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PhD Applied Ecology and Biotechnology
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Printed by: Flisa Trykkeri A/S

Place of publication: Elverum

Faculty of Applied Ecology, Agricultural Sciences and Biotechnology Inland Norway University of Applied Sciences, Department of Forestry and Wildlife Management, Evenstad.

PhD Thesis in Applied Ecology and Biotechnology no. 29

ISBN printed version: 978-82-8380-364-8

ISBN digital version: 978-82-8380-365-5

ISSN printed version: 2703-819X

ISSN printed version: 2703-8181

“Each of these species is masterpiece of evolution. Each has persisted for thousands to millions of years. Each is exquisitely adapted to the environment in which it lives, interlocked with other species to form ecosystems upon which our own lives depend in ways we have not begun even to imagine.”

− Edward O. Wilson, biologist

To my family,

Sammendrag

Arters demografi og funksjonelle roller i miljøet er styrt av samspillet mellom individers iboende tilstand, abiotiske og biotiske forhold, inkludert mellom-artsforhold som vert-parasitt interaksjoner. Slike interaksjoner har generelt blitt undersøkt med langtidsserier og de mange ulike faktorene er lite vurdert. I denne avhandlingen, så har jeg brukt en multi-faktoriell nisje-basert tilnærming for å predikere små pattedyrs proksimate respons på miljø-, klima- og antropogene faktorer, og deres tidsromlige påvirkning på vert-parasitt-sykdom-mønstre. Det ble gjennomført levende fangst av småpattedyrsamfunnet på tvers av breddegrader (Norge og Italia) og over en høydegradient (fra 500 til 2500 moh.). Med et feltdesign som baserte seg på manipulering av mattilgjengelighet, og transekter langs heterogene habitat, vurderte jeg driverne i småpattedyrsamfunnets komposisjon og demografi, og kaskadeeffektene fra antall parasitter og overføring av både vanlige og nye patogener. Jeg fant at småpattedyrs overlevelse var avhengig av en iboende sesongsyklusitet og at den kun ble forsterket av mattilgjengelighet ved krevende værforhold. Likevel så var populasjonsstørrelsen hovedsakelig styrt av klimatiske begrensninger og mattilgjengelighet. Når opportunistiske og dominerende gnagere og ikke-dominer arter var sympatriske, så viste de motsatte demografiske tendenser i forhold til mattilgang der de ikke-dominerende artene fikk redusert overlevelse og populasjonsstørrelse, som indikerer utnyttelses- og interferenskonkurranse (**Paper I**). Når romlige komponenter i disse mønstrene ble kontrollert for, så fant jeg at mattilgangen for gnagere også reduserte deres romlige utbredelsesområde. Etterhvert som den lokale tettheten av de mest opportunistiske artene økte og jevnheten (evenness) ble redusert, så forsterket høye flått-tettheter gnagernes flåttbelastning, spesielt hos tyngre individer. I sum så vil en ansamling av primær- og sekundærverter på matstasjoner, og mer generelt i antropogene fragmenterte landskap, trolig øke sannsynligheten for å kunne fullføre flåttenes livssyklus. Dette induserer en forsterket flåttbelastningen i enkle vertssamfunn (**Paper II**). Langs den alpine høydegradienten fant vi støtte for at flått kan trives i et mildere klima som forårsaket av klimaendringer. I tråd med dette kan vertssamfunnets komposisjon og utbredelse over høydegradienten bli påvirket av klimarelaterte forhold. I samsvar med dette, fant jeg at generalister (som klatremus) også var utbredt på høyereliggende områder, de var sympatriske med arter mer tilpasset høyereliggende klima som snømarkmus og markmus. Denne endringen i distribusjonen av generalister og vektorer, var også fulgt av utbredelsen av vanlige gnager- og vektorbærte patogener som syntes å være fordelt på ulike høydenivåer, et aspekt som trenger videre studie (**Paper III**). Noen protozoer, som *Hepatozoon* spp. (**Paper IV**), kan spille en sterk epidemiologisk rolle gjennom næringsnettet. For å konkludere, denne avhandlingen har belyst flere økologiske komplekse forhold omkring små pattedyr i sårbare økosystem. På makro- og mikroskala, brukte vi eksperiment for å demonstrere konsekvenser fra klima og menneskelige forstyrrelser på små pattedyrs samfunn og populasjoner, og deres implikasjoner for menneskers og økosystemers helse.

Abstract

The demography of species and their functional role in the environment are governed by the interplay between individual internal state, external abiotic and biotic conditions, as well as by interspecific interactions, including host-parasite relationships. Generally, these interactions in wild systems have been investigated by long-term studies, and the different components were so far rarely evaluated *ensemble*. In this dissertation, I implemented a multi-factorial niche-based approach to predict small mammals' proximate responses to environmental, climatic and anthropic factors, and their implications for host-parasite-disease spatio-temporal patterns. For this purpose, live-trapping targeting the small mammal community was performed across a wide latitudinal (Norway and Italy) and altitudinal (from 500 to 2500 m a.s.l.) gradient. Through field experimental designs based on manipulation of anthropogenic food availability, and longitudinal transects across heterogeneous habitats, I assessed the drivers of small mammal community composition and demography, and the cascading effects on the parasitic load and the circulation of common and emergent pathogens. I found that small mammal survival depended on intrinsic seasonal cycles and was enhanced by food availability only where harsh climate conditions occurred. Conversely, population size was mainly determined by climate constraints and food availability. When opportunistic, dominant rodent and subordinate vole species were sympatric, they showed opposite demographic trends in presence of anthropogenic food, with the subordinate species decreasing both survival and population size, likely pointing at exploitative and interference competition (**Paper I**). When the spatial components of these patterns were explicitly accounted for, I found that rodents also decreased their spatial range in presence of anthropogenic food. As the local density of the most opportunistic species increased and evenness decreased, their tick burden was amplified at high tick environmental density, especially in heavier individuals, and diluted at lower tick availability. To sum up, the overlap and aggregation of primary and secondary hosts at feeding sites, and more in general in anthropic, fragmented landscapes, likely enhances the completion of the tick life-cycle, inducing tick-burden amplification in a simplified community of hosts (**Paper II**). Ticks could also thrive in milder climatic conditions due to climate change, as observed along the Alpine altitudinal gradient. Similarly, the composition and altitudinal distribution of the host community can be affected by climate-related abiotic conditions. In accordance with this expectation, I detected the occurrence of generalist species (e.g. bank vole) also at high altitudes, so resulting sympatric with harsh climate specialists, such as snow and field voles. This upward distributional shift of generalist species, and vectors alongside, were also accompanied by circulation of common rodent- and vector-borne pathogens that seemed to show altitudinal segregation, an aspect that needs further investigation (**Paper III**). Among these, some protozoans, such as *Hepatozoon* spp. (**Paper IV**), that may hold a strong epidemiological role along the food web. In conclusion, this dissertation elucidated some complex ecological relationships that involve small mammals in sensitive ecosystems. At macro- and micro-scale, we experimentally demonstrated the cascading consequences of climate and anthropic disturbances on small mammal communities and populations, and their implications for the health of humans and ecosystems.

List of Papers

This dissertation is based on the following original publications and manuscripts, referred to Roman numerals in the text.

Paper I

Ferrari Giulia, Devineau Olivier, Tagliapietra Valentina, Johnsen Kaja, Ossi Federico and Cagnacci Francesca. (2022). Food resources drive rodent population demography mediated by seasonality and inter-specific competition. *bioRxiv*. Complete manuscript ready for submission. https://www.biorxiv.org/content/10.1101/2022.05.07.491005v1

Paper II

Ferrari Giulia, Cagnacci Francesca, Tagliapietra Valentina, Savazza Sara and Devineau Olivier. Is environmental heterogeneity driving host-parasite interactions? Evidence from a field manipulation in a deer-rodent-tick system. Complete manuscript ready for submission.

Paper III

Ferrari Giulia, Cagnacci Francesca, Devineau Olivier, Girardi Matteo, Rosso Fausta, Rizzoli Annapaola, Arnoldi Daniele, Inama Enrico and Tagliapietra Valentina. Small mammals' assemblage along an altitudinal gradient and consequences on rodent and vector-borne diseases. The manuscript is in early stage of preparation not shared with co-authors

Paper IV

Ferrari Giulia, Girardi Matteo, Cagnacci Francesca, Devineau Olivier, and Tagliapietra Valentina. (2022). First Record of *Hepatozoon* spp. in Alpine Wild Rodents: Implications and Perspectives for Transmission Dynamics across the Food Web. *Microorganisms* 10 (4): 712. https://doi.org/10.3390/microorganisms10040712

Abbreviations

EVT: Evenstad study area (Norway)

CEV: Cembra Valley study area (Italy)

CAV: Calamento Valley study area (Italy)

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1. Introduction

1.1 Background

The concept of ecological niche

The *ecological niche* represents a fundamental milestone in ecology and evolution, although it has been revised numerous times and in many ways (Sales et al. 2021). Originally, the term 'niche' stems from a suite of ecological requirements (e.g. average temperature, solar radiation, precipitation, terrain aspect) within which a species is capable of surviving and reproducing without immigrational subsidy (Grinnell 1917). Later, Elton (1927) placed the individual species within a community, proposing that niche was defined by the role, function and impact of species within the environment. Therefore, the former definition considers the autecological and physiological approach (*environmental niche*), involving scenopoetic abiotic variables not affected by the focal species; whereas, the latter definition is more related to the trophic levels of the food chain (*trophic niche*), involving resources potentially consumed by the species (Guisan and Thuiller 2005). Even after, Hutchinson (1957, 1978) combined the two previous visions by providing a new formalization of the term, defining the niche as a multidimensional hypervolume that permits species to exist. Accordingly to Hutchinson's view, organisms dynamically occupy a distinct area, called *fundamental niche*, in compliance with a set of abiotic conditions that enhance fitness properties. When multiple species co-occur in the same space, they ought to systematically adapt their behaviour and physiology to the environment and to the periodic seasonal rhythms (White and Hastings 2020). In this sense, species reshape their fundamental niche into a *realized niche* that represents the synergism between multi-species interactions and the changing environment, producing cascading effects on niche breadth actually observed in nature (Sexton et al. 2017). Hutchinson's niche concept was enforced by MacArthur and Levins (1967), for which the overall number of species in a community is related to the range of available resources and with niche breadth of each species (*competition-centered niche theory* and *resource utilization distribution*). However, all these definitions of niche did not explicitly consider the demographic outlook that was incorporated only by Maguire (1973) (*demographic niche*). In the 21st century, several models, such as *mechanistic theory* (Chase and Leibold 2003), *neutral theory* (Hubbell 2011) and *BAM diagrams* (Soberon and Peterson 2005), revised and extended the classical niche concept, including those limitations occurring at multiple spatial scales, such as immigration, dispersal and competition (Pulliam 2000, Soberón 2007).

The per-se ecological niche concept provides a useful framework for understanding: (i), whether a system equally combines intrinsic factors with extrinsic variability of the environment; and (ii), how a species relates with and to those extrinsic factors, through both effect and response interactions. However, despite the long historical course this concept still remains debated (Mcinerny and Etienne 2012a, 2012b).

Altered ecosystems under global change

Environmental heterogeneity is an umbrella term that incorporates the complexity, diversity, variability and structure of abiotic (e.g. climate conditions, soil properties and topography) and biotic factors (e.g. land cover configuration, vegetation composition and structure) (Stein et al. 2014). Being ubiquitous and uneven, availability and heterogeneity of resources, as well as favourable abiotic conditions, reflect into species' individual fitness by providing breeding sites, food resources and shelters from predators, parasites and adverse climate, ultimately affecting niche relationships both in time and in space (Bar-Massada 2015). Therefore, the scale and the grain of temporal and spatial heterogeneity, related also to life span, movement and dispersal ability of organisms, may favour or limit niche breadth.

Recently, environmental heterogeneity has been even more re-shaped by environmental stressors, such as weather extremes arising from climate change and human-induced land-use perturbations (e.g. habitat loss, degradation, fragmentation) (Côté et al. 2016). In particular, when limits of fundamental niche are surpassed, species track the new environmental optimum by adapting their niche breadth either via genetic evolution and phenotypic plasticity (Radchuk et al. 2019) or through distributional shifts (Huntley et al. 2010). These responses can occur both at individual and population levels (Parmesan 2006, Urban et al. 2016), and may involve those generalist species that are more tolerant to habitat and climate disturbances. Conversely, specialist species that are characterized by narrow niche breadth are more sensitive to those changes and may not be able to track their environmental optimum, thus resulting overwhelmed. For instance, milder climatic conditions (IPCC 2022), as well as an increase in resource availability (Bogdziewicz et al. 2021), have promoted upward and northward shifts of distributional ranges in generalist species (Moritz et al. 2008, Myers et al. 2009), creating cascading effects on interspecific encounters and causing biotic homogenization (Singleton et al. 2005, Clavel et al. 2011). These adaptive responses may emerge also under anthropic disturbance, with artificial supplemental feeding, a widespread practice used in Europe and North America for management purposes (Milner et al. 2014), that mimics the overabundance of food resources. Wildlife food supplementation may indeed affect species demography (Rémy et al. 2013, Johnsen et al. 2016), spatial (Ossi et al. 2017, Ranc et al. 2020) and foraging (Felton et al. 2017) behaviour, and aggregation of individuals (Yoccoz et al. 2001, Ossi et al. 2020). These effects have community implications, increasing intra- and inter-specific contact rates (Côté 2000, Saetnan and Skarpe 2006) and favouring generalist and opportunistic species.

The cascading consequences of these altered processes emerged especially in the complexity and ecosystem functioning of the most exposed and threatened ecosystems, i.e. arctic and alpine zones, which represent therefore unique settings for studying the underpinning mechanisms of global change. Specifically, these environments are characterized by high rates of local endemism, and great biodiversity and their biological and chemical processes experience high temperature sensitivity (Ernakovich et al. 2014, Elsen and Tingley 2015).

Small mammals as a model system

Due to their wide distribution across diverse habitats and numerous trophic niches and their rapid pace of life-history events, small mammals are considered informative models to track environmental effects on individual fitness, demography and, ultimately, population and community dynamics. In contrast to slow-living large mammals that tend to have a delayed effect of extrinsic perturbations on individual fitness and demography (Norin and Metcalfe 2019), small mammals (e.g. voles, mice and shrews) experience immediate functional and numerical responses to climatic and environmental changes. Indeed, they are key components for ecosystem functioning (Johnson and O'Neil 2001). They disperse seeds, pollen and fungal spores (Nystuen et al. 2014), contribute to vegetation composition and forest regeneration with selective grazing, and stir soil with tunnels and burrows (Davidson et al. 2012). Further, small mammals represent both preys (Fuller and Sievert 2001) and predators (Bradley and Marzluff 2003) along the trophic chain and act as hosts for many ecto-parasites and a multitude of zoonotic and non-zoonotic pathogens (Medlock et al. 2013, Dahmana et al. 2020).

