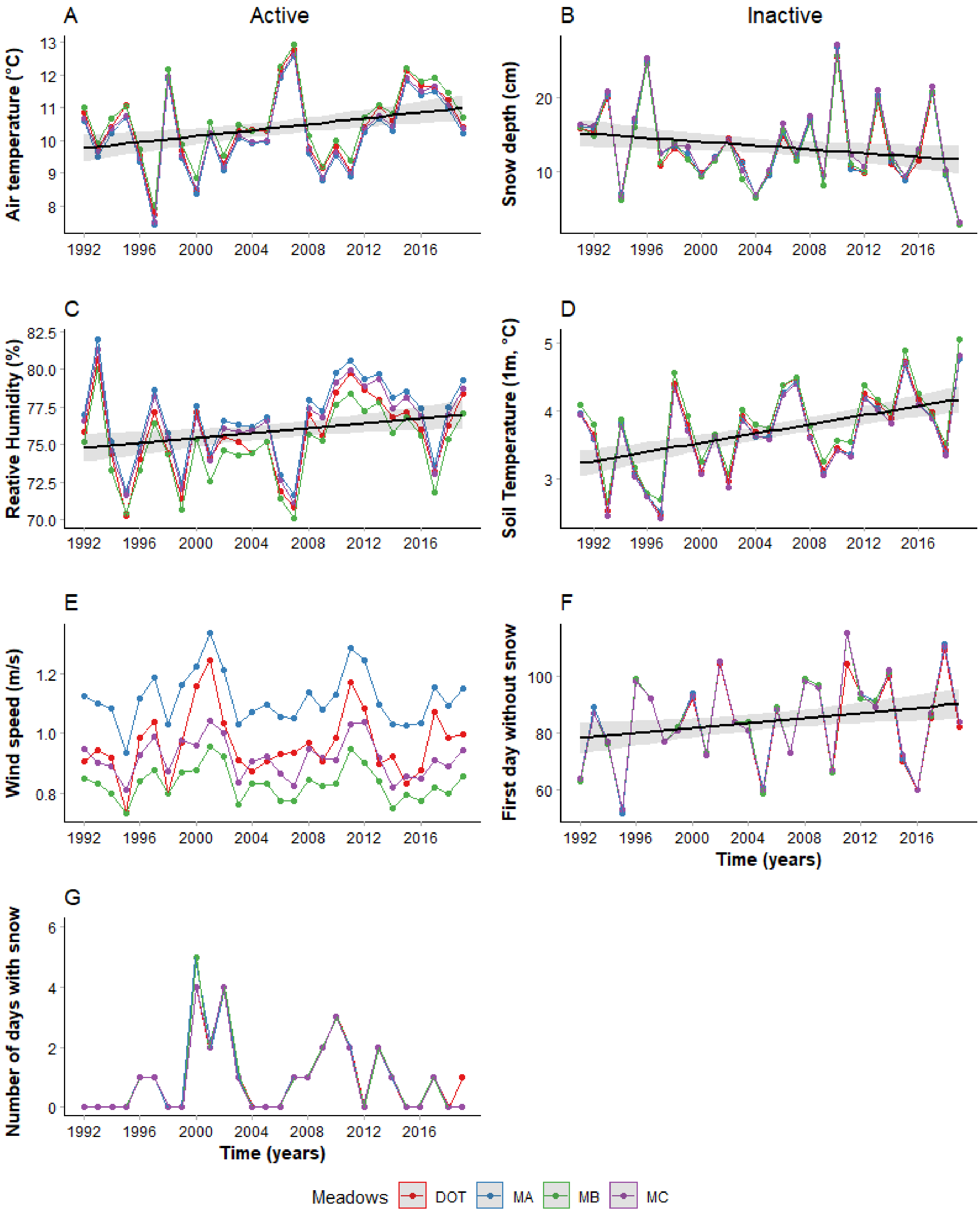


Figure 67. Long-term directional changes in climate. *Linear temporal trends of annual mean values of climate variables averaged by meadow on the four different meadows (MA, MB, MC, DOT). Plotted lines correspond to significant temporal trends and their 95% confidence interval (shaded area).*

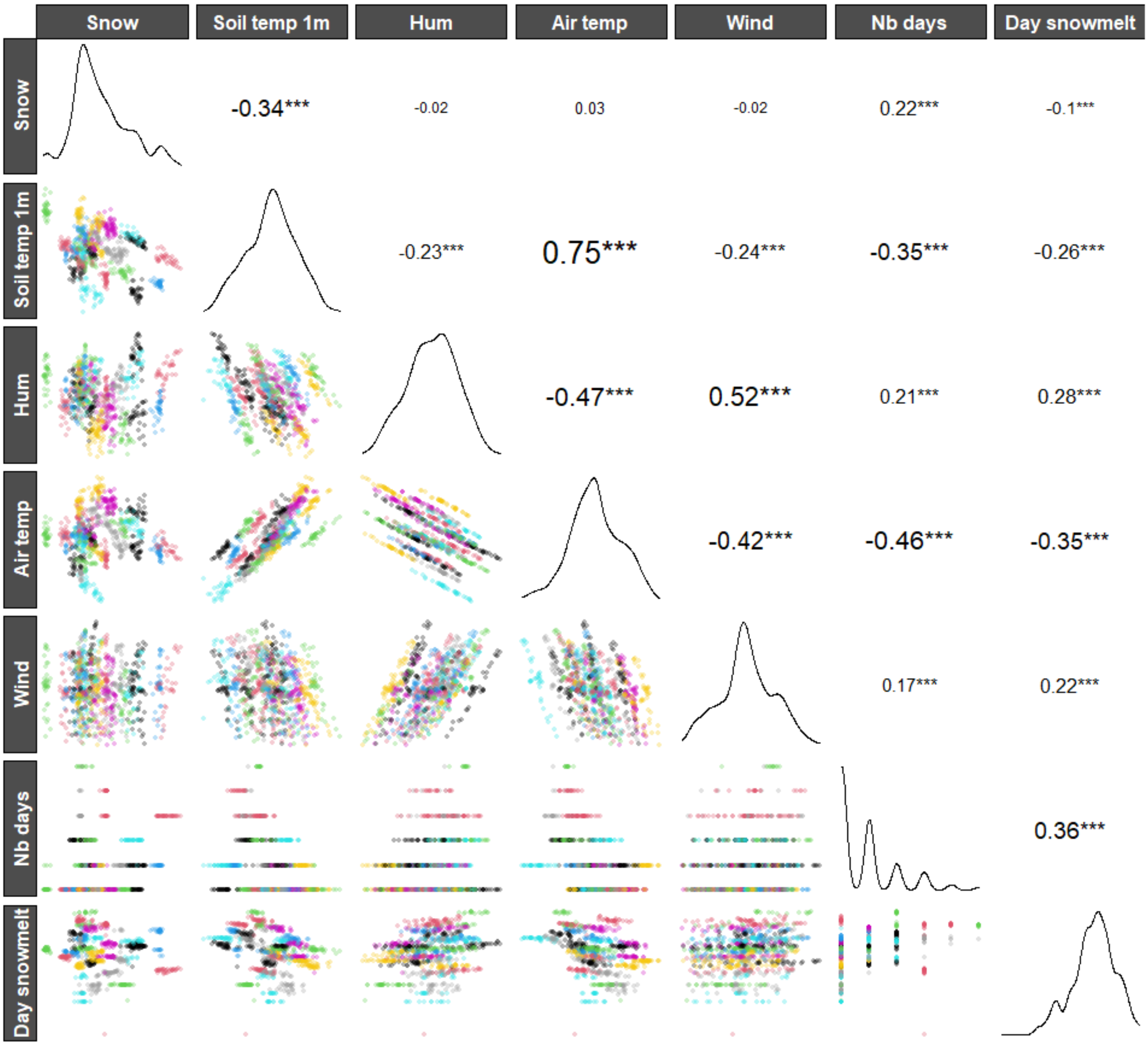


Figure 68. Correlations between the different climate variables. *The correlation matrix was established from climate variables used in the analyses on the link between climate and emergence date, averaged by year and cluster. Colors correspond to the different clusters (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).*

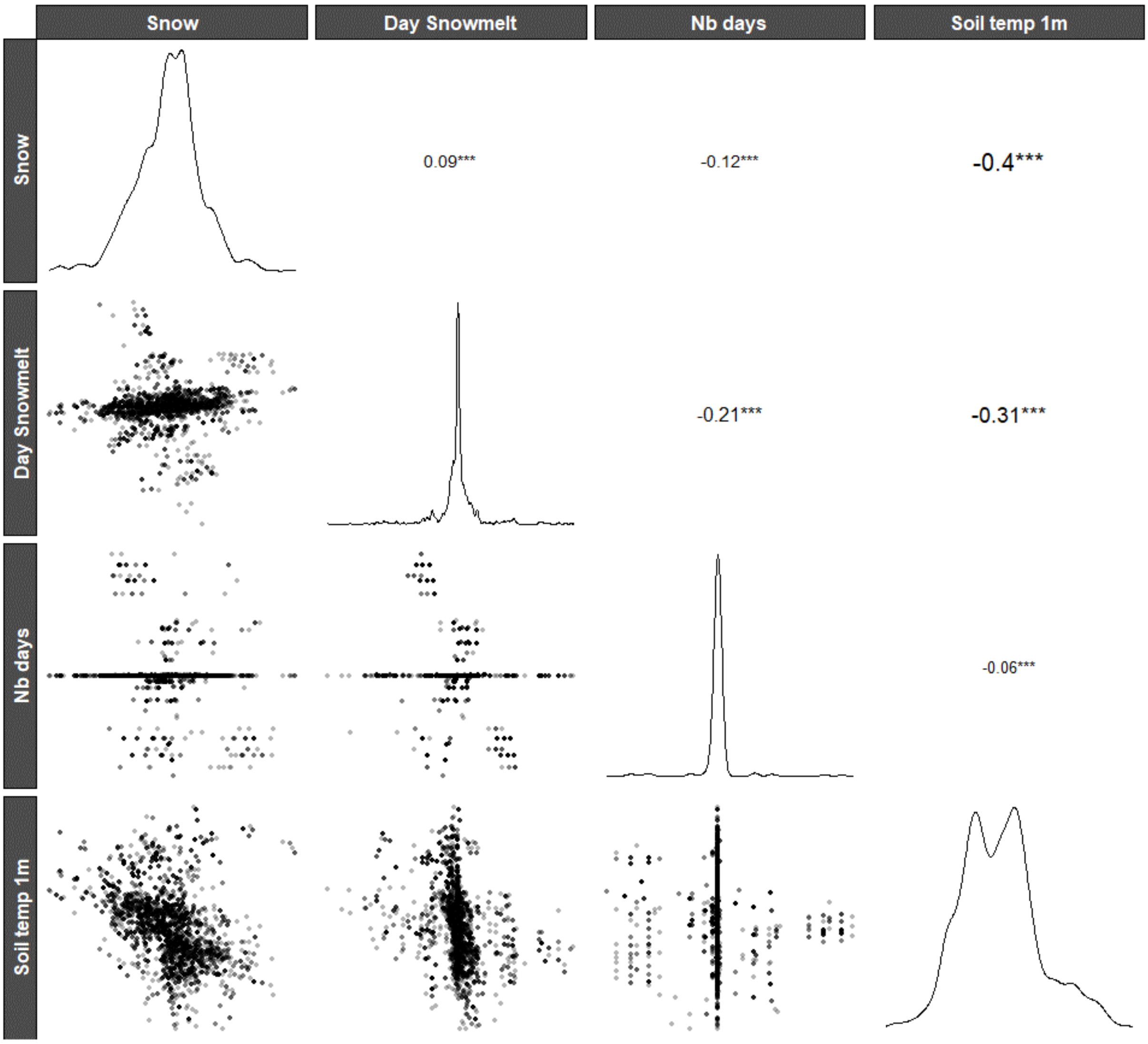


Figure 69. Spatial correlations between the different climate variables. *The correlation matrix was established from climate variables used in climate – ED analyses and centered by year (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).*

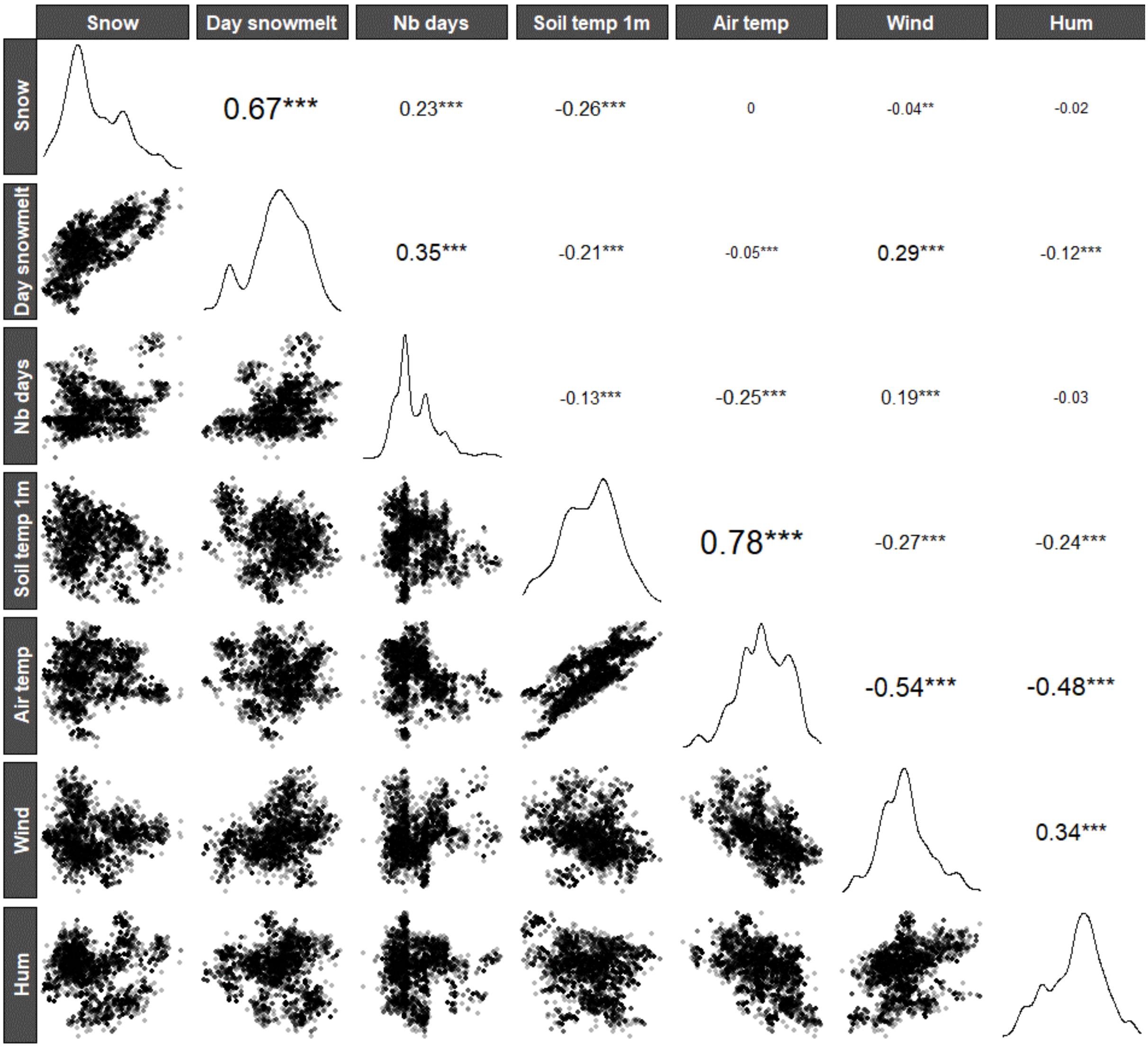
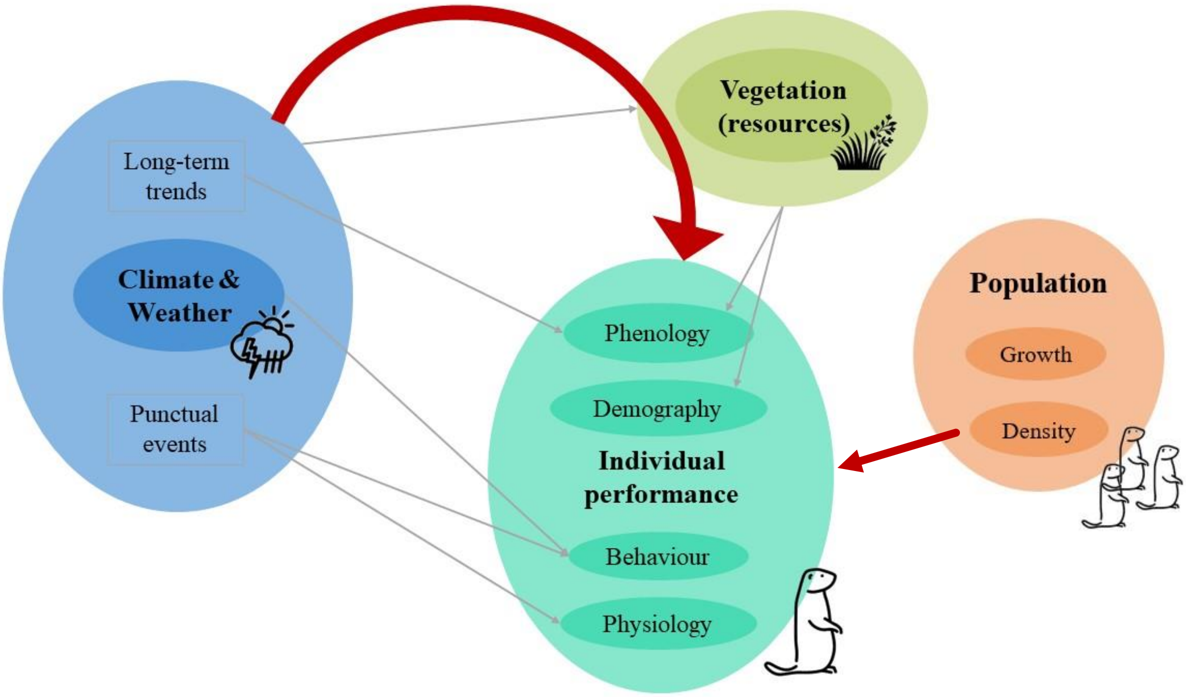


Figure 70. Temporal correlations between the different climate variables. *The correlation matrix was established from climate variables used in climate – ED analyses and centered by cluster (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).*

From interannual variations and long-term trends in climate to variations in resources and population density:

How do population size, demography, functional traits, and vegetation conditions vary temporally? How do they covary?

We found relationships between Columbian ground squirrels' phenology, and spatial and temporal variations in several climate variables. Whereas we hypothesized that climate effects on the animals during the active season are mediated partly through their effects on the quantity and quality of food resources and energy available to the animals, we did not consider vegetation directly. In this last chapter, I examined the joint effects of variations in vegetation and population density, on population functional traits and demographic rates (see red arrow on the illustration below).



CHAPTER 4

Food, density, and demography

Population density and vegetation resources influence demography in a hibernating herbivorous mammal

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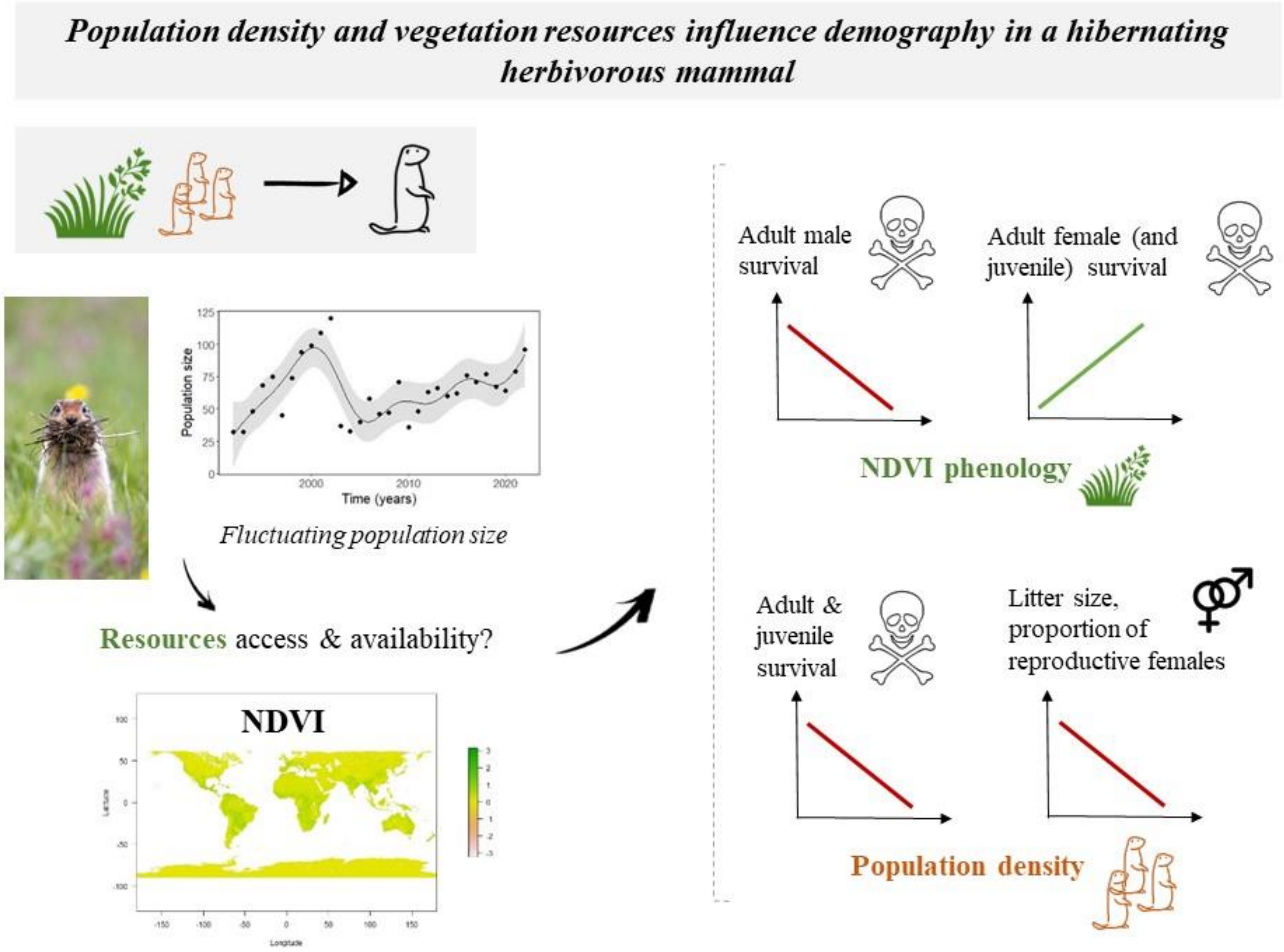
[Article submitted to *Oecologia*]

A. <u>Abstract</u>

Demography of herbivorous mammal populations may be affected by changes in predation, population density, harvesting, and climate. Whereas numerous studies have focused on the effect of single environmental variables on individual demographic rates, attempts to integrate the consequences of several environmental variables on numerous functional traits and demographic rates are rare. Over a 31-year period, we examined how forage availability (vegetation assessed through NDVI) and population density affected the functional traits and demographic rates of a population of Columbian ground squirrels (*Urocitellus columbianus*), an herbivorous hibernating rodent. We focused on mean population phenology, body mass, breeding success and survival. We found a negative effect of population density on demographic rates, including on breeding success and pup and adult survival to the next year. We found diverging effects of vegetation phenology on demographic rates: negative effects of earlier phenology on male survival, but positive effects on female (and marginally juvenile) survival. Interestingly, neither population density nor vegetation affected either population phenology or body condition in the following year. Nor did it affect the mean female somatic investment (mass gain over a season). Whereas our study provides evidence for important ecological interactions, showing that vegetation and population density variations affect animal demography (and differently depending on sex and breeding categories), the complex links between functional traits, demographic rates, and population dynamics still need to be explored.

Keywords: demographic rates, food, functional traits, herbivore, hibernation, mammal.

B. Graphical abstract



C. Introduction

Understanding the processes that influence animal life history and demography is a central focus in ecology. These processes have been especially well studied in rodents and other mammals (Blair 1953, Erb et al. 2001). They include competition for resources (e.g. forage such as herbage), predation, parasites, and diseases, and are often multifactorial in nature (Wallner 1987, Abbiati et al. 1992). For instance, variation in competition for food implies changes in the number of competitors (population density), or in the amount, availability and quality of food resources, or both (Putman et al. 1996, Solberg et al. 2001, Lima et al. 2003, Saether et al. 2004, Le Galliard et al. 2010).

Resource availability can have strong effects on consumer body mass, survival, and reproductive timing or output (also known as the "bottom-up effect", Gruner et al. 2008, Preisser 2008). For primary consumers (herbivores) vegetation biomass, quality, rate of growth, and phenology (*i.e.*, the timing of vegetation growth) should be important factors affecting functional traits and demographic rates, especially in seasonal environments where few resources are available in winter (Hansson 1971, O'Connell 1989, Jędrzejewski and Jędrzejewska 1996, Korslund and Steen 2006). In such environments, life cycles have often evolved so that reproduction coincides with brief periods of high resource availability in the environment (Williams et al. 2017c, Rehnus et al. 2020). Species that use daily foraging during such productive periods to support reproduction are termed "income breeders" (Jönsson 1997, Broussard et al. 2005).

The dynamics of vegetation growth are important aspects of these periods of high resource availability. Vegetation growth, quality, and timing are strongly influenced by climate (Voigt et al. 2003, Rosenblatt and Schmitz 2016). Vegetation growth is usually higher with warmer temperatures and increased precipitations (He et al. 2012, Verbyla and Kurkowski 2019), making it susceptible both to interannual weather variability and climate change. Further, with global warming, a general advancement of plant phenology has been observed in a variety of ecosystems (Cleland et al. 2007, Piao et al. 2019). Yet, non-concomitant changes in the timing of different trophic levels can lead to mismatches between periods of optimal food availability and the timing of reproduction, negatively affecting individual reproductive success and survival, with consequences for population size (Plard et al. 2014) and viability (Cohen et al. 2018, Radchuk et al. 2019, Schano et al. 2021). Changes are particularly marked in Arctic and alpine ecosystems, where climate is rapidly changing (Walther et al. 2005, Finch 2012, Gottfried et al. 2012, Thackeray et al. 2010, Zheng et al. 2022). This is critical for hibernating species inhabiting those regions, since they depend on the phenology, amount and quality of vegetation growth during the temporally restricted food bursts of the active season to reproduce and prepare for subsequent hibernation (Tchabovsky et al. 2005).

Besides the influences of food production (timing and quantity), the access to resources in a given area often depends on population density (Clutton-Brock and Harvey 1978). Intra-specific competition for resources is a mechanism which may compound the negative effects of phenological mismatches between resource availability and species reproduction, depending on yearly changes in population density (Bomford & Redhead, 1987; Reed et al., 2015; Ross et al., 2018). Population density has also been widely shown to affect reproduction and survival, including through effects on individual stress and body condition (Arcese and Smith 1988, Eccard and Ylönen 2001, Bonenfant et al. 2002, Creel et al. 2013, Tveraa et al. 2013, Reed et al. 2015, but see (Boonstra and Boag 1992). However, to date, comprehensive studies investigating forage availability and population density effects on several functional traits and demographic rates simultaneously are scarce (Violle et al. 2007, Gamelon et al. 2017).

We described temporal trends in functional traits and demographic rates of a population of a small hibernating rodent, the Columbian ground squirrel (*Urocitellus columbianus*), over a 31-year period. We tested the hypothesis that changes in resource availability and accessibility affected the functional traits and demographic rates Specifically, we tested how resource availability (estimated using the Normalized Difference Vegetation Index, NDVI; Pettorelli et al. 2005, 2011, Hurley et al. 2014, Rézouki et al. 2016) and population density influenced juvenile and adult survival, breeding success, phenology, and body condition of individuals in our population. To do so, we (1) described population density, (2) described yearly fluctuations in vegetation phenology and biomass, (3) described temporal trends in functional traits and demographic rates, and (4) evaluated the contributions of forage availability and population density to these temporal trends of functional traits and demographic rates.

Columbian ground squirrels are hibernating, colonial rodents that inhabit open meadows of the Rocky Mountains (Elliott and Flinders 1991). Their active period is notably short as they hibernate from August to April of the following year, about 70% of the year (Dobson et al. 1992). Resource consumption is important during the spring and summer activity period, when reproduction and fattening for subsequent hibernation occur (Young 1990b). Experimental studies have shown that food supplementation during the active period increases juvenile survival, the size of litters produced, and adult body mass (Dobson and Kjelgaard 1985a, 1985b, Dobson et al. 1986, Dobson and Murie 1987, Dobson 1988). Moreover, seasonal climate influences female annual fitness, a positive association being found between annual fitness, and spring temperature and early summer rainfall (Lane et al. 2012, Dobson et al. 2016). The underlying assumption is that such climate conditions should provide favorable conditions for grass and herbs growth, as suggested by subsequent positive impacts on reproduction and survival (Ritchie 1988, 1990). Similarly, climate conditions during the active season affect ground squirrels as they enter hibernation, by modifying the extent of reserves that are stored as fats before hibernation (Zammuto and Millar 1985).

The demographics and size of Columbian ground squirrel populations are primarly regulated by food availability (Dobson and Kjelgaard 1985a, Dobson 1995, Dobson and Oli 2001a). Thus, relaxing competition for food (*e.g.* in years of low population density) was expected to increase individual success, through higher mass gain over the season and consequently better survival, as well as higher reproductive success. These expectations were tested by comparing population density to the demographic rates and functional traits. In addition, resource availability was expected to have direct effects on individual condition. Active seasons with early and high vegetation growth and biomass that are timed with the reproductive and hibernating phenology of ground squirrels were expected to lead to higher body mass, reproductive success, and survival for this primarily income breeder (Dobson et al. 1999, Broussard et al. 2005). We used NDVI to calculate vegetation indices reflecting the timing and rate of spring greening-up, as well as yearly overall vegetation biomass, to disentangle these processes. Our expectations about the effects of vegetation were tested by comparing indices based on NDVI to the demographic rates and functional traits.

Carry-over effects from a current active period to the subsequent annual active period were also expected. A low density population with less competition, associated with high forage growth/biomass, was expected to allow individuals to store more body reserves for hibernation, positively affecting individual mass the next spring (Murie and Boag 1984, Dobson et al. 1992, Rubach et al. 2016). We tested for these carry-over effects by examining whether current-year conditions appeared to influence subsequent-year traits of the ground squirrels. Such a mechanism might also influence how individuals adjust their emergence timing to yearly changes in environmental conditions (i.e. timing of snowmelt, Lane et al. 2012, Dobson et al. 2016, Tamian et al. 2022, Thompson et al. *In press*).

D. Materials & Methods

1. Population density

Each year on MB, from 1992 to 2022, we counted all individuals in the population that emerged from hibernation, including male and female adults and yearlings, but excluding offspring born later within the year (pre-breeding census). Because the meadow was delimited to a fixed surface of ca. 2.6 ha, ‘population density’ has a consistent relationship to population size and both terms are used interchangeably in our study. Further, as the entire population was trapped each year (see in general materials and methods), no associated error had to be considered with the counts.

2. Yearly vegetation growth & biomass

To quantify yearly vegetation growth and biomass and examine its effects on ground squirrel demography and life history traits, we used the Normalized Difference Vegetation Index (NDVI) (Pettorelli et al. 2005, 2011, Hurley et al. 2014, Rézouki et al. 2016). We used and described the changes in the three different index calculated from NDVI values from 1991-2022: (1) mean yearly NDVI, (2) NDVI rate of increase within each year, and (3) an index of the seasonal phenology (starting week of the increase period of NDVI, details in general materials and methods). The phenology index might be particularly early compared to the ground squirrels’ phenology, but this is because it represents the overall greening of vegetation over a relatively large area. Here, we use it as an overall index of vegetation phenology to characterize interannual variations in green-up, as has been applied previously (Pettorelli et al. 2005).

3. Temporal changes in population demographic rates & functional traits

We examined how different demographic rates and functional traits varied over time. For demographic rates, we considered traits pertaining to reproduction (including mean annual litter size at weaning, mean juvenile survival of the offspring from weaning through their first hibernation, proportion of reproductive females in the population), and survival (including mean annual adult male and female survival, and yearling female survival). Survival could not be determined accurately for yearling males due to dispersal (Wiggett et al. 1989, Wiggett and Boag 1989, Festa-Bianchet and King 2011). The proportion of reproductive females in the population was calculated as the annual percentage of mature females that weaned a litter. For functional traits, we considered traits related to phenology (mean male and female dates of emergence from hibernation), or body condition averaged annually on the population (mean adult male and female body mass at emergence from hibernation, and mean mature female mass gain between emergence and weaning of offspring).