Extrinsic and intrinsic drivers of small mammals' populations

Based on the demographic niche concept, the overall niche of a species is formed by the combination of possibly divergent niches of the demographic vital rates (i.e. survival, fecundity, somatic growth) that change in response to environmental gradients (Pironon et al. 2018). Studies on small mammals were at the forefront in defining the interplay between top-down, bottom-up and density dependence processes, providing observational data under experimental and wild settings (Krebs and Myers 1974, Hansson and Henttonen 1988, Boonstra 1994, Cornulier et al. 2013; see reviews from Krebs 2013, Oli 2019, Andreassen et al. 2021).

Predicting the near- and long-term responses of small mammal demographic niche to environmental changes requires the understanding of relationships between demographic rates, per capita growth rate (Sibly and Hone 2002) and population density (Krebs 2002b) within the environment. Indeed, the population growth rate may show density-dependence, either direct or delayed (Batzli 1999), or density-independent stochasticity (Stenseth et al. 2002), which together regulate population returning to its equilibrium density (Krebs 2002a, Turchin 2003). The rate of population change is affected not only by current and past density but also by other intrinsic and extrinsic factors, whose interplay governs demographic rates. Intrinsic factors include those internal components making up the population, such as behavioural tendencies, sociality, life-history traits and physiological and genetic characteristics. For instance, social aggregation can promote winter survival thanks to thermoregulation (Sipari et al. 2016), but can also decrease litter size and breeding in females, although enhancing healthy offsprings (Gromov 2021). Body mass also can favour survival, especially during winter (Aars and Ims 2002), while dispersal animals may show a high mortality rate being more exposed to predators, but at the same time retained high resilience to habitat fragmentation (Rémy et al. 2014). Intrinsic effects interact with environmental extrinsic conditions, namely top-down (i.e. severe weather, predation, parasitism) and bottom-up (i.e. food quality and quantity, landscape shelters). The effect of predation on small mammal survival and abundance was extensively elucidated, also in interaction with food supply (Desy and Batzli 1989, Korpimaki et al. 2005, Korpela et al. 2014), which in turn has profound impacts on small mammal survival, breeding and density (Krebs and DeLong 1965, Hansen and Batzli 1978, Taitt and Krebs 1981, Rémy et al. 2013). Further, also climate constraints may either favour (Korslund and Steen 2006) or limit (Aars and Ims 2002, Kausrud et al. 2008) small mammal persistence and vital rates. As notable, studies observed a multitude of different demographic responses to intrinsic and extrinsic factors and therefore a niche-based approach that integrates the components of the ecological niche may solve the complexity that emerged in examining small mammal demography.

All these factors strongly shape the annual population pattern of small mammals, which is characterized by an alternation between breeding (i.e. from late spring to early autumn) and non-breeding (i.e. from late autumn to early spring) seasons, in temperate and polar regions (Eccard and Herde 2013). Specifically, in late spring, the decreased energetic costs due to warmer temperatures, together with the increase of food resources (Pearse et al. 2016), stimulate reproduction (Steinlechner and Puchalski 2003), which continues until autumn and during which early and later-born generations are produced. During the breeding season, deaths of newborn and immature animals, as well as high emigration rate of overwintered individuals (i.e. those that successfully survived throughout the previous winter) make the survival rate to collapse (Flowerdew et al. 1985). During the non-breeding season, the survival of overwintering late-born animals is higher, guaranteeing the bulk for the following year spring population. The demographic pattern governed by these variations of population rates typically shows its lowest in population size over winter, an increase in spring, a peak in late summer and a decline in autumn (Eccard and Herde 2013).

Competition in small mammals

Heterogeneous habitats offer a wide spectrum of colonization opportunities for a great number of small mammal species, which together create a suite of interactions which can trigger competitive mechanisms within ecological communities (Wiens 2011). Generally, sympatric species adjust their behavioural strategies, foraging activity (Zwolak et al. 2016), habitat selection (Hille and Mortelliti 2010) and activity patterns (Viviano et al. 2022) to partition their niche along trophic, spatial and temporal niche dimensions and thus facilitating coexistence (Schoener 1974). However, this mechanism may collapse either under strong environmental perturbations (Costa-Pereira et al. 2019) when species respond similarly to environmental niche requirements (e.g. food, mates, sites and shelters) or due to density-dependence mechanisms (Brook and Bradshaw 2006). These circumstances outset interspecific competition (Eccard and Ylönen 2003, 2007) that can emerge through exploitation or interference in small mammals. Exploitative competition describes a negative depletion of shared resources of one species to the other (classical exploitation; MacArthur and Levins 1967) or negative interaction between species that share a natural antagonistic factor (apparent competition; Holt and Bonsall 2017). Instead, behavioural interference involves direct interaction among species, with dominant ones inhibiting the ability of subordinate species to access the resources by obtaining dominance status (Grether et al. 2017). Although these two types of competition often overlap in nature, their effects are difficult to separate (Shenbrot and Krasnov 2002) and only some studies have been able to disentangle the two responses in small mammals through mathematical simulations or with experimental enclosures (Abrams 1998, Schmidt et al. 2005, Liesenjohann et al. 2011), but not in wild settings using an open experimental approach.

Parasitism and pathogens transmission mediated by small mammals

Parasitism can drive niche specialization (Britton and Andreou 2016), producing cascading effects on small mammals' ecological communities and ecosystem properties (Thomas et al. 2000). Small mammals are important hosts for ticks (represented by 907 species belonging mainly to *Argasidae* and *Ixodidae* families), which are obligate hematophagous ecto-parasites whose survival, as well as moulting across life stages and thus completion of their life cycle, depending on the successful exploitation of hosts (McCoy et al. 2013). The encounter between ticks and their hosts depends (i) on the ability of ticks to attach to the host and the strategy of hosts to avoid ticks, both driven by immunity, hormones and physiological conditions (Rosà et al. 2006); (ii) on the overlap of their spatial and temporal distribution (Raffel et al. 2008, Cagnacci et al. 2012), that can change based on home range size and activity patterns of hosts, as well as tick questing behaviour (Randolph 2004); (iii) on the host community composition and species abundance across environmental gradients (Dobson and Hudson 1986, Levine 2000). However, all these relationships have been usually considered separately, so the literature remained not conclusive in outlining the causal mechanisms that link host abundance with tick burden. This might be revealed by adopting a spatially explicit, ecosystemic approach accounting for the *ensemble* of potential drivers.

From an eco-epidemiological perspective, ticks are important vectors of a multitude of disease-causing pathogens infecting both domestic and wild animals, as well as humans (zoonotic spillover pathway) (Plowright et al. 2017). In particular, among the pathogens of the Palearctic region that are transmitted by hard ticks and small mammal hosts (Rizzoli et al. 2014), Tick-borne encephalitis virus (TBEV), causing tick-borne encephalitis (Riccardi et al. 2019), and *Borrelia burgdorferi* complex, which is involved in Lyme borreliosis (Steinbrink et al. 2022), have the greatest impact on human health. Ticks and small mammals can also harbour less hazardous vector-borne pathogens yet with medical and veterinary importance, such as *Anaplasma phagocytophilum*, a bacteria which leads to granulocytic anaplasmosis in human and wildlife (Stuen et al. 2013), and also protozoans of genus *Babesia*, that cause babesiosis in humans (Yabsley and Shock 2013). Interestingly, ticks are among the arthropod vectors that can infect small mammals with *Hepatozoon* spp. once ingested (Duscher et al. 2015), producing detrimental effects across the food web involving also prey-predator transmission. Beyond tick-borne pathogens, small mammals also promote the emergence of rodent-borne agents such as hantaviruses causing haemorrhagic fever with renal syndrome in Europe (Olsson et al. 2010).

These pathogens are mainly harboured by small mammals (Dahmana et al. 2020). Indeed, small mammals serve to maintain the infectious agents in the environment (paratenic role) and can have the competence to transmit them to other susceptible hosts and vectors (reservoir role). Several studies evaluated the spatial distribution or life-cycle of arthropod vectors and of hosts and others described the distribution of infectious agents (Mihalca and Sándor 2013, Medlock et al. 2013, Han et al. 2016). However, uncertainty remains on the relationship between species composition of natural communities of hosts and rates of pathogen circulation (Estrada-Peña et al. 2014, Valenzuela-Sánchez et al. 2021). Indeed, the novel associations between reservoir hosts and potentially infected vectors (Altizer et al. 2013, 2018) can ultimately boost vector-borne pathogens' circulation and spreading within the food web and the ecosystem (Johnson et al. 2015). The dynamics of hosts-parasites-pathogens associations could be further complicated by climate change, because milder climatic conditions and shifts of host availability in space and time may re-shape the ecological niche of ticks, opening relevant eco-epidemiological concerns about the alteration of circulation of pathogens and therefore of disease transmission (Estrada-Peña 2008, Gilbert 2021). For instance, recent studies have observed a geographic expansion of ticks towards northern latitudes (De Pelsmaeker et al. 2021) and higher altitudes (Garcia-Vozmediano et al. 2020), which increased the occurrence of tick-borne diseases (Randolph 2001).

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1.2 Knowledge gap

Innumerable studies have contributed to assessing the effects of ecological intrinsic and extrinsic factors on (i) the determination of individual fate, (ii) the modulation of population demography and dynamics, and (iii) the composition of animal communities and the relationships therein (see Background sections above). In an eco-epidemiological framework, such fundamental ecological processes cascade on the dynamics of host-parasite associations, and ultimately the circulation of pathogens. Some of these links have not been disentangled, yet their understanding is becoming urgent under the current climate and global change. The increasing human footprint is rapidly modifying the environmental gradients, both in terms of climate and distribution and concentration of resources and habitats. Species respond differently to these modifications and consequently, we assist to population expansions or extirpations, species distributional shifts, and community modifications. As all these cascades on parasite environmental persistence, parasitic load, and pathogen transmission, new and old diseases emerge and spread. My dissertation aims to identify some missing links between the ecological underpinning processes and the host-parasite-pathogen patterns that we observe in changing heterogeneous landscapes. I have addressed such ambitious goal by applying a multi-factorial, niche-based approach and using field experimental designs. I have used small mammals as model species due to their key eco-systemic role, including as primary hosts for several ecto-parasites and pathogens, with specific, but not exclusive, focus on ticks and tick-borne-pathogens (see Background). In particular, I first adopted a niche-based approach to experimentally evaluate the demographic responses of rodents to the heterogeneity of climate and resource distribution, and the consequences of such responses on community composition and inter-specific relationships. Then, I explored the cascading effects of such ecological relationships considering rodents as hosts, through a spatially explicit analysis of the hosts-parasite association. I finally evaluated how these interactions emerge as distributional patterns of hosts, parasites and pathogens along an altitudinal gradient, projecting the vertical habitat transitions as prospective changes through time under climate change. I articulated these tasks within a conceptual workflow and four specific objectives, corresponding to the four papers composing this thesis that I present in the next paragraph.

1.3 Objectives and content of the thesis

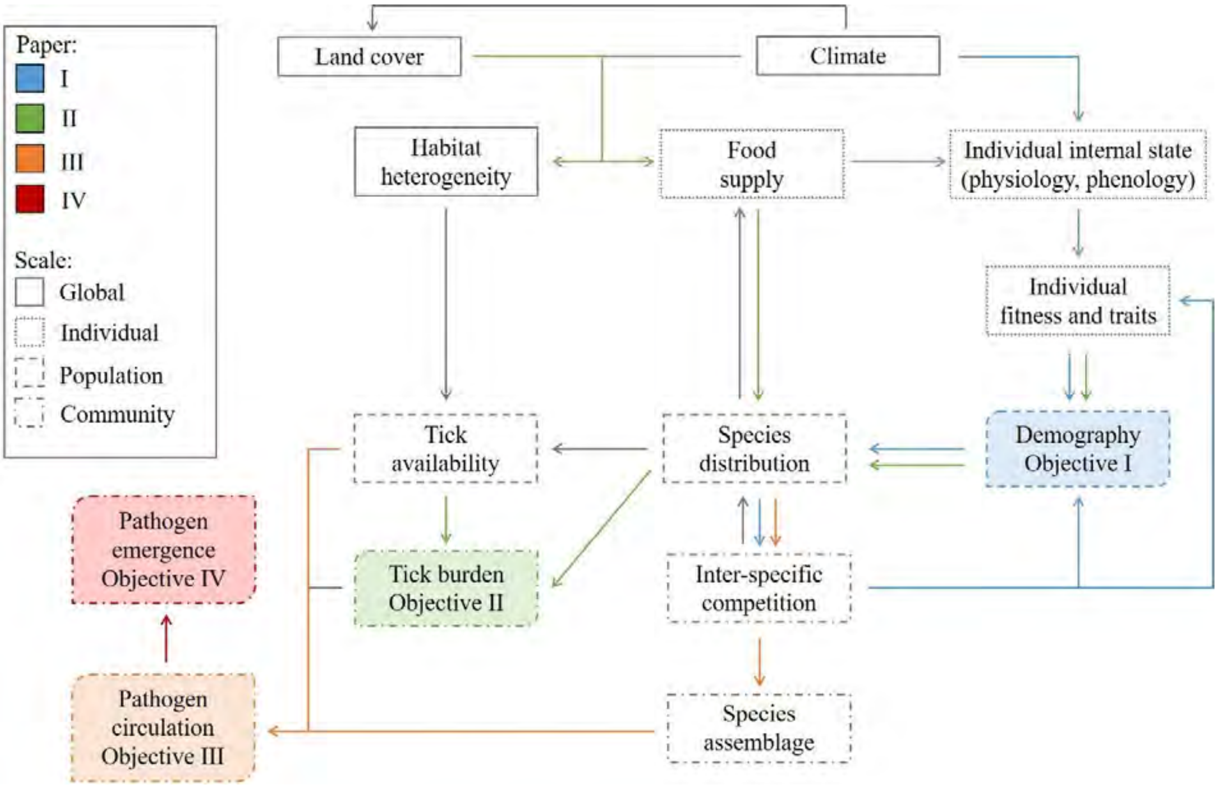

Figure 1: Eco-epidemiological processes supporting the workflow of the PhD thesis. Intrinsic, extrinsic factors and biotic interactions underpin host-parasite-pathogen patterns. These interactions cascade one onto another and emerge at different ecological scales. Boxes represent the factors at different scales (individual: dotted boxes; population: dashed boxes; community: dotted-dashed boxes; global: solid boxes), while the arrows indicate the relationships between them. The colour shades of boxes and arrows identify the ecological processes addressed by specific objectives that were at the basis of the papers included in the Thesis. In particular, blue: Paper I; green: Paper II; orange: Paper III and red: Paper IV.

The main objectives of this PhD dissertation were: (i) to assess the effects of environmental heterogeneity, in terms of climate and availability of trophic resources, on woodland rodent demography and species interactions by applying a niche-based approach; (ii) to track how these effects cascade on host-tick pairings within a multi-host system exposed to human impact; (iii) to describe the distributional patterns of rodent hosts, parasites and pathogens along an altitudinal gradient exposed to climate change; (iv) to assess the emergence of non-zoonotic vector-borne rodent pathogens of high ecosystemic relevance across gradients of environmental heterogeneity. I addressed these objectives using live-trapping data of small mammals across wide latitudinal and altitudinal gradients and applying serological and molecular laboratory analyses, as well as spatial implicit and spatially explicit capture-mark-recapture models, multivariate statistics and generalized linear modelling.

Specifically, the thesis is accompanied by four papers, each of which aimed to assess the above-proposed objectives (see Figure 1). In **Paper I**, I disentangled the effects of bottom-up (i.e. food availability), top-down (i.e. climate severity) extrinsic factors, as well as seasonal intrinsic cycles, and inter-specific competition on rodent demographic rates and patterns (**objective i**). To achieve this objective, we deployed control/treatment designs of food resource availability at two latitudinal extremes of the boreal-temperate gradient (Italy and Norway) to contemporarily measure the effect of climatic severity (longitudinal comparison) and food availability (food manipulation), while controlling for seasonal variation and co-occurrence of sympatric species. In **Paper II**, I investigated the role of environmental heterogeneity, in terms of availability and distribution of anthropogenic food and vegetation cover in multi-host systems (ungulate feeding sites), on rodent space use patterns and local density, and consequently evaluated the cascading effects on the intensity of tick burden (**objective ii**). To do so, we applied spatially explicit capture recapture models and measurements of the covariates, while accounting for other determinants, including host individual traits and tick availability in the environment. In **Paper III**, I evaluated the small mammal assemblage composition along an Alpine altitudinal gradient of habitats and the relation between host distribution and occurrence of infectious pathogens (**objectives iii**). To do so, we live-trapped and sampled small mammal species and evaluated their association along the aforementioned altitudinal transitions, as well as the occurrence and rate of infection of rodent- and vector-borne pathogens. In **Paper IV**, I assessed the occurrence and prevalence of protozoans belonging to *Hepatozoon* genus, a non-zoonotic micro-organism of wide ecosystemic significance, across the Alpine altitudinal gradient and under a diverse degree of anthropic pressure (**objective iv**). To do so, we examined the effectiveness of the PCR-based method coupled with sequencing for detecting these protozoans across a broad spectrum of small mammal hosts in wild and peri-urban areas.

2. Material and Methods

2.1 Study areas

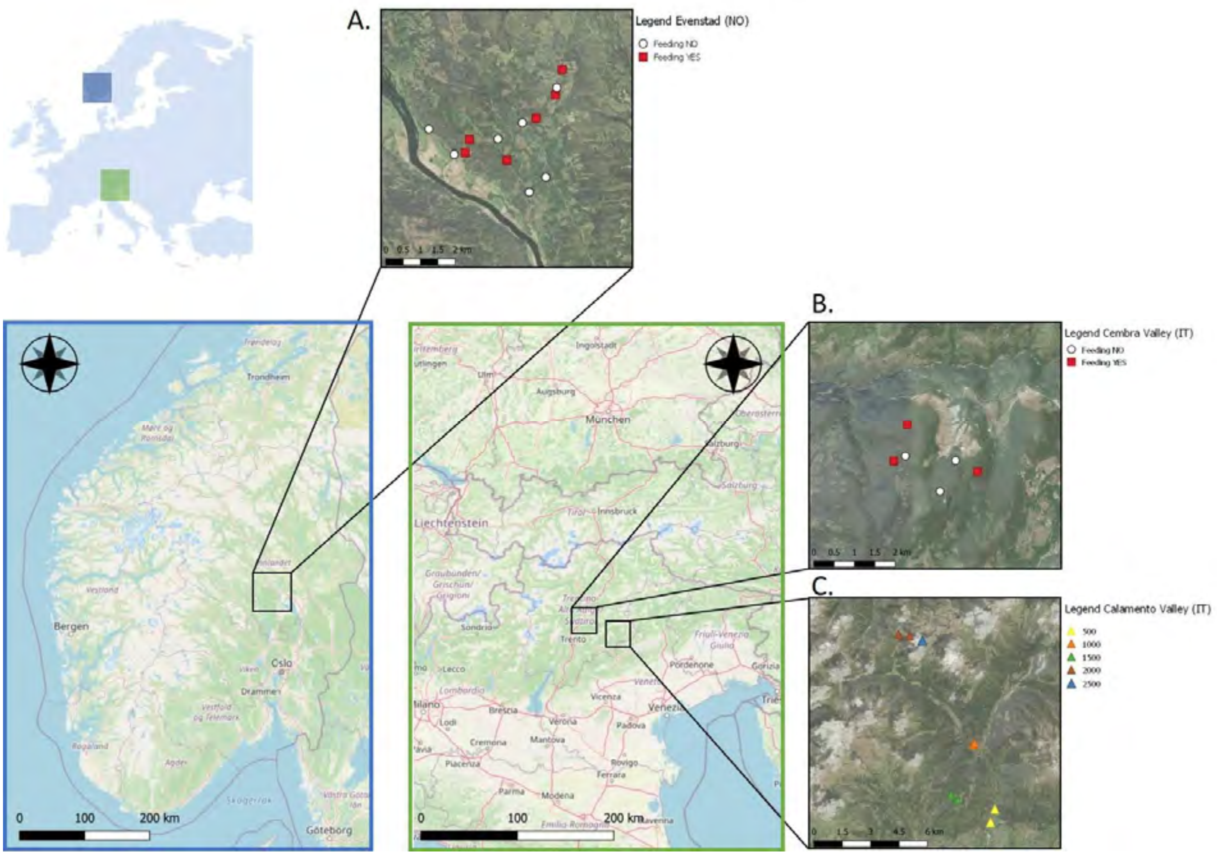

Figure 2: Locations of the study areas in Norway and in Italy. Evenstad (EVT; Norway) (Panel A) is included in Paper I, Cembra Valley (CEV; Italy) (Panel B) in Paper I, II, IV and Calamento Valley (CAV; Italy) (Panel C) in Paper III and IV. Red square: trapping grids with supplemental food, white circle: trapping grids without supplemental food; triangles: trapping grids along an altitudinal gradient (500-2500 m a.s.l.).

The data sampling was carried out in two areas located at the extremes of the boreal-temperate climatic gradient, namely Norway and Italy (Figure 2). In South-East Norway, small mammal trapping was performed at Evenstad study area (61.00N – 11.00E; Stor Evdal municipality, Inland - Norway). Here the climate is characterized by a strong seasonality with cold and snowy winters and mild and short summers (boreal climate, *sensu* Köppen-Geiger classification, Kottek et al., 2006) and the landscape is mainly dominated by *Picea abies* and *Pinus sylvestris* with bilberry in the shrub cover and mosses in the ground layer. Data from Evenstad study area were investigated in Paper I.

The Italian study areas were located in the North-Eastern Italian Alps, namely Cembra Valley and Calamento Valley. Cembra Valley (46.13020N – 11.17843E; Albiano municipality, Autonomous Province of Trento - Italy) comprises peri-urban areas with an alternation of small villages and areas of anthropic employment (quarries and forest harvest) and semi-naturalized forests. The seasonal shift is gradual with short and cool winters and warm and flourishing summers (temperate warm-alpine climate, *sensu* Köppen-Geiger classification, Kottek et al., 2006), which at the moderate altitudes (1000 m a.sl.) corresponds to secondary growth broad-leaved (*Fagus sylvatica*, *Quercus petraea*) and coniferous (*Pinus sylvestris*, *Abies alba*, *Picea abies*) mixed forests. Data collected in this study area were investigated in Paper I, II and IV.

Calamento Valley (46.12187 – 11.48985, Telve municipality, Autonomous Province of Trento - Italy) is included in the Lagorai massif and results relatively undisturbed, with few anthropic activities (traditional cattle husbandry and seasonal tourism). It is characterized by a rapid altitudinal gradient corresponding to the full Alpine vegetation succession, from broad-leaved woodland and mixed forest to conifer and stands forest, shrubs, and alpine prairies. Data from Calamento Valley was considered in Paper III and IV.

From here below we will use abbreviations for referring to these study areas, and specifically EVT for Evenstad, CEV for Cembra Valley and CAV for Calamento Valley.

2.2 Experimental designs

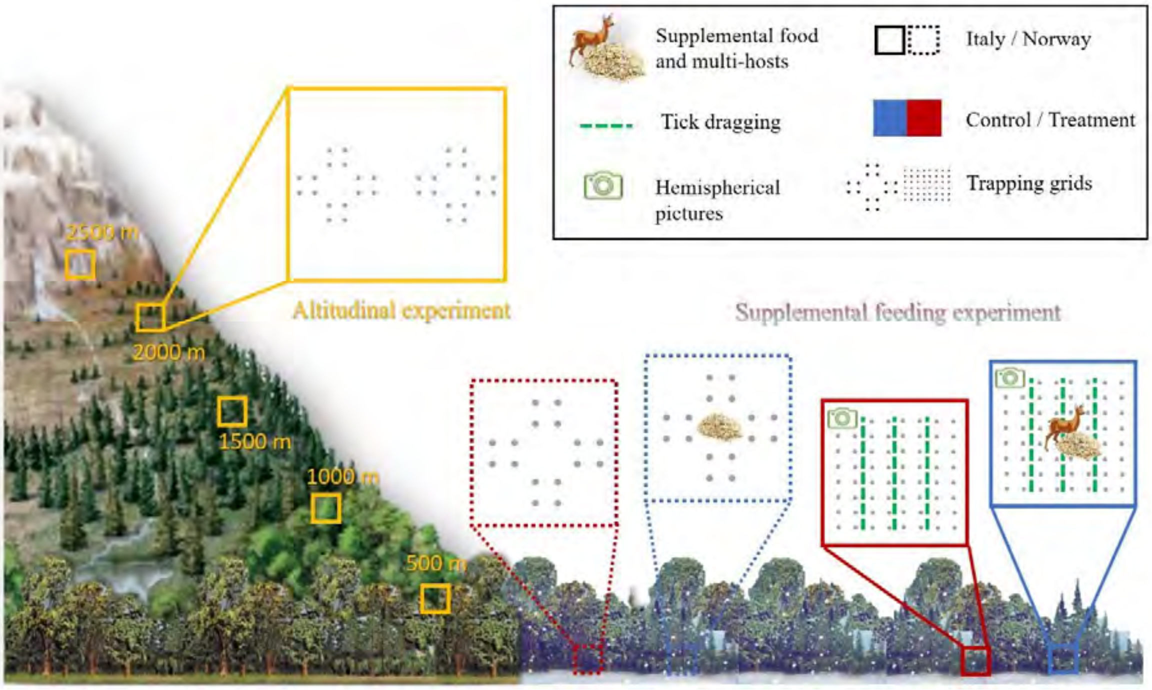

Figure 3: Experimental designs considered in Paper I, II, III, and IV. The supplemental feeding experiment (blue and red colours) was deployed in Norway (Evenstad study area, dotted lines) and in Italian Alps (Cembra Valley study area, solid lines). The altitudinal experiment (Calamento Valley study area, yellow colour) included 5 altitudinal belts of 500 m, from 500 m a.s.l. to 2500 m a.s.l.

2.2.1 Supplemental feeding experiment (Paper I and II)

To evaluate the demographic implications of extrinsic and intrinsic factors on small mammal populations (Paper I), we undertook experimental food manipulations in natural settings across a latitudinal gradient (Norway and Italy), which was used as a proxy for climatic severity (Figure 2A and Figure 2B). In particular, to assess the effect of resource availability, we provided *ad libitum* supplemental food in some trapping occasions and/or sites (‘Feeding yes’, or ‘Treatment’), but not in others (‘Feeding no’, or ‘Control’) both in Norway and in Italy. Specifically, in Norway, we deployed thirteen trapping grids and performed monthly captures from 2013 to 2015 by following a temporal control/treatment approach. Here, supplemental food (a mixture of oat and sunflowers) was provided *ad libitum* next to each trap during some trapping sessions (state of the sites: ‘Feeding yes’), while depletion of food occurred in other sessions (state of the sites: ‘Feeding no’) (see Johnsen et al. 2016 for details) (Figure 3). In Italy, we applied a control/treatment approach following a spatial resolution, by providing *ad libitum* corn supply in Treatment grids (sites: ‘Feeding yes’), while the Control grids (sites: ‘Feeding no’) lacked of food during the entire sampling period (Figure 3). In three Control and three Treatment grids small mammal monitoring was performed from 2019 to 2021, at monthly or bi-monthly intervals during winter and summer seasons, respectively (see 2.4 for *Small mammals’ trapping*).

In CEV, the supplemental food was dispensed through ungulate feeding sites located at the centre of each Treatment trapping grid, while the other three Control grids were located at least 500 m from any feeding site. Feeding sites are typically shaped as wooden hopper dispensers that provide a continuous supply of corn accessible all year round by target species, such as roe and red deer (*Capreolus capreolus* and *Cervus elaphus*), and non-target mammals (*Meles meles*, *Sciurus vulgaris*, Muridae, Arvicolinae) and birds (*Garrulus glandarius*, *Columba palumbus*) (Ranc et al. 2020). For these reasons, supplemental feeding sites permit not only testing the effect of food resource availability (Paper I), but also represent focal areas where large (deer) and small (rodents) hosts of ticks show the tendency of aggregation, and high density, which in turn enhance ticks to complete their life-cycle (Paper II) (Figure 4).

Figure 4: Pictures collected from camera traps located at ungulate feeding sites in Cembra Valley (Italy) (courtesy from 2C2T project). Both small mammals (bottom left side of picture A. and picture B.) and roe deer (right side of picture A.) were detected.

2.2.2 Altitudinal experiment (Paper III, IV)

Due to the rapid changes in physical, topographic and climatic condition over a relatively short spatial distance, a mosaic of habitats succeed along the altitudinal gradient of mountainous areas (Nagy et al. 2003). By following a wide altitudinal range (every 500 m, from 500 m a.s.l. to 2500 m a.s.l., Körner 2007), we identified five altitudinal belts in CAV, each of which is characterized by a specific Alpine habitat (Figure 2C and Figure 3). In particular, natural woodlands change along with altitude, from lowland and montane forest formations to dwarf shrubs (belts: 500 m a.s.l., 1000 m a.s.l. and 1500 m a.s.l.). Then above the treeline, forests give the way to grass–heath, sedge–heath, and moss–heath formations (belt: 2000 m a.s.l.), and finally, to open grasslands and rocky habitats (belt: 2500 m a.s.l.).

In each of the five alpine altitudinal belts, we deployed two trapping grids spaced at least 500 m one from each other. We live-trapped small mammals and evaluated ecto-parasites' load in 2019 and 2020 at monthly intervals from May to October, to assess small mammals' assemblage and pathogens' distribution across the Alpine altitudinal gradient (Paper III, IV). Capture of the small mammals and parasites' load assessment followed the same trapping procedure applied also for the winter experiment (see 2.4 for *Small mammals' trapping*).