We tested patterns of change in demographic rates and functional traits over the 31 years of study (from 1992-2022; except for a few missing data in 2020 due to the COVID-19 pandemic), using generalized additive mixed models (GAMMs). We applied the "gamm" function in R ("mgcv" package, Wood 2017), with year as an independent variable.

4. Common trend analyses

We used a Dynamic Factor Analysis (DFA) ('MARSS' function of the 'MARSS' package in R) to examine common trends between population functional traits and demographic rates and their relationships with population density and vegetation (Zuur et al. 2003). DFA looks for common trends in multivariate time series and allows testing for important covariates which might be thought to influence time-series. Because of the seasonal biology of ground squirrels (cycles of hibernation and active seasons), we separated our analyses into **two different models**: *Model 1* considered the effect of the **preceding** year (population density and vegetation indices) on hibernation emergence date and mass. *Model 2* considered the effect of the **current** year (population density and vegetation variables) on the other demographic rates and functional traits (litter size at weaning, mean juvenile survival, proportion of reproductive females in the population, mean female mass gain over the active season, mean adult male, adult female, and yearling female survival). To compare effect sizes, all independent variables were standardized prior to analyses.

Explanatory variables (population density and the 3 vegetation indices) were treated as covariates in the DFA models. For models 1 and 2, we ran a model selection procedure based on Akaike's information criterion corrected for small sample sizes (AICc). Candidate models were built based on their error matrix structures (diagonal and equal, diagonal and unequal, equal variance covariance, and unconstrained, see details in Supplementary Materials: Figure 76), the inclusion or not of the different covariates (from no covariates to all four: population density, mean NDVI, NDVI slope, NDVI phenology), and the numbers of common trends (from 1 to k-1, k being the number of explained variables). The model with the lowest AICc was considered the best fit. We also presented competing models that were similar in terms of strength of evidence, with a ΔAICc < 2 and AIC weights > 0.1 (W_i, corresponding to 10%, Wagenmakers and Farrell 2004, Burnham et al. 2011).

E. Results

1. Population density

Over the 31-year long-term monitoring, overall mean spring population density (= population size on the constant 2.6 ha) averaged 64 ± 4 animals, ranging from 32 (1992 and 1993) to 120 (2002) individuals. Population density exhibited a strong increase from 1993-2002 and a sudden drop between the active period of 2002 and the active period of 2003 (from 120 to 37 individuals, GAM; edf = 7.868, F = 4.369, $p = 0.002$, $R^2 = 0.54$, Deviance explained = 65.8%, n = 31, Figure 71). After 2003, population size varied more moderately, generally recovering slowly from 33 individuals in 2004 to 96 individuals in 2022. On average, the population was composed of 61% ± 1% females (ranging from 44% to 81%). Over the 31 years of monitoring, there were between 14 and 77 females and between 7 and 43 males in the population.

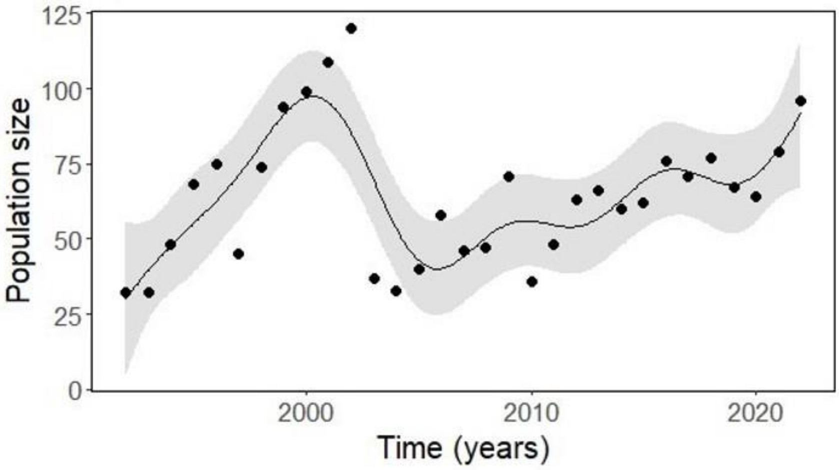


Figure 71. Total population size. *Number of individuals in the population reflecting the population size and therefore density (the total surface is not changing) over the years, along with the fitted line and 95% confidence interval from GAM.*

2. Yearly vegetation growth & biomass

Overall, the mean NDVI averaged from weekly values during the animal's active period (from the first emergences: week 14, to the approximate period of immergence: week 34) was 0.218 ± 0.008, ranging from 0.118 (1994) to 0.303 (2022) per year (n = 31 years). Years of high NDVI reflected "greener" years of overall higher productivity and biomass (Pettorelli et al. 2005), such as 2001, 2003, 2006, 2007, 2015, and 2022 (Figure 72.A). Contrarily, 1994 and 2021 were especially low in terms of NDVI during the active period of animals. Vegetation growth rate (NDVI slope) was also variable over the years, averaging at a slope of 0.018 ± 0.001, ranging from 0.007 (1992) to 0.033 (2011). As an example, 2002, 2011, and 2022 were years during which the NDVI increased rapidly, corresponding to rapid vegetation growth on the meadow (Figure 72.B). Oppositely, the growing slopes for the first years of the long-term study (1992, 1993, 1994), and for 2013 and 2014 were relatively low. The index used to reflect vegetation phenology varied greatly, from week 1 (January 1st) to week 26 (June 22nd) over the course of the study, overall averaging at week 14 (March 30th) ± 1 week. Vegetation green-up started particularly early in 1999, 2004, 2010, and 2016, as reflected by the phenology index (Figure 72.C).

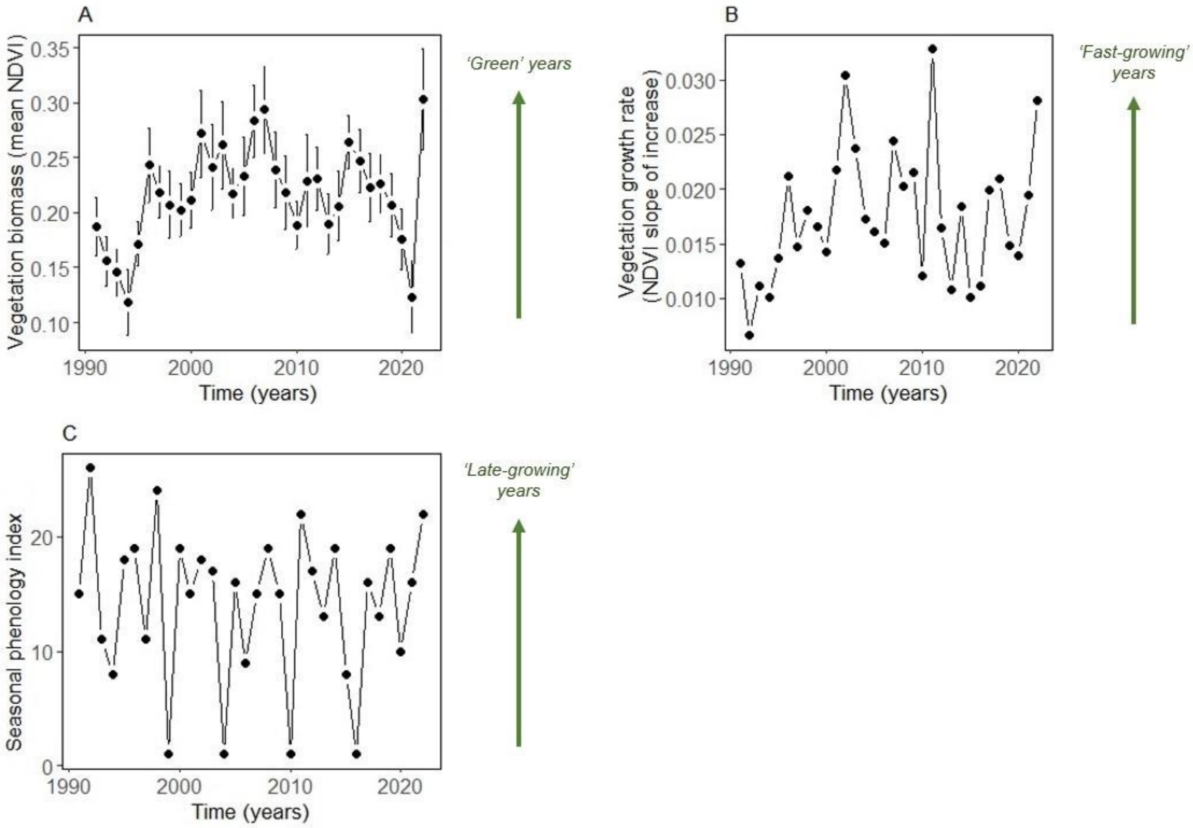


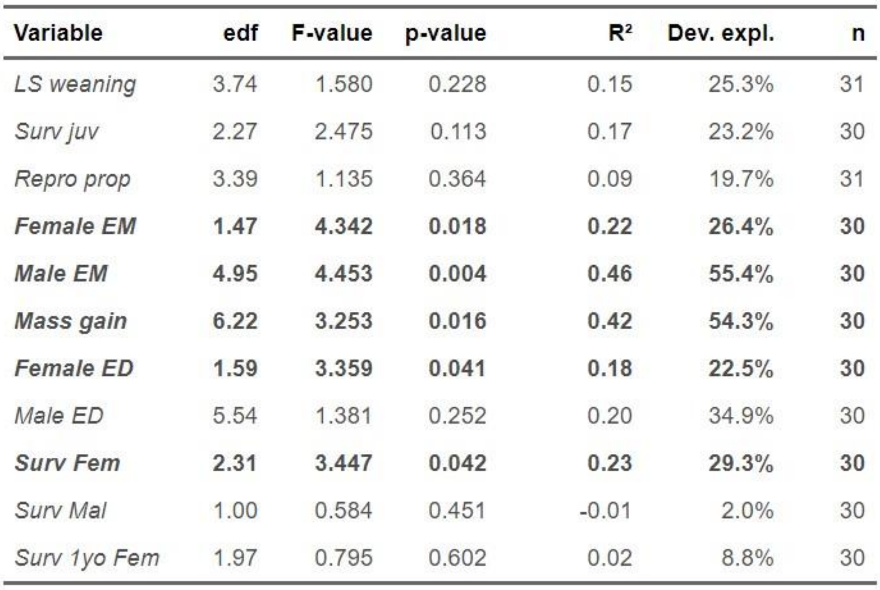
Figure 72. Fluctuations in vegetation biomass and growth over a 31-year period. *(A) Yearly vegetation biomass was estimated from the mean weekly value ± standard error of NDVI (greenness) over the active season of the squirrels, (B) vegetation rate of growth was estimated from the slope NDVI increase in each year, and (C) phenology index was defined as the week at which NDVI started increasing in each year (see Methods).*

3. Temporal changes in population demographic rates & functional traits

Demographic rates and functional traits exhibited marked interannual variations over the course of the study (see Table 7 with GAM results).

Table 7. Statistical results of temporal changes in population demographic rates and functional traits. *Statistics were extracted from each GAM of the different traits at the population level as a function of the year. An effective degree of freedom (edf) of one corresponds to a linear relationship, while higher edfs describe wavier curves. Variables that were*

statistically significant at the p ≤ 0.05 level appear in bold. The last columns (R², Dev. expl., n) correspond to the adjusted r-squared, the percentage of deviance explained, and the number of observations (years).



Variable	edf	F-value	p-value	R²	Dev. expl.	n
LS weaning	3.74	1.580	0.228	0.15	25.3%	31
Surv juv	2.27	2.475	0.113	0.17	23.2%	30
Repro prop	3.39	1.135	0.364	0.09	19.7%	31
Female EM	**1.47**	**4.342**	**0.018**	**0.22**	**26.4%**	**30**
Male EM	**4.95**	**4.453**	**0.004**	**0.46**	**55.4%**	**30**
Mass gain	**6.22**	**3.253**	**0.016**	**0.42**	**54.3%**	**30**
Female ED	**1.59**	**3.359**	**0.041**	**0.18**	**22.5%**	**30**
Male ED	5.54	1.381	0.252	0.20	34.9%	30
Surv Fem	**2.31**	**3.447**	**0.042**	**0.23**	**29.3%**	**30**
Surv Mal	1.00	0.584	0.451	-0.01	2.0%	30
Surv 1yo Fem	1.97	0.795	0.602	0.02	8.8%	30

None of the reproductive variables (litter size at weaning, juvenile survival, and proportion of reproductive females) displayed significant temporal trends (Table 7, Figure 73.A, B, C), interannual variability being much more important than longer-term changes. Both female and male emergence mass exhibited a marked decrease (Figure 73.D, E) over the course of the study, being stronger and more fluctuating in males (-148g, -29%; despite an increase in the very first years followed by decreases of different amplitudes) than in females (-44g, -10%; linear decrease). Female mass gain between emergence and weaning first increased strongly (+ 12%, 1997-2004), and fluctuated before decreasing, importantly in later years (- 21% 2019-2022, Figure 73.F). Females emerged from hibernation increasingly from 1992 to 2011, but emergence date stabilized in the last five years (Figure 73.G). The delay in emergence from hibernation over the study was less marked in males, and consequently not significant (edf = 5.54, $p = 0.252$), and its variability seemed to increase from 2011-2017 as well (Figure 73.H). The temporal pattern of adult female survival was relatively stable, showing a slight decrease from 1992 to 2002 followed by a slight increase until 2022 (Figure 73.I). Although not significant, the temporal pattern of male survival was a decreasing linear trend over time (Table 7, edf = 1), but highly variable between years (from 12% to 100% survival, Figure 73.J). Finally, yearling female survival varied from 0% (2004) to 100% (1993, 1997, 1999, and 2007), but did not display a significant temporal trend (Table 7, Figure 73.K).

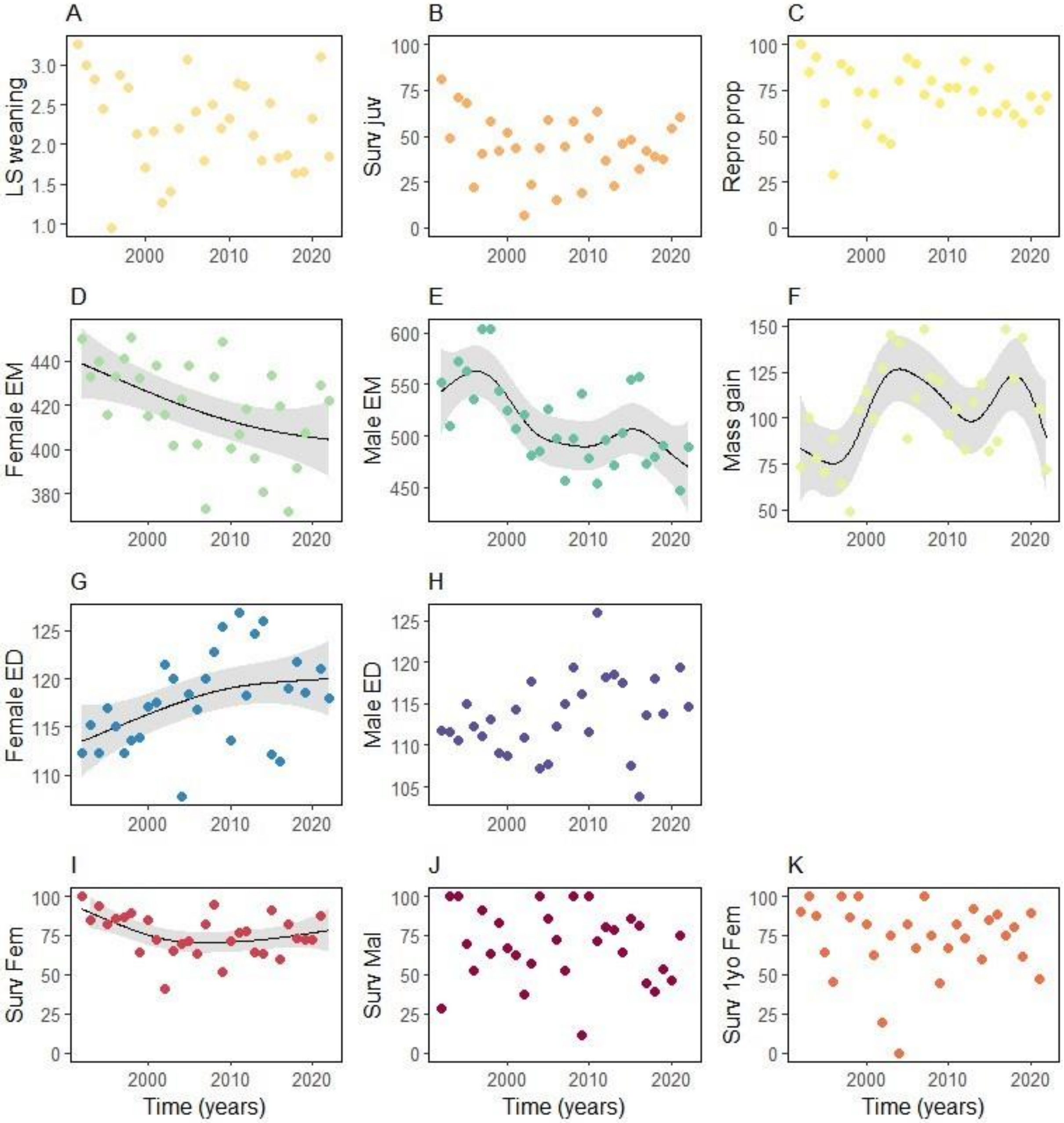
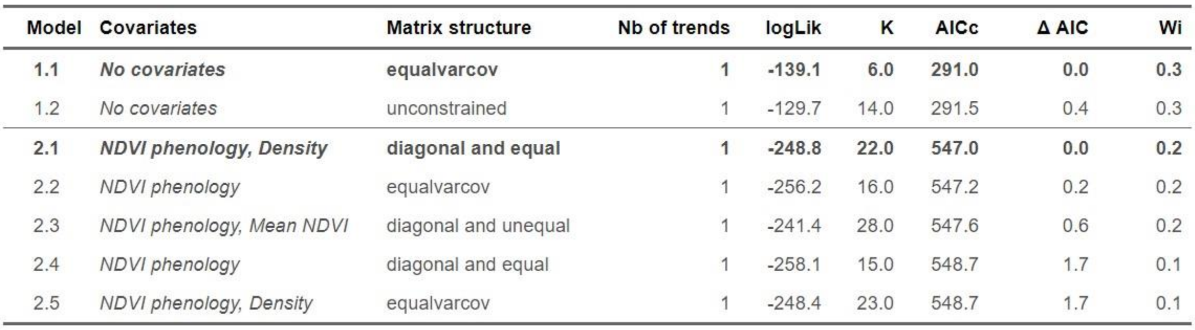


Figure 73. Temporal trends of population demographic rates and functional traits. *Temporal trends of (A) litter size, (B) juvenile survival, (C) reproduction proportion, (D) female emergence mass, (E) male emergence mass, (F) female mass gain, (G) female emergence date, (H) male emergence date, (I) adult female survival, (J) adult male survival, and (K) yearling female survival. Plotted lines correspond to significant temporal trends and their 95% confidence interval according to GAMMs, each colours correspond to variable colours in the DFA analysis.*

4. Common trend analyses

The first DFA model (*Model 1*) considered the effects of the previous year (population density and vegetation variables) on four functional traits. Model selection revealed the existence of one common trend in the four time-series of hibernation emergence date and mass of both males and females, but no influence of environmental covariates (population density and vegetation indices) from the preceding year (ΔAIC < 2, *Models 1.1* and *1.2* in Table 8).

Table 8. DFA model comparisons. *Based on the ΔAIC < 2, the best two models for Model 1 (Models 1.1 and 1.2, effects of density and vegetation indices in the* ***preceding*** *year on hibernation emergence date and mass) and the best five models for Model 2 (Models 2.1 to 2.5, effects of density and vegetation indices in the* ***current*** *year on litter size at weaning, mean juvenile survival, proportion of reproductive females in the population, mean female mass gain over the active season, mean adult male, adult female, and yearling female survival). The considered covariates were included, along with the structure of the matrix, the number of common trends found in the model, the log Likelihood, the number of parameters in the model (K), the AICc and ΔAIC (difference compared to the best model), and the AIC weight (Wi, probability of the model being the best model). Models with the lowest ΔAIC are indicated in bold. 'NDVI Phenology' was the seasonal vegetation phenology index and 'mean NDVI' estimated vegetation biomass.*



Model	Covariates	Matrix structure	Nb of trends	logLik	K	AICc	Δ AIC	Wi
1.1	***No covariates***	**equalvarcov**	**1**	**-139.1**	**6.0**	**291.0**	**0.0**	**0.3**
1.2	*No covariates*	unconstrained	1	-129.7	14.0	291.5	0.4	0.3
2.1	***NDVI phenology, Density***	**diagonal and equal**	**1**	**-248.8**	**22.0**	**547.0**	**0.0**	**0.2**
2.2	*NDVI phenology*	equalvarcov	1	-256.2	16.0	547.2	0.2	0.2
2.3	*NDVI phenology, Mean NDVI*	diagonal and unequal	1	-241.4	28.0	547.6	0.6	0.2
2.4	*NDVI phenology*	diagonal and equal	1	-258.1	15.0	548.7	1.7	0.1
2.5	*NDVI phenology, Density*	equalvarcov	1	-248.4	23.0	548.7	1.7	0.1

The two competing models differed only by the variance structure and the fit of the data, the model with an 'equal variance covariance' showing a much higher goodness of fit (Supplementary Materials: Figure 77). The common trend correlated positively with the trend for emergence dates of females and males, and negatively with the trend for emergence masses of female and males (|factor loadings| > 0.2, Figure 74), showing that males and females responded the same way but that mass and date displayed opposite temporal trends. This trend exhibited fluctuations but an overall increase from 1992-2013, a strong decrease in 2015-2016, and a stabilization at high values in recent years (Figure 74).

The second DFA model considered the effects of the current year (population density and vegetation variables) on seven demographic rates and functional traits. Model selection retained five competing models (ΔAIC < 2, *Models 2.1* to *2.5;* Table 8). All five models had one common trend for the seven traits and retained the NDVI phenology index as a covariate. While models differed in their matrix structure, models *2.1* and *2.5* also showed the importance of population density as a covariate and model *2.3* of mean NDVI. The common trend correlated positively with trends for litter size at weaning, the proportion of reproductive females, juvenile survival, yearling survival, and adult male and female survival. It correlated negatively with the trend for female mass gain between emergence and weaning (|factor loadings| > 0.2, Figure 74). The trend was generally stable through time, with short-term fluctuations and a large decrease in 2004 (Figure 74). The reconstruction of patterns in the data using these models (along with their 95% confidence intervals) appeared to closely fit raw data analyses (Supplementary Materials: Figure 78, *Models 2.1*, *2.2*, and *2.3*).

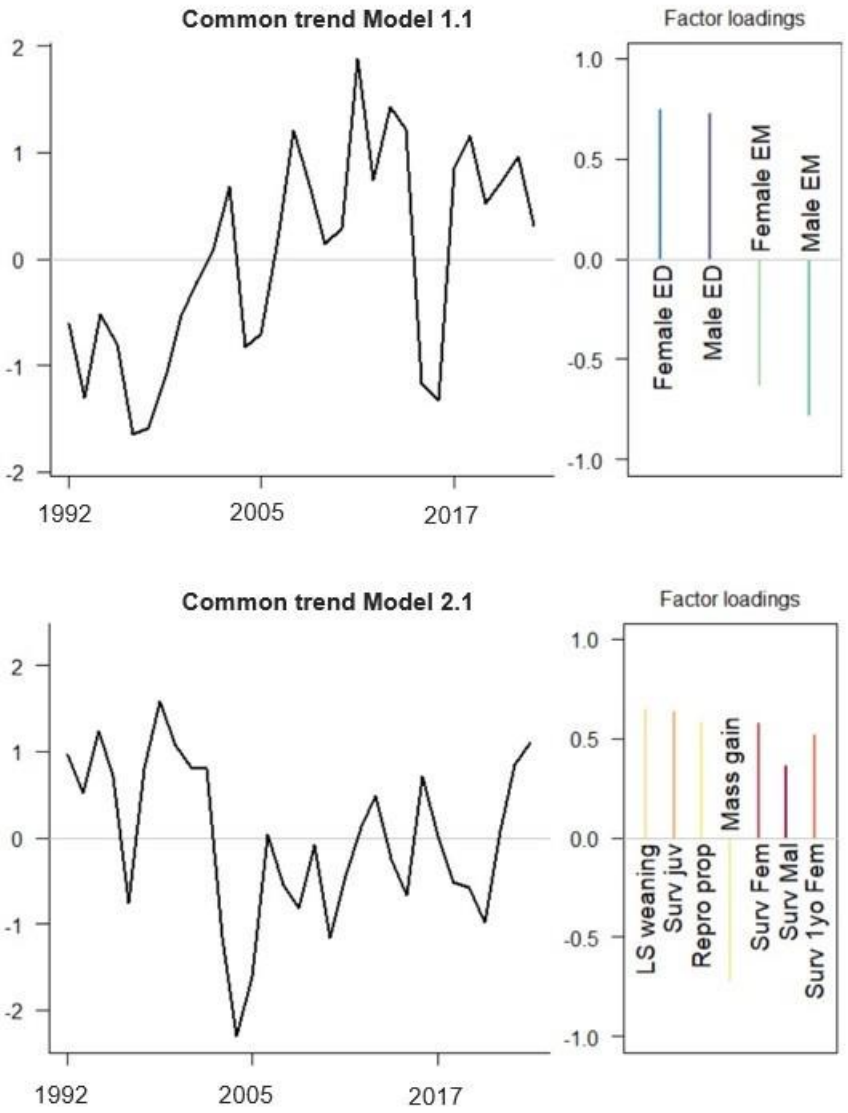


Figure 74. Common trends and associated variables for the best models. *Higher panel: Model 1.1 with the female and male emergence date (ED) and female and male emergence mass (EM). Lower panel: Model 2.1 with the litter size, juvenile survival, reproduction proportion, female mass gain, adult female survival, adult male survival, and yearling female survival. Positive loadings correspond to positive relationship with the trend, and negative loadings correspond to negative relationship with the trend (colored bars).*

Yearly population density was negatively related to all variables (although the effect on the survival of yearling females was not quite significant despite a large effect size), except for female mass gain (95% CI: [-0.282 – 0.532]) (Figure 75). The timing of NDVI increase was strongly and significantly negatively associated with the survival of adult males (Figure 75), and positively associated with the survival of adult females (95% CI: [0.009 – 0.580]) and juveniles (though not significantly, 95% CI: [-0.085 – 0.500]). To sum up, in years when population density was high, mean population reproductive and survival parameters were low. In years when vegetation grew relatively later, the survival of adult males was low, but the survival to the next year of adult females and juveniles born during the same season was high. Covariate effects regarding the timing of NDVI increase from *Model 2.2* to *2.5* were similar to effects described above (Supplementary Materials: Figure 79). In *Model 2.3*, mean NDVI (overall vegetation biomass) had a marked negative effect on litter size (though not statistically significant, mean effect = -0.41, 95% CI: [-0.696 – 0.057]) and a significant negative effect on juvenile survival, mean effect = -0.51 (95% CI: [-0.753 – -0.047], Supplementary Materials: Figure 79).

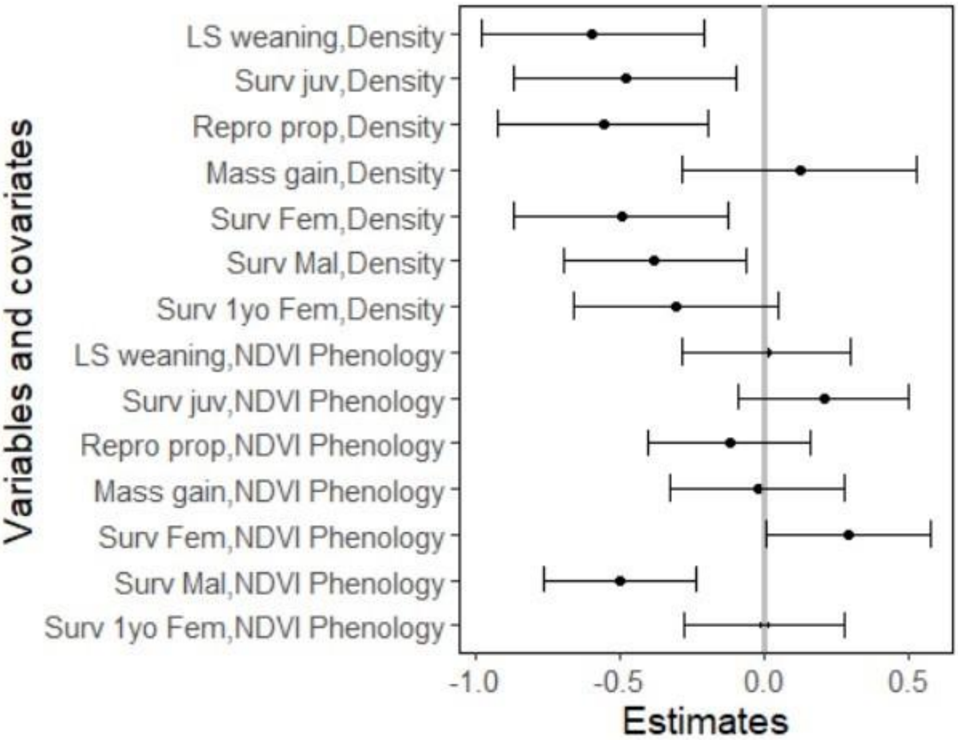


Figure 75. Vegetation phenology and density effects on demographic rates and functional traits. *Estimates and 95% confidence intervals from the best DFA model (2.1), representing the effect of covariates (NDVI phenology which corresponds to index of NDVI increase and density) on demographic rates and functional traits (litter size, juvenile survival, reproduction proportion, female mass gain, adult female survival, adult male survival, and yearling female survival).*

F. Discussion

Whereas the effects of climate or population density on population vital rates (survival and reproduction) (Bomford and Redhead 1987, Ross et al. 2018) or population growth rates (Reed et al. 2015) have often been examined in wild animals, fewer explorations exist into the life history and demographic processes underlying these effects (Oli and Dobson 2003, Jenouvrier et al. 2018). Here, we examined the effects of forage availability and population density on the demographic rates (yearling and adult survival, breeding success) and functional traits (phenology, body condition) of a Columbian ground squirrel population over a 31-year period. Our results showed a general negative effect of population density on vital rates in the ground squirrel population. However, the effects of vegetation on demographic rates were varied and sometimes opposite in direction depending on the sex or age categories. Similarly, despite effects of both density and vegetation on population demography, we did not find evidence for carry-over effects of density and vegetation on functional traits (emergence phenology and body condition) in the following year.

1. Changes in demographic rates & functional traits

Population density markedly varied over the course of our study, and exhibited a strong increase followed by an abrupt fall and a progressive recovery. Given the observed changes in population density, we could expect high variability in vital rates. We found little evidence for long-term changes in demographic rates, except perhaps in females, for whom survival slightly decreased over the study. However, interannual variability in demographic rates was strong, especially in adult male survival, yearling female survival, juvenile survival, and in litter size. By contrast, adult female survival exhibited relatively low interannual variation (see also Viblanc et al. 2022), as would be expected from long-lived species where adult survival may be buffered by life-history trade-offs against temporal variability (*viz.*, the "ecological canalization hypothesis" of Gaillard et al. 1998, Gaillard and Yoccoz 2003). Such buffering might not apply to males, since they have highly variable reproductive success (can produce up to 30 pups in one season, Raveh et al. 2010); as well, we found their survival to vary strongly among years.

Next, we investigated the temporal variability of functional traits, which are typically highly variable and responsive to environmental stochasticity (Jenouvrier et al. 2018). We found a common trend between emergence dates of males and females (Thompson et al. *In press*), and this trend was inversely related to emergence mass. This means that individuals emerged later but lighter in some years, but earlier and heavier in others. In years when emergence from hibernation was delayed, likely due to unfavorable microclimatic conditions (Tamian et al. 2022), the inactive period spent fasting below ground would have lengthened, leading individuals to have fewer body reserves when they first came out of hibernation. Additionally, female ground squirrels emerged later and lighter from hibernation in recent years, showing changes in functional traits over time, suggesting plastic or micro-evolutionary responses to changes in the environment (Williams et al. 2017b, Lane et al. 2019, Bonamour et al. 2020). We also found strong interannual variation in female mass gain (somatic investment) over the breeding season (between emergence and weaning of the offspring). Since mass gain varied between means of less than 75g to more than 125g, but the length of this period varied little (from 53 to 57 days on average; Supplementary Materials: Figure 80), the strong interannual variation observed was more likely related to changes in food availability/quality/phenology or to changes in energy allocation between soma and reproduction than changes in amount of time needed to accumulate body mass.