2.3 Vegetation cover at fine-scale (Paper II)

When considering environmental heterogeneity, spatial scale depends on both species' taxonomic group and on the study aim and determines the type of structural variables that have to be chosen to reflect habitat requirements (Tews et al. 2004). Being small mammals and ecto-parasites strongly affected by microclimate and microhabitat (Estrada-Peña 2008, Hille and Mortelliti 2010), in CEV we assessed vegetation cover at ground level at each trap station. In this way, besides tree canopy cover, also fine-scale understory components (e.g. leaves, branches, stalks of understory) were detected. Specifically, we applied a standardized and objective approach, consisting of a camera system (Nikon Coolpix 4500) with fish-eye lens (Nikon Fish-eye Converter FC-E8) that retrieved digital hemispherical photographs (DHPs) of the vegetation structure from the ground. In particular, we collected one DHP per season following a standardized protocol. To extract fine-scale vegetation cover estimates, we processed all DHPs using CAN-EYE software (version 6.495, National Institute of Agricultural Research, Toulouse, France), which ensures efficiency and accuracy, as well as traceability by avoiding the confounding observer's effect (Chianucci and Cutini 2012).

2.4 Small mammals' trapping (Paper I, II, III, IV)

Small mammal trapping was conducted in the three study areas (EVT, CEV and CAV). In EVT (Paper I) and in CAV (Paper III, IV), trapping grids carried out a cross-shaped design, with 16 trap stations placed 15 m and 7.5 m apart depending on the external and internal trap position (total grid area: 3600 m^2). Meanwhile in CEV (Paper I, II, IV), animals were caught in square-grids of 8 x 8 traps with 10 m of inter-trap distance (total grid area: 4900 m^2). In all study areas, trapping sessions lasted 4 days/3 nights per month, while traps were checked every 12 hours at EVT and every 24 hours in CEV and CAV. The traps were left open during non-trapping periods to habituate animals to their presence. Each trap consisted of a standard Ugglan Multiple Live Trap (model 2, Granhab, Sweden), filled with hay during the cold season, and baited with carrot slices and seeds (oat in Norway, sunflower in Italy). Standard capture-mark-recapture techniques (CMR) were adopted (Pollock et al. 1990, Amstrup et al. 2005). Each animal was individually marked with a Passive Integrated Transponder (PIT) tag (Trovan® Ltd., UK), and standard information were recorded for each capture (Figure 5). In Italy only, blood (from the retro-orbital sinus, using micro-haematocrit capillary), tissue samples (tail tip and ear biopsy, using sterile scissors and disposable ear punch) and faecal pellets (from the trap, using sterile tweezers) were collected for further laboratory analyses and stored at -80°C (blood, ear biopsy and faeces) or in alcohol (tail tip). All animal handling procedures and ethical issues were approved by the regional or local Wildlife Management Committees.

Figure *5: Bank* vole (*Myodes glareolus*) captured at Cembra Valley (Italy) during winter 2020, using Ugglan Multiple Live Trap (© Simone Dal Farra).

2.5 Parasites' monitoring (Paper II, III)

Habitat characteristics affect ticks questing behaviour and vital parameters (Ginsberg et al. 2020), while climate mostly influences tick abundance and distribution (Randolph 2004). Further also mean tick burden (number of attached ticks on hosts) is essential to evaluate parasite distribution among hosts (Rosà et al. 2006). We evaluated tick population using the number of ticks collected per unit of distance from tick dragging but also assessed tick burden by performing tick screening from captured small mammals.

Monitoring of tick populations was performed only in CEV (Paper II). Each of the six trapping grids was visited once per year (May 2019 and May 2020). The collection of host-seeking ticks was carried out in a standard way by dragging 1 m^2 white flannel cloth through vegetation and leaf litter. The cloth was pulled behind the investigator along 3 transects of 70 m per grid following the rodents' trap line and inspected for ticks every 5-10 m, accordingly to vegetation cover. Tick larvae, nymphs and adults attached to the cloth were counted and separated based on sex. Number of ticks collected by dragging was used to infer tick density at grid level per m^2.

Additionally, during a capture event in both CEV and CAV (Paper II and III), each small mammal individual was screened for the presence of ecto-parasites (i.e. ticks, fleas, mites and mobile mites). In particular, for ticks, we discriminated life stages (i.e. larval, nymphal and adult stage), and we recorded the body position in which the tick was found attached on host (i.e. head, body and ears), as well as free nymph and co-feeding between larvae and nymphs. The mean number of attached ticks for each host determined the individual tick burden.

2.6 Laboratory analyses

2.6.1 Small mammal species identification (Paper I, II, III, IV)

Ear biopsies collected in Italy were used for epidemiological analyses (see sections 2.6.2 and 2.6.3), as well as to genetic determine those small mammal species that were morphological similar (i.e. *Apodemus* spp., *Microtus* spp. and shrews).

For DNA extraction, ear samples were incubated overnight at 56°C and isolated using DNeasy 96 Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. For species genetic identification, we performed conventional polymerase chain reaction (PCR) method on extracted DNA (Table 1b and Table 1c) using specific primers (Table 1a) to target the cytochrome b in yellow-necked mouse (*Apodemus flavicollis*) and wood mouse (*Apodemus sylvaticus*) and to identify shrew and *Microtus* species (Irwin et al. 1991). The discrimination between the two *Apodemus* species was confirmed using the Qiagen QIAxcel® capillary electrophoresis system. Meanwhile for shrews and *Microtus* spp., after controlling the success of amplification through electrophoresis, the PCR products were purified using ExoSAP-IT (USB, Cleveland, OH, USA) and then sequenced using Sanger Sequencing. The DNA sequences were analysed and aligned using Sequencher software version 5.4.6 (Gene Codes Corp., Ann Arbor, Mich.) and Clustal X software version 2.0 and subsequently compared with the data stored in the GenBank database with Basic Local Alignment Search Tool (Blast; online version).

2.6.2 Serological screening (Paper III)

To detect the presence of antibodies against flaviviruses (Tick-borne encephalitis virus, TBEV) and orthohantaviruses (Puumala, PUUV and Dobrava-Belgrade virus, DOBV) in small mammals, we analysed sera samples through Indirect Immunofluorescence Assay (IFA). IFA is based on two types of antibodies, unconjugated primary antibody and fluorophore-conjugated secondary anti-antibody, which target the presence of primary antibody through immunofluorescence (Niedrig et al. 2007). All blood samples were centrifuged at 12,320 g for 12 min to separate the serum from other blood components (white and red cells, clotting factors and platelets). Sera samples were diluted 1/20 according to the manufacturer's protocol (HaartBio Ltd., Helsinki, Finland) with phosphate buffered saline (PBS) and were then added to slides provided by the Department of Virology, University of Helsinki, Finland. These slides contained cellular viral antigens that can bind any potential primary antibody against the virus and may occur in infected sera samples. Slides with diluted sera were incubated at 37°C for 30 min in a stove and three washes with PBS were performed. A secondary anti-mouse antibody, which carries the fluorophore, was then added to each serum sample to highlight the binding between the primary antibody and antigen. Further, incubation in stove and three PBS washes were performed for the second time. Immunofluorescence of positive samples was then detected with a fluorescence microscope. For each stock of samples, two negative (PBS with anti-mouse either anti-human antibodies) and one positive (human antibodies against TBEV, PUUV or DOBV with anti-human antibodies) controls were included (Tagliapietra et al. 2018, Kallio-Kokko et al. 2022).

Table 1: PCR-based method for detection of small mammals’ species. Specifically, specific primers (Table 1a) were used for each PCR reaction (Table 1b), that followed temperature cycling profile (Table 1c). ‘-‘: represents reagent not included.

Table 1a	Primer F	Primer R
A. flavicollis	FLADN 5'–GCG TAT GCA AAT AGG AAG TAC– 3'	FLAUP 5'–AGC TAC ACT AAC ACG TTT C– 3'
A. sylvaticus	SYLDN 5’ –TTA ATA TGG GGT GGG GTG TTA– 3'	SYLUP 5'–GAG GAG GAT TCT CAG TAG AC– 3'
Microtus spp. and shrews	H427 5'–TCA GAA TGA TAT TTG TCC TCA– 3'	L79 5'–AAC ATC TCA GCA TGA TGAA – 3'

Table 1b	Final volume (µl)	DNA volume (µl)	H2O (µl)	Reaction buffer (µl)	25 mM MgCl2 (µl)	10 mg/ml BSA (µl)	10 pmol/µl Primer F (µl)	10 pmol/µl Primer R (µl)	10 mM dNTPs (µl)	5 U of GoTaq (µl)
A. flavicollis	50	2	28.75	10	6	-	1	1	1	0.25
A. sylvaticus	50	2	26.85	10	6	-	1	1	1	0.25
Microtus spp. and shrews	50	2	30.75	10	4	-	1	1	1	0.25

Table 1c	Initial temperature (°C)	Denaturation temperature (°C)	Annealing temperature (°C)	Extension temperature (°C)	Final temperature (°C)	Number of cycles
A. flavicollis and *A. sylvaticus*	95 x 2'	95 x 20"	58 x 30"	72 x 1' 30"	72 x 5'	33
Microtus spp. and shrews	95 x 2'	95 x 20"	58 x 30"	72 x 1' 30"	72 x 5'	40

2.6.3 Molecular screening (Paper III, IV)

To assess the occurrence of protozoa (*Babesia* spp., *Hepatozoon* spp.) and bacteria (*Anaplasma* spp. and *Borrelia* spp.) in small mammal hosts, we used DNA extracted from ear biopsy (section 2.6.1) and we performed conventional polymerase chain reaction (PCR) method, followed by sequencing of the amplified DNA.

Pathogens' occurrence was detected by amplification of specific genes (18S rRNA for *Hepatozoon* spp. and *Babesia* spp., 16S rRNA gene for *Anaplasma* spp. and *OspC* gene for *Borrelia* spp.) through PCR reactions (Table 2b) using specific primers (except for *Hepatozoon* spp.; Table 2a) and following temperature cycling profile (Table 2c). For *Anaplasma* spp. amplification of long fragment was performed applying nested PCR with two types of primers, while for other pathogens single PCR was conducted. Visualization of the success of amplification was confirmed using the Qiagen QIAxcel® capillary electrophoresis system. Positive PCR products were purified by enzymatic ExoSAP-IT (USB, Cleveland, OH, USA) according to the manufacturer's instructions and then sequenced using Sanger Sequencing. The DNA sequences were analysed and aligned using Sequencher software version 5.4.6 (Gene Codes Corp., Ann Arbor, Mich.) and Clustal X software version 2.0 and subsequently compared with the data stored in the GenBank database with Basic Local Alignment Search Tool (Blast; online version).

Table 2: PCR-based method for detection of *Babesia* spp., *Borrelia* spp., *Anaplasma* spp. and *Hepatozoon* spp. from small mammal ear biopsy. Specifically, specific primers (but not for *Hepatozoon* spp.; Table 2a) were used for each PCR reactions (Table 2b), that followed temperature cycling profile (Table 2c).

Table 2a	Primer F	Primer R
Babesia spp. and *Hepatozoon* spp.	BN2 5'–TAG TTT ATG GTT AGG ACT ACG– 3'	BJ1 5'–GTC TTG TAA TTG GAA TGA TGG – 3'
Anaplasma spp. 1 PCR	ge3a 5'–CAC ATG CAA GTC GAA CGG ATT ATT C– 3'	ge10r 5''–TTC CGT TAA GAA GGA TCT AAT CTC C– 3'
Anaplasma spp. 2 PCR	ge9f 5'–AAC GGA TTA TTC TTT ATA GCT TGCT– 3'	ge2 5'–GGC AGT ATT AAA AGC AGC TCC AGG– 3'
Borrelia spp.	OspC F 5'– AAA GAA TAC ATT AAG TGC GAT ATT– 3'	OspC R 5'–GGG CTT GTA AGC TCT TTA ACT– 3'

Table 2b	Final volume (µl)	DNA volume (µl)	H2O (µl)	Reaction buffer (µl)	25 mM MgCl2 (µl)	10 mg/ml BSA (µl)	10 pmol/µl Primer F (µl)	10 pmol/µl Primer R (µl)	10 mM dNTPs (µl)	5 U of GoTaq (µl)
Babesia spp.	50	5	21.25	10	8	0.5	2	2	1	0.25
Hepatozoon spp.	50	1	28.25	10	6	0.5	1.5	1.5	1	0.25
Anaplasma spp. 1 PCR	50	2	29.75	10	4	0	1.5	1.5	1	0.25
Anaplasma spp. 2 PCR	50	2	30.75	10	4	0	1	1	1	0.25
Borrelia spp.	50	5	24.75	10	5	0	2	2	1	0.25

Table 2c	Initial temperature (°C)	Denaturation temperature (°C)	Annealing temperature (°C)	Extension temperature (°C)	Final temperature (°C)	Number of cycles
Babesia spp.	95 x 2'	95 x 20''	53 x 30''	72 x 40''	72 x 5'	40
Hepatozoon spp.	95 x 2'	95 x 20''	46 x 15''	72 x 50''	72 x 5'	40
Anaplasma spp. 1 PCR	95 x 2'	95 x 30''	54 x 30''	72 x 40''	72 x 5'	40
Anaplasma spp. 2 PCR	95 x 2'	95 x 30''	54 x 30''	72 x 40''	72 x 5'	40
Borrelia spp.	95 x 2'	95 x 30''	54 x 30''	72 x 1'15''	72 x 5'	45

2.7 Data management and EuroSmallMammals

2.7.1 Data processing, relational database and quality protocols

All data collected during this PhD both from fieldwork and obtained from laboratory analyses were stored in the relational database created specifically for the EuroSmallMammals initiative (see section 2.7.2). In this way, we have supported the concept of “data sharing readiness” (Michener 2015) and envisioned their future reuse within the scientific community.

The database was built under open software platforms (Django, PostgreSQL, PostGIS; Urbano and Cagnacci 2014) and is based on relational architecture, which permits to manage high quantity of complex data (see Figure 6). Before storing the data in the database, they passed from harmonization process and quality checks performed through formal and partially automated controls (Urbano and Cagnacci 2021), necessary to detect errors, inconsistencies and lacking or suspicious information. Also, robustness and consistency checks were applied once the data were stored in the database.

The core information of the database is the capture event (‘sampling’) and the captured animal (‘animal’). Beyond the capture-recapture data collected during the capture event (see records of ‘sampling’ look-up table in Figure 6) and the animal characteristics, the database includes also other aspects covered during a small mammal study. For instance, biological samples (‘collected sample’), morphological measures (‘body measure’) and parasitological data (‘tick position’), which are collected during the capture event. Further, also tables to store data from pathogens’ screening (‘laboratory analyses’) and animals’ dissection (‘autopsy’) are included. Moreover, the database stores information on the trapping protocol (‘sampling design’) and the geographic and topographic characteristics of the trapping grids (‘plot’) and of the study areas (‘site’). We decide to create also microclimatic (‘weather’) and microhabitat (‘land-use’, ‘vegetation’, ‘productivity’) tables.

The structure of the database with the multiple look-up tables is illustrated in Figure 6.

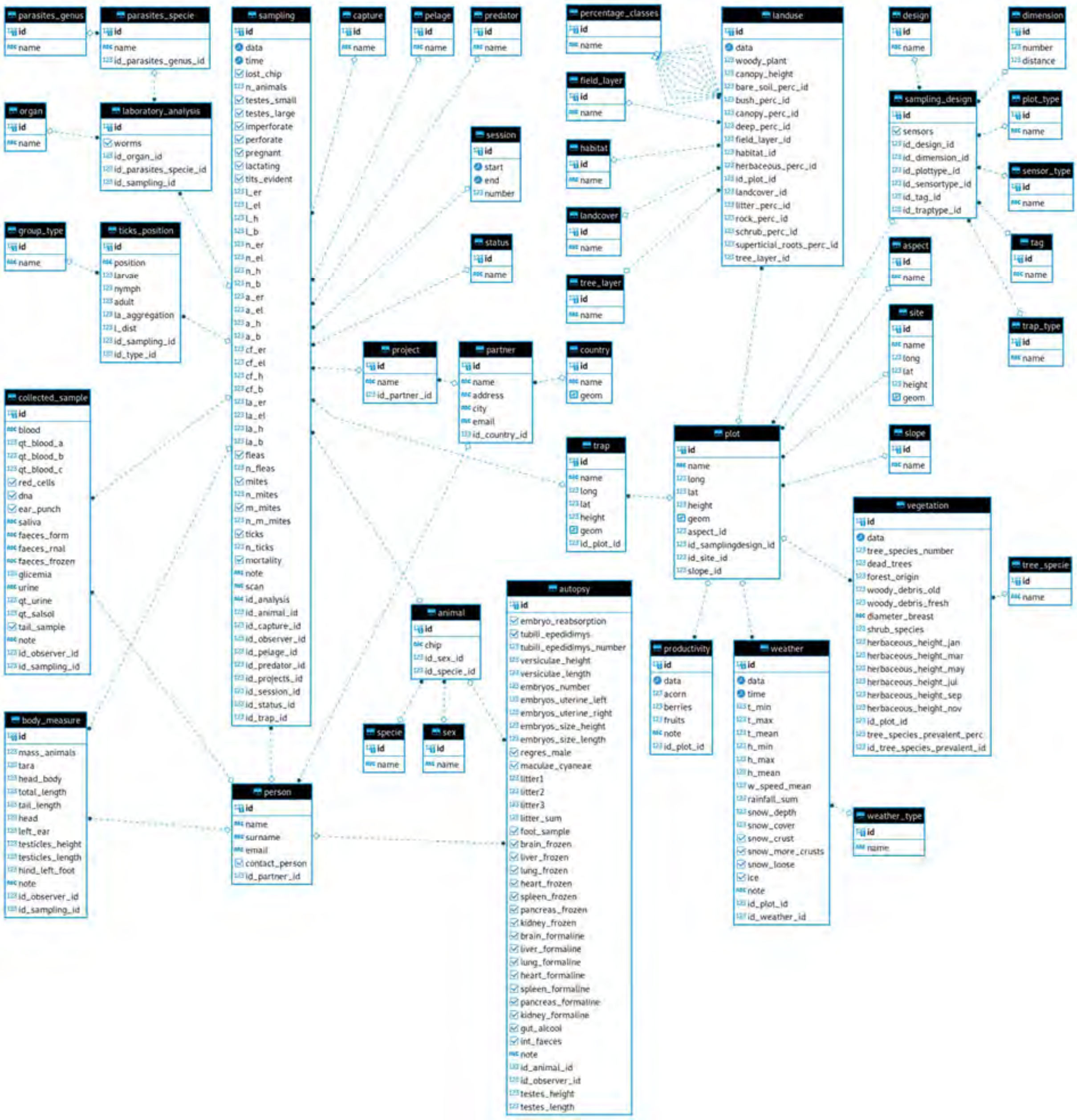

Figure 6: EuroSmallMammals database model structure.

2.7.2 EuroSmallMammals initiative

Beyond the benefits derived from storing data within a repository (see 2.7.1 section), we invested time to laid the basis for a new European network, which aims in pioneering collaborative science and data sharing in small mammals' ecology. In this sense, we took advantage of the collaboration between Inland Norway University of Applied Sciences and Edmund Mach Foundation to found a new branch within the Euromammals project (https://euromammals.org/; Urbano and Cagnacci 2021) dedicated to small mammals. In particular, EuroSmallMammals has the intent of promoting collaborative open science and networking among scientists involved in small mammal ecology across Europe, for connecting different bottom-up projects and addressing unforeseen questions on small mammals' ecology.

EuroSmallMammals collaborative network is constantly expanding throughout Europe (see Table 3) and involves partners that cover different fields of interest, e.g. population demography and dynamics, epidemiology, conservation genetics, community composition, prey-predator dynamics, and landscape ecology. To promote transparency and reliability among partners, as other Euromammals groups, EuroSmallMammals consortium is based on a form of collaboration that embraces the entire scientific process and is confirmed every time a new theme of research is raised (Terms of Use signed by all partners). Further, EuroSmallMammals relies on an open data repository (see section 2.7.1 and Figure 6 for the database) that stores the data shared by the partners to make them reliable, consistently curated and analysis-ready (Reichman et al. 2011, Costello et al. 2018). Other forms of interaction are also considered relevant within the initiative. For instance, a mailing list including all partners was created for improving efficiency in communication but also for remaining in contact about diverse small mammals-related topics. Additionally, we created a specific website (https://eurosmallmammals.org/), which includes the project aim, scientific purposes and outputs, as well as private pagers used by the partners to have access to the database (future implementation).

To date (April 2022) EuroSmallMammals consortium includes 15 European partners covering 9 countries (Spain and Andorra, France, Germany, Italy, Norway, Poland, Czech Republic, and Albania) extended across a wide gradient of environmental, topographic and climatic contexts (see map Figure 7). The data shared by now (see Table 3), and partially stored in the database, span from 1990 to 2019 and include 48 small mammal species and 70562 capture events across 113 study areas. Other partners, which only recently joined the network, are preparing their data that are going to be included in the database.

By scaling up the knowledge from the individual to the ecosystem (Hampton et al. 2013) and by sharing data and expertise on small mammals' populations, the EuroSmallMammals initiative could give the opportunity to fill some important knowledge gaps and provide novel perspectives to evaluate the effect of global changes on species distribution, ecosystems functioning and eco-epidemiological aspects.

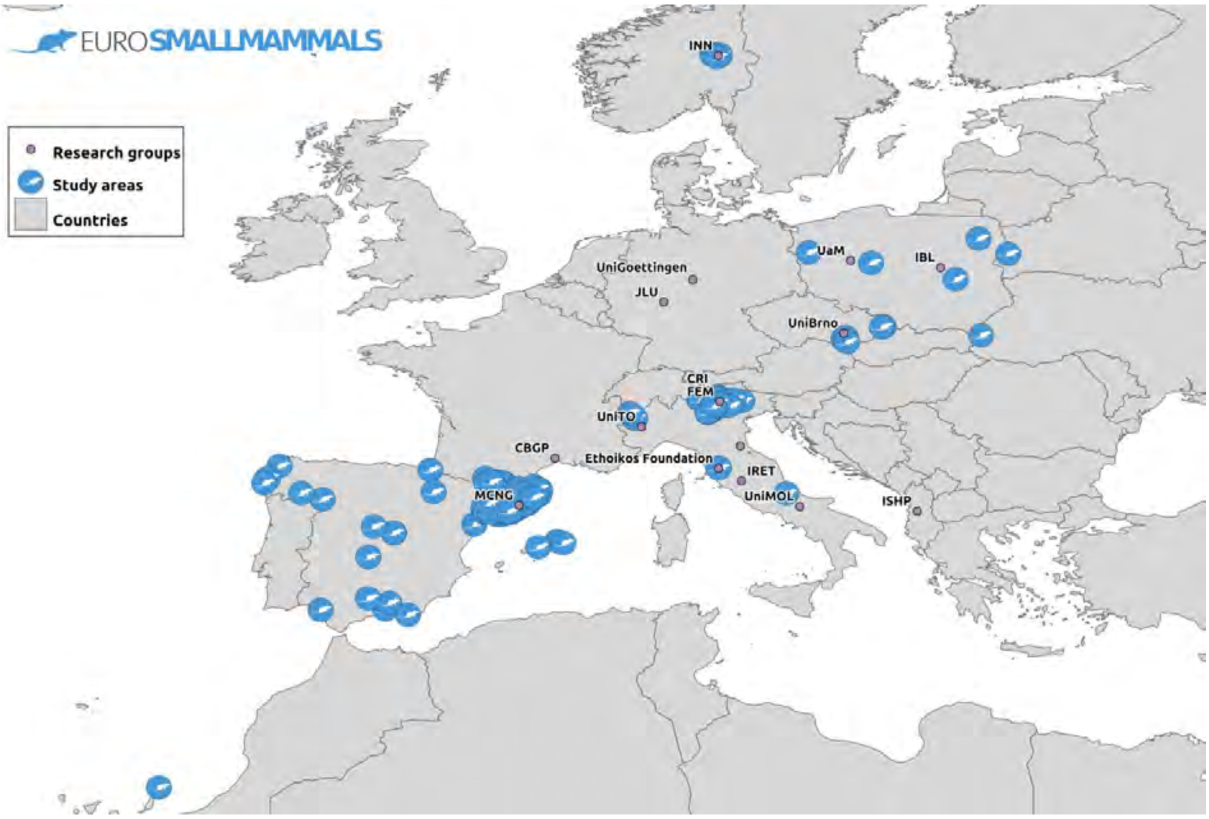

Figure 7: Distribution of the EuroSmallMammals partners, as of June 2022.

Table 3: Summary of the data collected so far (April 2022) from EuroSmallMammals partners. Other partners did not yet share raw data but signed the Terms of Use for collaboration.

Country	Region	Year of sampling	#Study areas	#Species	#Capture events
Czech Republic	Lowland	2002 - 2012	3	14	7437
	Mountain	2005 - 2018	2	16	2137
Italy	Abruzzo	1990 - 1995 2000 - 2005	1	8	3340
	Piemonte/Valle d'Aosta	2015	3	19	1184
	Toscana	2011 - 2014	1	3	2890
	Trentino	2019 - 2021	2	11	2247
Norway	Birkebeinervegen	2011 - 2019	1	7	1137
	Innlandet	2013 - 2016 2017 - 2019	3	5	11158
Poland	Western Poland	2010 - 2013	1	9	8765
	Biebrza	2003-2019	2	12	3488
	Bialowieza Forest	2017 - 2019	1	6	689
Spain and Andorra	across Spain and Andorra	2008 - 2020	93	22	26079

2.8 Statistical methodologies

2.8.1 Multistate Open Robust Design Models (Paper I)

Our field protocol defined by daily secondary occasions within monthly primary ones corresponds to the so-called robust design framework (Pollock 1982). Therefore, we used a multistate open robust design (MSORD) approach to estimate demographic parameters (Kendall and Nichols 2002, Cooch and White 2019) in EVT and CEV study areas. We modelled demographic parameters, namely true and apparent survival (S and φ), capture and arrival probability (p and $pent$) and transition probability (ψ), in dependence on extrinsic bottom-down (i.e. food availability) and top-down (i.e. climate severity) factors, while controlling for seasonal variation and sympatric species co-occurrence. From these models, we derived also monthly population size. We fitted the models with the RMark (Laake 2013) and used the AICc (corrected

Akaike’s Information Criterion) for model selection (Burnham and Anderson 2002) with a threshold of ΔAICc ≤ 4.

2.8.2 Spatially Explicit Capture Recapture Models (Paper II)

We used spatially explicit capture-recapture (SECR) models (Borchers and Efford 2008) to estimate rodent density in CEV study area, across the primary occasions and separately for the rodent species, *Apodemus* spp. and *Myodes glareolus*. We combined the spatial information of grid array and trap detectors with the capture-recapture history of animals, also overlapping a habitat mask based on vegetation cover, to estimate SECR parameters: encounter rate (*g0*), spatial range (σ) and density (*D*) (Efford 2018). This set of detection parameters was modelled in dependence of spatial (i.e. supplemental food availability and vegetation cover) and temporal (i.e. primary occasions) covariates using exponential detection function. All models were fitted with secr (Efford 2020) and we performed model selection based on AICc scores.

2.8.3 Generalized Linear Mixed Models (Paper II, III and IV)

Within the thesis, we have applied Generalized Linear Mixed Models (GLMMs; Bolker et al. 2009) and Generalized Linear Models (GLMs; Crawley 2013) to assess the variation of response variables in dependence on explanatory variables that were biologically meaningful to address our working hypotheses.

In Paper II, we selected *a priori* the data collected during summer and on *Apodemus* spp. in CEV study area and we modelled tick burden as a function of vegetation cover at ground level, rodent density, controlling for tick density in the environment and body condition. We assumed negative binomial distribution of errors and fitted random effect on trap and primary trapping occasions. In Paper III, we investigated the role of altitude in shaping small mammal abundance by fitting GLMMs with negative binomial distribution on small mammal abundance, considering the altitude as the main covariate, together with small mammal species, while we controlled for temporal variation of year and trapping month. Finally, we included the trapping grid as random effect. Moreover, we also assessed the effect of altitude, small mammal species and abundance on the probability of infection of small mammals with *Hepatozoon* spp. and *Borrelia* spp. The infection with *Hepatozoon* spp. was evaluated by fitting GLMMs with binomial distribution of errors and random effect on trap, while infection with *Borrelia* spp. was modelled with GLMs, applying binomial distribution of errors on the probability of infection. In Paper IV, we focused only on *Hepatozoon* spp. to evaluate the role of rodent species and environmental context on the probability of infection with these parasites, controlling for life history traits. Specifically, we applied two analytical designs: (i), we evaluated the effect of anthropogenic pressure on rodent infection by comparing the study areas (CEV: high pressure; CAV: low pressure), only for homogeneous altitude; (ii), we assessed how the probability of infection would vary across species along the alpine altitudinal gradient in particularly wild context (CAV). To this end, for both analyses, we fitted GLMMs with binomial distribution of errors to the probability of infection, in dependence on anthropogenic pressure and altitudinal belts, respectively, for the two designs, as the main covariates together with species. Finally, to account for spatial autocorrelation between captures we included grid as the most parsimonious random effect.

In all the cases described, starting from the full model, we performed a model selection ranked based on the AICc. The model with the lower AICc was selected as the best model.

2.8.4 Stepwise Redundancy Analyses (Paper III)

In Paper III, we examined the relationship between small mammals' assemblage and alpine altitude with ordination method. Since the length of the first axis of the Detrended Correspondence Analysis (Hill and Gauch 1980) was below three standard deviation units, we applied a Stepwise Redundancy Analyses (RDA; (ter Braak 1995) to determine the pattern of variation in small mammal species and assemblage across altitudes.

2.8.5 Pathogens' prevalence (Paper III, IV)

In Paper III and IV, we estimated prevalence of rodent and vector-borne pathogens with 95% Confidence Interval (CI) by using EpiR package (Carstensen et al. 2021) for each small mammal species in both CEV and CAV study areas. We applied the Two Proportion Z-test to analyse the differences in prevalence rates among rodent species only for *Hepatozoon* spp.

3. Results and Discussion

3.1 Monitoring data

3.1.1 Small mammals' captures

Overall in Norway, at EVT study area, 983 bank voles (*Myodes glareolus*) were captured accounting for 4156 total capture events from December 2013 to August 2015.