2. Density-dependence and access to resources

In years when population density was high, mean litter size, the proportion of females reproducing, and the survival of all individuals were low, at least to some degree. Increased population density might translate into increasing competition between individuals leading to a decreased access to resources or higher aggression rates, stress and energy expenditure, and ultimately decreased survival and reproduction, as shown in other ground squirrel populations (Boag and Murie 1981) and in other species (Arcese and Smith 1988, Karels and Boonstra 2000, Bretagnolle et al. 2008, Reed et al. 2015, Allison and Conway 2022). Similarly, the reproductive success of reindeer (*Rangifer tarandus*) has been shown to respond both to population density and vegetation green-up (Tveraa et al. 2013). Finally, we did not find density dependence for functional traits, such as emergence phenology and mass, and female mass gain between emergence and weaning. This could reflect a trade-off where females invest less in reproduction (Rubach et al. 2016), or shift their allocation strategy (e.g. by modulating offspring sex ratio; Barra et al. 2021, Kanaziz et al. 2022), to invest more into their soma and maintain their mass gain through the season when competition is high.

3. Differential effects of vegetation phenology on survival between sexes & reproductive status

Our index of vegetation phenology had a strong influence on demographic rates: in years when the initial NDVI increase was late, adult male survival was low, but the survival of adult females and juveniles born during the active season was high. Columbian ground squirrels are mostly income breeders (Broussard et al. 2005) that rely heavily on environmental resources during their short (4-mo.) breeding and subsequent active season. Previous experimental food-supplementation and empirical studies on Columbian ground-squirrels had highlighted the importance of food resources for life-histories of this primarily income breeder but only in terms of quantity, with no regards to phenology and potential mismatch effects (Dobson and Kjelgaard 1985a, 1985b, Dobson et al. 1986, Dobson 1988, 1995, Ritchie 1990). The observed differences between males and females in the association of vegetation onset and survival might be explained by differential energy requirements at different times of the breeding season. For males that emerge from hibernation first (Murie and Harris 1982, Tamian et al. 2022) and establish territorial boundaries by competing with other males as soon as they emerge (high competition for mating; (Murie and Harris 1978, Manno and Dobson 2008, Raveh et al. 2010), high resource and energy availability early in the season is critically important to mating, and thus reproductive success.

In contrast, yearling, non-reproductive individuals, and adult females were not under the same energetic constraints as males, and consequently were less affected by the phenology of initial green-up. Furthermore, the most energetically-constraining period for female mammals is during lactation (Festa-Bianchet and Boag 1982, Kenagy and Barnes 1988, King et al. 1991, Speakman 2008), and females should benefit most from forage conditions later in the season. Similarly, later vegetation growth might be beneficial for juveniles born in the season which need to store enough energy between their weaning and the start of hibernation (Murie and Boag 1984, Neuhaus 2000). Indeed, later vegetation growth could allow females and juveniles to match their most-energy-demanding period with the early vegetation succession stage of potentially higher quality.

Here, demographic and functional variables were density-dependent and vegetation phenology had an effect on demographic traits, but these effects differed according to individual sex and breeding status, possibly due to their different energetic requirements. The integration of several biological variables allowed us to disentangle the respective effects of population density and forage availability on ground squirrel demography. In a relatively closed population (which is mostly our case, since only yearling males emigrate), environmental effects can only influence population growth through changes in demographic rates (survival and reproduction, Greenwood 1980, Dobson 1982). Future studies could focus on the relative contribution of traits and demographic rates to population growth rate, allowing to test for environmental effects on population dynamics in this seasonal mammalian species by considering its underlying demographic and functional mechanisms.

G. Acknowledgements

We are grateful to JO Murie for initiating the ground squirrel long-term study, for his critical comments on the study, and for his precious advice and council throughout the years. We are grateful to the Biogeoscience Institute, University of Calgary (E.A. Johnson and S. Vamosi, Directors; Judy Mappin-Buchannan and Adrienne Cunnings, Managers of the Kananaskis Field Stations; Kathreen Ruckstuhl, faculty member responsible for the R.B. Miller Field Station) for housing and facilities during fieldwork. We are especially grateful to E.A. Johnson for his continued support throughout the years. We are grateful to our colleagues and friends P. Neuhaus and K. Ruckstuhl for their critical insights during fieldwork, and to P. Neuhaus for his expert advice on ground squirrel ecology. The fieldwork was aided by many volunteers and students over the years, and we thank them for their excellent efforts. This research was supported by a USA National Science Foundation grant (DEB-0089473) and a fellowship grant from the Institute of Advanced Studies of the University of Strasbourg to F.S.D., a Natural Sciences and Engineering Research Council of Canada grant to J.O. Murie, a CNRS Projet International de Coopération Scientifique grant (PICS-07143), an AXA Postdoctoral Research Fellowship, and a Fyssen Foundation research grant to V.A.V., and an Initiative d’Excellence (IDEX attractivité) research grant to C. Saraux. F.S.D. thanks the Région Grand Est and the Eurométropole de Strasbourg for the award of a Gutenberg

Excellence Chair during the time of writing. A.T. was supported by a PhD scholarship from the Ministère de l'Enseignement Supérieur, de la Recherche et de l'Innovation.

H. Supplementary materials

$$R_1 = \begin{bmatrix} \sigma_1 & 0 & 0 & 0 \\ 0 & \sigma_1 & 0 & 0 \\ 0 & 0 & \sigma_1 & 0 \\ 0 & 0 & 0 & \sigma_1 \end{bmatrix} \qquad R_2 = \begin{bmatrix} \sigma_1 & 0 & 0 & 0 \\ 0 & \sigma_2 & 0 & 0 \\ 0 & 0 & \sigma_3 & 0 \\ 0 & 0 & 0 & \sigma_4 \end{bmatrix}$$

$$R_3 = \begin{bmatrix} \sigma & \gamma & \gamma & \gamma \\ \gamma & \sigma & \gamma & \gamma \\ \gamma & \gamma & \sigma & \gamma \\ \gamma & \gamma & \gamma & \sigma \end{bmatrix} \qquad R_4 = \begin{bmatrix} \sigma_1 & 0 & 0 & 0 \\ 0 & \sigma_2 & 0 & \gamma_{2,4} \\ 0 & 0 & \sigma_3 & 0 \\ 0 & \gamma_{2,4} & 0 & \sigma_4 \end{bmatrix}$$

Figure 76. Four possible matrixes for model comparisons in DFA. *As described in the article in the material section "Common trend analyses", we used the Dynamic Factor Analysis (DFA) for detecting common trends in multivariate time-series. We compared models with different covariates, different number of trends, and four different structures for the covariance matrix of the error term (Zuur et al. 2003). The different matrix structures are represented above (for example with n = 4): diagonal and equal (R_1), diagonal and unequal (R_2), equal variance covariance (R_3), and unconstrained (R_4).*

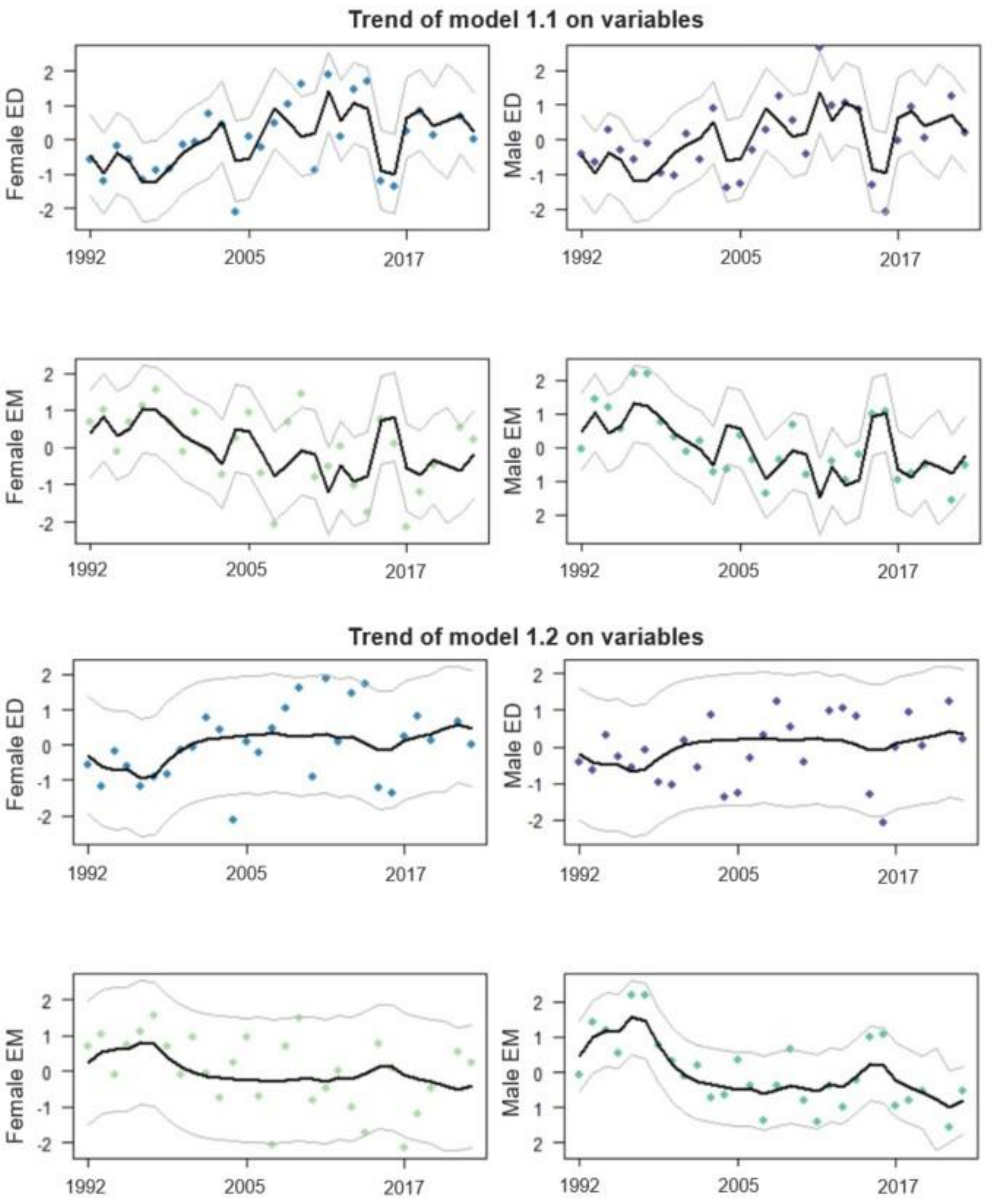


Figure 77. Common trends found in Models 1.1 (upper part) and 1.2 (lower part), ∆AIC < 2 and Wi > 0.1. *Functional traits are plotted over time (colored dots), with the common trend (black line) and the 95% confidence interval (upper and lower grey lines).*

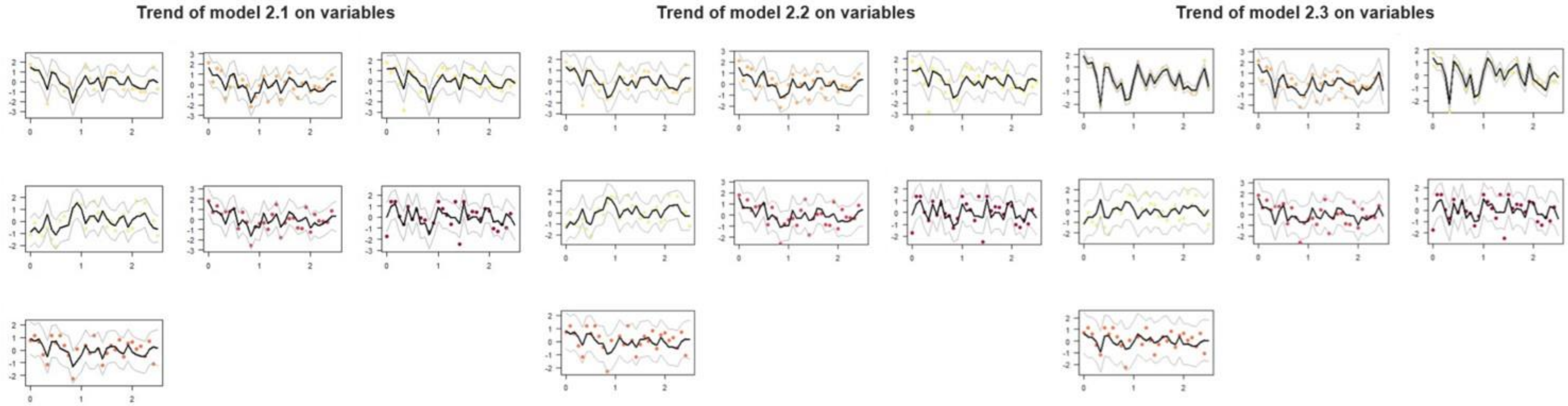
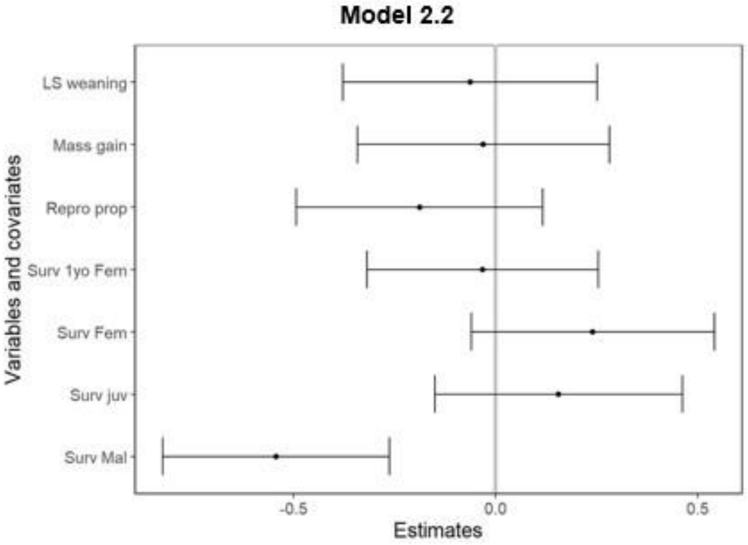


Figure 78. Common trends found in Models 2.1 (upper part), 2.2 (middle part), and 2.3 (lower part), ∆AIC < 2 and Wi > 0.1. *Functional traits are plotted over time (colored dots), with the common trend (black line) and the 95% confidence interval (upper and lower grey lines). The patterns of common trends found in the best three models are similar.*



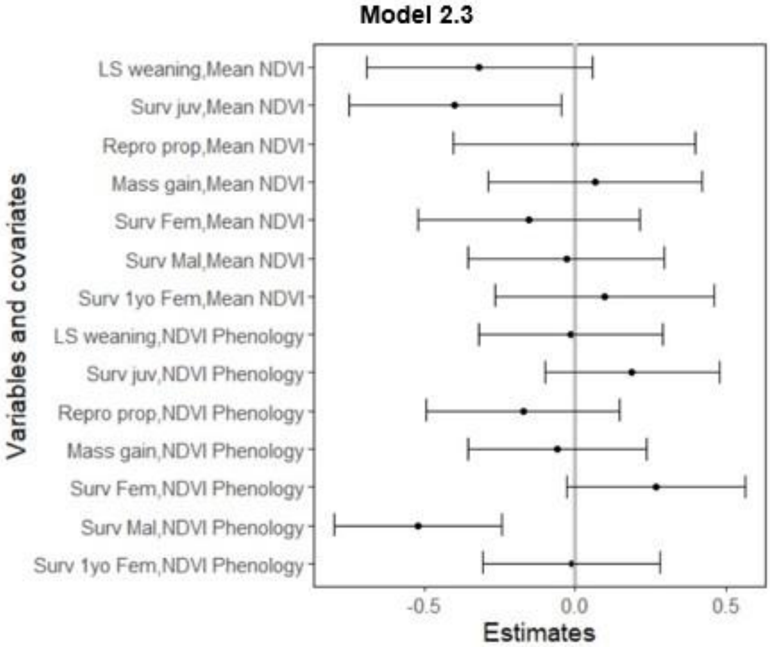


Figure 79. Mean NDVI and phenology effects on functional traits. *Estimates and 95% confidence intervals from the second and third best DFA model (ΔAIC < 2 and Wi > 0.1,* models 2.2 *and* 2.3*), representing the effect of covariates (*model 2.2*: NDVI phenology only, and* model 2.3*: NDVI phenology and mean NDVI) on functional traits (litter size, juvenile survival, reproduction proportion, female mass gain, adult female survival, adult male survival, and yearling female survival). Effects of NDVI phenology are highly similar between the best model (*2.1*) and the second and third best models (*2.2 *and* 2.3*).*

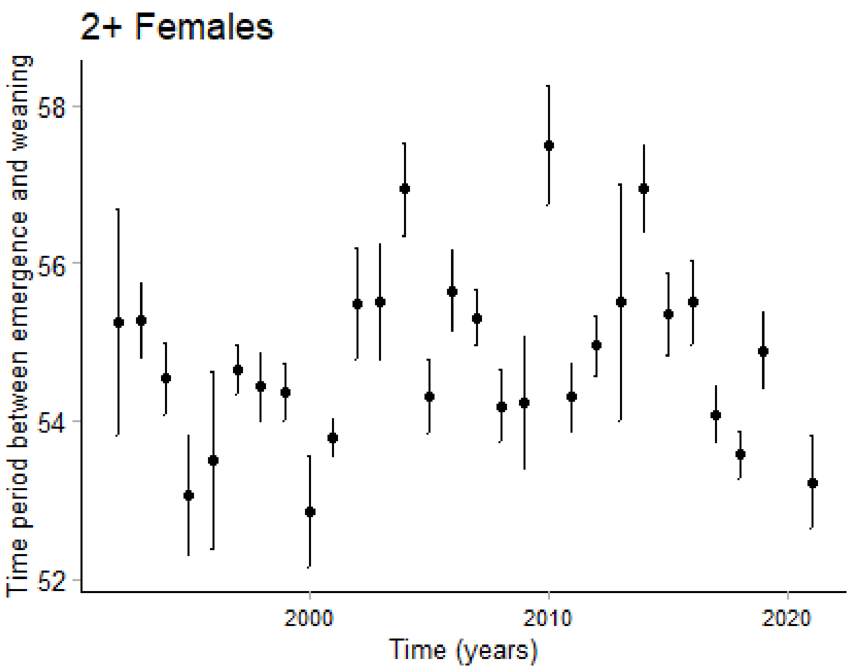
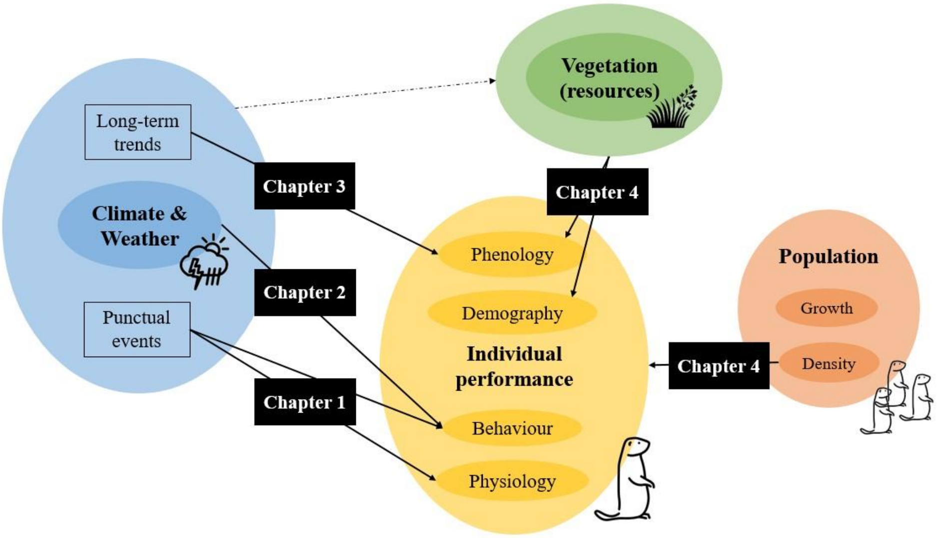


Figure 80. *Annual mean number of days between the emergence date and the weaning date of adult females is represented, along with standard errors.*

Environmental variations and effects on Columbian ground squirrels: results synthesis, limitations, and perspectives

General discussion and conclusion

In this thesis, I examined individual behavioral and physiological responses to punctual events of inclement weather or elevated temperatures during the breeding season, individual and population phenological responses to interannual variation and long-term trends in climate, and population functional trait and demographic rate responses to interannual variations in resources and population density (see flow diagram below).



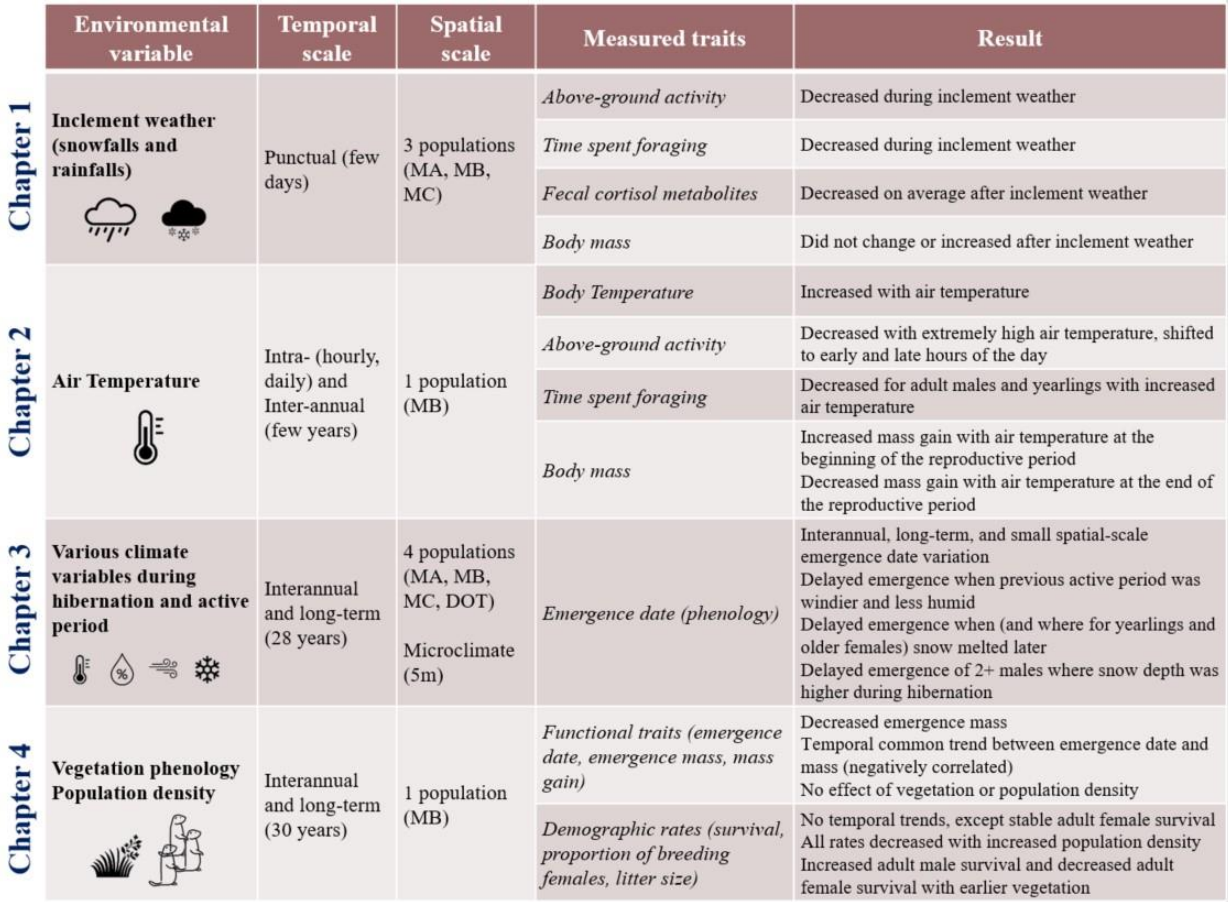
In the next section (general discussion and conclusion), results from the different chapter are summarized, a critical view of the various methodologies is presented, and further studies to continue this work are proposed.

GENERAL DISCUSSION & CONCLUSION

A. Synthesis of the questions, hypotheses, & results

In this thesis, I showed that, whereas Columbian ground squirrels responded to changes in climate and weather variations through marked behavioral/physiological plasticity, the demographic rates of their population nonetheless strongly varied with population density and resource fluctuations. Before discussing the extent and potential limits of this plasticity, I briefly summarize the results of the different chapters in Table 9, focusing on the environmental variables examined, the temporal and spatial scale considered, and the traits measured in each chapter. The main question, which was to evaluate to what extent environmental variations are affecting living organisms in the context of strong seasonal adaptations and current climate changes, is discussed below, in addition to methodological limitations and future perspectives.

Table 9. Summary of the principal results uncovered in my PhD thesis. *Environmental variables, temporal and spatial scales, and phenotypic traits considered in the analyses, along with the different chapter results are presented.*



	Environmental variable	Temporal scale	Spatial scale	Measured traits	Result
Chapter 1	**Inclement weather (snowfalls and rainfalls)**	Punctual (few days)	3 populations (MA, MB, MC)	*Above-ground activity*	Decreased during inclement weather
				Time spent foraging	Decreased during inclement weather
				Fecal cortisol metabolites	Decreased on average after inclement weather
				Body mass	Did not change or increased after inclement weather
Chapter 2	**Air Temperature**	Intra- (hourly, daily) and Inter-annual (few years)	1 population (MB)	*Body Temperature*	Increased with air temperature
				Above-ground activity	Decreased with extremely high air temperature, shifted to early and late hours of the day
				Time spent foraging	Decreased for adult males and yearlings with increased air temperature
				Body mass	Increased mass gain with air temperature at the beginning of the reproductive period Decreased mass gain with air temperature at the end of the reproductive period
Chapter 3	**Various climate variables during hibernation and active period**	Interannual and long-term (28 years)	4 populations (MA, MB, MC, DOT) Microclimate (5m)	*Emergence date (phenology)*	Interannual, long-term, and small spatial-scale emergence date variation Delayed emergence when previous active period was windier and less humid Delayed emergence when (and where for yearlings and older females) snow melted later Delayed emergence of 2+ males where snow depth was higher during hibernation
Chapter 4	**Vegetation phenology** **Population density**	Interannual and long-term (30 years)	1 population (MB)	*Functional traits (emergence date, emergence mass, mass gain)*	Decreased emergence mass Temporal common trend between emergence date and mass (negatively correlated) No effect of vegetation or population density
				Demographic rates (survival, proportion of breeding females, litter size)	No temporal trends, except stable adult female survival All rates decreased with increased population density Increased adult male survival and decreased adult female survival with earlier vegetation

B. Discussion on environmental adaptations and their limitations

1. Environmental fluctuations

As presented in the introduction, heterothermic endotherms living in fluctuating ecosystems (*i.e.* mountains) should be well adapted to face environmental variations and stochasticity (Baythavong 2011, Molina-Montenegro and Naya 2012, Burggren 2018). We found that, in general, ground squirrels appeared to be able to cope relatively well with weather variations, both with short punctual events of inclement weather and with interannual and long-term changes in climate. When experiencing short snowfalls and rainfalls or elevated temperatures, they were able to thermoregulate behaviourally, relying on their underground habitat to avoid harsh conditions (Chapter 1 and 2). They were also able to decrease their metabolism to minimize energy consumption. Such weather events, sometimes refered to as "extremes", are often considered as stressful for animals (Bize et al. 2010, Wingfield et al. 2017). But what is actually stress? Whereas stress is usually considered negative, it is foremost an adaptive response for animals to cope with noxious stimuli threatening organisms' survival and homeostasis, or perceived to do so (Romero 2004, Boonstra 2013). In the case of species living in a variable, unpredictable, environment, we would expect physiological (including adaptive stress responses) and behavioral traits enhancing individuals' fitness to be selected. Additionally, the high interannual and spatial variation in emergence date, and its relationship to climate variables, highlight the phenotypic plasticity of CGS in a highly variable environment (Chapter 3). This plasticity, along with plasticity in the relative timing of breeding, initial maternal mass, and reproductive and somatic effort (Dobson et al. 2023), suggest that they can cope with a certain degree of variability.

Whereas we found that ground squirrels also responded to long-term changes, inter-, and intra-annual variation, the environmental cues used to do so are still not clearly understood. Especially for these hibernating and ground-dwelling organisms, where soil temperatures and light may be buffered. Other environmental variables such as the moisture content of the burrow might be an important indicator of external conditions (see example in Romañach et al. 2005), and remains to be studied (which could be possible near future at the study site thanks to micro_era5 or weather stations soil sensor).

Even if the cues are not clearly established, at least for the hibernation period of their life cycle, these animals clearly adjust to both short and long-term environmental variation. We found metabolic and thermoregulatory adatations through physiological and behavioral plasticity both when facing rain and snow storms, or extreme heat conditions. However, their body mass was still negatively affected by elevated temperatures (Chapter 2). Thus, the question remains to know if phenotypic plasticity will be sufficient to cope with a predicted increase in extreme heat waves and overall global warming, which will depend on the frequency, intensity, and length of such punctual events.

Regarding rain and snow storms, the observed events in our studies were relatively short but intense, and did not negatively affect individual body mass. However, one previous example showed that during a prolonged snow period, body mass might be strongly affected, and further negatively influence survival and/or breeding success (Neuhaus et al. 1999). Thus, the length and frequency of punctual weather events might be more important than intensity regarding energy transfers in these heterotherms thanks to the high buffering capacity of their underground habitat. An increase in the frequency of these extreme events, as predicted under future climate change, thus challenges animals' foraging and energy acquisition (Stott 2016, van de Pol et al. 2017, Stillman 2019, Seneviratne et al. 2021), highlighting the need to integrate changes in climate variability rather than just changes in mean climate in future work (van de Pol et al. 2017). Future studies, as suggested below in the perspectives, should focus on bioenergetics and physiological thermal challenges to understand the full picture of heterotherms thermoregulation, especially when facing increased temperatures (Levy et al. 2019, Briscoe et al. 2023).

2. Long-term changes

We found some phenotypic traits (emergence date, emergence mass) to exhibit directional temporal trends, ground squirrels emerging later (despite differences among sex/age groups and populations) and leaner over time (Chapter 3 & 4). We question what could be the long-term effect in a directionally changing environment, getting warmer and more variable. Emergence date being a trait under directional selection, earlier emergence phenotypes are favored (see Annex II), as should be earlier breeding (genetic association between the emergence date and mating date in Lane et al. 2011, see Figure 81 below for illustration of correlation).

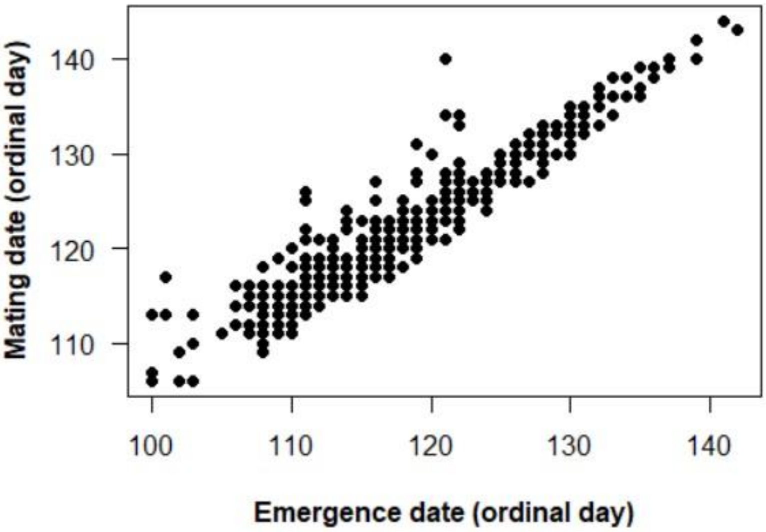


Figure 81. Relationship between the emergence date from hibernation and the mating date of female Columbian ground squirrels in MB population. *The mating date (unique oestrous day of each female determined by observations and genital inspection) was strongly correlated to the emergence date (cor. = 0.94, p < 0.001, n = 744 observations, N = 245 females, and 30 years) and the delay between emergence and mating averaged 4 ± 0.1 days over the 30 years (1992 – 2022).*

If the active season lengthens, as found in Yellow-bellied marmots (*Marmota flaviventris*), positive effects on survival and breeding success might be expected (Inouye et al. 2000, Ozgul et al. 2010). However, we found that Columbian ground squirrels delayed their emergence date, potentially shortening their active season (even if the emergence date is only one of the two indices of their phenology, and we were unfortunately unable to estimate their full active season length in the absence of immergence date). In this case, we might predict future challenges for animals to reproduce and fatten sufficiently before hibernation in an extremely short period. It appears difficult for animals to modify the duration of their hibernation period nonetheless, as hibernation surplus energy might not be used to shorten hibernation but to rewarm more frequently and to allow shallower torpor bouts (Bieber et al. 2014, Allison et al. 2023). Finally, hibernation conditions might change as well, and especially changes in soil thermal insulation in the absence of snow cover may challenge the hibernation energetics of montane hibernators (Kearney 2020, Chmura et al. 2023).