Conversely, in the two Italian study areas, we captured a broad spectrum of small mammal species. In particular, in CEV, a total of 507 individuals, belonging to yellow-necked mouse (*Apodemus flavicollis*), wood mouse (*Apodemus sylvaticus*) and bank vole (*M. glareolus*) were captured 1422 times during the period February 2019 – April 2021. In CAV, 322 individuals belonging to snow vole (*Chionomys nivalis*), field vole (*Microtus arvalis*, *Mi. agrestis*, *Mi. subterraneus*), mice (*A. flavicollis* and *A. sylvaticus*) and bank vole (*M. glareolus*) were captured 823 times in total from May to October 2019 and 2020. Additionally in CAV and CEV, shrews were identified as common shrew (*Sorex araneus*), alpine shrew (*S. alpinus*), pygmy shrew (*S. minutus*) and bicoloured-shrew (*Crocidura leucodon*) (see Table 4, for details).

3.1.2 Ticks and pathogens data

In Italian study areas, a total of 5573 (1790 in CEV and 3783 in CAV) ticks belonging to the genus *Ixodes* were counted on captured rodents. In particular, we recorded 5462 larvae (98.0%), 109 nymphs (1.9%) and only sporadic adults (2 adults, one in CEV and one in CAV). We also recorded the number of co-feeding groups, (i.e. when ticks at nymphal and larval stages feed in spatio temporal proximity to each other on the same host) (see Table 5). Moreover, in CEV, we estimated an average density of 0.1 ticks/m^2 in May 2019 and 0.07 ticks/m^2 in May 2020.

From blood and ear tissue analysed samples, we detected occurrence of viruses (Tick-borne encephalitis, TBEV; Dobrava-Belgrade virus, DOBV; and Puumala virus, PUUV), bacteria (*Borrelia* spp. and *Anaplasma* spp.) and protozoans (*Hepatozoon* spp. and *Babesia* spp.) in both CEV and CAV. Specifically, we found rare prevalence of TBEV, PUUV and DOBV, while we recorded high prevalence of *Hepatozoon* spp. and *Borrelia* spp. Infection rates with *Babesia* and *Anaplasma* were low (see Table 6 for details).

Table 4: Number of captured small mammal individuals in the three study areas (EVT = Evenstad – Norway, 2013-2015; CEV = Cembra Valley and CAV = Calamento Valley – Italy, 2019-2021) considering species, altitude (m a.s.l.) and food experimental manipulation (Yes = supplemental food, No = no supplemental food, - = no experiment).

Study area	Altitude (m a.s.l)	Feeding	*A. flavicollis*	*A. sylvaticus*	*M. glareolus*	*C. nivalis*	*Mi. arvalis*	*Mi. agrestis*	*Mi. subterraneus*	*S. araneus*	*S. alpinus*	*S. minutus*	*Cr. leucodon*
EVT								0	0	0	0	0	0
	255 – 700	No	0	0	537	0	0	0	0	0	0	0	0
	255 – 700	Yes	0	0	446	0	0	0	0	0	0	0	0
Total			0	0	983	0	0	0	0	0	0	0	0
CEV													
	1000	No	156	8	82	0	0	0	0	1	0	0	0
	1000	Yes	232	0	29	0	0	0	0	1	0	0	0
Total			388	8	111	0	0	0	0	0	0	0	0
CAV													
	500	-	80	2	0	0	0	0	0	0	0	0	2
	1000	-	45	0	52	0	0	0	0	3	2	1	0
	1500	-	34	0	9	0	0	1	0	6	0	0	0
	2000	-	2	0	33	16	2	0	2	19	1	0	0
	2500	-	0	0	1	8	0	0	0	1	0	0	0
Total			161	2	95	24	2	1	2	29	3	1	2

Table 5: Total ticks based on their life stages and co-feeding groups recorded in the Italian study areas (CEV = Cembra Valley and CAV = Calamento Valley) in 2019-2021. Altitude (m a.s.l.) and food experimental manipulation (Yes = supplemental food, No = no supplemental food, - = no experiment) are showed.

Study area	Altitude (m a.s.l.)	Feeding	Total ticks	Total larvae	Total nymphs	Total adults	Co-feeding
CEV							
	1000	No	673	645	28	0	4
	1000	Yes	1117	1099	17	1	3
Total			1790	1744	45	1	7
CAV							
	500	-	2197	2170	27	0	12
	1000	-	1415	1384	30	1	14
	1500	-	144	140	4	0	0
	2000	-	21	18	3	0	2
	2500	-	6	6	0	0	0
Total			3783	3718	64	1	28

Table 6: Results (positive cases / total analysed samples and prevalence in brackets) of the serological and molecular screening of pathogens detected in small mammals in the Italian study areas (CEV = Cembra Valley and CAV = Calamento Valley) in 2019-2021. Altitude (m a.s.l.) and food experimental manipulation (Yes = supplemental food, No = no supplemental food, - = no experiment) are showed. TBEV = tick-borne encephalitis virus; DOBV = Dobrava-Belgrade hantavirus; PUUV = Puumala hantavirus.

Study area	Altitude (m a.s.l.)	Feeding	TBEV	DOBV	PUUV	*Hepatozoon* spp.	*Babesia microti.*	*Anaplasma phagocytophilum.*	*Borrelia* spp.
CEV									
	1000	No	4/456 (0.87)	3/258 (1.16)	3/198 (1.51)	114/245 (46.53)	5/245 (2.04)	7/245 (2.85)	42/245 (17.14)
	1000	Yes	2/361 (0.55)	5/321 (1.55)	0/42	75/261 (28.73)	3/261 (1.14)	0/261	34/261 (13.02)
Total			6/817 (0.73)	8/579 (1.38)	3/240 (1.25)	189/506 (37.35)	8/506 (1.58)	7/506 (1.38)	76/506 (15.01)
CAV									
	500	-	0/150	2/150 (1.33)	0/1	13/89 (14.60)	0/89	0/89	17/89 (19.10)
	1000	-	0/145	0/60	0/86	47/103 (45.63)	0/103	4/103 (3.88)	24/103 (23.30)
	1500	-	0/64	0/49	0/16	11/50 (22.00)	0/50	0/50	4/50 (8.00)
	2000	-	0/66	1/19 (5.26)	1/64 (1.56)	24/72 (33.33)	7/72 (9.72)	17/72 (23.61)	0/72
	2500	-	0/15	2/15 (13.33)	2/15 (13.33)	3/10 (30.00)	3/10 (30.00)	2/10 (20.00)	0/10
Total			0/440	5/293 (1.70)	3/182 (1.65)	98/324 (30.24)	10/324 (3.08)	23/324 (7.09)	45/324 (13.88)

3.2 Food resources drive rodent population demography mediated by seasonality and inter-specific competition (Paper I)

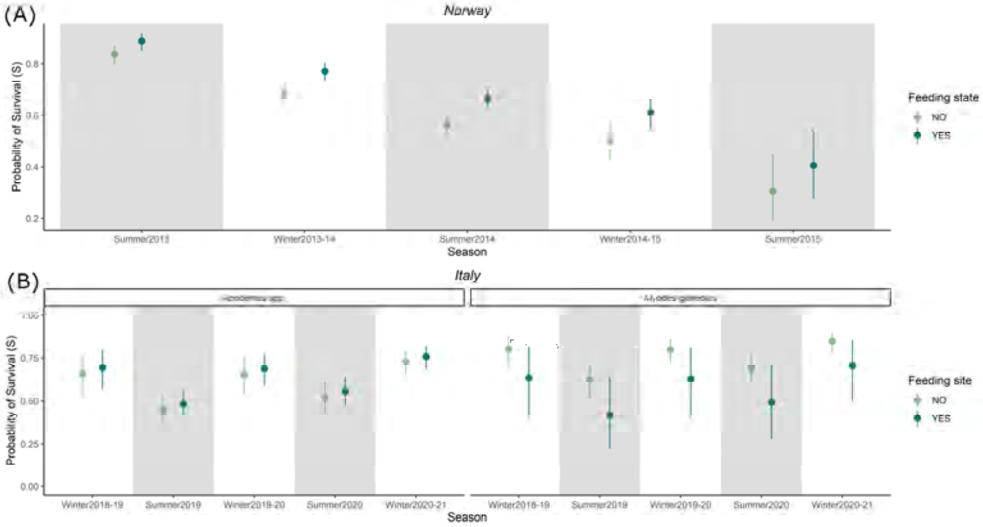

Figure 8: Survival estimates (S) of rodents in Norway (Evenstad, EVT) (**A**) and Italy (Cembra Valley, CEV) (**B**) by species and seasons, under food manipulation (supplemental food available = dark green; no supplemental food = light green) conditions ('Feeding state' for Norway and 'Feeding site' for Italy). Summer months are grey-shaded.

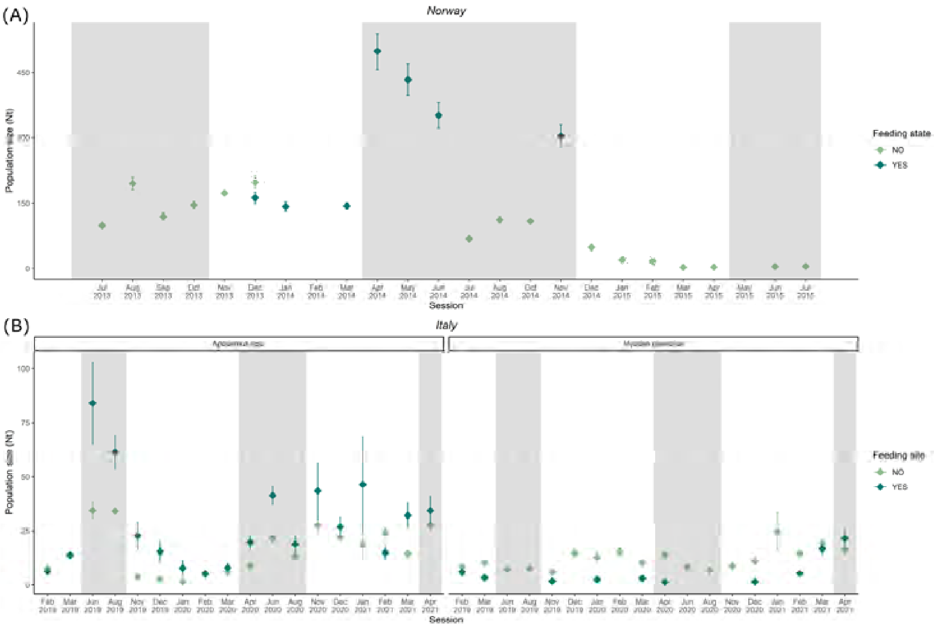

Figure 9: Population size (Nt) of rodents in Norway (Evenstad, EVT), 2013-2015 (**A**) and in Italy (Cembra Valley, CEV), 2019-2021 (**B**), under food manipulation (supplemental food available = dark green; no supplemental food = light green) conditions ('Feeding state' for Norway and 'Feeding site' for Italy). Summer months are grey-shaded.

By considering a niche-based framework, we found evidence that bottom-up processes, and specifically food availability, crucially affect woodland rodent demography, and that this effect is mediated by top-down extrinsic drivers, such as climate severity, as well as by intrinsic seasonal cycles and the demography of competitor species. We observed similar seasonal patterns of survival at both latitudes and across species (Figure 8A and Figure 8B). In particular, survival increased in winter and decreased in summer, suggesting that it was governed by physiological and behavioural seasonal cycles (Eccard and Herde 2013, Olenev and Grigorkina 2014). Meanwhile, the effect of food availability on survival emerged when unfavourable conditions occurred. Specifically, with harsh climate, *M. glareolus* survival increased with food availability in Norway (Figure 8A) because its energetic and nutritional needs were fulfilled by supplemental food (Ylönen and Eccard 2004, Ergon et al. 2011). Observing the population size, we assessed that population of *Apodemus* spp. in Italy and of *M. glareolus* in Norway complied with the typical rodent demography (Figure 9A and Figure 9B), with an increase during summer and a decline during winter (Gasperini et al. 2016, Johnsen et al. 2018). Meanwhile, *M. glareolus* in Italy exhibited an opposite pattern, with an increase in winter and a decline in summer, suggesting a niche partitioning over the temporal scale (Viviano et al. 2022), since this generalist species is able to exploit extreme abiotic winter conditions (Markova et al. 2018). These demographic patterns were generally enhanced by food resource availability (Figure 9A and Figure 9B), probably due to reduction of home ranges (Tioli et al. 2009) that may alter dispersal mechanisms (Le Galliard et al. 2012).

However, the benefits ensured by food availability both on survival and on population size collapsed when sympatric species co-occurred, as observed in Italy (Figure 8B and Figure 9B). In particular, where supplemental food was provided, niche partitioning between *Apodemus* spp. and *M. glareolus* failed and triggered antagonistic interactions between them. Specifically, supplemental food favoured *Apodemus* spp., promoting abundance and so buffering the winter effect. On the contrary, at feeding sites, demography of *M. glareolus* resulted depressed (a decrease in survival and nearly absent abundance). Thus, where food was abundant and confined, the dominant *Apodemus* spp. outcompeted the subordinate *M. glareolus* (Grüm and Bujalska 2000) by means of behavioural interference (i.e. decrease in population size; Figure 9B) coupled with exploitative competition (i.e. depressed survival; Figure 8B).

3.3 Is environmental heterogeneity driving host-parasite interactions? Evidence from a field manipulation in a deer-rodent-tick system (Paper II)

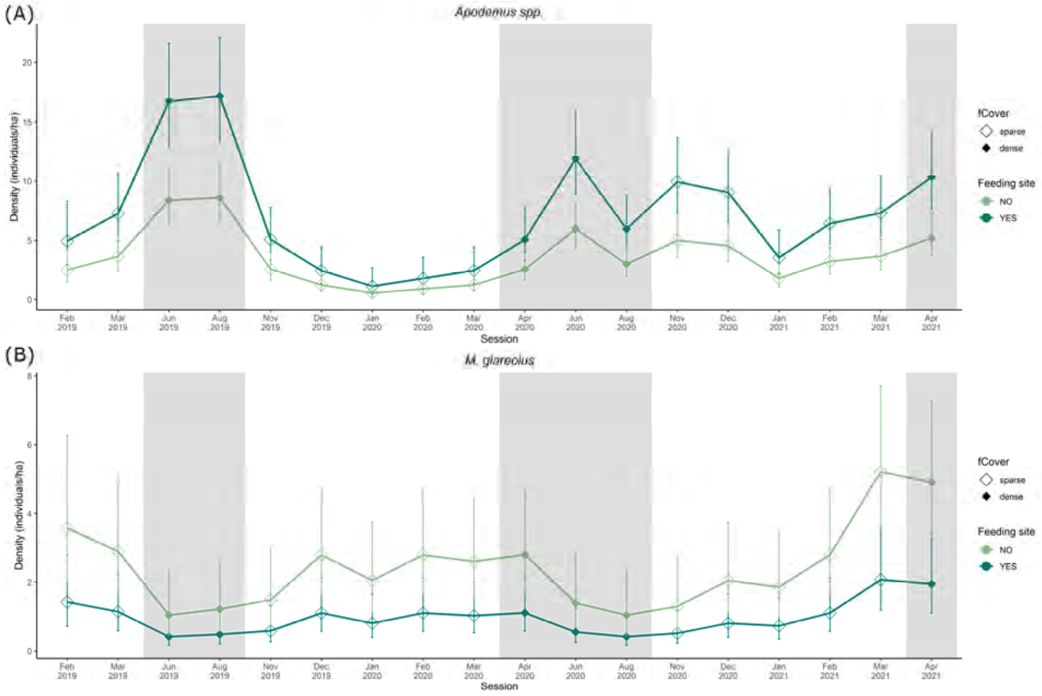

Figure 10: Density (*D*) of *Apodemus* spp. (**A**) and *M. glareolus* (**B**) in Cembra Valley (CEV), Italy, 2019-2021 at Treatment (‘Feeding site: yes’; dark green) and Control (‘Feeding site: no’; light green) sites and under varying vegetation cover (‘fCover’; dense 67% = filled diamonds; sparse 47% = empty diamonds). Summer months are grey-shaded.

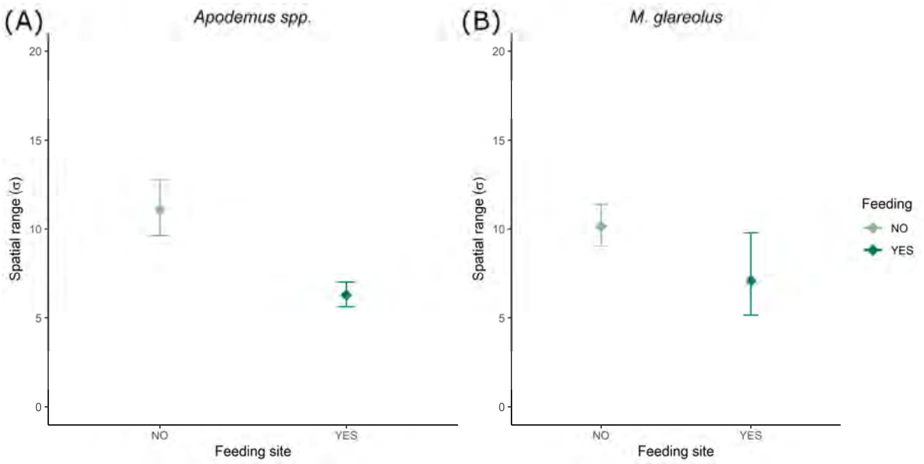

Figure 11: Estimates of spatial range (σ) in *Apodemus* spp. (**A**) and *M. glareolus* (**B**) in Cembra Valley (CEV), Italy, 2019-2021, at Treatment (‘Feeding site: yes’; dark green) and Control (‘Feeding site: no’; light green) sites.

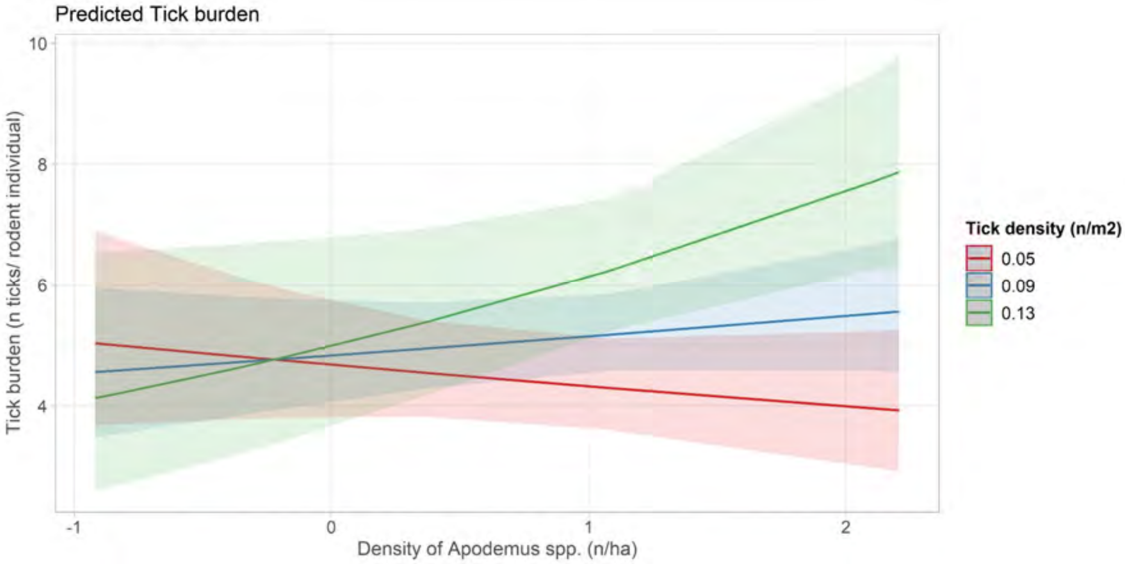

Figure 12: Predictive plot showing the effect of the interaction between rodent density and tick density on individual tick burden. The coloured lines denote the effects for three values of tick density chosen for visualisation (mean value ± standard deviation), with the corresponding coloured shaded areas referring to the 95% confidence intervals.

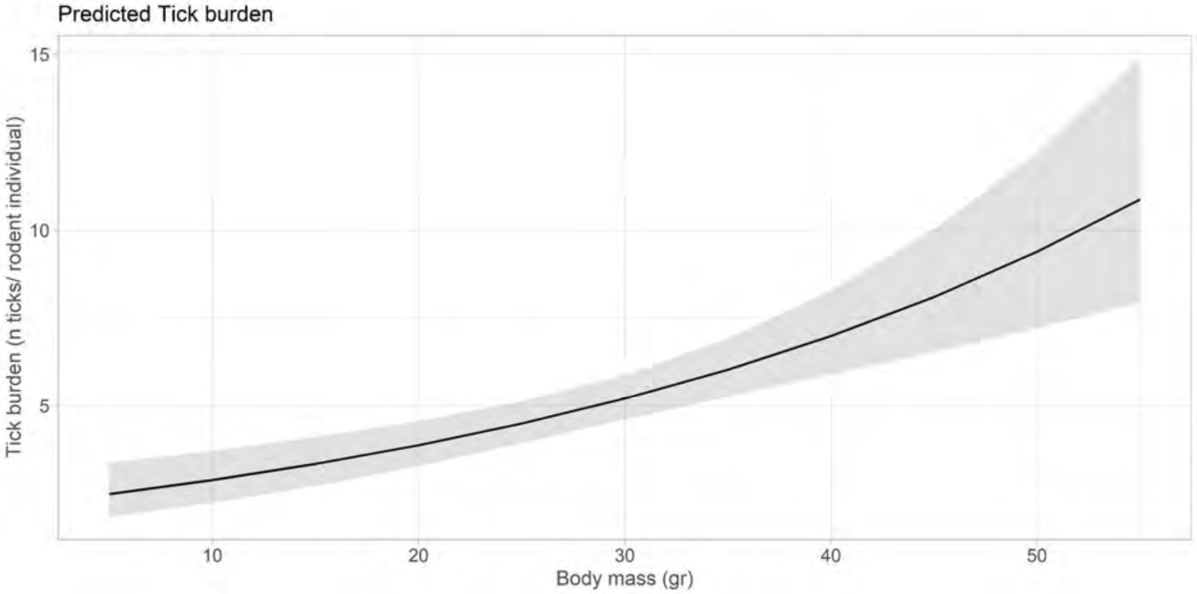

Figure 13: Predictive plot showing the effect of host body mass on individual tick burden. The black line is the average value while the grey shadow represents the 95% confidence intervals.

In Paper II, we highlighted the cascading mechanisms underpinning host and tick association, confirming that tick burden represents the obvious, but central factor measuring the parasitic interaction with the harbouring hosts. Specifically, when food availability was abundant and concentrated (e.g. at ungulate feeding sites) animals tended to decrease their dispersal range and mobility during foraging, thus minimizing predator encounters (Mazurkiewicz and Rajska-Jurgiel 1998). This effect was observed at sites with anthropogenic food availability, where local density of rodents (spatially determined) was promoted by restricted home ranges and low mobility (Figure 10 and Figure 11) (Stradiotto et al. 2009). However, anthropogenic food affected also rodent community (low evenness) with dominant and abundant *Apodemus* spp. depressing density of *M. glareolus* (Figure 10). This finding supported the competition hypothesis proposed in Paper I.

We found that as density and aggregation of *Apodemus* spp. increased, tick burden was amplified, but only at high environmental tick density (Figure 12). The latter condition was likely favoured by conditions enhancing the completion of the tick life-cycle, e.g. where secondary hosts (deer) aggregation and low evenness of primary hosts (rodents) co-occur (Tagliapietra et al. 2011, Cagnacci et al. 2012), i.e. at ungulate feeding sites (i.e. where food is abundant and clumped). Indeed, at ungulate feeding sites we observed a low evenness of rodent community, with high density and aggregation of rodent species more susceptible to ticks attachment (i.e. *Apodemus* spp.). At these sites, roe deer (*Capreolus capreolus*), i.e. secondary hosts, were also observed to aggregate in space and time (Ossi et al. 2020). Conversely, we obtained evidence that in areas with low tick density in the environment, tick burden is predicted to decline following the ‘encounter-dilution effect’ (Côté and Poulin 1995, Krasnov et al. 2007).

Further, in accordance with other studies (Harrison et al. 2010), we found that body mass was positively correlated with tick burden (Figure 13). Finally, high vegetation cover at the fine-scale was correlated with high population density of rodent species, as a likely perceived protection from predation risk (Loggins et al. 2019), while in compliance with other studies (Ginsberg et al. 2020), vegetation cover did not affect tick burden on hosts.

3.4 Small mammals' assemblage along an altitudinal gradient and consequences on rodent and vector-borne diseases (Paper III)

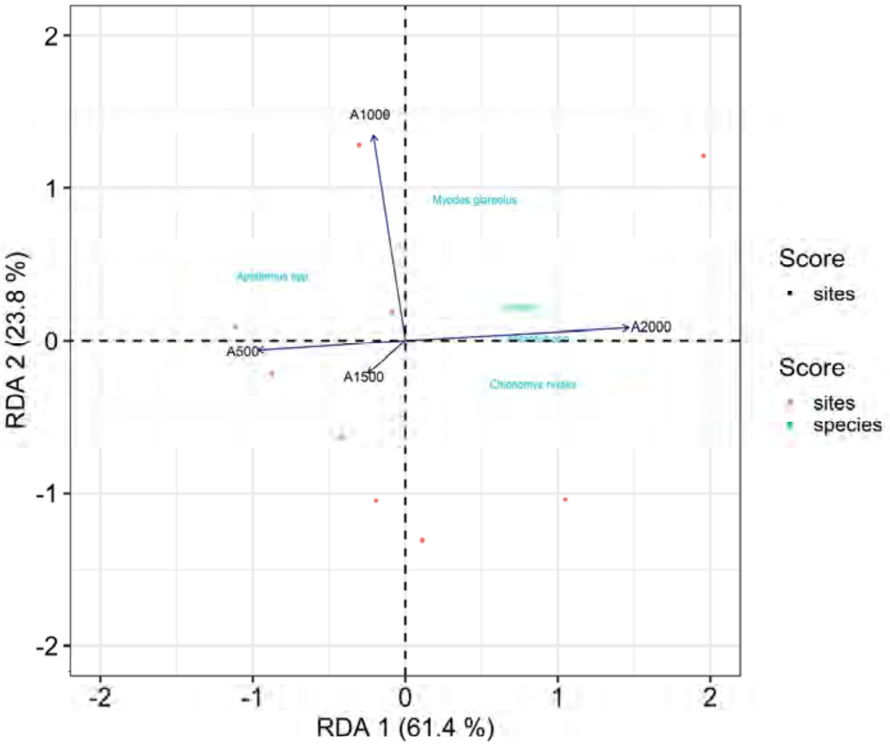

Figure 14: Biplot of the relationship between altitudes and small mammals, as determined by Stepwise Redundancy Analysis (RDA). The length and direction of each vector are proportional to the strength of the association between the altitude and the RDA axes. Data collected in Calamento Valley, CAV, (Italy) 2019-2020.

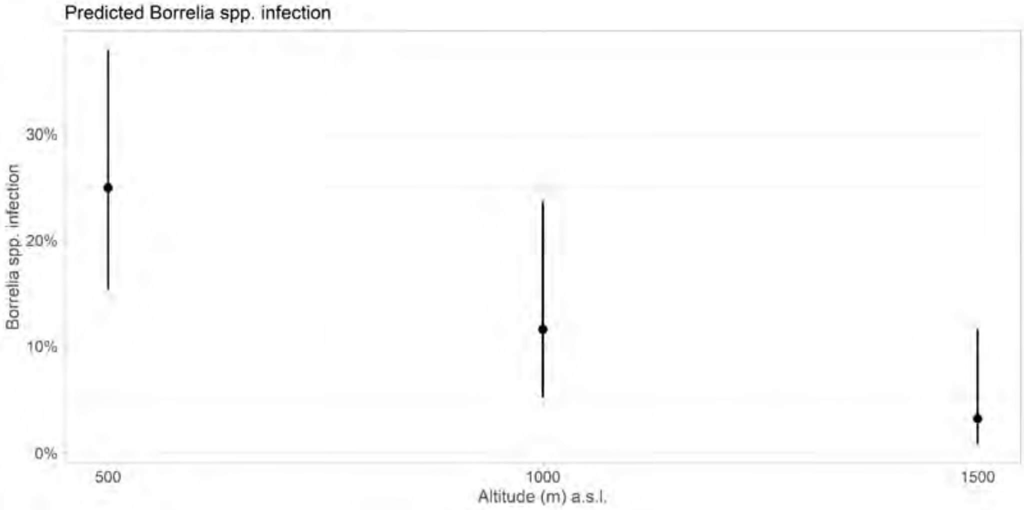

Figure 15: Predictive plot showing the effect of altitude on *Borrelia* spp. probability of infection, after controlling for the effects of other covariates in the Generalized Linear Models. Data collected in Calamento Valley (CAV) study area, 2019-2020.

In Paper III, we assessed that small mammals' assemblage depended on habitat typology and on species' functional role (Figure 14). Indeed, we found an evident division between species belonging to *Muridae* and *Arvicolinae* families, the former more related to lower altitudes and woodland forests, the latter covering the entire alpine succession of habitats (Flowerdew et al. 1985). Among *Arvicolinae*, specialist species, such as *Microtus* spp. and *C. nivalis*, selected ecotone zones and habitats at extreme altitudes (Nappi 2002). Conversely, *M. glareolus* was often associated with wood forests. This generalist species was also able to exploit a wider range of alpine habitats, expanding its distribution toward high altitudes. Indeed, the extreme altitudes of the Alpine succession are now characterized by milder climate (Nogués-Bravo et al. 2007). We argue that this climatic trend may be promoting the geographic expansion of some hosts (Ehrlén and Morris 2015), especially the most generalist, and consequently of parasites with the role of pathogens' vectors (Garcia-Vozmediano et al. 2020). Indeed, all detected vector-borne pathogens were tightly related to the generalist *M. glareolus* (Table 6), confirming its wide susceptibility and competence (Rizzoli et al. 2014). Finally, we observed that common rodent- and vector-borne pathogens seemed to show altitudinal segregation, an aspect that needs further investigation (Figure 15) by applying more complex analytical approaches.