Ideally, the different observations on individual phenotypic plasticity and long-term trends could be combined into an integrative approach to further test environmental variability influences on life histories and population dynamics, but it would not allow understanding detailed behavioral and physiological mechanisms. These methodological trade-offs are discussed below.

3. An integrative approach?

In this thesis, I examined the effects of environmental variation on the phenotype and demography of Columbian ground squirrels, at different temporal and spatial scales. I used a multifactorial approach that considered multiple ecological variables (punctual weather events, climate change and variability, thermal conditions, resources availability, competition) and responses of animals to environmental variation. Environmental effects are numerous, may be direct or indirect, and at different time (and spatial) scales. They also may be immediate or through carry-over effects. To answer this, I used a dual approach: (1) integrative analyses, such as in chapters 3 and 4, where I looked either at the effects of several environmental variables at different spatial and temporal scales on one parameter (the hibernation emergence date) or included as many population functional traits into single analyses to better understand the temporal fluctuations of the population, and (2) mechanistic analyses to understand specifically the behavioral and physiological responses to weather fluctuations (chapters 1 and 2). While integrative approaches enabled me to study concurrent effects and partition the variance between them, it lacked the fine degree of details needed to shed light on the mechanisms behind those effects.

One example of integration is to try to partition variance in population dynamics explained by environmental *vs*. other effects. Demographic rates of Columbian ground squirrels did not show directional temporal trends over the 30 years of our study, and population size (of the MB population) was variable, but stable (see Figure 71 in Chapter 4). Contrarily to what was suggested before (Lane et al. 2012), this species does not seem particularly vulnerable to current climate change, but the question remains on how this might change in the future, following directional trends in climate. To apprehend the full understanding of population dynamics, we could focus on population growth rate, its relation to environmental variables, and the relative contribution of the different demographics rates. This could be done through population modeling integrating demography and environmental effects on population dynamics (see examples in Gamelon et al. 2017, Jenouvrier et al. 2018, Paniw et al. 2020). However, these approaches lack the integration of detailed behavioral (or physiological) mechanisms and causal links between resources, animal activity and foraging, and consequences on demography.

In an integrative approach, the questions remain to know which scale, either temporal or spatial, should be considered? Here, it is worth noting that environmental variables (climate, vegetation, density) were considered during distinct phases in the biological cycle of ground squirrels: during the preceding active period to test for carry-over effects, during hibernation, and during the active period of animals. Winter conditions, that can have important effects on species and population as illustrated on Figure 82, are more rarely included in ecological studies (Williams et al. 2015).

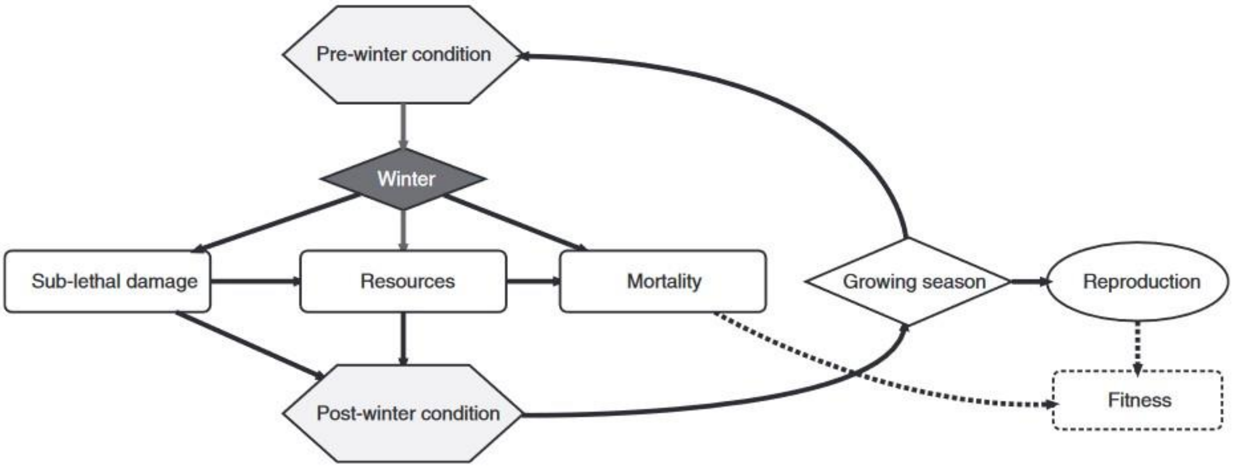


Figure 82. Integration of winter process into growing-season biology. *The black diamond "winter" represents the abiotic conditions that are experienced during winter (hibernation) and the white diamond represents growing (active) season performances (from Williams et al. 2015).*

In addition, we accounted for small-scale variations in climate that may influence animals differently (in chapter 3). Indeed, relatively close populations (few kilometers away) did not exhibit the same phenological patterns, which were related to spatial heterogeneity in climate conditions. Despite the recent advances to easily access fine-scale environmental data (Kemppinen et al. 2023), conditions experienced by animals are still rarely assessed from a microclimate perspective. Nonetheless, this can have important biological consequences (Woods et al. 2015, Høyvik Hilde et al. 2016, Maclean et al. 2017) and may help to identify intraspecific contrasts in climate-change responses. Additionally, we found temporal variability in space used by ground squirrels to forage (in relation to thermal conditions, chapter 2), which attests as to the local heterogeneity in environmental conditions, including resources. Our understanding of local conditions' effects on montane mammals would greatly benefit from a global characterization of the microhabitat, even within one population, including the precise description of resources. All these future directions are discussed below in the perspectives, but some general limits first need to be noted.

C. Critical views & methodological limitations

First, even if long-term monitoring of wild animal populations requires an substantial commitment of time, energy, and funding, 30-year is relatively short for a "long-term" study, especially from an evolutionary perspective (Clutton-Brock and Sheldon 2010). Climate changes are happening at geological time scales, and depending on the time scale considered for biological studies, the strength of the directional effects may vary (Cusser et al. 2021). For example, our analyses on hibernation phenology conducted on several populations and over 30 years led to more contrasted results than those from a similar study of only MB, over a shorter time scale (Lane et al. 2012). Thrity years also represent only 30 data points when considering yearly values (*e.g.* for breeding success, survival, etc.) and is rather poor regarding statistical power, especially to run integrative studies investigating simultaneously the effect of several environmental parameters. Second, these Columbian ground squirrel populations are partly favored by cattle grazing: the meadows are maintained open thanks to grazing (happening late in the ground squirrel active season; from mid-June), and cattle may affect vegetation and forage composition. Finally, it has to be noted that our human presence may always affect wild animals, but that, from our perspective, these effects are difficult to estimate, evaluate and consider. From a recurrent monitoring, animals might habituate to human presence, which on one hand allow them to exhibit "normal" behaviors in the presence of observers, but on the other hand may affect their behavioral and physiological response (*e.g.* stress response) or apply a selective pressure by selecting only individuals being habituated. In the analyses conducted here, some methodological limits are also important to raise.

1. Climate & vegetation data accessibility

When examining environmental conditions over large time scales, data accessibility is an important challenge, especially when research questions on different topics where not initiated at the same time (single-species monitoring *vs* ecosystem assessment, including abiotic variables). Compared to in-situ records (weather stations, loggers, vegetation samplings), satellite data prove very useful to go back in time and access global historical records, and these are easily accessible on the internet (*e.g.* climate and weather data or NDVI from governmental platforms). However, the spatial scale at which satellite imagery is estimated is usually large and leads to coarse resolutions. When studying small animals that have a relatively small/localized home range, satellite data averaged over several kilometers or hundreds of kilometers tend to homogenize spatial heterogeneity, and do often not reflect the actual environmental conditions experienced by the animals.

Microclimate modelling offers an interesting solution to downscale past records of satellite data into smaller spatial scales, to better represent the local conditions experienced by organisms. Weather can be modeled at high spatial and temporal resolution (*e.g.* every hour and every 5m in our case) and allow to access past climate. However, the use of a model to estimate climate variables needs to be validated against empirical measures, at the location of the study site. Indeed, the microclimate model also uses satellite data, either from the NOAA (NCEP data, US) or from Copernicus (era5 data, EU). We validated the model at our study site and, overall, most climate variables were well correlated although era5-modeled data (*micro_era5* function) seemed better correlated to empirical data than NCEP-modeled data. Unfortunately, when I first started my PhD, only NCEP data were available, but we adapted and used era5 in the last conducted study (chapter 2). In addition, the microclimate model used here does not downscale precipitations (rain) that remain coarse-gridded. This may influence snow modeling that requires further empirical validation, but an improvement of the model can be done by adding a local time series of rain. While this is promising for future research (as we now have a weather station or even studies after 2008 with Sheep River station), we sere unable to go back further in time so that long-term studies (chapter 3) had to be done with a coarse resolution for rainfall. Regarding snow, our attempts to empirically measure winter snow-cover unfortunately failed (camera traps deployed overwinter randomly stopped, probably because of extremely low temperatures, when nobody was able to go in the field), so that further validations of the snow cover should be conducted.

Modeled climate variables, such as temperatures, depend on the specified percentage of shade. Because Columbian ground squirrels live on open meadows, we ran the model without any shade provided. Yet, we showed in chapter 2 that individuals might exploit the outskirt of the meadows during period of intense heat, quite possibly to benefit from the shade of the trees. Having a detailed map of the % of shade on the meadow would thus be useful to improve the model. Further complicating this, shade might be dynamic and change within a season for small animals such as Columbian ground squirrels, due to vegetation growth. Because it can be difficult to evaluate shade percentage, especially regarding short vegetation (*i.e.* shade from growing grasses during the season), we could use small light loggers at the height of animals. Light intensity differences between loggers under the grass and a reference logger positioned higher above ground should indicate the percentage of shade due to vegetation. We tried this protocol in the field, but only in 2022 at the center of the meadow in locations where grass growth remained low, and it did not lead to conclusive results. Indeed, light intensities were globally always high, as loggers were directly exposed facing the sky, even when located in the vegetation. This experience further shows that grass shade might not be high in the center of the meadow, but more crucial on the edges, depending on the hour of the day and the time of the season. It was too late to repeat the experiment in the timeframe of my thesis, but would be interesting to implement for further analyses using the microclimate model.

In the absence of vegetation monitoring over the historical data, we used NDVI to access ground squirrels' resources. On one hand, NDVI spatial resolution around 1 km, thereby encompassing meadows, forests etc. in that zone. *In-situ* measurements (vegetation height, greenness of the meadow base don pictures) were implemented from 2022 to validate derived variables from NDVI at a small scale, such as phenology. Based on our 2 years of data, we found that NDVI dynamic was a good reflection of grass height and greenness. On the other hand, NDVI is only a global index of greenness, which does not necessarily reflect the actual plant community, plant composition, or quality of the vegetation. To answer these questions in more details, I developed a protocol of *in-situ* vegetation sampling that started in 2023 and evaluated vegetation characteristics (composition, energy density, water content, etc., more details provided in the perspectives below).

To conclude, the installation of local weather stations and the implementation of systematic empirical vegetation measurements allowed the acquisition of data to validate the different global indices used in my thesis, but also to initiate a larger assessment of the animal's ecosystem and its variations.

2. Correlative approach

To test for biological effects of environmental variation, we conducted various analyses that were mainly correlations. In the wild, it can be difficult to manipulate environmental parameters, although food availability and population density have been experimentally modified before in different studies (Dobson and Kjelgaard 1985a, 1985b, Both 1998, Karels and Boonstra 2000, Blix et al. 2012, Travis et al. 2023). Regarding weather conditions, however, it becomes more difficult to control conditions, especially during prolonged periods (*e.g.* hibernation). Thus, I tested correlative links between environmental and biological variables, which allows testing many different relationships, but does not prove a causal relationship between two variables. For example, climate variables might covary (Supplementary materials in Chapter 3: Figure 68), or be correlated to other latent environmental parameters that were not considered. Several examples of spurious correlations are well known, as for instance the age of Miss America and the number of murders by Steam, Hot Vapors, and Hot Objects in the USA (Figure 83 below).

Figure 83. *Example of spurious correlation: temporal changes in the age of Miss America and in the number of murders by steam, hot vapors, and hot objects in USA (assessed from Spurious Correlations).*

Thus, a correlative approach, which first needs nuanced interpretations from results, also needs to be complemented by experimental studies manipulating environmental parameters. Experimental modifications of climate have been conducted for plants (seedlings) by modifying temperature and rainfalls (Lloret et al. 2004, del Cacho et al. 2012), but this kind of design applied to (wild) animals remains rare (Raffaelli and Moller 1999, Bestion et al. 2015). Regarding Columbian ground squirrels, reciprocal translocation experiments to test for elevation effect on emergence date have been conducted (Lane et al. 2019), which allows inference of the extent of phenotypic plasticity, but not the direct effect of climate. Future experimental studies could modify thermal conditions during the hibernation of animals, by artificially adding or removing the amount of snow on the ground. The implementation of temperature loggers inside burrows could allow monitoring the empirical winter soil temperatures, and test for differences in phenology among experimental designs (with snow all winter, without snow, with partially removed snow). One current study I am involved in with Dr. Tim J. Karels and Dr. Rudy Boonstra aims at examining spatial differences in Arctic ground squirrels habitat and especially in snow-accumulation, and their consequences on hibernation mass dynamics and survival.

3. Strengths & drawbacks of Fecal Cortisol Metabolites (FCM)

In Chapter 1, we tested if ground squirrels were affected by inclement weather and if their “stress levels” or metabolism was modified following inclement weather. We found a decreased mobilization of stored resources as indicated by lower fecal cortisol metabolites in the hours/days following periods of inclement weather. Fecal cortisol metabolites reflect a “baseline” level in the body (several hours before defecation and sampling) and require a non-invasive easy sampling, but are **metabolites**, meaning that part of hormones might have been metabolized by the body before (primarily by the liver and other organs, and then by bacterial enzymes in the intestine, Taylor 1971). Metabolites might also be partly reabsorbed before being released in the feces after a time delay (Palme 2019), see Figure 84 for a representation.

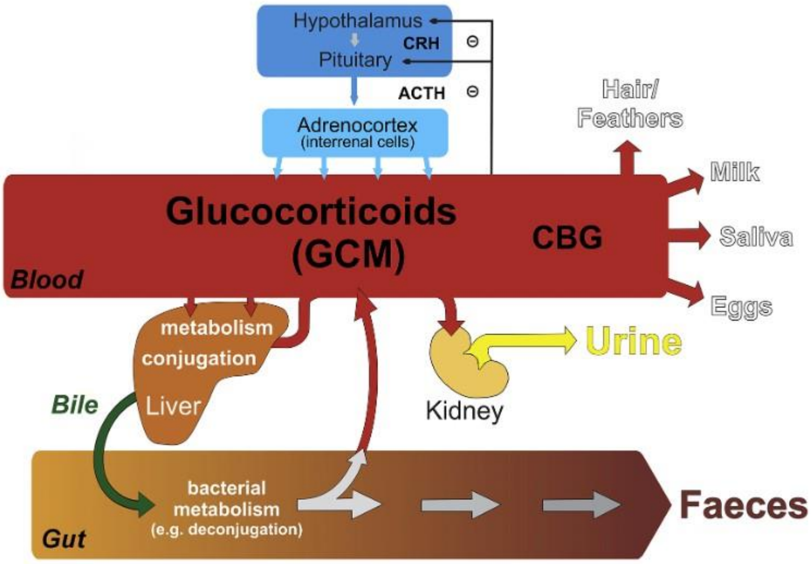


Figure 84. *Metabolic pathways of glucocorticoids (from Palme 2019).*

Thus, while FCM levels give us a reliable index of cortisol secretion, they are sensitive to numerous parameters, including the gut passage time and other individual differences (Palme et al. 1996, Price et al. 2019). One solution would be to measure glucocorticoids in other tissues, such as blood, saliva, or especially hairs. Glucocoticoids diffuse into growing hair from the follicular capillaries (Sheriff et al. 2011, Dantzer et al. 2014, Gormally and Romero 2020), and compared to blood or saliva measures, hair measurement allows inference of the amount fixed at specific times when hair grow, reflecting physiological response to stressors on a longer time scale (*i.e.* several weeks). However, this method also has limits since it requires knowledge on hair growing speed and renewal period. In the end, the choice of the methodology depends on the question asked, and whether the examined responses are expected to reflect acute or chronic stress responses (Boonstra 2013).

Finally, FCM levels were measured through enzyme immunoassays, which are easier to implement compared to radioimmunoassay that requires expensive equipment and special permits/laboratories for handling and measuring radioactivity (Palme 2019). More generally, immunoassays do not require knowledge of the chemical identity of the measured metabolites in order to measure it, compared to chromatographic methods coupled with mass spectrometry (Kersey and Dehnhard 2014, Wudy et al. 2018). On the other hand, mass spectrometry methods provide structural information and higher specificity, along with the ability to measure several metabolites simultaneously and can be used to assess several physiological variables (metabolites) simultaneously (Wudy et al. 2018).

Indeed, whereas the "stress response" is frequently assessed only through glucocorticoid levels, other physiological markers of the animal response facing a "stressor" (noxious stimuli, Romero 2004) are as important as glucocorticoids (Breuner et al. 2013, MacDougall-Shackleton et al. 2019, Lemonnier et al. 2022). For instance, the physiological state of an individual can also be measured through energy expenditure, immune responses, oxidative stress, or telomere dynamics (Davis et al. 2008, Monaghan 2014, Pepper et al. 2018, Angelier et al. 2019).

The methodologies used, from the general approach to environmental and physiological measurements, present respective limits that were considered in result interpretations. It does not invalid conclusions, but rather suggests further ideas to apply to future studies around the main question of environmental variation effects on animal phenotype. Different perspectives and future studies, sometimes partly examined, are presented below.

D. Perspectives & Future studies

Numerous aspects of this thesis might be developed further in future studies, either in the field of physiological ecology or general ecology. First, a better understanding of individual bioenergetics facing elevated temperatures would allow predictions of animal responses and challenges to heat stress. Second, accurately describing vegetation (main food and water ground squirrel resource) and its seasonal and interannual variations would allow a better understanding of the link between animals' energy/water requirements and the energy/water available in their environment. Finally, whereas the relationships between environmental variables and functional traits and demographic rates were tested, the direct influence of vegetation and population density on population dynamics was not examined. It would help to identify the extrinsic factors affecting population dynamics, as a complement to the demographic contributions to population regulation.

1. Assessing bioenergetics

In the context of climate change, our understanding on how small mammals, and especially hibernating rodents, cope with elevated temperatures would greatly benefit from ecophysiological and energetic analyses (Levy et al. 2019, Briscoe et al. 2023). Columbian ground squirrels exhibited physiological and behavioral plasticity: their subcutaneous temperature increased with ambient temperature, their above-ground activity decreased with extremely high temperature, as well as their foraging behavior. However, the animals' subcutaneous temperature does not necessarily reflect the core body temperature, and we were not able to examine detailed ground squirrels energy and water transfers when facing elevated temperature.

We could take a variety of field measurements and use modelling to calculate energy and water losses (Bernstein et al. 1977, Porter and Kearney 2009). One example would be the use of endotherm models to calculate animal energy and water costs to maintain a certain body temperature under local microclimate conditions. The endotherm models from NicheMapR (Porter and Mitchell 2006, Porter and Kearney 2009, Mathewson and Porter 2013, Kearney et al. 2021) allow to run mechanistic modeling of the heat budget of an organism in a given microclimate. It can compute the metabolic rate, the water loss rate, the fur temperature, and the skin temperature of an organism from its morphological and physiological traits. This would allow us to predict the costs of endothermy or water requirements in future climate from knowledge on animals' energy expenditure.

An endotherm model application to the Columbian ground squirrels would need the assessment of several morphological and physiological parameters, documenting their body mass, shape, insulation (fat and fur characteristics), body temperature, minimal metabolic rate. Some morphological characteristics are already known (masses, shape, and metabolic rate variations, Elliott and Flinders 1991, Skibiel et al. 2013, Roth et al. 2019), but would gain in accuracy by accounting for inter-individual variation within a population. In addition, fur parameters could be measured relatively easily in the field in the coming years, and body temperatures could be assessed by thermal cameras. By running the model, we could then predict the metabolic rate and evaporative water loss of individuals under different weather-conditions scenarios, complementing the behavioral responses described in this thesis. Because we know physiological parameters can vary (*e.g.* body temperature), the model would need to be calibrated and validated with different environmental and individuals states. An example of the endotherm model application on small mammals is the study conducted by Levy et al. (2019) simulating costs of activity of diurnal and nocturnal rodents in the context of climate change (Figure 85). This study highlights that the costs of endothermy may become lower at night, leading to the suggestion that some diurnal mammals may shift to a more nocturnal lifestyle in the future.

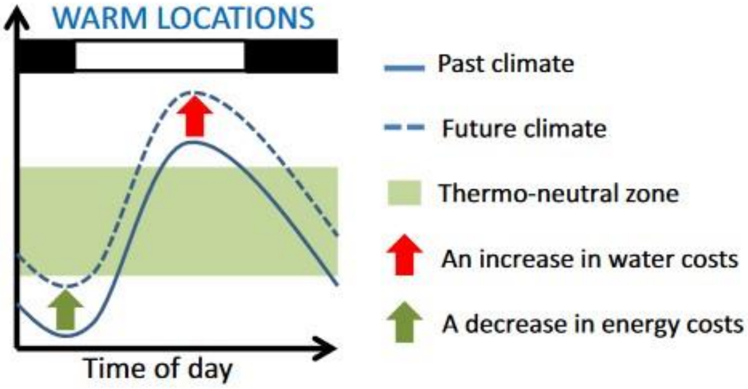


Figure 85. Example of challenges happening for animals at warm locations in the context of climate warming. *Higher temperatures will decrease costs of nocturnal activity (green arrow), but increase water loss during diurnal activity (temperatures above the thermoneutral zone, red arrow, adapted from Levy et al. 2019).*

Outputs and work from the endotherm models might also be linked to the dynamic energy budget (DEB) theory (Kooijman 2010, Kearney et al. 2021), which is based on the processes of substrate uptake and use by organisms. DEB models aim to describe all dynamics of energy assimilation and utilization, as well as processes of growth, development, and maintenance according to two external parameters: temperature and resource availability. In this objective, precise prediction in energy dynamics would gain from a more accurate assessment of vegetation, the main resource of Columbian ground squirrels.

2. Assessing vegetation quality

The Normalized Difference Vegetation Index, used in this thesis as vegetation index, is an indicator of the level of greenness in a given area. Derived indices from the NDVI, widely used in ecological studies (Pettorelli et al. 2005, 2011), can give us indication on the phenology of the growing season, as well as speed and “quantity” of vegetation growth. One surprising result is that we found negative effects of greening on breeding success (supplementary materials in Chapter 4: Figure 79). Indeed, in highly “green” years (high mean NDVI), litters were smaller and juveniles survived less well to the end of their first hibernation. In addition, we found that during years characterized by fast-growing vegetation, vegetation biomass was higher, suggesting that either the vegetation was turning greener as the season advanced, or that changes in plant communities dominating the meadow occurred (Heim et al. 2021).

Columbian ground squirrels are selective foragers (Ritchie 1988, 1990), known to prefer forbs over grasses (Harestad 1986, Bennett 1999). However, the actual role played by food “quality” in affecting demographic rates and body condition is still unclear. A higher NDVI and greener vegetation could reflect plant species with more fibers and lignin, being less digestible and of lower quality (Drożdż 1968, Veloso and Bozinovic 1993, Bennett 1999, Moore and Jung 2001).

Thus, the quality of forage for animals might be reflected by the diversity of dietary components within the colony, associated with differences in digestibility, elements (secondary compounds, minerals, polyunsaturated fats), and water content according to species, but also differences according to vegetation age and height (Bintz 1984, Bergeron and Jodoin 1987, Frank 1994). As a preliminary analysis and as an internship project I co-supervised during 2023, every week we sampled vegetation in 7 different 10 × 10 m-quadrats (from 4 May to 29 June). We weighed vegetation immediately after sampling and after lyophilization (35°C during 48h) to estimate the water content and its change throughout the growing season. As first results, the water content of vegetation increased during the first period of the active season, before decreasing from the 22nd week of 2023 (which corresponds to around the 1st of June, Figure 86).

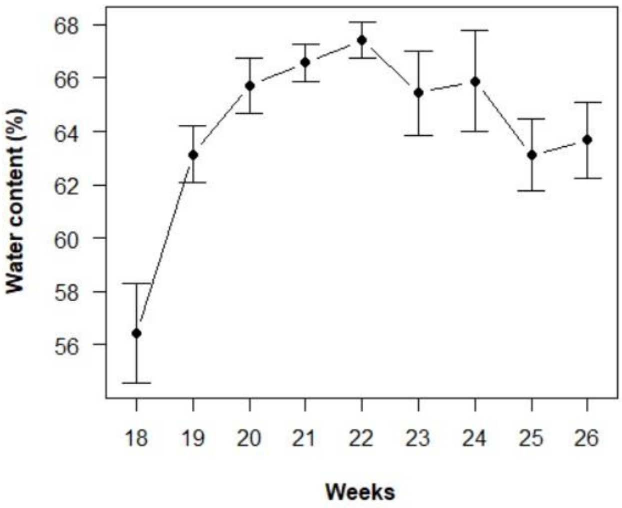


Figure 86. *Mean water content (± SE) of vegetation, estimated as percentage of the total wet mass (water mass = wet mass – dry mass), and sampled from 7 quadrats on MB during 2023 active season.*

In addition, we ran energy-density analyses using a bomb-calorimeter (Calorimeter Parr 6200) to evaluate the energy content of vegetation, and test whether it changed between the beginning and the end of the season. Briefly, a bomb-calorimeter measures the heat of combustion of the dried and grinded vegetation samples to obtain their energy density. While analyses are still in progress, first results indicate that the energy content of vegetation did not seem to change importantly over the active season of ground squirrels (Figure 87).

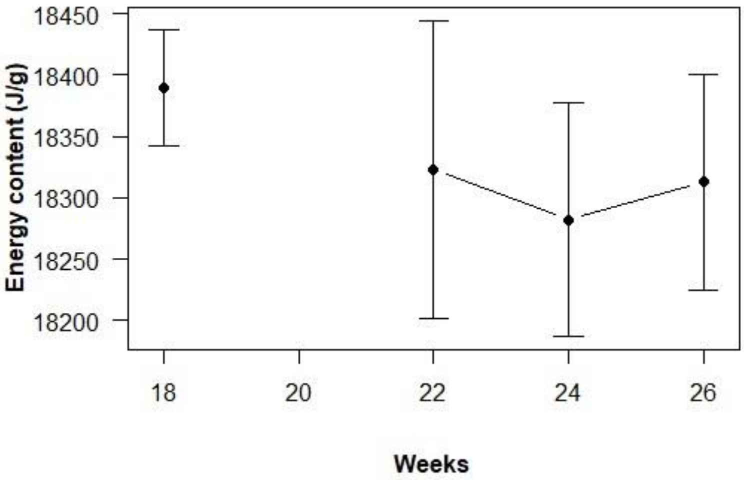


Figure 87. *Mean energy content (± SE) in Joules per gram of dry mass for vegetation samples from week 18 (n = 5), 22 (n = 6), 24 (n = 5), and 26 (n = 7) during 2023 active season. Only a small subset of all the vegetation samples were analysed (explaining the variable samples sizes), and some of them were run in duplicates or triplicates.*

However, this was expressed in energy per dry mass and the water content increased during the season, indicating that the energy gained from vegetation globally decreased along the growing season. Thus, individuals would need to forage more to acquire the same amount of energy, when above-ground activity may be additionally limited by upper thermal constraints. Furthermore, the decreases in vegetation water content toward the end of the active season (from week 22) raises the question of water constraints, especially during the lactating period of breeding females. Indeed, this decreases from week 22 (end of May – beginning of June 2023) corresponds to the week when a start in the decline of vegetation greenness was observed, and when the slope of increase of vegetation height decreased (see red lines on Figure 88 below). In particular, in 2023 we observed particularly high temperatures compared to previous years, and while we do not have access to water content in 2022, we can see that vegetation dynamics (greenness and height) differed between 2022 and 2023. Thus, these water constraints might be exacerbated during periods of increased heat, which are likely to increase in the context of climate change and potentially become a new constraint for animals.

Water constraints during lactation might be critical for breeding females (Speakman 2008, Zhao et al. 2020) but we observed important heat effects on yearling individuals, probably due to less favorable surface/volume ratios. Thus, it may be even more challenging for offspring born during the year, which are particularly small but require high energy amounts for their first hibernation.

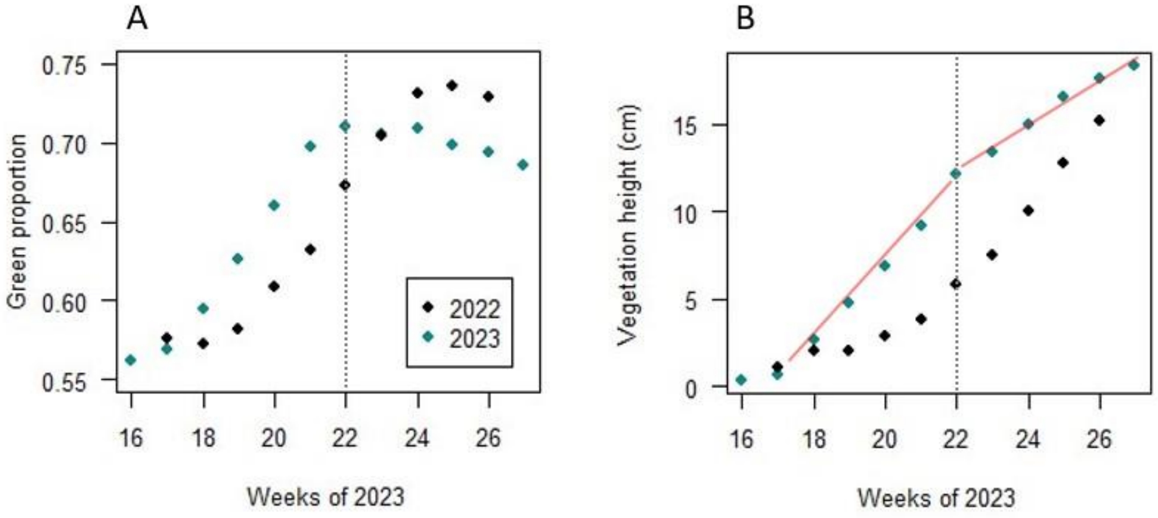


Figure 88. Green proportion of vegetation and vegetation height during 2023 active season on MB. *Green proportion is calculated from pictures taken on the meadow, and vegetation measured on graduated poles positioned on the meadow (details in general materials and methods). The vertical dashed line represents week 22 (end of May – beginning of June) when both vegetation greenness and height dynamics exhibited a change in the increasing slope.*

Overall, the question around water constraints remains relatively understudied in the literature (Bintz 1984, Fuller et al. 2020, 2021). Finally, it would be interesting to evaluate spatial differences in vegetation water and energy content. Indeed, Columbian ground squirrels are matrilineal and strongly philopatric, where females are settled on a given territory (Murie and Harris 1978, Festa-Bianchet and Boag 1982, Harris and Murie 1984, Arnaud et al. 2012). The questions remains to know to which extent individual (matrilineal) variations are linked to local spatial variations in forage quality on their territory, whether strong microhabitat quality differences occur within a same population, and whether it can be linked to changes in foraging locations during the reproductive season. Our field observations suggest that despite this strong territoriality, females range widely over the populations during their foraging activities, suggesting that dietary/water requirements may supersede the potential costs of territorial aggression. Additional analyses on plant digestibility would also be interesting to conduct, in order to evaluate the amount of energy contained in vegetation that animals are actually able to access and use. Indeed, all these resource parameters may have significant effects on individual performance and further consequences on population dynamics.

3. Assessing population dynamics

a) Influences of vegetation and population density on population growth rate

In Chapter 4, we described variations in population size on MB, without actually assessing population dynamics. In addition, we examined the link between vegetation and population density and functional and demographic rates, but vegetation effects on population growth or density-dependence of the population growth rate were not examined (see numbers with blue and red backgrounds on Figure 89 below).

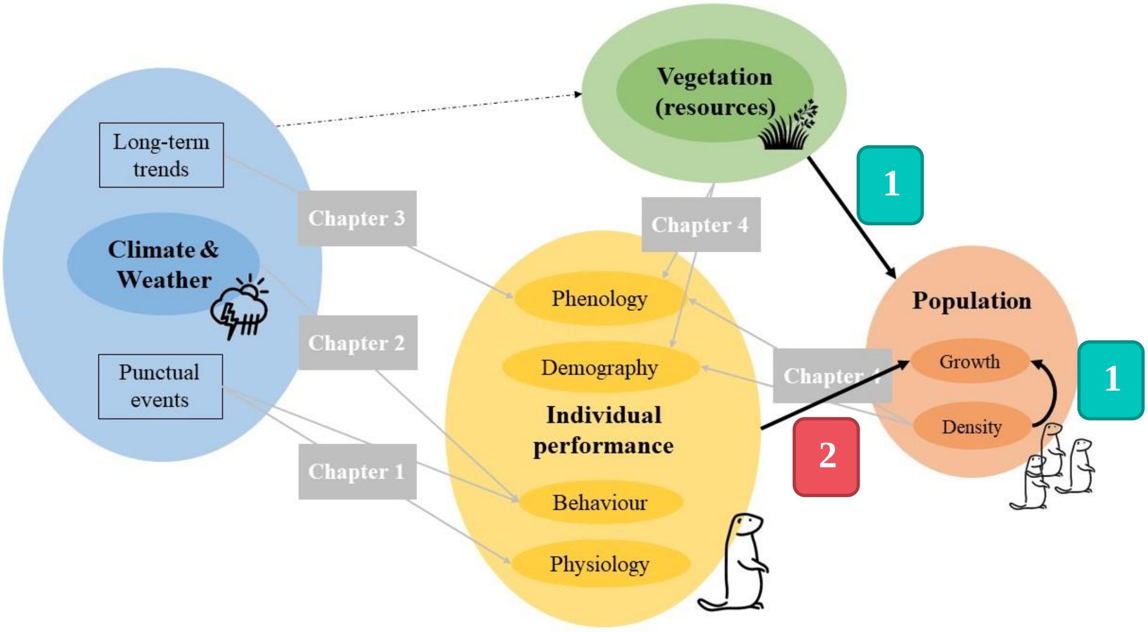


Figure 89. Flow diagram of the thesis questions and different chapters (grey boxes and arrows) along with further perspectives (black arrows). *First, the resources and population density influences on population growth might be examined (blue boxes), and second the demographic contribution to population dynamics may be evaluated (red box).*

Below, I use a simple calculation of the population growth rate (finite rate of growth, λ) to test for density-dependence and relationships with vegetation (NDVI) indices presented in the general materials and methods and used in the analyses in Chapter 4. To do so, I calculate population growth rate from year_t to year_{t+1} as N_{t+1}/N_t where N was population size.

Over the 30 years of study, the finite population growth rate, termed lambda (λ), was on average 1.1 ± 0.05, reflecting an overall stability of the population (no significant trend in GAM; edf = 1, F = 0.325, $p = 0.573$, R^2adj = -0.024, Deviance explained = 1.2%, n = 30 years). However, this growth rate ranged from 0.3 (between 2002 and 2003) to 1.6 (between 1997 and 1998), showing marked interannual variability among years of negative and positive growth (see Figure 90). Besides the sudden drop between 2002 and 2003, population growth rate was particularly low between 2009 and 2010 (0.5), and between 1996 and 1997 (0.6). It was also particularly high in 1997-1998 (1.7), 1993-1994 (1.5), 2005-2006 (1.4), and 2008-2009 (1.5). Finally, the variability in growth rates has been lower since 2010.

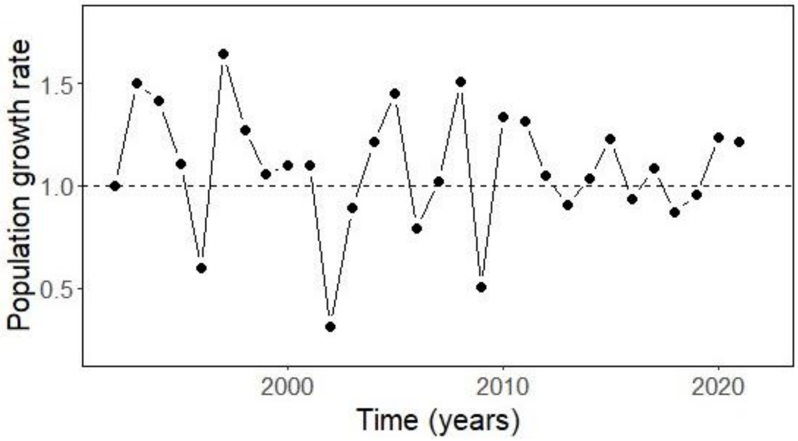


Figure 90. Population growth rate (Nt+1/Nt) of MB population between 1992-2022*. It increased population size if > 1 and decreased population size if < 1 (dashed horizontal line).*

To test for a yearly effect of vegetation on population growth rate, I included the three NDVI variables (vegetation biomass or mean NDVI, vegetation growth rate, and onset of vegetation growth) as independent variables in a linear model (LM). Independent variables were tested for multicollinearity (all VIFs <3, Zuur et al. 2010). To allow comparisons of effect sizes, NDVI variables were standardized beforehand.

To test for density-dependence, I used a derived Gompertz model (Lebreton and Gimenez 2013). Specifically, I tested if the slope of $Log(N_{t+1}) \sim Log(N_t)$ was < 1. A slope equal to 1 shows a linear relationship between $Log(N_{t+1})$ and $Log(N_t)$, and no evidence for density-dependence. In contrast, a slope < 1 indicates lower population growth rates at high population density, highlighting density-dependence (while slopes higher than 1 could indicate some sort of facilitation). Thus, I ran a non-linear least squares (NLS) analysis with $Log(N_{t+1}) \sim (1-b) * Log(N_t) + a$ to test if b was significantly positive (Lebreton and Gimenez 2013). Because this was a one-tailed test, the *P*-value was divided by 2.

Population growth rate was negatively correlated with vegetation biomass ($r = -0.074 \pm 0.064$, $t = -1.154$, $p = 0.259$), vegetation growth rate ($r = -0.065 \pm 0.067$, $t = -0.963$, $p = 0.344$), and the onset of vegetation growth ($r = -0.024 \pm 0.058$, $t = -0.419$, $p = 0.679$), although none of these effects were statistically significant (n = 30 years, Figure 91.A, B, and C).

Regarding density-dependence, the fitted Gompertz model showed a slope of $Log(N_{t+1}) \sim Log(N_t)$ significantly < 1 (NLS, b>0, $b = 0.476 \pm 0.156$, $t = 3.061$, $p = 0.002$, n = 30 years, Figure 91.D) revealing a negative effect of population density on population growth.

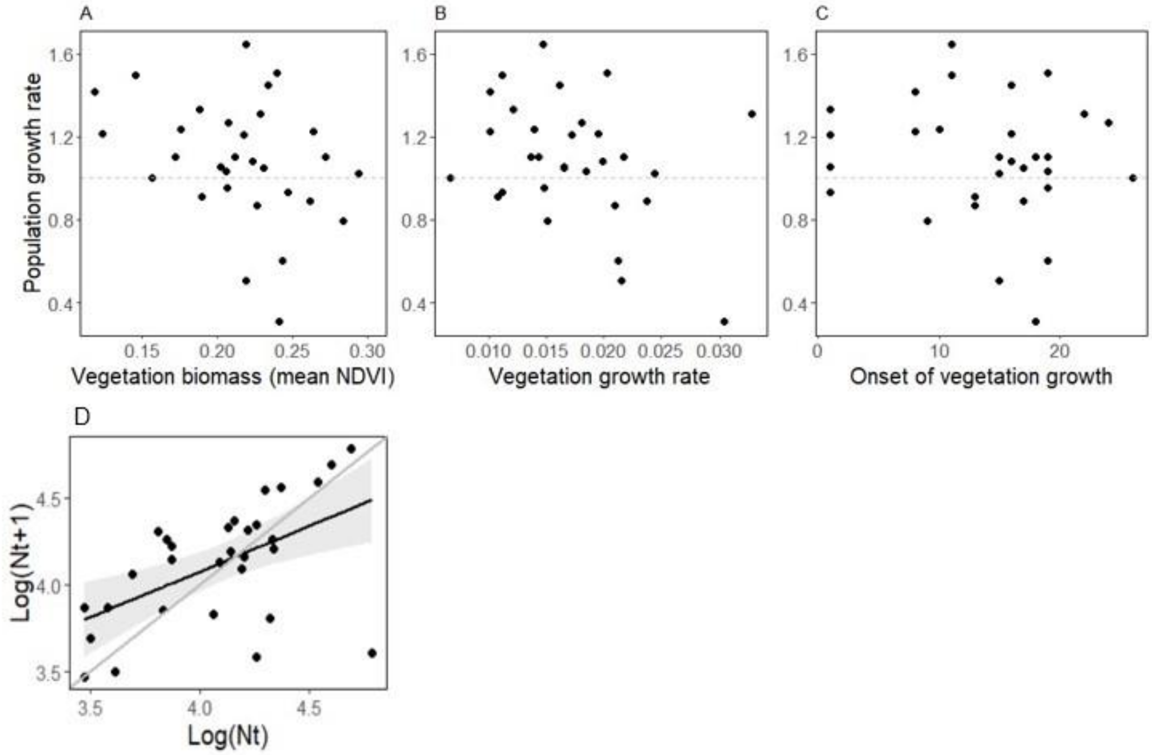


Figure 91. Vegetation effects on population growth rate and density-dependence. *Population growth rate from year t to year t+1 as a function of (A) vegetation biomass (weekly mean NDVI), (B) vegetation growth rate, and (C) onset of vegetation growth of year t. (D) Relationship between Log(Nt+1) and Log(Nt) and significant slope (black line) along with 95% confidence interval (shaded area). The estimated slope is below one (grey diagonal), indicating density-dependence.*

These results show that the dynamics of the population were negatively influenced by population density, as were the demographic rates (survival, breeding success, see Chapter 4 results). As described in Chapter 4, increased population density might translate into increased competition between individuals and negative effects on demography. In contrast, non-significant effect of vegetation indices on population growth rate might be explained by the differential effects of vegetation phenology on adult male and female survival that would balance themselves. Whereas changes in population growth are most strongly related to changes in demographic rates (Lebreton and Clobert 1991, Gaillard et al. 1998, Sæther and Bakke 2000, Lima et al. 2003, Oli and Armitage 2004, Ozgul et al. 2004, Sim et al. 2011, Griffith et al. 2016), the total growth rate calculated here does not integrate the respective contributions of different demographic rates to population growth, and differences between sex/age/breeding status categories. Thus, it might be important to examine the relationships between demographic rates and population growth (see below).

b) Demographic contributions to population growth rate

As a first simple solution, I applied a linear model with the population growth rate calculated as described before as the response variable, and the different demographic rates presented in chapter 4 as explanatory variables (litter size at weaning, survival of yearling females, survival of adult females, survival of adult males, juvenile survival). Independent variables were tested for multicollinearity (all VIFs <3, Zuur et al. 2010) and to allow comparisons of effect sizes, demographic variables were standardized beforehand.

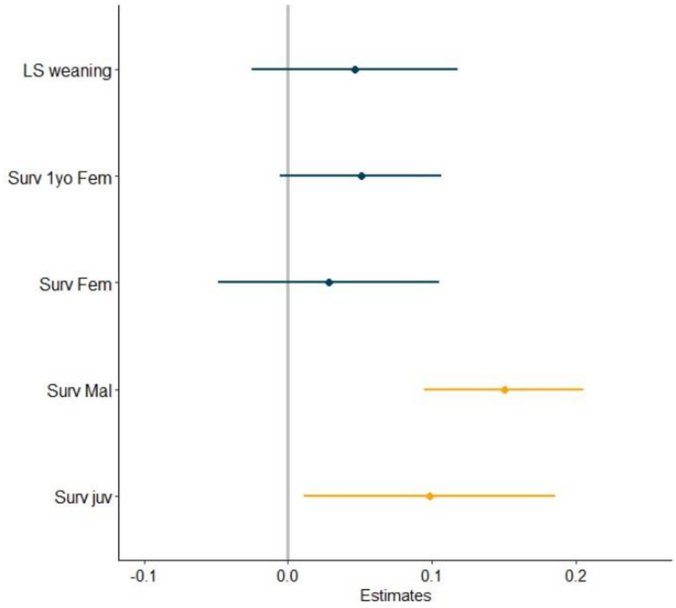


Figure 92. Effects of different demographic rates on the population growth rate. *Estimates and 95% confidence intervals from the LM are presented (significant effect in orange), demographic variables were standardized and variance inflation factors were verified.*

Adult male and juvenile survival contributed the most to population growth (LM, males: t = 5.577, $p < 0.001$, n = 30, juveniles: t = 2.327, $p = 0.028$, n = 30, Figure 92), meaning that population growth on MB was mainly regulated by the survival of adult males and juveniles through their first hibernation. If vegetation phenology is delayed and density increases, male survival may decrease (see chapter 4 results) and negatively affect population growth. This would be partly coherent with previous results in Columbian ground squirrels, showing that the most influential demographic rates were age at maturity, juvenile survival, and fertility (Dobson and Oli 2001b), but which was conducted on females only and so could not detect the important influence of male survival.

To examine these relationships in more detail, it would be interesting to use matrix population models instead of the simple population index to analyze population growth and declines (Caswell 2000), and to focus more effort on understanding male-related fluctuations (survival, dispersal, etc.). In addition, variance in population growth rate calculated from matrix population models can be decomposed into different contributions of variance in the vital rates (Life Table Response Experiments, LTREs, Caswell 1989, 1996). Running these analyses on different periods of population dynamics could help in elucidating the relative contribution of factors to either growth or declines (for example one period from 1992 – 2002 before the crash, between 2002 – 2005 during the population crash, and from 2005 – 2023 during the last and slow increase in population size, visible on Figure 71).

Further, environmental variables can be integrated in the models to evaluate the amount of variance in population growth explained by environmental variation (Jenouvrier et al. 2015, 2018, Gamelon et al. 2017). This would help further understanding the extrinsic and intrinsic variables relative contributions to population regulation and dynamics.

E. Conclusion

To conclude, this thesis examined various biological variables of Columbian ground squirrels in responses to climate, resource, and density variations. It helped us understand to what extent small hibernating rodents inhabiting montane ecosystems are adapted to their environment, especially in the context of global change. However, one might differentiate climate change effects on montane versus tropical hibernators, where seasonal and environmental constraints might strongly differ (lack of research on tropical hibernators, Wells et al. 2022) or regarding species living at higher altitudes or latitudes (Sheriff et al. 2012, Chmura et al. 2023). Short events of inclement weather did not seem to have detrimental consequences on their condition, whereas high temperatures may threaten their ability to forage and accumulate body mass during the short active period and before entering hibernation. Other small rodents or mammals, living in open area might answer similarly to environmental fluctuations (Goldberg and Conway 2021), if they have the possibility to thermoregulate behaviorally or physiologically.

Population dynamics of other small herbivores living in colonies may likely be regulated by animal density, and under constraints of resource variations. However, most of the research aiming to understand the challenges around ecological energetics and hibernation phenology examines rodent hibernators (ground squirrels, chipmunks, dormice, marmots), and other taxa remain understudied (*e.g.* temperate-zone insectivorous bats, Willis 2017).

Finally, whereas the access of small-scale climate conditions was possible, further studies would gain accessing local variations in vegetation and resources conditions to comprehend the spatial heterogeneity and organisms' microhabitats.

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ANNEXES

A. Annex I

Microclimate, an inseparable part of ecology and biogeography

Running Head: *Microclimate ecology and biogeography*

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**Shared first-authorship*

[In revision in *Global Ecology and Biogeography*]

Biosketch

The authors are participants of the Microclimate Ecology and Biogeography conference held in Antwerp, Belgium in 2022. Together they collaboratively wrote this perspective paper that brings together 97 experts and their views on the recent advancements and knowledge gaps in terrestrial microclimate. The paper was coordinated by Julia Kemppinen, Jonas Lembrechts, Koenraad Van Meerbeek, and Pieter De Frenne, and writing different sections was led by Jofre Carnicer, Nathalie Chardon, Paul Kardol, Jonathan Lenoir, Daijun Liu, Ilya Maclean, Jan Pergl, Patrick Saccone, Rebecca Senior, Ting Shen, Sandra Słowińska, Vigdis Vandvik, and Jonathan von Oppen. For more details on authors' statistics and how the work was organised, please see Supplementary information Figures S1-3.

Abstract

Brief introduction: what are microclimates and why are they important?

Microclimate science has developed into a global discipline. Microclimate science is increasingly used to understand and mitigate climate and biodiversity shifts. Here, we provide an overview of the current status of microclimate ecology and biogeography in terrestrial ecosystems, and where this field is heading next.

Microclimate investigations in ecology and biogeography

We highlight the latest research on interactions between microclimates and organisms, including how microclimates influence individuals, and through them populations, communities, and entire ecosystems and their processes. We also briefly discuss recent research on how organisms shape microclimates from the tropics to the poles.

Microclimate applications in ecosystem management

Microclimates are also important in ecosystem management under climate change. We showcase new research in microclimate management with examples from biodiversity conservation, forestry, and urban ecology. We discuss the importance of microrefugia in conservation and how to promote microclimate heterogeneity.

Methods for microclimate science

We showcase the recent advances in data acquisition, such as novel field sensors and remote sensing methods. We discuss microclimate modelling, mapping, and data processing, including accessibility of modelling tools, advantages of mechanistic and statistical modelling, and solutions for computational challenges that have pushed the state-of-the-art of the field.

What's next?

We identify major knowledge gaps that need to be filled for further advancing microclimate investigations, applications, and methods. These gaps include spatiotemporal scaling of microclimate data, mismatches between macroclimate and microclimate in predicting responses of organisms to climate change, and the need for more evidence on the outcomes of microclimate management.

Keywords

animal ecology, biodiversity, biogeography, climate change, data acquisition, ecosystem management, plant ecology, microclimate, modelling

Brief introduction: what are microclimates and why are they important?

Microclimates refer to the local climate conditions that organisms and ecosystems are exposed to (Bramer *et al.*, 2018). In terrestrial ecosystems, microclimates often differ strongly from the macroclimate, that is, the climate representative of a large geographic region. Microclimates are chiefly mediated by topography, vegetation, and soil, and they are a combination of local temperature, water (precipitation, air humidity, water availability), solar radiation, cloud, wind, and evaporation conditions (Bramer *et al.*, 2018). This fine-scale variation of microclimates is not captured by coarse-resolution macroclimatic data, because microclimates can vary over very short spatial and temporal extents. As such, microclimates influence the ecophysiology of individuals, and the dynamics of populations, communities, and ecosystems across biomes.

Microclimates enable organisms to develop, survive, and reproduce, for instance, below and near soil surface, and in tree canopies and cavities in an otherwise unsuitable macroclimate (Bramer *et al.*, 2018). Conversely, the same organisms can be absent in places where the microclimatic extremes exceed their limits. Additionally, microclimates dictate many ecosystem functions and processes, such as biogeochemical cycles. These local climatic conditions can be captured by microclimatic measurements, not by standard weather stations above short grass in the open (Bramer *et al.*, 2018). Thus, merging microclimate methods with ecological and biogeographic investigations and applications can provide valuable insights.

Recently, methods have become widely available for ecologists and biogeographers to inspect their study objects in relation to microclimates at high spatio-temporal resolutions and at large spatial and temporal extents (Lembrechts *et al.*, 2019b). Consequently, microclimate science has rapidly shown its high relevance to ecological and biogeographical investigations and applications (Bramer *et al.*, 2018; De Frenne *et al.*, 2021). Now, microclimate science is recognised as an integral component of ecology and biogeography, and is used to investigate local ecological manifestations of the global climate and biodiversity patterns (Zellweger *et al.*, 2020a; Riddell *et al.*, 2021), and to improve ecosystem management (Hylander *et al.*, 2022).

Microclimate science has a long tradition. Already in the mid 20th century, microclimatology was identified as an important subfield of meteorology, with clear repercussions for ecology and biogeography (Geiger, 1942a, 1951). The physics of microclimate (Baum & Court, 1949), the appropriate spatial scale, and the challenges of measuring microclimates (Geiger, 1951; Royal E. Shanks, 1956) have been studied for decades. Recent reviews have highlighted the importance of microclimate over macroclimate (Bramer *et al.*, 2018), and discussed microclimate in relation to remote sensing (Zellweger *et al.*, 2019), measurement techniques (Maclean *et al.*, 2021), species distribution modelling (Lembrechts *et al.*, 2019b), and forest ecology (De Frenne *et al.*, 2021). Following these examples, we consider that the microclimate scales and boundaries are highly dependent on the ecological context (Potter *et al.*, 2013; Pincebourde & Woods, 2020), e.g., ranging from minutes and cubic millimetres for within-leaf herbivore insects to monthly averages and hectares for understory communities in forests (Pincebourde & Woods, 2020; Zellweger *et al.*, 2020b).

Here, our aim is to provide an overview of the current status of microclimate ecology and biogeography, and where this field is going next from the perspective of a wide range of ecologists and biogeographers investigating diverse topics related to terrestrial microclimates (read more about the authors in Supplementary information Figures S1-3). In this perspective article, we focus on terrestrial ecosystems, because the physics behind microclimate buffering are entirely different in aquatic ecosystems. We showcase when and how incorporating microclimate science into ecological and biogeographical questions can increase knowledge and predictability of fine-scale phenomena and processes that generate larger or even global patterns. Recently, microclimate science has taken major strides forward, especially at the following three frontiers: 1) investigations of microclimate ecology and biogeography, 2) microclimate applications in ecosystem management, and 3) methods in microclimate science. For each of these themes, we identify a set of knowledge gaps to fill before microclimate data and concepts become a common option in ecology, biogeography, and beyond, from fine scale to the global scale. We herewith highlight the maturation of microclimate ecology and biogeography into a global discipline.

Microclimate investigations in ecology and biogeography

Organisms drive microclimates

Organisms play a pivotal role in shaping microclimates and have the capacity to establish mosaics of microclimates within ecosystems (Figure 1). For instance, grass and forest canopies create distinct microclimatic gradients (Vandvik *et al.*, 2020; De Frenne *et al.*, 2021). These canopies introduce both vertical and horizontal gradients in microclimates within relatively small geographic spaces (Ozanne *et al.*, 2003). Consequently, microclimates can serve as mediators in mitigating the impacts of climate change on organisms in the understory (Dobrowski *et al.*, 2015). This, in turn, allows various species (e.g., insects, frogs, and epiphytes) to adapt their distributions in response to broader climate shifts associated with changes in elevation or latitude (Scheffers *et al.*, 2013; Basham *et al.*, 2023). Also, the influence of animals on microclimates encompasses a wide spectrum, from large herbivores affecting microclimates through grazing and trampling on vegetation to insects regulating their nest temperatures through wing fanning and building temperature-modulating mounds (Jones & Oldroyd, 2006; Joseph *et al.*, 2016; Gordon *et al.*, 2023). These examples highlight the diverse ways in which organisms actively shape microclimates.

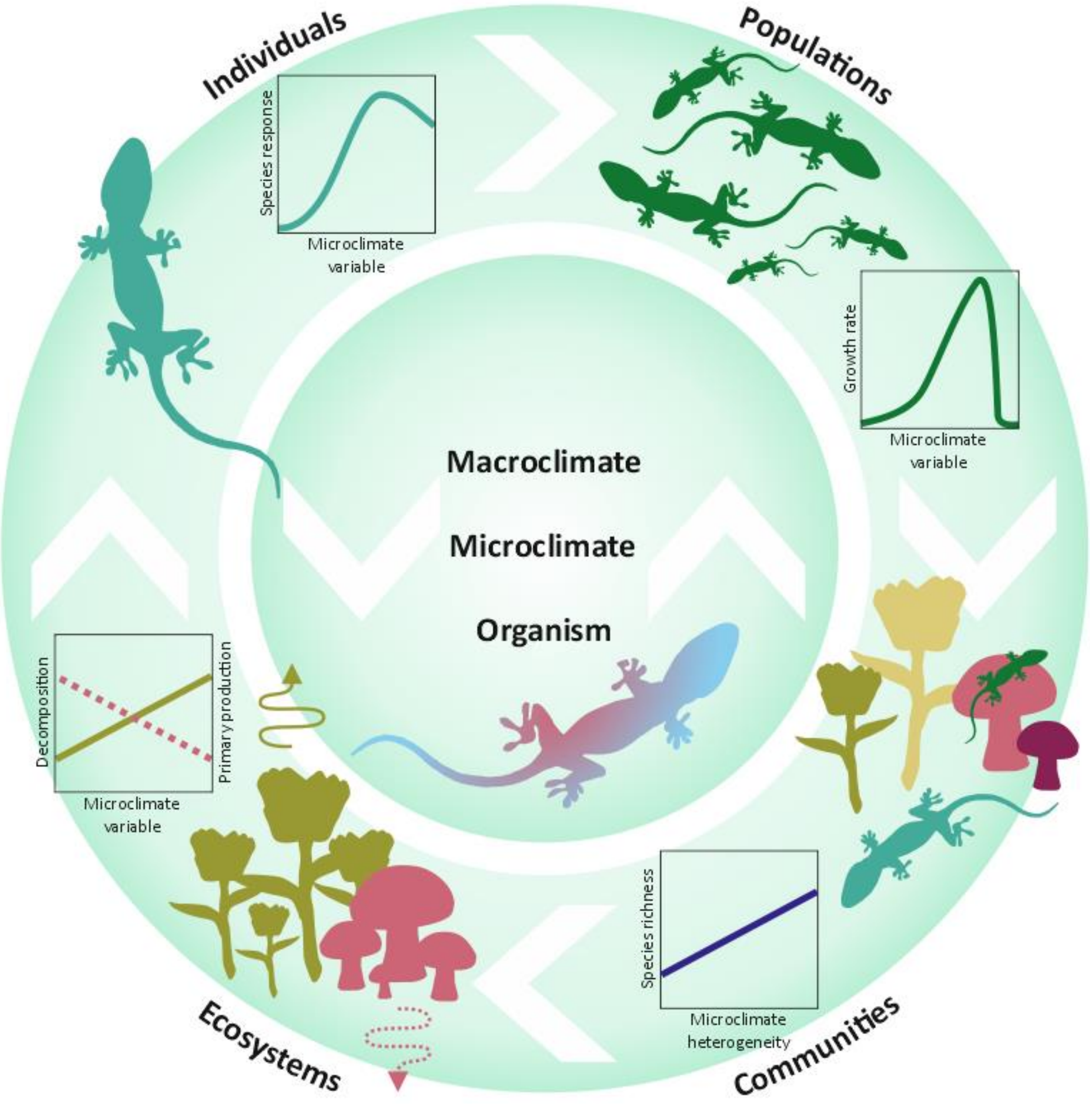


Figure 1. Microclimate investigations in ecology and biogeography. *The conceptual figure highlights that microclimate is the link between macroclimate and the ecophysiology of organisms. We show examples of how microclimates influence individuals, populations, communities, and ecosystems and their processes.*

Microclimates influence individuals and populations

Microclimates have a profound influence on all organisms by shaping their ecological interactions and geographic distributions. Microclimates exert a multifaceted influence on individuals, impacting their performance (Poorter *et al.*, 2019), structural characteristics (Kemppinen & Niittynen, 2022), organs (Opedal *et al.*, 2015), and cellular functions (Zweifel *et al.*, 2007). The influence of microclimates in the context of biophysical ecology is evident for ectothermic organisms, given that their thermoregulatory behaviour and temperature-dependent sex determination are governed by microclimatic conditions (Sears *et al.*, 2016; Carter & Janzen, 2021; Stark *et al.*, 2023). Insects provide excellent examples of how microclimates influence entire populations through individual responses. For instance, darker ants tend to dominate tree canopies due to melanism, which provides protection against UV radiation and reduces moisture loss (Law *et al.*, 2020). Additionally, the vertical variation in microclimatic conditions within forests promotes the evolution of thermal performance and desiccation resistance in ant populations (Bujan *et al.*, 2016; Kaspari *et al.*, 2016). These biophysical adaptations are closely linked to the organisms' ability to withstand thermal, hydrological, and light-related stressors. Furthermore, thermal tolerance has an impact on biodiversity at a global scale (Trew & Maclean, 2021) and can serve as a predictor of species performance, behaviour, and adaptability. This is evident in butterflies (Pincebourde & Casas, 2019; von Schmalensee *et al.*, 2021), birds (Kim *et al.*, 2022), and plants (Bert *et al.*, 2022), among other taxa. Consequently, microclimate models are used in various applications within the field of biophysical ecology (Sears *et al.*, 2016; Carter & Janzen, 2021; Briscoe *et al.*, 2022), serving as valuable tools for understanding and predicting interactions between organisms and their environmental conditions.

Microclimates shape populations by directly influencing demographic rates through individual recruitment and survival (Oldfather & Ackerly, 2019; Goodwin & Brown, 2023). This phenomenon is evident in plant populations, where crucial processes like seed germination and seedling establishment depend on specific temperature, humidity, and light conditions (Davis *et al.*, 2016; Graae *et al.*, 2022). Furthermore, the growth and mortality rates of plants are linked to water availability (Liu *et al.*, 2018), a factor that also controls the regeneration of trees after disturbances (Lloret *et al.*, 2004; Thom *et al.*, 2022). In animal populations, microclimates influence behavioural responses. For instance, butterflies employ strategies such as clustering at different heights in trees to avoid frost (Brower *et al.*, 2011), while birds take into account wind characteristics when selecting nest sites (Momberg *et al.*, 2023). Moreover, microclimates control fine-scale variations in phenology (Tansey *et al.*, 2017).

Microclimates structure communities

The individual-level effects of microclimates ultimately shape the composition and dynamics of communities. Microclimates serve as an important determinant in structuring communities, by influencing both species distributions and patterns of species richness (le Roux *et al.*, 2013; Checa *et al.*, 2014; Niittynen *et al.*, 2020; Momberg *et al.*, 2021; Ma *et al.*, 2022). Within plant communities, microclimates shape species richness, turnover, and the composition of vascular plants (Opedal *et al.*, 2015; Shen *et al.*, 2022b), bryophytes (Man *et al.*, 2022; Shen *et al.*, 2022a), and lichens (Kemppinen *et al.*, 2019). The heterogeneity of microclimates mediates how species respond to climate change (Zellweger *et al.*, 2020a) (see also (Bertrand *et al.*, 2016)), and it also plays a critical role in the context of land use changes (Christiansen et al. 2022). Consequently, the incorporation of microclimate data is crucial for increasing ecological realism of species distribution models (Haesen *et al.*, 2023b), including models on reptile (Stickley & Fraterrigo, 2023), bird (Massimino *et al.*, 2020), as well as vascular plant, bryophyte, and lichen species (Niittynen & Luoto, 2018).

Species interactions are influenced by microclimate conditions through a variety of mechanisms, encompassing behavioural, phenological, and ecophysiological processes. For example, microclimates significantly shape the habitat preferences of insects (Carnicer *et al.*, 2019; Vives-Ingla *et al.*, 2023) and influences the timing of plant phenological events (Kankaanpää *et al.*, 2018), ultimately leading to cascading effects on community structures across multiple trophic levels (Kankaanpää *et al.*, 2020). In forests, microclimates can significantly modify species interactions by altering phenological responses, influencing the development of chemical defence traits, impacting colonisation patterns, and competitive processes (Greiser *et al.*, 2021; Sanczuk *et al.*, 2021; Willems *et al.*, 2021). Furthermore, microclimates play a critical role in determining facilitation. For instance, shrubs and cushion plants modify their below-canopy microclimates which facilitate the growth of seedlings (Cavieres *et al.*, 2014; Vega-Álvarez *et al.*, 2019).

Microclimates control and create ecosystems

Microclimates control ecosystem processes, the most essential of these being the cycles of energy, water, and matter, such as the carbon cycle (Cahoon *et al.*, 2012; Gora *et al.*, 2019; Meeussen *et al.*, 2021). Microclimates can regulate litter decomposition (Chen *et al.*, 2018), heterotrophic and autotrophic soil respiration (Fernández-Alonso *et al.*, 2018), and photosynthesis (Poorter *et al.*, 2019). Hence, microclimatic temperatures drive biogeochemical cycles, such as greenhouse gas fluxes, and fine-scale moisture conditions determine local methane sinks and sources (Virkkala *et al.* Preprint). Overall, microclimates are important to consider in investigating ecosystem processes, since they regulate resources for primary production and regulate many ecosystem functions.

Through the many impacts on plant and animal individuals, populations, and communities, microclimates support microrefugia, small ecosystems buffered from climate change. In microrefugia, temporal changes in local temperature, water, and light conditions are smaller than in the surrounding areas (Ashcroft, 2010; Keppel *et al.*, 2012; McLaughlin *et al.*, 2017). Thus, microrefugia can buffer climate change impacts (Morelli *et al.*, 2020), and preserve biodiversity and ecosystem functions (Ashcroft, 2010; Ellis & Eaton, 2021). Microrefugia affect seed survival and plant growth, and can create opportunities for animals to hide, feed, and reproduce (Checa *et al.*, 2014; Frey *et al.*, 2016a; Lucid *et al.*, 2021). Microrefugia can be identified using thermal imaging (Hoffrén & García, 2023), high-resolution gridded microclimate products (Haesen *et al.*, 2023b), topographic data (Ashcroft *et al.*, 2012; Meineri & Hylander, 2017), or exploring disjunct populations (Finocchiaro *et al.*, 2023). Overall, microrefugia can shape species redistributions under climate change (Lenoir *et al.*, 2017; Stark *et al.*, 2022). Thus, microrefugia are important for maintaining biodiversity (Dobrowski, 2011; Suggitt *et al.*, 2018; Maclean & Early, 2023), and consequently, for nature conservation (Ackerly *et al.*, 2020), and can have the same importance as larger ecosystem management activities (Thorne *et al.*, 2020).

Microclimate applications in ecosystem management

Microclimates are pivotal in ecosystem management, especially in the face of climate change (Figure 2). The question of how management practices affect microclimates has been discussed for decades (Kraus, 1911; Geiger, 1942b). Similarly, managing microclimates has long been part of land-use practices, especially in agriculture. In agriculture, microclimates can be managed, for example, by planting shade trees for enhancing growing conditions of crops, such as coffee and vanilla (Beer *et al.*, 1998; Lin *et al.*, 2008). Microclimate management can help pest management by creating microclimates beneficial for retaining natural enemies (Begg *et al.*, 2017), and planting trees or small forest patches can also benefit agrobiodiversity (Wurz *et al.*, 2022). Overall, more focus has recently been drawn to managing microclimates for mitigating climate change and for promoting and protecting biodiversity.

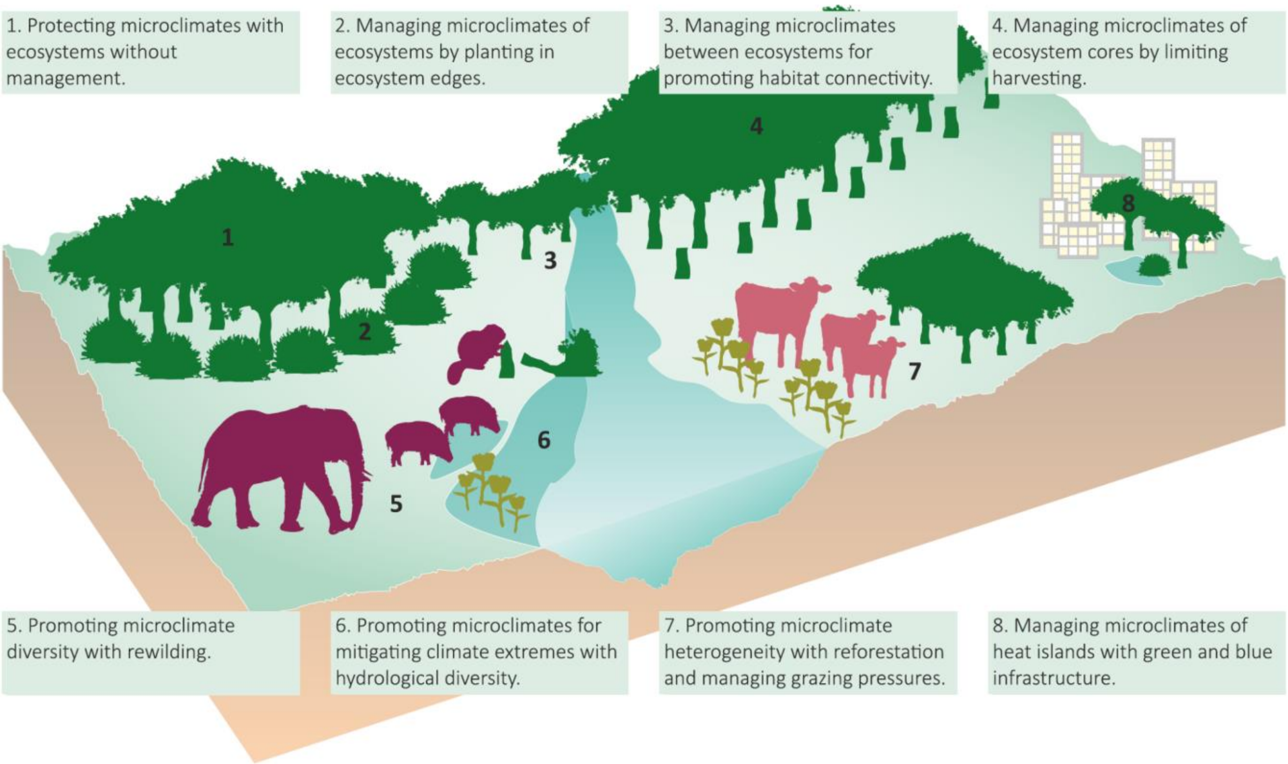


Figure 2. Microclimate applications in ecosystem management. *The conceptual figure presents examples of biodiversity conservation, forestry, and urban ecology maintaining and promoting microclimate heterogeneity for the benefit of biodiversity.*

Microclimate management in biodiversity conservation

Microclimate management is crucial for protecting biodiversity under climate change (Greenwood *et al.*, 2016) and land use change (Williamson *et al.*, 2021). Microclimate heterogeneity is an indicator of microrefugia (Keppel *et al.*, 2015), and can reduce extinction risks (Moritz & Agudo, 2013; Suggitt *et al.*, 2018). Microclimate heterogeneity can be increased by altering vegetation structure (Curtis & Isaac, 2015; Hylander *et al.*, 2022). Vegetation structure can be modified using silvicultural practices, managing grazing pressure by livestock, and trophic rewilding with wild megafauna (Thers *et al.*, 2019; Malhi *et al.*, 2022). For example, beaver constructions buffer microclimates from extreme fluctuations by increasing hydrological connectivity and creating floodplains (Weber *et al.*, 2017; Larsen *et al.*, 2021). Also, elephants, wild boars, horses, and donkeys engineer microclimates by grazing and trampling on vegetation, and modifying topography and water availability (Sandom *et al.*, 2013; Lundgren *et al.*, 2021; Gordon *et al.*, 2023). Maintaining and creating microclimate heterogeneity and habitat connectivity is an effective basis for future-proofing ecosystems which increases resilience to climate change (Hylander *et al.*, 2022; Maclean & Early, 2023; Stark *et al.*, 2023). Moreover, knowledge and data on microclimate heterogeneity can help identify organisms and ecosystems most vulnerable to climate change (McCullough *et al.*, 2016), and when combined with biophysical ecology, this knowledge can improve and create new management practices to promote biodiversity (Briscoe *et al.*, 2022; Ononye *et al.*, 2023; Welman & Pichegru, 2023).

Microclimate management is used for buffering against gradual environmental change and short-term climate extremes, such as heat waves or droughts, and this increases resistance and enables proactive transformation of managed ecosystems (Brang *et al.*, 2014; Hylander *et al.*, 2022). Proactive transformation considers protection of cool microclimates which promote microrefugia (Schmalholz & Hylander, 2011; Hylander *et al.*, 2022). Microclimate management is constantly evolving (Kermavnar *et al.*, 2020; Thom *et al.*, 2020), and increasingly applied within principles of close-to-nature management (Brang *et al.*, 2014; Hylander *et al.*, 2022). For example, in selective logging the post-logging recovery of forest microclimates can be rapid (Senior *et al.*, 2018; Mollinari *et al.*, 2019). This suggests that, in contrast to clear-cutting, selective-logging can provide timber while maintaining microclimate heterogeneity, if logging rotations allow sufficient space and time for regeneration of understorey vegetation (Menge *et al.*, 2023).

Microclimate management in forestry

Forestry is an excellent example of how ecosystem management affects microclimate heterogeneity (Scheffers *et al.*, 2017; Menge *et al.*, 2023). In forestry, microclimates are managed to reduce insect outbreaks (Kautz *et al.*, 2013), support tree regeneration (Thom *et al.*, 2022), and reduce frost damage (Örlander, 1993). Forest microclimates are affected by the diversity in tree species, forest structures, management practices (e.g., thinning), and distance to forest edge (Geiger, 1942b; Chen *et al.*, 1993; Meeussen *et al.*, 2021). For example, cool and wet microclimates are lost when humid tropical forests are degraded (Senior *et al.*, 2017), even where tree cover remains, such as within tree plantations (Luskin & Potts, 2011) and selectively logged forests (Blonder *et al.*, 2018). This is severe because it decreases the capacity of the forest to buffer climate change impacts and maintain biodiversity (Scheffers *et al.*, 2014). Old-growth forests with diverse microclimatic conditions are especially important for climate change mitigation and biodiversity conservation (Norris *et al.*, 2011; Frey *et al.*, 2016b; Wolf *et al.*, 2021). However, as temperatures increase and water availability is more limited, forests can lose their capacity to buffer climate extremes (Davis *et al.*, 2019). Knowledge and practices found in forestry can be further applied also in other anthropogenically modified environments.

Microclimate management in urban ecology

Increasing recognition of the importance of microclimates has led to a proactive approach also in urban ecology to achieve desired microclimate outcomes (Lai *et al.*, 2019). Microclimate heterogeneity is particularly important to consider in rapidly urbanising and densely populated areas (Hartig & Kahn, 2016; Souza *et al.*, 2016; Xue *et al.*, 2017). In urban ecosystems, microclimatic anomalies are driven by the lack of vegetation and abundance of impervious, dark surfaces, which create heat islands (Schwaab *et al.*, 2021; Iungman *et al.*, 2023). Urban heat islands shape organisms, as they have been shown to alter spider behaviour (de Tranaltes *et al.*, 2022), and result in diversity changes in plant, bird, and insect species (Aronson *et al.*, 2014; McGlynn *et al.*, 2019). Management practices can optimise microclimate conditions of urban heat islands by using green and blue infrastructure (Bowler *et al.*, 2010; Lin *et al.*, 2020), which consists of water bodies, green roofs and facades, street trees, and urban forests (Zölch, Teresa *et al.*, 2016; Taleghani, 2018; Lai *et al.*, 2019). Responses to green infrastructure are taxa-specific, but overall, green infrastructure can significantly benefit urban biodiversity

(Filazzola *et al.*, 2019), and also improve human thermal comfort and decrease human heat mortality in cities (Gillerot *et al.*, 2022; Iungman *et al.*, 2023).

Methods for microclimate science

Advances in data acquisition

Microclimate measurements rely largely on in-situ sensors for obtaining data on local temperature, water, solar radiation, cloud, wind, and evaporation conditions (Figure 3). In-situ sensors now form part of the toolkit of many ecological studies due to the improvements in chip devices, battery technology, cost-effectiveness, and the miniaturisation of sensors and their hardware (Mickley *et al.*, 2019; Wild *et al.*, 2019; Rebaudo *et al.*, 2023). Moreover, advancements in wireless communications, such as the 'internet of things' (Li *et al.*, 2015), and data transmission using cellular technology or potentially via satellite, increasingly allow the deployment of these devices in ad-hoc mesh networks across a landscape (Keitt & Abelson, 2021). Here, strategically planned study designs lay foundations for representative microclimate networks (Lembrechts *et al.*, 2021), and new methods are developed to make most of sparse microclimate ground data, such as signal processing theory, which leverages cyclic microclimate patterns and temporally downscales sparse time-series (von Schmalensee, 2023). Also, animal-borne microclimate sensor networks can provide a biological lens to obtaining microclimate data from land and air (Ellis-Soto *et al.*, 2023), and as a by-product, wildlife camera imagery can provide micrometeorological data on e.g., sunshine, snow, and hail (Alison *et al.*, 2023). However, the accuracy of low-cost loggers can be uncertain, and the reduction in size and costs affects measurement accuracy of accompanying sensors (Terando *et al.*, 2017; Maclean *et al.*, 2021). Therefore, it is often advisable to calibrate sensors against laboratory measurements (e.g. climatic chambers for temperature sensors), to validate sensors by comparing them to a reference, and also to inter-calibrate sensors by comparing them to each other (Heinonen *et al.*, 2014; Playà-Montmany & Tattersall, 2021). In the case of temperature measurements, standard weather station protocols including shading and ventilating thermometers often do not apply as measured microclimatic temperature variation mainly has it origin in low wind speed and variation in solar radiation (Terando *et al.*, 2017; Maclean *et al.*, 2021). Therefore, ultra-fine-wire thermocouples remain recommended for specific purposes, especially when sensors are subjected to direct sunlight (Maclean *et al.*, 2021). Hydric microclimate data can also be challenging to calibrate and validate, both for air and soil humidity measurements. For instance, measurements of soil moisture are influenced by soil heterogeneity and stoniness that affect sensor-soil contact (Robinson *et al.*, 2008; Wild *et al.*, 2019).

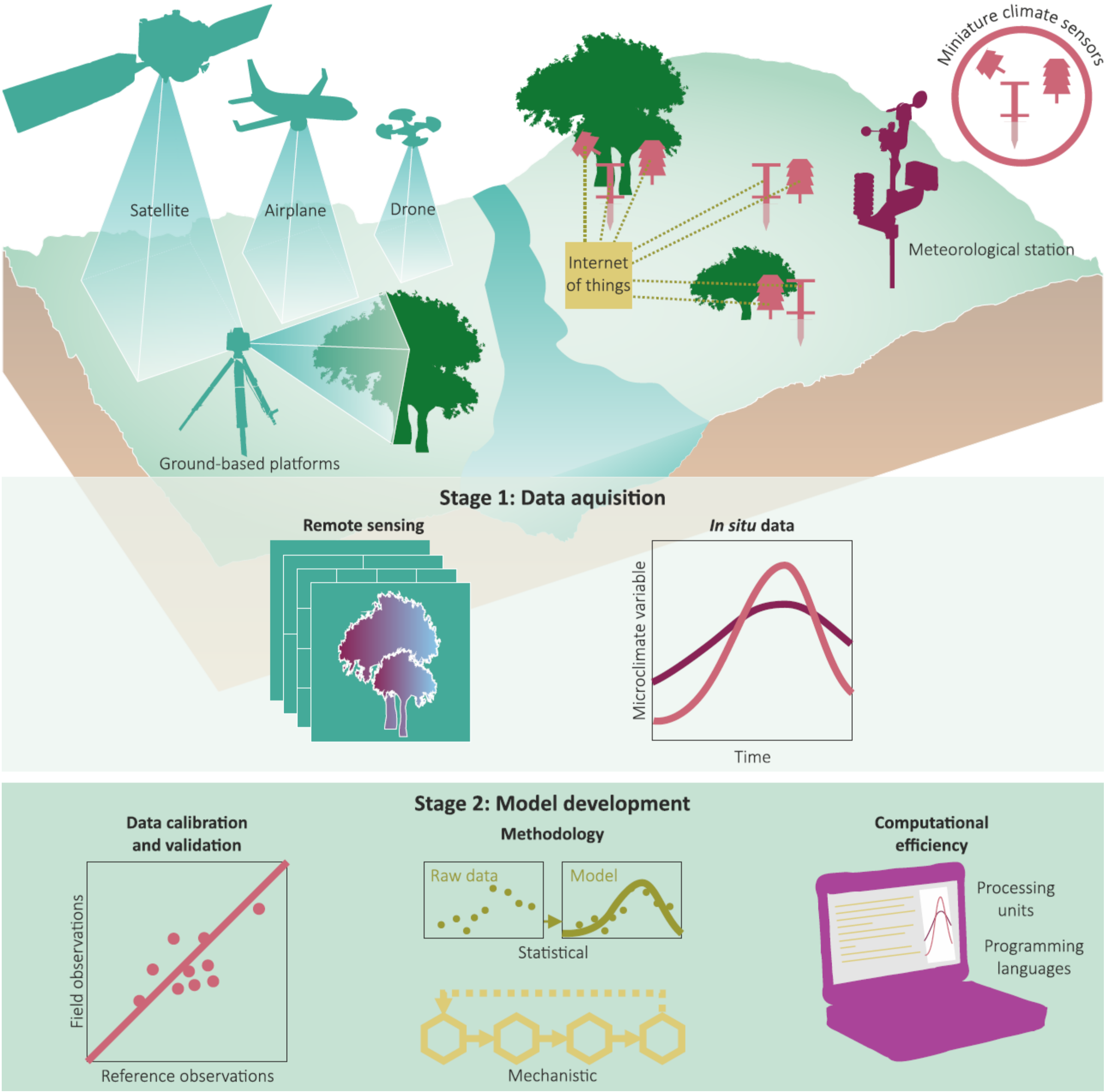


Figure 3. Methods for microclimate science. *This conceptual figure presents examples discussed in the main text on how microclimate data and its explanatory variables are acquired from remotely-sensed products and in situ measurements (Stage 1). We show examples of key areas where microclimate models have recently improved, from calibration to modelling methods and computational efficiency (Stage 2).*

Remote sensing allows researchers to capture leaf- to landscape-scale microclimate data with spatio-temporal representativeness, for instance on local temperature conditions (Faye *et al.*, 2016; Zellweger *et al.*, 2019). In structurally complex areas, such as forests, mountains, or cities, measurements from a small number of sensors over a short time period will fail to adequately capture the range of microclimate conditions present (Scherrer & Kãrner, 2009; Zhou *et al.*, 2011; De Frenne *et al.*, 2021). This limitation can be overcome by linking microclimate measurements with remote sensing data on key predictors of microclimates (e.g. Haesen et al. 2021): vegetation and topographic features, and also snow in seasonally snow covered areas. These data can be used for modelling microclimates across landscapes by filling the gaps between the microclimatic ground data. Spatially continuous structural or spectral data on vegetation and terrain structures can be obtained from satellites, aeroplanes, and unoccupied aerial vehicles (UAVs) mounted with, e.g., thermal imaging or light detection and ranging (LiDAR) sensors (LiDAR-derived topography and forest structure predict fine-scale variation in daily surface temperatures in oak savanna and conifer forest landscapes, 2019; Båserud *et al.*, 2020; Kašpar *et al.*, 2021). For instance, high-resolution LiDAR data are openly available for some countries, such as for >15 European countries (<5 m resolution) (Kakoulaki et al. 2021). Terrestrial and mobile remote-sensing platforms can overcome canopy occlusion by obtaining measurements from a large range of viewpoints inside the canopy (Disney, 2019; Calders *et al.*, 2020). UAVs enable obtaining data at even higher spatial resolution over limited spatial extents (Faye *et al.*, 2016; Duffy *et al.*, 2021; Hoffrén & García, 2023). Fusing these different types of remotely-sensed data with novel approaches of radiative transfer modelling through canopies offer interesting new avenues for microclimate ecology (Jonas *et al.*, 2020). Overall, there is great potential to exploit new modelling advances in further microclimate research.

Advances in microclimate modelling and data processing

Microclimate models tend to be based on mechanistic understanding of the physical processes governing the energy balance. These models owe their origins to the pioneering work on weather forecasting by Richardson (1922), who demonstrated the application of energy balance equations for modelling the turbulent mixing of the atmosphere-biosphere boundary, and microclimate modelling by Porter et al. (1973), who developed a general microclimate model for solving the heat and water budgets of organisms. Thus, the most recent developments are not in the modelling of microclimate itself, but rather in making complex models more accessible to a wider audience. Recently, a series of microclimate models have been written using the R programming environment (R Core Team, 2022), enabling easy application by ecologists (Kearney *et al.*, 2020; Maclean & Klinges, 2021). There are also guides with interactive visualisations for selecting and accessing microclimate data (Meyer *et al.*, 2023). In parallel, the climate modelling community has been including multi-layered canopy representations in multiple land surface models (CLM-ml, ORCHIDEE-CAN, CLM-FATES) (Lawrence *et al.*, 2019) allowing for point site evaluation of coarse microclimate data (Bonan *et al.*, 2021). Such models have the advantage to be directly embedded in earth system model frameworks, therefore opening avenues to study coupled vegetation-microclimate feedbacks.

Microclimate varies considerably at fine temporal resolutions (Bramer *et al.*, 2018). Therefore, mechanistic models are run in sub-daily time increments. It is, in turn, computationally challenging to model microclimate mechanistically over large areas, even with the ongoing rapid advances in computing power. Also, lack of data can hinder the use of mechanistic models that require a comprehensive set of predictors. In part for these reasons, ecologists and biogeographers have tended to seek statistical relationships between microclimates and their drivers, such as topography and vegetation features (Ashcroft *et al.*, 2009; LiDAR-derived topography and forest structure predict fine-scale variation in daily surface temperatures in oak savanna and conifer forest landscapes, 2019), or have sought to establish these relationships through machine learning (Haesen *et al.*, 2021; Lembrechts *et al.*, 2022). The advantage of statistical and machine learning approaches is that bioclimatic variables of interest are not always needed at high temporal resolution (Hijmans *et al.*, 2005), which can reduce the computational demands of the models. A significant drawback of statistical approaches is that the influence of variables used as predictors in statistical models, such as terrain and vegetation, vary in space and time. Thus, relationships derived at one location or time-period cannot necessarily be readily applied to others (Aalto *et al.*, 2022). This could be overcome by modelling spatiotemporally varying relationships, i.e., by using geographically weighted regression. Databases have emerged to provide the large precalculated microclimate datasets that are needed for modelling the relationships accurately, including for instance projections of past and future microclimates (Levy *et al.*, 2016), hourly estimates of historical microclimates (Kearney, 2019), and global soil temperatures (Lembrechts *et al.*, 2020a). However, the data can originate from different sources and require preprocessing. Also microclimate data processing has advanced, for instance, with the advent of automated R packages that are suited for gap filling, flagging erroneous measurements, calculation of summary statistics, and analysing thermal images (Senior *et al.*, 2019), and for microclimate data handling and standardised analyses (Man *et al.*, 2023).

The fusion of statistical and mechanistic approaches to model microclimates shows promise for developing mechanistically-informed and computationally efficient methods. The application of statistical model emulation techniques that reproduce the behaviour of more complex models using techniques routinely adopted in other areas of climate modelling could significantly reduce computational run times (Baker *et al.*, 2022). Further implementation requires a break-down of traditional barriers between disciplines as far apart as ecology, meteorology, and computer science (Briscoe *et al.*, 2023). Also, recent developments in hardware and software provide potential solutions to the computational challenge of modelling microclimates. First is the modern computationally efficient programming language, Julia (Bezanson *et al.*, 2018). Julia is similar to dynamic languages like Python and R, yet it compiles packages and user scripts down to machine code at run-time, thereby achieving speed comparable to Fortran or C++, and support for graphics processing unit-based programming geared at optimising parallel computing is under active development (Besard *et al.*, 2019; Schouten *et al.*, 2022). Second is the burgeoning computational infrastructure for model processing, development and testing. Central to this infrastructure is the growing availability of affordable cloud-based computing and storage for back-end processing. Coupled with databases for model testing and comparisons (see e.g. (Dietze *et al.*, 2021)), such frameworks provide a robust infrastructure for collaborative model development and processing at massive scales. These advancements in data collection, modelling, and processing collectively enable us to attain microclimatic data at increasingly finer spatio-temporal resolutions, aligning more and more closely with the scales at which organisms operate.

Finer resolution is not necessarily the better solution

Despite the importance of microclimates across many aspects of ecology and biogeography, we stress that a higher spatio-temporal resolution is not always necessary. Indeed, some organisms and ecosystem functions operate at spatial or temporal extents at which macroclimate data are more appropriate, thus, research questions do not automatically require a microclimate approach. In some cases, microclimate data did improve ecological models (forest plants, see (Haesen *et al.*, 2023b); and tundra plants see (Kemppinen *et al.*, 2021)), yet, one approach is not necessarily transferable to other organisms (Lembrechts *et al.*, 2019a). For instance, decade-long gridded air temperature data did outperform short-term soil temperature data in distribution models of bacterial membrane lipids with long-term stability in the soil (Halffman *et al.*, 2022), as patterns that form over decades or centuries do not relate to short-term microclimatic fluctuations. These examples highlight that methods, including microclimate data and tools, should always be hypothesis-driven and justified by ecological and biogeographical theory. In many cases, the use of macroclimate data can be sufficient, or macroclimate data could simply be downscaled using, for example, fine-scaled topographic proxies (Kusch & Davy, 2022). Therefore, the microclimate approach is not a default answer to all ecological and biogeographical questions.

What's next?

In this perspective paper, we showcased that microclimate ecology and biogeography have evolved into a distinct, global discipline. We highlighted the most substantial recent microclimate advances at the core of ecology and biogeography. Microclimate science is rooted in environmental biophysics and has recently experienced a surge of methodological progress, such as in logger autonomy, measurement accuracy, and computing power allowing advancements in microclimate investigations and applications. This recent unlocking of microclimatic data and knowledge is welcomed, as microclimates are inseparable from the physiological constraints of individuals, populations, communities, and ecosystems. Consequently, microclimates are also critical for understanding the influence of global change drivers, such as climate and land-use change on ecology and biogeography. As a result, microclimate science stands at the core of multiple important applications in ecosystem management, such as biodiversity conservation, forestry, and urban ecology. Nevertheless, major steps are also ahead for this emerging field to have it reach its full potential.

First of all, global microclimate research should be conscious of its biases. For instance, forest and tundra biomes are well represented in the microclimate literature, while microclimates matter to many terrestrial organisms across all terrestrial biomes. Secondly, it is also important to note that in the English-written scientific literature, microclimate ecology and biogeography is largely represented by studies, researchers, and institutions of European, North American, and Australian origin. We emphasise that these knowledge gaps and biases are important to consider in all future research that aims for a genuinely global coverage in microclimate investigations. This is key for making ecology and biogeography a more global endeavour (Nuñez *et al.*, 2021).

Knowledge gaps in microclimate investigations in ecology and biogeography

The mismatches between macroclimate and microclimate should be considered when predicting responses of organisms to climate change (Liancourt *et al.*, 2020; Zellweger *et al.*, 2020a). It is crucial to understand the influence of microclimates on organisms under climate change, but there are many remaining unknowns. This would require measuring and modelling the effects of all different microclimatic conditions that influence a given organism and its functions (Kemppinen & Niittynen, 2022). This could, for example, be achieved by coupling observational approaches with experiments, which would allow understanding the climatic optima and tolerance levels of the organism (Ripley *et al.*, 2020; Vandvik *et al.*, 2020). Also, mobile organisms can move between microclimates in search of more suitable conditions (Frey *et al.*, 2016a; Kim *et al.*, 2022), however, more investigation is needed to understand which organisms exploit microrefugia under climate change and why.

Microclimate science is increasingly incorporated into ecological and biogeographical questions at local to regional extents (De Frenne *et al.*, 2021), but questions of continental or global extents are rare (but see *e.g.*, Haesen *et al.*, 2023b; Risch *et al.*, 2023). Incorporating the principles and approaches of microclimate science into studies beyond local extents would call for improved global data integration. This would also require the harmonisation of measurement methods and increased monitoring of remote, undersampled areas and ecosystems, such as tropics, deserts and tundra. The first is partly hindered by lack of standard guidelines that would increase comparability of microclimate data (Maclean *et al.*, 2021), and the latter by the cost of microclimate sensors which is not globally accessible (Nuñez *et al.*, 2021). However, some microclimate products, such as models of soil and near-surface temperatures, have recently become openly available at continental and global extents (Haesen *et al.*, 2021; Lembrechts *et al.*, 2022).

Microclimate investigations on larger organisms and above-ground systems are plentiful, whereas, more research is needed on microclimate relationships of microorganisms and below-ground organisms and ecosystem processes. However, investigations in soil ecology are partly hindered due to a lack of high-resolution data on belowground microclimates (Eisenhauer *et al.*, 2022). Moreover, microclimates are crucial also for aquatic ecosystems, such as freshwater, riparian, coastal, and marine ecosystems (Judge *et al.*, 2018; Enriquez-Urzelai *et al.*, 2019; Bentley *et al.*, 2020; Nadeau *et al.*, 2022).

Knowledge gaps in microclimate applications in ecosystem management

More evidence is needed on the outcomes of microclimate management. This evidence should show when and where microclimate management is required for promoting and protecting biodiversity (Ellis, 2020; Tinya *et al.*, 2021). Currently, the evidence for microclimate management to build climate-resilient ecosystems is often theoretical (Morelli *et al.*, 2020; Hylander *et al.*, 2022), and therefore, additional data could strengthen these links.

There is a need for identifying general patterns of microclimate-organism relationships across and within ecosystems (Kemppinen *et al.*, 2021). For example, what makes microclimates act as microrefugia varies by site, by species, and potentially by life stage, each depending on different spatiotemporal factors and scales (Caron *et al.*, 2021; Greiser *et al.*, 2022). Thus, not all microrefugia are equally valuable for protecting biodiversity (Hylander *et al.*, 2015).

Microclimate science can be used beyond ecology and biogeography. This could lead to new knowledge and applications in microclimate ecology and urban ecology (Roman *et al.*, 2021; Iungman *et al.*, 2023), microclimate biogeography and agriculture (Gardner *et al.*, 2021), and microclimate biogeography and health geography (Paaijmans *et al.*, 2010; Wong & Jim, 2017; Wimberly *et al.*, 2020). Microclimate science can be used to address major societal challenges, such as health and well-being (Jenerette *et al.*, 2016; Gillerot *et al.*, 2022), green energy efficiency (Shafique *et al.*, 2020), and socioeconomic injustice (Ghosh *et al.*, 2022; Yin *et al.*, 2023). By embracing interdisciplinarity, microclimate science can be exploited in solving these crucial issues for an ecologically and socioeconomically sustainable future.

Knowledge gaps in methods for microclimate science

Methods for microclimate science should aim to achieve a more flexible spatio-temporal scaling of microclimate data. This entails developing a comprehensive library of gridded microclimate products that match the scale and extent required in specific research questions. However, pursuing higher resolutions is not valuable in itself in ecological and biogeographical investigations, as the inclusion of microclimate mechanisms, especially those non-linearly related to macroclimate, takes precedence over spatiotemporal resolution (Bennie *et al.*, 2014; Bütikofer *et al.*, 2020). Nonetheless, most existing products lack in at least one dimension, whether it be in spatial or temporal resolution, and/or mechanistic proximity. Enhancing these dimensions can be accomplished by integrating open access data platforms for in-situ data, such as the SoilTemp database (Lembrechts *et al.*, 2020b), gridded microclimate products generated for example through fusion with remote sensing and macroclimate products, such as CHELSA or WorldClim (Haesen *et al.*, 2023a), and increased efficiency and scalability of mechanistic microclimate models (Maclean & Klinges, 2021).

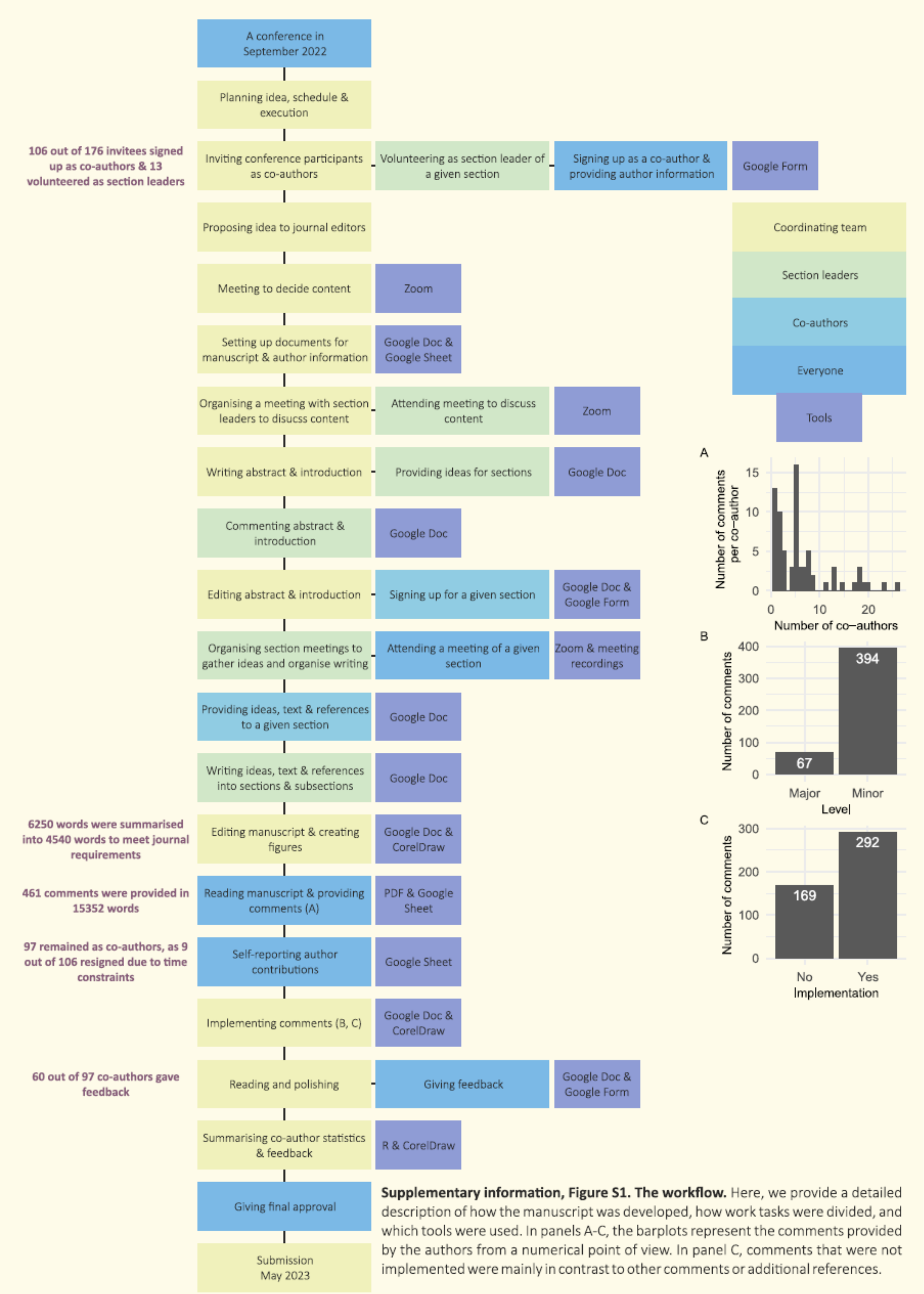
Importantly, microclimate data should evolve from stationary to dynamic products (Kearney *et al.*, 2020). For instance, future microclimatic data is largely lacking, since the currently available microclimate datasets with a broad spatial extent only provide bioclimatic variables for the present (Lembrechts *et al.*, 2022; Haesen *et al.*, 2023a). Ideally, datasets would also capture microclimates in all three dimensions of space. Ultimately, predictors used for modelling microclimates should be advanced to accommodate this progress (e.g., land-use change scenarios).

Integrating microclimate-vegetation feedback into global change biology is an important avenue (Bonan *et al.*, 2021). This could be further developed by coupling airborne laser scanning based single tree-delineation methods with radiative transfer and microclimate models

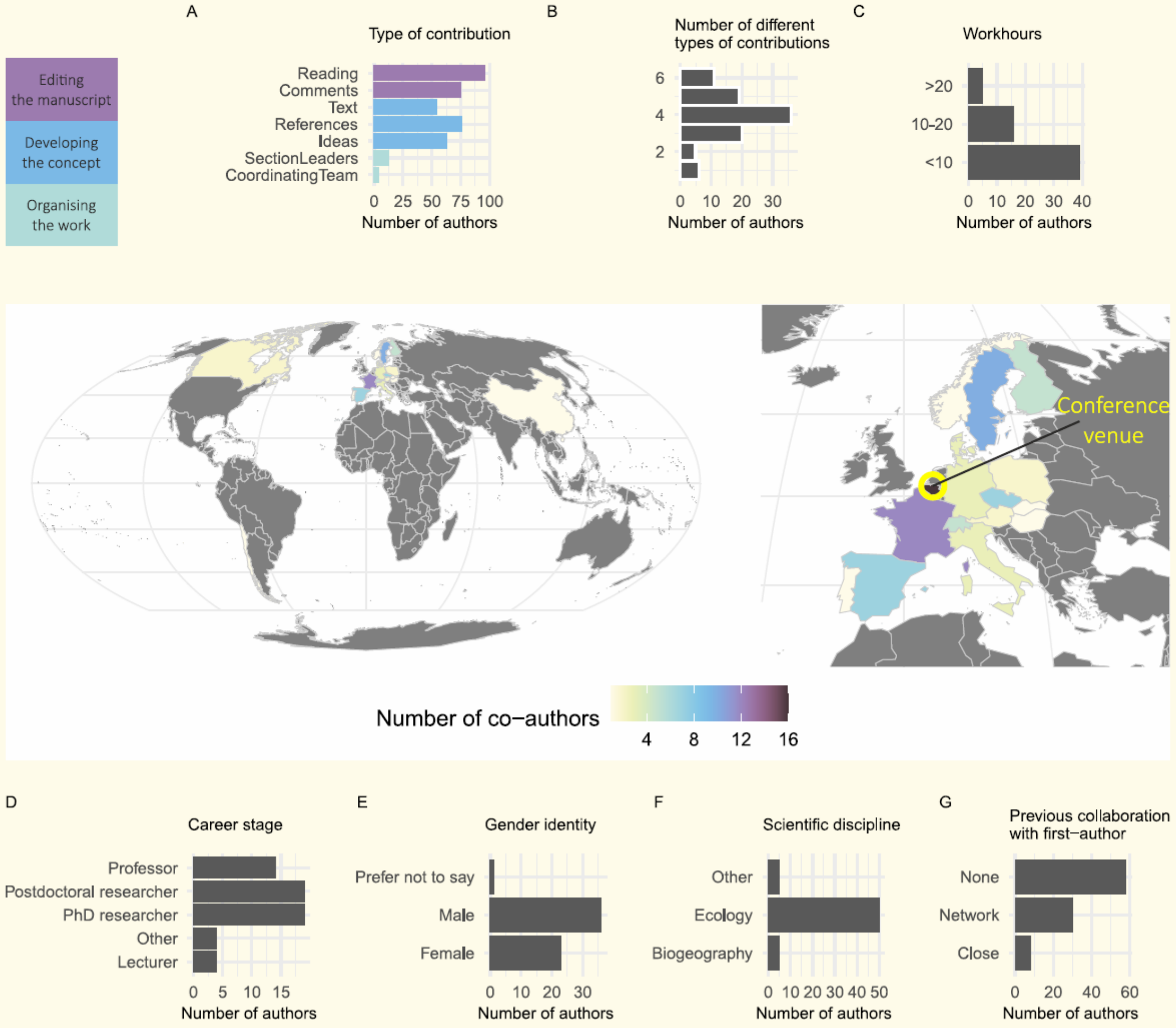
(Webster *et al.*, 2020). This would allow for spatially extensive and explicit simulations of microclimate dynamics under, for instance, different management regimes, natural disturbance dynamics, or climate scenarios.

We have demonstrated that endeavours in microclimate ecology and biogeography are worthwhile and can provide many new avenues for future research. The constantly evolving methods for microclimate science open new possibilities in the investigations of microclimate-organism relationships that can be further applied into ecosystem management, such as biodiversity conservation. We hope to have inspired fellow ecologists and biogeographers to find more ways to increase the awareness of microclimates and their importance in our fields and beyond.

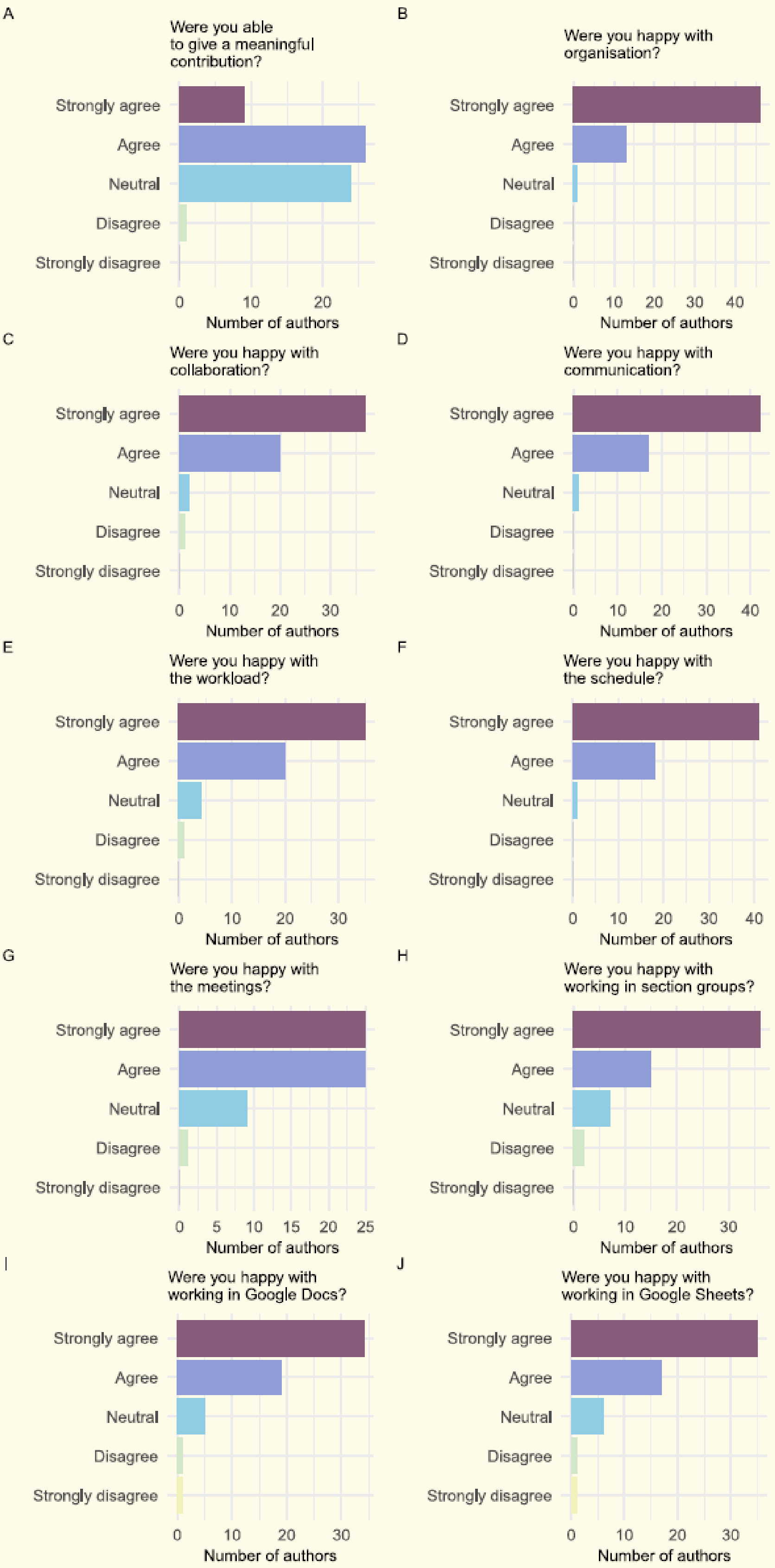
Supporting information



Supplementary information, Figure S1. The workflow. Here, we provide a detailed description of how the manuscript was developed, how work tasks were divided, and which tools were used. In panels A-C, the barplots represent the comments provided by the authors from a numerical point of view. In panel C, comments that were not implemented were mainly in contrast to other comments or additional references.



Supplementary information, Figure S2. The author demographics. Here, we provide background information on the authors of the manuscript. In panels A-B, the barplots represent how contributions were distributed among authors. The maps present the geographical distribution of the authors' institutions. Here, the yellow circle represents the location of the conference that initiated the manuscript. In panel G, the barplot represents collaborations with the first-author prior to the manuscript. Here, "Network" refers to collaboration via network publications, and "Close"refers to collaboration via other publications and projects. Note that 60 out of 97 authors gave information presented in panels C-F.



Supplementary information, Figure S3. Anonymous feedback from authors. Here, we provide a summary of the anonymously given feedback from the authors. In panels A-J, the barplots represent answers to the given questions. Note that 60 out of 97 authors gave feedback.

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B. Annex II

Behavioral Ecology and Sociobiology (2022) 76:79
https://doi.org/10.1007/s00265-022-03176-8

ORIGINAL ARTICLE

Measuring fitness and inferring natural selection from long-term field studies: different measures lead to nuanced conclusions

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Received: 27 October 2021 / Revised: 22 April 2022 / Accepted: 26 April 2022

Abstract

Measuring individual reproductive success in the wild is often achieved by counting the number of descendants produced by individuals. In seeking to understand how reproductive success can inform us about natural selection, however, we are faced with a conundrum. In terms of timing, what is the most relevant measure for examining selection? We might count the number of offspring born, surviving to the termination of parental care, surviving to adulthood, or only those surviving to themselves reproduce. Clearly, only the latter are passing on genes and traits to future generations, but this estimate may not always be available. So, are different estimates of fitness consistent? Do they provide us with similar inferences of selection on phenotypic traits? We examined these questions on a 29-year long-term study of individually monitored male and female Columbian ground squirrels (*Urocitellus columbianus*). We used the long-term data to calculate male and female fitness based on an annual measure of adult survival and the yearly production of offspring counted at the stages of birth, weaning, and yearling age. We then decomposed fitness into its constitutive elements including (1) adult survival to the next spring, and (2) the yearly production of offspring counted at the stages previously mentioned. We then compared fitness metrics to evaluate if they provided similar or contrasting information in the wild. Next, we used those fitness metrics to test for selection on the date of emergence from annual hibernation, a phenotypic trait previously shown to be highly variable, heritable, and associated with reproduction. Finally, we partitioned selection on emergence date into additive episodes of selection by looking at how selection changed from reproduction measured at birth, weaning, and when offspring reached yearling age. Overall, fitness metrics were well correlated, but correlations weakened the further offspring were counted from birth. We generally found directional selection for earlier emergence dates both in males and females. The strength of selection depended on which fitness metric was used. Most of the selection gradient on emergence date was explained by offspring born, and the selection differential was stronger in males than females. We evaluate how the choice of fitness metrics in life-history studies may nuance our inferences about natural selection.

Significance statement

This study explores how our inferences about natural selection acting on organismal traits vary depending on our choice of fitness metrics. Focusing on the timing of emergence from hibernation in Columbian ground squirrels, we show that directional selection for earlier emergence dates occurs, but the strength of selection depends on whether fitness is evaluated from offspring counted at birth, at weaning, or later in life. These results show that the choice of timing for fitness measurements may nuance inferences about natural selection in life-history studies.

Keywords Fitness · Life history · Reproductive success · Mating · Natural selection · Survival

Introduction

Understanding natural selection and the selective processes that shape the evolution of phenotypic traits in living organisms hinges upon our ability to measure and aptly capture the adaptive influence of traits with respect to specific environments (Endler 1986). Studies of natural selection can be broadly separated into those that model the evolutionary

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Communicated by M. Festa-Bianchet

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This article is a contribution to the Topical Collection Measuring individual reproductive success in the wild—Guest Editors: Marco Festa-Bianchet, Janet Mann

Extended author information available on the last page of the article process and those that attempt to measure the action of natural selection in populations in nature. Models of natural selection seek to reveal how the process works through mathematical expressions of change from one generation to the next (Wright 1931; Crow and Kimura 1970; Orr 2009). These models often assume that changes in trait frequencies are the result of natural selection, without the occurrence of other processes such as migration, mutation, drift, or epigenetic modifications of gene expression.

In contrast, evolutionary change in real populations may actually reflect a combination of these different processes. In addition, empirical studies of natural selection, in action in real populations, generally measure a change in trait frequencies from short-term measures of survival or reproduction. This is problematic for inferences on natural selection, since fitness advantages of particular trait values are often measured over a shorter time period than a complete generation (Lande and Arnold 1983; Kruuk et al. 2002; Dobson et al. 2017). Such studies measure fitness as success at surviving a storm or at producing juvenile offspring, and then compare the fitness metric to trait values, rather than using success at producing offspring that themselves reproduce in the next generation (but see Boyce and Perrins 1987).

Fitness is the complex metric by which one typically measures the adaptive value of organismal traits in evolutionary ecology. Studies that refine the definition of fitness or seek alternative measures of fitness are legion (Fisher 1930; Hamilton 1964; Grafen 1982, 2015; Endler 1986; Lucas et al. 1996; Queller 1996; Oli 2003; Qvarnström et al. 2006; van de Pol et al. 2006; Hunt and Hodgson 2010; Dobson et al. 2012; Zhang et al. 2015; Scranton et al. 2016; Harris et al. 2017; Rubach et al. 2020; Levin and Grafen 2021). These studies usually propose some practical means of measuring the propensity of a given gene, group of genes, trait, or group of traits, to spread through populations over generations (Sæther and Engen 2015). In the present paper, we use a trait-based definition of fitness: the change in frequencies of alternative forms of an organismal trait over time in a population (Dobson and Viblanc 2019). This definition presents the advantage that trait variation integrates sources of both genetic and non-genetic (e.g., early environmental or maternal effects affecting trait expression) variation. It further has the advantage of practicality since it is the phenotypic traits (e.g., individual mass, size, age) that are measured in field studies, with no assumption made about their underlying genetic or non-genetic architecture (e.g., the genetic variance–covariance matrix; Lande 1979; Arnold et al. 2008).

Regardless of the metric used, measuring fitness requires understanding how traits are transmitted through the population over time. In terms of evolution, it is the fitness of a trait form relative to others that counts (Ayala and Campbell 1974). For sexual organisms, such as many animals and plants, the transmission of traits is achieved through reproduction. Thus, most studies evaluate fitness by measuring the propensity of organisms to transmit traits through the number of offspring they produce. For iteroparous organisms, this includes successive reproductions, so that adult survival and longevity, as well as age at first reproduction and reproductive senescence, are also important components of fitness (Brommer et al. 2002; Oli et al. 2002; Bouwhuis et al. 2012). However, when measuring fitness, the most important issues are what ultimately influences changes in trait frequencies, and how our conclusions about trait evolution are shaped by the nature of our fitness measures.

If adaptive traits are those that spread in the population relative to other trait forms, then surely offspring that die before reproducing themselves do not contribute to the fitness of a given trait. Yet, for studies in nature, our ability to monitor individuals over time is often limited: many animal species disperse at a young age (Greenwood 1980; Dobson 1982), and for many species, age at maturity may be delayed (Cole 1954; Oli and Dobson 2003), so that the contribution of offspring to trait fitness through their own future reproduction is difficult to measure. Thus, as second-best proxies, studies of animals often rely on metrics such as clutch size, brood size, and litter size at birth or at offspring independence, to quantify the number of offspring produced that potentially can contribute to the next generation. Yet, because numerous offspring die at a young age, it is unclear how trait fitness is influenced by further selection or changed by environmental stochasticity as time goes by (Hadfield 2008). On one hand, trait fitness may be influenced by genetic correlations and selection on other traits than the one of interest. On the other hand, the further fitness is measured from the production of reproductive offspring, the more environmental variation may change the association between a phenotypic trait and realized fitness (the fitness of alternative traits when offspring begin reproduction).

The purpose of our study was to assess how the evaluation of fitness changed depending on the timing of measurement using a 29-year long-term data set of male and female Columbian ground squirrels (*Urocitellus columbianus*). Specifically, we were interested in understanding (1) how individual fitness varied depending on whether we considered the offspring born, weaned, and at yearling age; and (2) whether the association of a phenotypic trait and fitness changed depending on the timing of the fitness measurement. To do this, we measured individual survival and reproduction, and estimated the number of gene copies that a breeding parent passes on to the next year, by adding the parent's survival from the current year to the next (1 for surviving, 0 for not) to half the number of offspring it produced. We then compared this measure to other individuals in the population to obtain an estimate of annual fitness (Qvarnström et al. 2006). We also estimated fitness from adult annual survival and reproduction separately, and here the number of offspring was not halved (both annual fitness and these latter measures were used, for example, by Dobson et al. 2017). Offspring production may be measured at different time points, such as birth, weaning, or survival to a later period. We evaluated how important the choice of when to measure offspring production was with an examination of repeatability (the intraclass correlation coefficient "ICC," used as a measure of consistency) of annual and reproductive fitness measures when using the number of offspring measured at birth, weaning, and yearling age and investigated paired correlations between these.

We also evaluated how measuring fitness at different time periods, and using different metrics, affects inferences about natural selection. For illustrative purposes, we focused on a single trait, and examined the strength of selection on emergence date from hibernation, i.e., the date of spring emergence above ground from hibernation. For ground squirrels, this occurs close to the termination of torpor (Williams et al. 2014). We did not know the genetic variance–covariance matrix for traits associated with emergence from hibernation, and thus could not differentiate selection among genetically correlated traits (Lande and Arnold 1983). However, adult female Columbian ground squirrels go through a single day of estrus and mating each year, about 3–5 days after emergence from hibernation (Murie and Harris 1982). The genetic correlation between emergence and estrus dates was 0.98 ± 0.01 SE (Lane et al. 2011), indicating selection on one of these traits would undoubtedly influence selection on the other. Since our sample sizes were greatest for emergence date from hibernation, we chose this trait for analyses. Columbian ground squirrels are hibernating rodents, with a short and intense reproductive season lasting only a few months (Murie and Harris 1982; Dobson et al. 1992). The timing of emergence from hibernation is highly variable (Tamian et al. 2022), and previous studies have shown it to be both significantly heritable ($h^2 = 0.22$–0.34; Lane et al. 2011), and negatively associated with annual fitness when measured using offspring at emergence from their first hibernation, at about 1 year old (for adult females; Lane et al. 2012), prerequisite conditions for responding to selection. However, nothing is known about whether the strength or direction of selection gradients differs when the fitness for this trait is measured at different time periods, nor whether selection gradients exist for males. Thus, emergence date appeared to be a good candidate trait to investigate its association with different estimates of fitness measured from different life stages.

Materials and methods

Study species and long-term population monitoring

Columbian ground squirrels were studied from 1992 to 2020 in the Sheep River Provincial Park, Alberta, Canada (50° 38′ 10.73″ N; 114° 39′ 56.52″ W; 1524 m; 2.3 ha). Individuals were fitted with permanent numbered ear tags (#1-Monel metal tag; National Band and Tag Company, Newport, KY) when weaned (or at first capture for immigrants). Thus, it was not possible to record data blind because our study involved focal animals in the field. In each year of the study, the entire population was trapped at spring emergence using $13 \times 13 \times 40$ cm^3 live traps (Tomahawk Live Trap, Hazelhurst, WI, USA) baited with a small amount of peanut butter. Each individual was then dyed with a temporary unique dorsal mark (Clairol® Hydrience black hair dye N°52 Black Pearl, Clairol Inc., New York, USA) for identification during field observations. We followed ground squirrels daily throughout the breeding season to assess breeding phenology and success. Females copulated with multiple males within 3–5 days following emergence from hibernation, typically during a single day of estrus (Murie and Harris 1982; Raveh et al. 2010). We determined female mating date through behavioral observations and by inspecting female genitalia (presence of a copulatory plug or plug material in abdominal fur, or sperm in vaginal smears; Murie and Harris 1982). Following mating events, we identified female single-entrance nest burrows during gestation by visual observations of females stocking them with dry grass (Murie et al. 1998), and marked them with colored and flagged metal pins (1 m in length).

Females in the wild gave birth to an average of three (one to seven) blind and hairless offspring in a specially constructed nest burrow, after some 24 days of gestation (Dobson and Murie 1987; Murie 1995). From 2000 to 2016, we caught pregnant females within 2–3 days of expected parturition, about 21–22 days after mating, and brought them to an on-site field laboratory (Hare and Murie 1992). Females were housed indoor in polycarbonate microvent rat cages ($483 \times 267 \times 200$ mm; Allentown Caging Equipment Company, Allentown, NJ), supplied with wood shavings and newspaper as nesting material. Females were provided with apple, lettuce, and horse feed (EQuisine sweet show horse ration; Unifeed, Okotoks, Alberta, Canada) ad libitum. After parturition, offspring were sexed and a small tissue biopsy was acquired for paternity analyses (see below) by clipping a toenail bud as previously described by Hare and Murie (1992). We returned mothers and their offspring to flagged nest burrows, usually within a day of birth.

After a lactation period of approximately 27–28 days, offspring first emerged above ground around the time of weaning (Murie and Harris 1982). We trapped females and their entire litters the day the young first emerged. Mothers were determined by observation of the single lactating female that associated with the natal burrow from which young emerged. Juvenile ground squirrels hibernate within their colony of origin for the winter, and those juvenile males that emmigrate typically do so towards the end of the subsequent spring. Thus, in each year, we were able to recapture all yearling males and females that survived their first hibernation.

Paternity analyses

From 2001 to 2017, paternity was estimated following the methods of Raveh et al. (2010). Briefly, DNA was extracted from offspring, known mothers and potential fathers tissue biopsies using DNeasy Tissue extraction kits (Qiagen, Venlo, The Netherlands). We amplified 13 microsatellites with polymerase chain reaction (PCR), using primer pairs previously developed for *U. columbianus* GS12, GS14, GS17, GS20, GS22, GS25, and GS26 (Stevens et al. 1997); *Marmota marmota* BIBL18 (Goossens et al. 1998); MS41 and MS53 (Hanslik and Kruckenhauser 2000); and *Marmota caligata* 2g4, 2h6 (Kyle et al. 2004), and 2h4 (GenBank accession no. GQ294553) amplified polymorphic microsatellite loci. We used similar PCR conditions and cycling parameters as Kyle et al. (2004), but with an annealing temperature of 54 °C. We tested for deviation from Hardy–Weinberg equilibrium (HWE) at each locus within cohorts, and for linkage disequilibrium between pairs of loci within cohorts using exact tests.

Paternity assignment was done using CERVUS 3.0 (Marshall et al. 1998; Kalinowski et al. 2007). Paternity was assigned with 95–99% trio confidence (assumed dam–sire–offspring relationship). Analyses were conducted for each year separately. The input parameters for the simulation step of CERVUS were 10 000 cycles, 70 candidate fathers, 90% of the population sampled, and 1% genotyping error. Parental assignments were accepted when the offspring had no more than 2 mismatches with both parents.

Annual survival, reproduction, and fitness

We calculated annual survival ($Surv$), as adult survival from the spring mating period to the time of emergence in the next spring (1 for surviving, 0 for not surviving), separately for males and females. Second, we quantified annual reproduction (R), as the number of offspring measured at birth, weaning, and yearling age, separately for males and females. All immature individuals (i.e., females not observed mating or males with testes in abdominal position) were excluded from these calculations. However, individuals considered mature (females observed mating and males with testes in scrotal position) were all kept, even when their reproductive success was zero.

We calculated annual contributions to lifetime fitness, viz. annual fitness (λ_{an}), following the method of Qvarnström et al. (2006), as applied to ground squirrels by Lane et al. (2011, 2012) and Dobson et al. (2016, 2020):

$$\lambda_{an} = Surv + \frac{1}{2}R$$

For any given individual, R is halved since only half of an individual's genetic contribution is passed on to offspring.

The objective of our study was to determine whether fitness metrics were comparable when reproduction was measured at different time points. Thus, annual fitness was calculated based on R being measured as the number of offspring either produced at birth (λ_{anb}), or weaning (λ_{anw}), or surviving up to yearling age (λ_{any}).

Selection analyses

We tested for directional, stabilizing, or disruptive selection on emergence date using linear and quadratic regression on annual fitness λ_{an} (Lande and Arnold 1983). We further ran selection analyses decomposing λ_{an} into its constitutive elements including annual reproduction (fecundity selection) and annual survival (viability selection). Those three fitness metrics were calculated relative to the population in a given year by dividing them by the annual population mean for annual fitness, reproduction, and survival, respectively, and for males and females separately (Lande and Arnold 1983). As selection operates among individuals, we first centered emergence dates per year by subtracting from individual emergence dates the mean emergence date of the population in a given year, hence translating how early or late (number of days) an individual was compared to others in that same year.

We estimated (1) directional selection gradients (β) using univariate linear models, followed by (2) quadratic selection gradients (γ) from models that included both a linear and quadratic term (Lande and Arnold 1983; Arnold and Wade 1984a). The general form for these models is:

$$\omega = \alpha + \beta z + \varepsilon \qquad (1)$$

$$\omega = \alpha + \beta' z + \frac{1}{2}\gamma z^2 + \varepsilon \qquad (2)$$

where ω is the considered measure of fitness (i.e., either λ_{an}, R or $Surv$), α is the intercept, z the phenotypic trait (here centered emergence date), and ε an error term. Note that γ coefficients are reported as Lande and Arnold (1983)'s original formulation and do not require doubling to be interpreted as stabilizing or disruptive selection gradients (Stinchcombe et al. 2008). We used linear mixed models to account for individual ID as a repeated random term, and individual age as a random factor because of known fitness differences that occur with age (Broussard et al. 2003; Raveh et al. 2010). Statistical significance of the selection gradients for viability was estimated with generalized linear mixed models (GLMMs) with a binomial error structure (Garant et al. 2007; Dobson et al. 2017). Directional selection is indicated by significant linear coefficients (β), the sign of the coefficients indicating the direction of selection. Stabilizing or disruptive selection occurs when γ is significantly < 0 or > 0, respectively (Lande and Arnold 1983; McGraw and Caswell 1996). We ran the analyses separately in males and females because the variance of fitness metrics was far greater in males than females (Jones et al. 2012).

Partitioning selection into additive episodes

Following Arnold and Wade (1984a, b), we partitioned selection on emergence date into additive episodes of selection by looking at how selection changed from reproduction measured at birth, weaning, and when offspring reached yearling age. To do so, we separated reproduction into three distinct biological episodes: production of offspring at birth, offspring survival from birth to weaning, and offspring survival from weaning to yearling age. To better understand how each of these episodes contributed to the total selection, we estimated selection differentials for each of them. Selection differentials represent the change in the mean value of a phenotypic character (here emergence date) produced by selection. Because reproductive success at yearling age can be decomposed in the product of the number of offspring born and the number surviving in the two following episodes, selection differentials corresponding to each of the episodes should sum to the total selection differential. Selection differentials are thus presented for each episode in absolute values (shift of the emergence date in number of days), but also as a % of contribution to total selection.

Statistics

All analyses were done in R v. 4.0.2 (R Core Team 2020). The 95% confidence intervals for male and female survival were obtained by parametric bootstrapping (10,000 simulations, 50% of the individuals resampled each time). The consistency of fitness metrics (repeatability, or intraclass correlation coefficient (ICC)) was calculated at different stages using the "rptR" package in R (Stoffel et al. 2017), $\mathrm{ICC} = \frac{V_G}{V_P} = \frac{V_G}{V_G+V_R}$, where V_G is the among-individual variance, V_R is the within-individual (or residual) variance, and V_P is the total variance in fitness. The ICC for annual fecundity (number of offspring) was estimated using a Generalized Linear Model with a Poisson distribution (Stoffel et al. 2017), as appropriate when working with count data (we added + 1 to offspring numbers to avoid zero values). Correlation between fitness metrics measured at different time points are Spearman's rank correlation tests, as not all distributions were Gaussian. Because information at birth and paternity analyses were not available in all years, sample sizes vary and are indicated as n the number of reproductive events, N_1 the number of individuals, and N_2 the number of years in the results.

Results

Male and female annual survival

Sexually mature (scrotal) male survival from one breeding season to the next was on average 65% ($\mathrm{CI}_{95} = [59\% - 71\%]$, $n = 209$ reproductive events, $N_1 = 78$, $N_2 = 21$). In contrast, sexually mature females (i.e., those that estrus cycled and mated) had survival rates of 73% ($\mathrm{CI}_{95} = [70\% - 76\%]$, $n = 732$ reproductive events, $N_1 = 223$, $N_2 = 28$), on average.

Male and female annual fecundity

Adult male annual reproductive success varied from 0 to 29 offspring born (mean ± SD $= 6.2 \pm 5.7$, $n = 154$ reproductive events, $N_1 = 61$ fathers, $N_2 = 16$ years), 0 to 26 offspring weaned (4.2 ± 4.8, $n = 190$, $N_1 = 73$, $N_2 = 19$), and 0 to 16 offspring surviving to yearling age (1.7 ± 2.6, $n = 190$, $N_1 = 73$, $N_2 = 19$). Adult female annual reproductive success was lower, and varied from 0 to 7 offspring born (3.0 ± 1.2, $n = 454$ reproductive events, $N_1 = 167$ mothers, $N_2 = 21$ years), 0 to 7 offspring weaned (2.1 ± 1.5, $n = 759$, $N_1 = 228$, $N_2 = 29$), and 0 to 5 offspring surviving to yearling age (0.9 ± 1.1, $n = 732$, $N_1 = 223$, $N_2 = 28$). Overall, offspring survival from birth to weaning was 75% ($\mathrm{CI}_{95} = [72\% - 78\%]$, $N = 684$) for male offspring and 77% ($\mathrm{CI}_{95} = [72\% - 78\%]$, $N = 655$) for female offspring; and from weaning to yearling age 43% ($\mathrm{CI}_{95} = [40\% - 47\%]$, $N = 775$) for male offspring and 42% ($\mathrm{CI}_{95} = [39\% - 46\%]$, $N = 742$) for female offspring. Annual fecundity was strongly consistent in males ($\mathrm{ICC} = 0.71$, $\mathrm{CI}_{95} = [0.60 - 0.78]$), but less so in females ($\mathrm{ICC} = 0.08$, $\mathrm{CI}_{95} = [0.03 - 0.13]$).

Male and female annual fitness

When calculated using all of the offspring born (λ_{anb}), weaned (λ_{anw}), or surviving to yearling age (λ_{any}), annual fitness was strongly consistent in males (ICC = 0.67, CI_{95} = [0.60 – 0.73]), but less so in females (ICC = 0.49, CI_{95} = [0.45 – 0.54]). The correlation between annual fitness metrics generally weakened as the period between life stages increased, from 0.95 between birth and weaning to 0.73 between birth and yearling age in males (Fig. 1A) and from 0.74 to 0.65 in females (Fig. 1B).

Selection on emergence date

Annual fitness (λ_{an}) selection

In males, we found directional selection for earlier relative emergence date when annual fitness was calculated based on offspring born ($\beta = -0.024$; Fig. 2A), and on offspring surviving to weaning ($\beta = -0.018$; Fig. 2C), but not when calculated based on offspring that survived to yearling age ($\beta = -0.007$; Fig. 2E) (Table 1). The strength of selection appeared to decrease from $|\beta\lambda_{anb}| > |\beta\lambda_{anw}| > |\beta\lambda_{any}|$. In females, we also found directional selection for earlier relative emergence date when annual fitness was calculated based on offspring born ($\beta = -0.007$; Fig. 2B), and on offspring surviving to yearling age ($\beta = -0.013$; Fig. 2F), but not when calculated based on offspring that survived to weaning ($\beta = -0.004$; Fig. 2D). Note that the directional selection coefficient for relative emergence date almost doubled for females between annual fitness calculated based on offspring born compared to when fitness was based on offspring surviving to yearling age (−0.013/−0.007 = 1.86), but decreased in males (−0.007/−0.024 = 0.29).

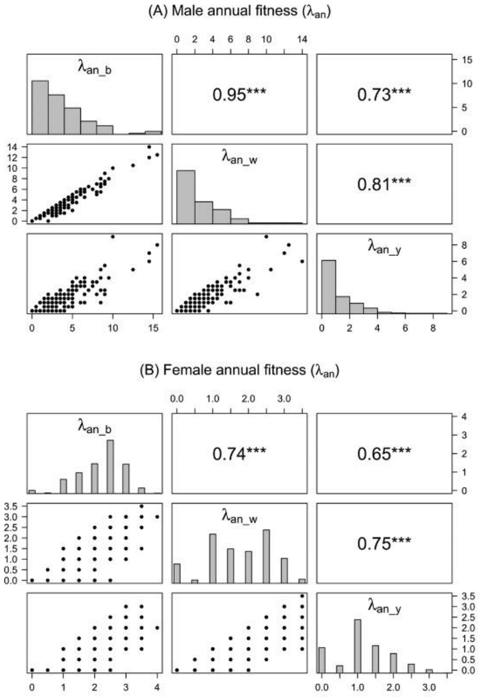

Fig. 1 Pairwise correlation plots for (**A**) male and (**B**) female annual fitness metrics calculated from offspring counted at birth (λ_{an_b}), weaning (λ_{an_w}), or surviving to yearling age (λ_{an_y}). The distribution of data is given on the diagonal. Significant Spearman's correlation coefficients are given for $^{***}P < 0.001$

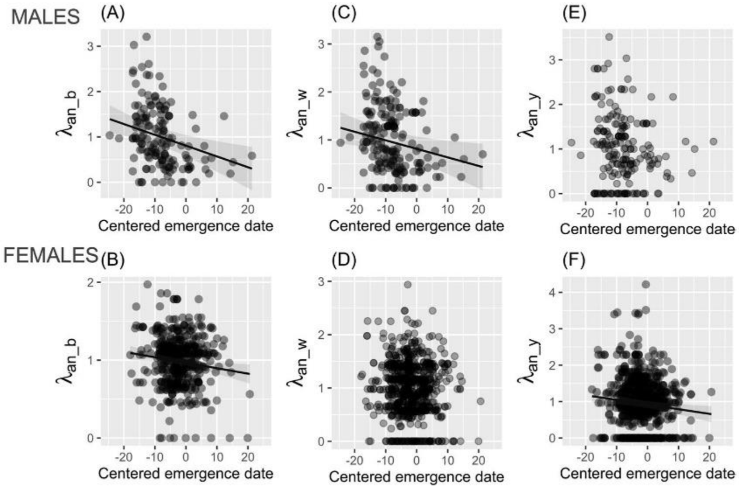

Fig. 2 Selection on emergence date from regression of annual fitness (λ_{an}) on year-centered emergence dates in males (top panels) and females (bottom panels). Fitness was calculated for fecundity based on offspring counted at birth (λ_{an_b}) (**A, B**), weaning (λ_{an_w}) (**C, D**), or yearling age (λ_{an_y}) (**E, F**). Significant regressions are indicated by black lines, and the gray ribbons represent 95% confidence intervals

Both in adult males and adult females, we found no evidence of substantial disruptive or stabilizing selection on relative emergence date, regardless of whether annual fitness was measured based on offspring born, or on offspring surviving to weaning, or yearling age (Table 1).

Viability selection

In males, we found directional viability selection for later emergence date ($\beta = +0.022$), but no evidence of non-linear viability selection on emergence date (Table 1). In females, there was no evidence of significant directional viability selection, but a suggestion of weak stabilizing viability selection on emergence date that approached significance (Table 1).

Fecundity selection

In males, we found directional selection for earlier emergence date when fecundity was calculated from the number of offspring born ($\beta = -0.032$, Fig. 3A), weaned ($\beta = -0.033$, Fig. 3C), and surviving to yearling age ($\beta = -0.033$, Fig. 3E) (Table 1). Selection gradients were of similar magnitude regardless of the period considered. We found no evidence of disruptive or stabilizing selection regardless of whether fecundity was calculated from the number of offspring born, weaned, or surviving to yearling age (Table 1).

In females, there was mild directional selection for earlier relative emergence date when fecundity was calculated from the number of offspring born ($\beta = -0.007$, Fig. 3B), but not when calculated from offspring weaned ($\beta = +0.002$, Fig. 3C), or surviving to yearling age ($\beta = -0.012$) (Table 1). However, we found weak stabilizing selection for emergence date based on offspring surviving to yearling age that approached significance ($\gamma = -0.005$; Table 1, Fig. 3E).

Partitioning selection into additive episodes

In both males and females, selection on the number of offspring born provided a major selective advantage favoring an earlier emergence date (Table 2). For males, selection on the number of offspring born accounted for 93% of the total selection differential and shifted the mean emergence date by 2.5 days, while selection acting between birth and weaning or between weaning and yearling age were minor, accounting for only 5 and 2% of the total selection differentials. For females, selection differentials were much lower than in males (0.4 days vs. 2.6 days shift due to total selection). While selection on the number of offspring born, as well as from weaning to yearling, also shifted emergence dates earlier (by 0.6 and 0.3 days respectively), the selection from birth to weaning counteracted this by shifting emergence dates later by 0.5 days.

Discussion

Our results in the Columbian ground squirrels show that, overall, measures of fitness are relatively well correlated when fitness is measured from offspring counted at birth, at weaning, and at yearling age. In general, annual measures appeared quite strongly inter-correlated at different stages. Not surprisingly, the correlation between fitness measures changed, generally waning over the progression of life stages of the offspring. Overall, the strongest correlations were for fitness measured from birth and weaning offspring numbers, and associations became weaker, as time went by (from birth to yearling surviving offspring). These results might have been expected, since offspring mortality was low between birth and weaning, and much higher between weaning and yearling age.

Columbian ground squirrel offspring are raised over a short lactation period of 27 days (Murie and Harris 1982), during which they are secluded in specialized burrow systems and protected by their mothers (Murie and Harris 1988). As expected, mortality is relatively low during this period (roughly 25%). After weaning, they only have a few weeks of foraging to grow and gain sufficient body mass to survive their first hibernation (Dobson 1992; Dobson et al. 1992). Offspring with insufficient body condition are unlikely to survive to the next spring (Murie and Boag 1984; Dobson et al. 1986). Thus, not surprisingly, overwinter mortality of juveniles is high (> 50%) (Dobson and Murie 1987; Zammuto 1987; Neuhaus and Pelletier 2001), and the correlation between fitness metrics wanes rapidly as the period between life stages of the offspring increases. Nonetheless, it is noteworthy that for annual fitness measures these correlations remain relatively high (> 0.60), so that measuring fitness from the number of offspring weaned in a given year already appears as a reasonable first approximation for estimating fitness. For instance, the correlation between annual fitness measured at weaning and when offspring were yearlings was 0.81 for adult males and 0.75 for adult females.

Sources of variation concerning the changes in correlation between measures between birth and yearling age of offspring remain to be determined. Such variations could include stochastic events that occur on an annual scale and accumulate over time, or other sources of biological variation known to affect early offspring survival such as changes in the social environments (e.g., kin numbers; Viblanc et al. 2010; Barra et al. 2021). It is possible that selection on the offspring before they begin to reproduce at a later time could operate in a way that could weaken or strengthen associations of fitness estimates (whether or not an evolutionary response to selection occurs). Nonetheless, it is noteworthy that, overall, the correlations between fitness measured from

Table 1 Linear (β) and non-linear (γ) selection gradients for viability selection (adult survival), fecundity selection (adult reproduction, number of offspring produced), and annual fitness selection (λ_{an}, see methods) on emergence date in Columbian ground squirrels. Coefficients estimated from LMMs are provided with their standard errors. Significant coefficients at $P < 0.05$ are indicated by an asterisk. Significance of coefficients was obtained with Gaussian error structure for fecundity and annual fitness, and binomial error structure for viability

		Males			Females		
Viability		*Coefficient ± SE*	*z*	*P*	*Coefficient ± SE*	*z*	*P*
Linear	β (ED)	0.022 ± 0.009	2.650	0.008*	−0.009 ± 0.004	−1.507	0.132
Non-linear	β (ED)	0.022 ± 0.012	1.584	0.113	−0.016 ± 0.006	−1.857	0.063
	γ (ED^2)	−0.000 ± 0.002	−0.410	0.682	−0.001 ± 0.001	−1.909	0.056
Fecundity		*Coefficient ± SE*	*t*	*P*	*Coefficient ± SE*	*t*	*P*
Birth							
Linear	β	−0.032 ± 0.009	−3.675	< 0.001*	−0.007 ± 0.003	−1.932	0.054
Non-linear	β	−0.027 ± 0.010	−2.593	0.010*	−0.008 ± 0.004	−2.243	0.025*
	γ	0.001 ± 0.001	1.020	0.309	−0.001 ± 0.001	−1.806	0.072
Weaning							
Linear	β	−0.033 ± 0.010	−3.410	< 0.001*	0.002 ± 0.005	0.344	0.731
Non-linear	β	−0.027 ± 0.011	−2.368	0.019*	0.001 ± 0.005	0.114	0.909
	γ	0.001 ± 0.001	0.962	0.337	−0.001 ± 0.001	−1.017	0.310
Yearling							
Linear	β	−0.033 ± 0.015	−2.180	0.031*	−0.012 ± 0.010	−1.119	0.264
Non-linear	β	−0.024 ± 0.018	−1.337	0.183	−0.017 ± 0.011	−1.568	0.117
	γ	0.002 ± 0.002	0.912	0.363	−0.005 ± 0.002	−2.101	0.036*
Annual fitness		*Coefficient ± SE*	*t*	*P*	*Coefficient ± SE*	*t*	*P*
Birth							
Linear	β	−0.024 ± 0.007	−3.211	0.002*	−0.007 ± 0.003	−2.624	0.009*
Non-linear	β	−0.017 ± 0.009	−2.022	0.045*	−0.008 ± 0.003	−2.819	0.005*
	γ	0.002 ± 0.001	1.362	0.175	−0.001 ± 0.001	−1.602	0.110
Weaning							
Linear	β	−0.018 ± 0.007	−2.405	0.017*	−0.004 ± 0.003	−1.116	0.265
Non-linear	β	−0.012 ± 0.009	−1.341	0.181	−0.005 ± 0.003	−1.376	0.169
	γ	0.001 ± 0.001	1.166	0.245	−0.001 ± 0.001	−1.318	0.188
Yearling							
Linear	β	−0.007 ± 0.008	−0.858	0.392	−0.013 ± 0.004	−2.873	0.004*
Non-linear	β	−0.000 ± 0.010	−0.048	0.962	−0.014 ± 0.004	−3.188	0.001*
	γ	0.002 ± 0.001	1.161	0.247	−0.002 ± 0.001	−1.719	0.086

offspring counted at birth, weaning, or yearling age were all high (> 0.65) for annual fitness measures, suggesting that measuring fitness from offspring surviving to the first year is a good proxy to measuring actual fitness from offspring susceptible themselves to passing on traits (as for adult females in Lane et al. 2012; Dobson et al. 2016).

How did measuring fitness from an annual perspective at different time points affect our inferences on natural selection? To answer this question, we focused on the entire data set, calculating fitness from both male and female offspring, and regressed individual annual fitness on the date of emergence from hibernation, a phenotypic trait known to be heritable (Lane et al. 2011), responsive to environmental fluctuation (Dobson 1988), and negatively associated with fitness (Lane et al. 2012). There appeared to be little or no stabilizing or disruptive selection (no quadratic effects), but only directional selection, acting on emergence date. In general, individuals emerging earlier from hibernation had higher estimated fitness, and this was especially true for males.

In males, selection coefficients decreased when based on measurements from offspring counted at birth, weaning, and yearling age. This likely occurred because of relatively strong mortality during the juvenile period, and supports the hypothesis that environmental stochasticity may dilute the association between a phenotypic trait and fitness due

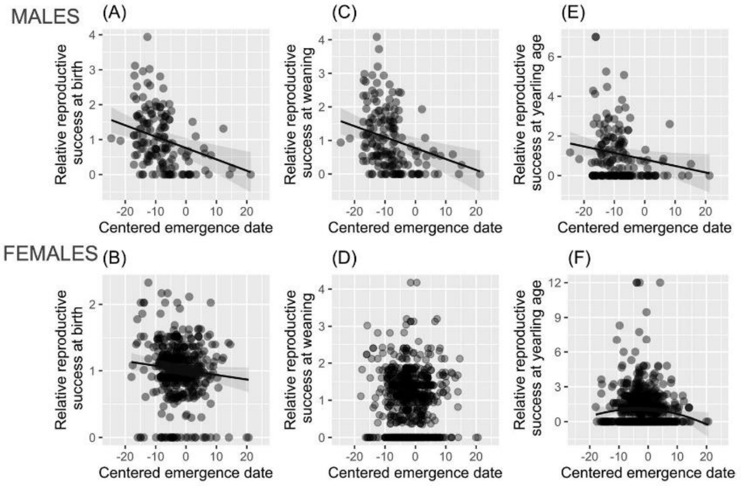

Fig. 3 Fecundity selection on emergence date from regression of relative annual reproductive success on year-centered emergence dates in males (top panels) and females (bottom panels). Fecundity was calculated from offspring counted at birth (**A, B**), weaning (**C, D**), or surviving to yearling age (**E, F**). Significant regressions are indicated by black lines, and gray ribbons represent 95% confidence intervals

to the passage of time, since the environmental event (viz., emergence from hibernation) increases. This is important, because although the directional selection coefficients measured at different time points are consistent in sign (i.e., negative), the conclusions made would differ substantially. For instance, in males, based on fitness calculated from the number of offspring counted at weaning, significant linear (β) coefficients would give rise to a conclusion of directional selection (Lande and Arnold 1983) acting on emergence date, whereas non-significant coefficients based on fitness calculated from the number of offspring counted at yearling age would not (if relying on significance thresholds, but see below). Similarly, in females and based on statistical significance, one might conclude on directional selection on emergence when using offspring counted at birth, but not when using offspring counted at weaning or at a yearling age, despite the selection gradients being strongest when using the number of offspring that reach a yearling age (Table 1).

Decomposing annual fitness into its annual survival and reproductive components generally revealed consistent patterns. Interestingly, we found mild positive directional viability selection on emergence date for males, but no viability selection for females: males that emerged later had better annual survival. Positive selection on viability in males might occur because males emerge from hibernation early to establish mating territories (Manno and Dobson 2008), but early emergence has a survival cost (Turbill et al. 2011; Constant et al. 2020). For females, a weaker pattern of selective advantage was revealed for earlier emergence from hibernation, but the pattern was only significant when the combined index of annual fitness was used. In general, for both sexes, fecundity selection on emergence date revealed overall similar patterns as annual fitness, except perhaps for females where a mild stabilizing selection was found using offspring that survive to yearling age as the fecundity fitness estimate.

When fecundity selection was separated into distinct episodes of selection (Arnold and Wade 1984a, b), we found that selection at birth accounted for the vast majority of the selection differentials on emergence date both in males and females. For males' dates of emergence from hibernation, selection based on the number of young at birth was the strongest (by nearly 2.5 days, Table 2), and might have reflected sexual selection (primarily number of mates) and maternal effects during gestation. Selection during lactation, based on numbers of offspring at weaning, however, was weak. This is consistent with the males contributing virtually nothing to parental care in this species, and reproductive success being determined foremost by the number of mated females early on in the season (CS et al., unpublished data). Finally, ecological influences on male date of emergence from hibernation may have been best reflected by the numbers of offspring that survived their first hibernation, but this influence was trivial. For females, selection on emergence from hibernation was relatively weak and only approached significance. Partitioning this pattern into episodes of selection did not appear to produce important insights, other than a slight fitness advantage of emerging earlier from hibernation (by less than half a day).

Taken together, our results over a 29-year long-term study of Columbian ground squirrels showed that annual fitness measures, whereas generally closely related, may lead to nuanced conclusions on the strength (but not direction) of selection acting on heritable phenotypic traits. We

Table 2 Episodes of fecundity selection (Arnold and Wade 1984a, b). The trait under selection was date of emergence from hibernation, an important phenological trait that is strongly associated with successful reproduction and fitness (Lane et al. 2012). Selection differentials are given in days

	MALES		FEMALES	
	Selection differential S_k	Contribution to total selection	Selection differential S_k	Contribution to total selection
Selection at birth # Offspring born	−2.435	93%	−0.593	144%
Selection from birth to weaning % Surviving offspring birth to weaning	−0.142	5%	0.492	-120%
Selection from weaning to yearling age % Surviving offspring weaning to yearling age	−0.043	2%	−0.310	75%
Total selection	−2.619	100%	−0.411	100%

documented an overall dilution of fitness associations, and in most cases a waning association between fitness and emergence date, as time passed likely due to added stochastic processes affecting offspring survival. Importantly, focusing on the significance of associations between phenotypic traits and fitness led to contrasting conclusions depending on when fitness is measured. The values of the selection coefficients, however, were fairly consistent using different fitness estimates. Taking a step back from a traditional hypothesis-testing approach and focusing on the magnitude of effect sizes, as is more and more frequently recognized in evolutionary ecology (Yoccoz 1991; de Valpine 2014), likely provides more meaningful information on the strength and patterns of selection acting on phenotypic traits in living organisms.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00265-022-03176-8.

Acknowledgements We are grateful to J. Mann and M. Festa-Bianchet for inviting us to contribute to this topical collection on "Measuring individual reproductive success in the wild." We sincerely thank M. Morrissey, M. Festa-Bianchet, and an anonymous reviewer for thoughtful and helpful comments on a previous version of the paper. We are grateful to Alberta Parks, and Alberta Environment, Fish & Wildlife, for granting us access to the study sites and support with the long-term research. The University of Calgary Biogeoscience Institute provided housing at the R. B. Miller field station during data collection in Sheep River Provincial Park (AB, Canada). We are grateful to E. A. Johnson and S. Vamosi (Directors), J. Mappan-Buchanan and A. Cunnings (Station Managers), and K. Ruckstuhl (Faculty Responsible) for providing us with field camp and laboratory facilities over the years, and to P. Neuhaus and K. Rucktuhl for their continued support, help, and discussions in the field. We are especially grateful to E. A. Johnson for his continued support on the ground squirrel long-term research throughout the years.

Funding The research was funded by a Natural Sciences and Engineering Research Council of Canada grant to JOM, a USA National Science Foundation grant (DEB-0089473) to FSD, a CNRS Projet International de Coopération Scientifique grant (PICS-07143) and a research grant from the Fondation Fyssen to VAV, a National Science and Engineering Research Council of Canada grant to DWC (Discovery Grant RGPIN-2018–04354), and by a fellowship grant from the Institute of Advanced Studies of the University of Strasbourg to FSD and VAV. FSD received from the Région Grand Est and the Eurométropole de Strasbourg the award of a Gutenberg Excellence Chair during the time of writing.

Data availability All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declarations

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures carried out in the field and laboratory were approved by Auburn University Institutional Animal Care and Use Committee (IACUC) and by the University of Calgary. Authorization for conducting research and collecting samples in the Sheep River Provincial Park was obtained from Alberta Environment and Parks and Alberta Fish & Wildlife.

Conflict of interest The authors declare no competing interests.

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RÉSUMÉ EN FRANÇAIS

Effets des variations climatiques et de disponibilité alimentaire sur les réponses phénotypiques et démographiques d’un rongeur hibernant, l’écureuil terrestre du Columbia

A. Introduction

Le changement climatique à long-terme et l’occurrence d’évènements climatiques ponctuels extrêmes imposent une contrainte forte sur la productivité des écosystèmes, et sur les espèces en dépendant. Comprendre les effets directs du climat sur le comportement et la physiologie des individus, ainsi que les effets indirects agissant au travers de la modification des ressources alimentaires, est nécessaire afin d’anticiper les trajectoires des populations face au changement climatique. Or, peu d’études s’intéressent actuellement, de manière intégrative, conjointement aux échelles individuelles (comportement, physiologie) et populationnelles (démographie). Par ailleurs, si, au cours des dernières décennies, de nombreuses études ont considéré les effets du climat sur les organismes, elles l’ont généralement fait en utilisant des données climatiques globales provenant de satellites ou de stations météorologiques souvent éloignées des zones d’études. Or, dans de nombreux écosystèmes (ex. en montagne), le climat est susceptible de varier fortement sur de courtes échelles spatiales, des populations avoisinantes étant soumises à des microclimats bien distincts. L’utilisation de modèles microclimatiques à haute résolution spatiale et temporelle permet d’appréhender l’environnement local ressenti par les animaux (ex. températures du sol pendant l’hibernation), afin de comprendre à fine échelle les effets du climat dans un écosystème donné.

C’est dans ce cadre que mon travail de thèse s’intéresse à (1) caractériser le microclimat et les conditions locales de végétation; et (2) étudier leurs effets sur le comportement, la physiologie et la démographie du spermophile du Columbia (*Urocitellus columbianus*), un écureuil terrestre hibernant, vivant dans les montagnes Rocheuses d’Amérique du Nord. En particulier, je m’intéresse à comprendre les effets (*i*) du microclimat sur la date d’émergence d’hibernation (un indice de la phénologie, c.-à-d. la synchronisation des cycles de vie des individus), (*ii*) des perturbations météorologiques, telles que les tempêtes de neige et de pluie, sur le comportement et le stress physiologique des individus, (*iii*) des conditions climatiques locales sur le comportement de fourragement et la condition corporelle des individus, (*iv*) des ressources (abondance et phénologie de la végétation) et de la densité de population sur la démographie. Le spermophile du Columbia est un modèle animal idéal pour adresser ces questions : ce petit rongeur hibernant présente une période d'activité réduite (mi-Avril à fin Juillet), au cours de laquelle il doit se reproduire et constituer suffisamment de réserves énergétiques avant l'hibernation suivante. Ainsi, les ressources disponibles (végétation pour cet animal principalement herbivore) pendant la période de reproduction constituent un facteur déterminant pour cet animal. Par ailleurs, les conditions climatiques pendant cette période peuvent aussi directement influencer le comportement et la physiologie des individus en diminuant, par exemple, le temps passé à se nourrir en conditions de stress thermique. Toutefois, l'importance du climat et des ressources par rapport à d'autres facteurs, tels que la compétition intraspécifique (densité de population), dans l'explication du succès reproducteur des animaux, de la survie adulte et juvénile, et la démographie reste largement inexplorée. Enfin, les conditions climatiques pendant l'hibernation peuvent aussi affecter directement la dépense énergétique des animaux, modifiant leur phénologie (ex. la sortie d'hibernation), et leurs conséquences restent à déterminer.

B. Méthodologie

Quatre populations voisines de spermophiles sont suivies annuellement depuis plus de 30 ans en Alberta, Canada (50°38'N, 114°39'W; 1540 m d'altitude). Les animaux sont identifiés individuellement de leur naissance à leur mort, et des données sur leur phénologie (date d'émergence d'hibernation, date de reproduction) et leur physiologie (masse à l'émergence, gain de masse pendant la saison), ainsi que sur la démographie de la population (taux de natalité, survie des jeunes et des adultes, taille de la population) sont collectées chaque année. En complément de ce suivi récurrent et dans le cadre de ma thèse, j'ai ajouté des observations comportementales (lors de 3 saisons de terrain en 2019, 2022 et 2023) et des prélèvements fécaux (non-invasifs) lors d'évènements météorologiques précis (2 tempêtes de neige en 2021 et 2022, et 1 forte chute de pluie en 2022) afin d'étudier les réponses des individus à ces perturbations. Les observations permettent de quantifier le temps que les animaux passent en dehors du terrier et à se nourrir, ainsi que leur budget-temps comportemental. L'analyse des échantillons fécaux, que j'ai effectuée à l'Université de Toronto, m'a permis d'obtenir les niveaux de stress (concentration des métabolites fécaux du cortisol) des animaux avant et après chaque perturbation (n = 463 échantillons). En parallèle, des données environnementales (météorologiques et de végétation) ont été obtenues de différentes manières. Premièrement, un modèle microclimatique récent et encore peu appliqué a permis de reconstituer en continu, toute les heures, les conditions thermiques (température) de l'air et du sol, les précipitations (y compris de neige), l'humidité et le vent sur nos sites d'étude de 1992 à 2022, à une échelle spatiale inégalée (5m). Une validation *in-situ,* a été permise grâce à l'installation de 4 stations météorologiques en 2021 sur les 4 prairies suivies. En utilisant l'indice de végétation par différence normalisée (NDVI) mesuré par image satellitaires, j'ai construit 3 indices représentant la biomasse, la phénologie et la croissance de la végétation, indices que j'ai ensuite validés par la mesure in situ de la croissance et du verdissement (via analyse d'images) de la végétation sur le site d'étude en 2022 et 2023. Ainsi, des données environnementales (modélisées et satellites, corrélées avec des enregistrements locaux) sont disponibles pour toute la période d'étude et permettent de caractériser le microclimat et les conditions locales de végétation. A partir de la richesse et de la diversité de ces données environnementales et biologiques, j'ai réalisé plusieurs analyses statistiques afin d'intégrer les différents effets de l'environnement sur les animaux.

C. Résultats

Premièrement, mes analyses révèlent un lien étroit entre variables microclimatiques modélisées sur les sites d'étude pendant l'hibernation et date d'émergence d'hibernation des individus. De manière importante, ces analyses confirment l'existence de microclimats et de réponses contrastées entre populations vivant dans des zones géographiquement proches (quelques kilomètres). En moyenne, les animaux émergent plus tard d'hibernation les années, ou dans des zones d'habitat, où la neige fond tardivement. Cette synchronisation pourrait permettre aux individus d'éviter les périodes/zones encore enneigées à la sortie d'hibernation, profitant ainsi de manière optimale de la végétation. Deuxièmement, mes résultats montrent que les tempêtes de neige et de pluie au cours de la reproduction modifient fortement le comportement des animaux : en conditions de pluie ou de neige les animaux restent plusieurs jours à l'abri sans se nourrir, à un moment où l'apport alimentaire est crucial. Curieusement, ces périodes ne sont pas caractérisées par une augmentation du stress physiologique des animaux mais plutôt par une diminution de celui-ci, ni par une perte de masse marquée, suggérant que ces animaux se sont adaptés à un environnement variable et sont capables de faire face à des perturbations climatiques ponctuelles. Troisièmement, des analyses montrent une plascticité comportementale des individus en condition de fortes chaleurs : une augmentation de la température sous-cutanné compensée par une augmentation du temps passé dans le terrier, une diminution du temps passé à fourrager ainsi qu'un déplacement des activités vers les zones ombragées de la prairie et les heures les moins chaudes de la journée (début et fin de journée). Quatrièmement, la croissance de la population au cours des 30 années d'étude semble principalement affectée par la densité-dépendance, la densité de population influençant négativement les traits démographiques (survie et reproduction), suggérant une compétition accrue pour les ressources lorsque la densité augmente. L'effet de la phénologie de la végétation (croissance avancée ou tardive dans l'année) sur la survie des individus, quant à lui, s'inverse en fonction des individus, probablement en raison de contraintes énergétiques différentes. Une croissance tardive serait négative pour les mâles adultes, très actifs au début de la période d'activité pour la reproduction, mais positive pour les femelles adultes allaitant les jeunes plus tard dans la saison.

D. Discussion & Conclusions

Ainsi, l'analyse parallèle d'importants jeux de données environnementales et biologiques m'a permis d'établir de clairs liens entre les variations environnementales et le phénotype des animaux vivant dans ces conditions. En revanche, si mes résultats documentent un rôle important des conditions microclimatiques et des ressources sur les traits comportementaux, la survie, et la démographie, l'indice de végétation (NDVI) utilisé dans mes études ne fournit pas d'informations sur la qualité des ressources, jouant un rôle possiblement important pour la reproduction et l'accumulation de réserve chez ces herbivores. Il reste donc essentiel de décrire la qualité des ressources (alimentation et eau) disponibles et consommées par les individus. Ces résultats pourraient permettre de prédire les impacts d'éventuels changements des communautés végétales sur les consommateurs primaires, et être appliqués à d'autres espèces vivant dans les écosystèmes montagneux.

De plus, la contribution de variables climatiques sur la dynamique des populations n'a pas été examinée, et une étude plus approfondie pourrait permettre de mieux comprendre les liens entre changements environnementaux et dynamique de population chez cette espèce, en utilisant des modèles populationnels complexes. Finalement, il serait également intéressant de comprendre dans quelle mesure le phénotype et le comportement des individus est modulé plutôt par des effets génétiques ou par des effets environnementaux, notamment grâce à des modèles animaux d'analyses de génétique quantitative.

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Phenotypic and demographic responses to climate and resources variation in a hibernating rodent, the Columbian ground squirrel

Abstract

The abiotic environment, including climate conditions, may affects organism in multiple ways: by modifying their behaviour or physiology in the short-term, by having long-term evolutionary consequences, or by modifying resources availability. The consideration of both punctual and long-term climate effects have rarely been assessed, especially regarding seasonal species. My PhD aimed to evaluate microclimate and resources (vegetation) effects on a hibernating and herbivorous mammal (Columbian ground squirrels, *Urocitellus columbianus*) living in the Rocky Mountains and with a short active period. Thanks to a 30-years population monitoring, effects of climate and vegetation variations on individual behaviour and physiology, as well as demographic consequences (young and adult survival, reproductive success), were measured. Whereas this species seemed well adapted to short climate event (snow and rainfalls), responses to changes in climate, vegetation, and population density were observed on the long-term. Punctual and long-term climate change effects on individuals was described, and now the questions remain on what can be the links between resource availability, quality, and individual and population performance.

Résumé

L’environnement abiotique, notamment les conditions climatiques, peut affecter les organismes de multiples manières : sur le court terme en modifiant leur comportement ou physiologie, sur le long-terme en ayant des conséquences évolutives, ou encore à travers les modifications des ressources disponibles. La différenciation des effets des évènements ponctuels ou des tendances à long-terme est peu connue, en particulier chez les espèces saisonnières. L’objectif de la thèse est donc d’évaluer les effets du microclimat et des ressources (végétation) sur un mammifère hibernant principalement herbivore (Ecureuil terrestre du Columbia, *Urocitellus columbianus*), vivant dans les montagnes Rocheuses et dont la période d’activité est très courte. A l’aide d’un suivi individuel à long-terme (>30 ans), les effets des variations climatiques et végétales sur le comportement et la physiologie du stress des individus, et les conséquences sur la démographie (survie des jeunes et des adultes, succès de la reproduction) sont mesurés. Cette espèce semble plutôt bien adaptée à des courts évènements climatiques (chute de neige, forte pluie) pendant la période d’activité, mais répondent également aux variations climatiques locales et aux changements temporels de végétation et de densité de population. Ayant mis en évidence des effets climatiques ponctuels et à long-terme sur les individus, il reste à comprendre quels sont les liens précis entre la disponibilité et la qualité des ressources, et la performance des individus ou des populations.