3.5 First record of *Hepatozoon* spp. in Alpine Wild Rodents: Implications and Perspectives for Transmission Dynamics across the Food Web (Paper IV)

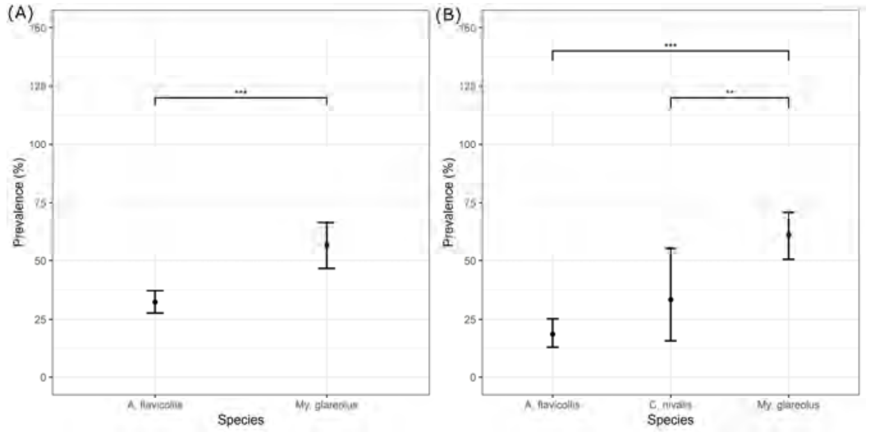

Figure 16: Difference of prevalence rates of *Hepatozoon* spp. among rodent species in Cembra Valley, CEV (A) and in Calamento Valley, CAV (B), Italy (2019-2021). Vertical bars represent the 95% confidence interval. Horizontal bars refer to significant differences, with ***=significance level <0.001; **=significance level <0.05.

By coupling molecular screening approaches with less invasive sampling methods, in Paper IV we reported for the first time the occurrence of *Hepatozoon* spp. in wild rodents in the Italian Alps (see Figure 16 and Table 6). In particular, we detected these parasites in their most common Alpine rodent hosts, *M. glareolus* (61.0%) and A. *flavicollis* (18.4%) (Hamšíková et al. 2016, Baltrūnaitė et al. 2020) (CAV, Figure 16B). In addition, this is their first identification in *A. sylvaticus* (30.0%) using molecular approaches (Healing 1981) and in *C. nivalis* (33.3%), at the highest altitudes ever recorded for the occurrence of this parasite (2000 – 2500 m a.s.l.). Our results suggest that *Hepatozoon* spp. are common in Alpine environments, where the occurring milder climate and a broad spectrum of interactive and mobile hosts (Gilbert 2021) may have favoured their circulation and spread. The low host specificity of *Hepatozoon* spp. (Laakkonen et al. 2001) may also indicate that these parasites persist in the environment sustained by small mammal hosts that act as paratenic and reservoir hosts. Also in peri-urban areas (CEV, Figure 16A), *M. glareolus* resulted the most infected species (56.7%), although also *A. flavicollis* retained a high prevalence (32.2%). These conditions may increase the risk of spillover events among wildlife and domestic animals (Almberg et al. 2012), but also may induce relevant cascading effects on predators across the food web (Maia et al. 2014).

General conclusion and perspectives

In this dissertation, I have empirically confirmed through a latitudinal niche-based comparison that intrinsic and extrinsic factors interplayed in governing small mammal demographic rates and patterns (Krebs 2013). I evidenced that the intensity of the demographic causal relationships tightly depends on the environmental context. Indeed, species-specific intrinsic seasonal cycles that usually govern demography (Flowerdew et al. 1985, Eccard and Herde 2013) were affected by supplemental food resources availability only under harsh climatic conditions for what concerns survival, while population abundance was enhanced by food across contexts. However, the stability of these regulatory mechanisms, which permit niche partitioning among sympatric species (Chase and Leibold 2003), failed when interspecific competition arose, triggered by clumped overabundance of food resources. This resulted in a modification of the realized niche of subordinate species (e.g. bank vole) to the advantage of opportunistic and dominant ones (e.g. woodland mice), a probable scenario which can also be predicted under climate warming. These findings thus highlighted the complexity of such demographic relationships, which can be revealed only using a niche-based framework that simultaneously accounts for the relative impacts of intrinsic, extrinsic factors and biotic interactions on small mammals' demography.

When I explicitly accounted for the spatial components of these demographic patterns, I found that small mammals decreased their spatial range in presence of anthropogenic supplemental food. In turn, the spatio-temporal variation of small mammal species assemblage and population density, driven by supplemental food, showed cascading effects on their parasite burden. By considering ungulate feeding sites as an experimental source of concentrated food, I elucidated the mechanisms underpinning the pathway of deer-rodent-tick association with respect to food resources distribution, complementing what emerged in oak forest monitoring acorn production (Ostfeld et al. 2006). In particular, I identified local density of hosts and environmental tick density, mediated by hosts' body condition as the main drivers of tick burden variation in rodents, in turn, regulated by environmental heterogeneity and availability of food resources. Our study system can be considered as an experimental simplification of what is observed in human-dominated landscapes, especially in residential areas (VanAcker et al. 2019), where high local density and low evenness of hosts enhance the completion of tick life-cycle may create a 'self-inflating' system boosting their tick burden. These findings would therefore open concerns about the increase of enzootic hazards and human exposure to tick-borne disease risk in those focal areas (Altizer et al. 2018, Diuk-Wasser et al. 2021).

Further to these experimentally controlled conditions, I therefore attempted to link small mammals' assemblage with rodent- and tick-borne pathogens' occurrence along a complex gradient of environmental heterogeneity such as the Alpine altitudinal gradient. Although the pathogens were unevenly distributed across alpine habitats, an aspect that needs further investigation, they were all harboured by bank vole as the main competent reservoir. This work may preliminarily corroborate the hypothesis for which climate-driven expansion of habitat generalist species, such as bank vole may introduce new pathogens in the sylvatic cycle. Indeed, climate change promotes communities dominated by generalists, which, first, force specialists to move to the edges of their distribution (Clavel et al. 2011), and second, carry the most viral pathogens (Yabsley and Shock 2013, Rizzoli et al. 2014, Rosso et al. 2017).

High tick burden on rodents leads to an increased probability of host infection by tick-borne pathogens (Calabrese et al. 2011). However, beyond transmission occurring through ticks' bites, infections may emerge through direct ingestion of infected ticks (Johnson et al. 2010), such as for *Hepatozoon* spp. By applying an accurate identification (via PCR-based methods coupled with sequencing), I successfully detected *Hepatozoon* spp. in a wide range of small mammals' species, and in susceptible Alpine hosts for the very first time. Therefore, I contributed to the understanding of *Hepatozoon* spp. distribution across habitats so far unexplored, such as the Italian Alps, and across a broad range of small mammal hosts. These findings may suggest that the widespread occurrence of this parasite is linked to the expanding availability of arthropod vectors, in turn, due to the variations in temperature and moisture observed under climate change (Gilbert 2021). *Hepatozoon* spp. distribution across a wide range of small mammal hosts and alpine habitats may therefore represent the starting point to evaluate the ecological role of these parasites and other pathogen distributional shifts in habitats particularly exposed to climate change (Battisti et al. 2020).

Overall, this dissertation represents an 'eco-epidemiological bridge' between classical pillars of ecological research, such as fitness (survival), spatio-temporal demographic cycles and inter-specific competition, and current directions of investigation, which include anthropogenic effects, and environmental heterogeneity gradients. To do so, I evaluated habitat heterogeneity and the role of availability and concentration of food resources at multiple scales, by investigating community distribution and species demography of small mammals across latitudinal and altitudinal gradients, as well as by applying field experiments in wild settings. I elucidated the cascading effects of the ecological relationships between environmental factors and small mammal populations on the dynamics of host-parasite association, and ultimately on the emergence and occurrence of rodent- and vector-borne pathogens. I investigated these complex ecological relationships by adopting a niche-based multi-factorial approach, coupled with advanced analytical tools and biological techniques. Despite the advanced provided by regional and local study empirical designs, long-term comparative studies are likely crucial to generalize these relationships and obtain robust predictions within the context of climate change and habitat loss. To this end, collaborative projects, such as EuroSmallMammals, may open future opportunities to scale up knowledge from local to global scale (Urbano and Cagnacci 2021), taking into consideration climate instability.

Acknowledgments

This work was financially supported by the PhD Programme of Inland Norway University of Applied Sciences, Faculty of Applied Ecology, Agricultural Sciences and Biotechnology and by Fondazione Edmund Mach, Research and Innovation Centre. The multi-disciplinary and the international contexts deeply rooted in both institutions characterized this research project.

I want to express my deepest gratitude to my supervisors, Olivier Devineau (main supervisor), Valentina Tagliapietra (co-supervisor) and Francesca Cagnacci (co-supervisor), for the invaluable moments we passed together discussing on science and for the immense knowledge you transmitted to me. You allowed me to grow both professionally and personally, thank you for always being there. To Olivier, for your patience during data analysis and the constant support you gave me during the entire PhD period, with the hope of working together ever more in person and no more through remote calls. To Valentina, for sharing with me your decennial experience on these tiny creatures (ticks included), and for spending together unforgettable days both in the labs and, especially during trapping, also under snowfall on Ziolera peak. To Francesca, for accompanying me during this memorable path, encouraging during the difficult periods and teaching so much about collaboration in science, empathy and the extraordinary world, which is animal ecology; you remain a guide and a friend. Finally, I want to thank the always beloved Harry P. Andreassen, with whom I had not have the possibility to share these moments, but the small time we had together remained a milestone in my professional life.

I would like to thank Daniele, Enrico, Federico, Michele, Sara, Simone and Valentina for your great effort during small mammals' trapping and fieldwork in general. Wherever we were, from the Cembra woodlands to the higher peaks in Calamento, with any type of weather, those memories and laughing will last in my memory, without forgetting our 'end-up-something' dinners.

A sincere thank goes to Matteo and Fausta, you were extremely patient and thanks to the knowledge you have transmitted to me on laboratory analyses, I was able to enforce and expand my professional background.

A special thank goes to the colleagues from the Applied Ecology, Animal Ecology and Conservation Genetics units, you are so many and I owe something to each of you.

Among others, I am grateful to the friends and colleagues from Evenstad, Magne, Kaja, Ilaria, Natalia, Giorgia and Jeremy, and from the FabLab, Andrea, Steffen, Johannes, Benjamin, Charlotte, Simone and Gioele, for your valuable scientific support along this period and for sharing memorable time whether we were in the office or during skiing, hiking, biking, swimming or barbecuing.

I am extremely grateful to people from the administration/department/innovation centre, and among others, Elisabetta, Marisa, Tiziana, Lisbeth, Ragnhild and Henriette for their constant availability and kindness, helping me with any doubts I had (and they were many) and their inestimable support during Covid-19 pandemic.

My gratitude goes also to Francesca, Luca and Ferdinando, and the partners of EuroSmallMammals, you have trusted in this idea and permitted the creation of this project. I am looking forward to deeply collaborating with you to promote open and innovative science on small mammals' ecology.

Finally, my deepest grateful goes to my family and my husband, and here I want to switch to my Italian... Ai miei genitori e a mia sorella, che hanno reso possible tutto cio', sostenendomi da sempre ed invogliandomi a perseguire i miei sogni, trasmettendomi la loro sconfinata passione per la natura. Infine al mio caro Federico, grazie per aver condiviso con me questa esperienza straordinaria, seppur con alcuni momenti bui (rottura di ginocchio e di una caviglia, ed una pandemia). Mi sei sempre stato accanto rendendo questo percorso ancora piu' speciale ed unico, rimarra' un ricordo intenso collegato con la nostra passione per la montagna.

Giulia Ferrari

Ferrari Giulia, 10th May 2022

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Dissertation articles

I

Food resources drive rodent population demography mediated by seasonality and inter-specific competition

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Complete manuscript ready for submission

Abstract

1. As fast reproducing species, rodents present proximate numerical responses to resource availability that have been assessed by experimental manipulation of food, with contrasting results. Other intrinsic and extrinsic factors, such as climate severity, species life cycles, and sympatry of potential competitors in the community, may interplay to modulate such responses, but their effects have rarely been evaluated *ensemble*.
2. We applied a niche-based approach to experimentally determine the effect of bottom-up (food availability) and top-down (climate severity) extrinsic factors, as well as intrinsic seasonal cycles, on rodent demography, also in presence of sympatry between species in the community.
3. To this end, we live-trapped rodents at two latitudinal extremes of the boreal-temperate gradient (Italian Alps and Norway) deploying control/treatment designs of food manipulation. We applied a multistate open robust design model to estimate population patterns and survival rate.
4. Yellow-necked and wood mouse (*Apodemus* spp.) were sympatric with bank vole in Italy, while the latter was the only species trapped in Norway. At northern latitudes, where harsher climatic conditions occurred, vole survival was principally regulated by intrinsic seasonal cycles, with a positive effect of food also on population abundance. At southern latitudes, mice and voles exhibited asynchronous population patterns across seasons, with survival depending from seasonal cycles. When concentrated *ad libitum* food was experimentally provided, though, population size and survival of voles strongly decreased, while mice abundance benefited from food supplementation.
5. Our results evidence that rodent demography is regulated by a combination of top-down (climate severity) and bottom-up (food availability) extrinsic factors, together with intrinsic seasonal ones. Moreover, we showed that the seasonal niche partitioning of mice

and voles could be disrupted by availability of abundant resources that favour the demography of the more opportunistic *Apodemus* spp. at the expense of *Myodes glareolus*, suggesting competitive mechanisms. We conclude putting our results in the context of climate change, where shifts in vegetation productivity may affect the diversity of the rodent community via demographic effects.

Keywords: *Apodemus* spp., assemblage, demography, interspecific competition, latitudinal gradient, *Myodes glareolus*, seasonality, supplemental feeding.

1 Introduction

Mammalian populations can be placed along a fast-slow continuum according to their life-history traits (Oli, 2004), metabolism (Lovegrove, 2003) and fertility rate (Oli & Dobson, 2003). Extrinsic factors (bottom-up e.g. food availability, top-down e.g. climate severity or predation), together with intrinsic mechanisms (seasonal cycles, density-dependence), control the dynamics of both large, 'slow-living', and small, 'fast-living' mammals (Odden et al., 2014; Wolff, 1997). However, large mammals have lower basal metabolic rate being related with body mass (Schmidt-Nielsen & Knut, 1984; C. R. White & Kearney, 2013), so that extrinsic perturbations tend to have a delayed effect on individual fitness (e.g. carry-over effect, Harrison et al. 2011; metabolic plasticity, Norin & Metcalfe 2019), hence demography (elasticity patterns, Heppell et al., 2000). Conversely, small mammals show an r-selection strategy with a rapid pace in life-history events (e.g., earlier maturity, higher reproductive rate, shorter generation times and higher basal metabolic rate) (Boyce, 1984), therefore experiencing proximate functional and numerical responses to climatic and environmental changes, such as masting (Zwolak, Bogdziewicz, & Rychlik, 2016), and habitat fragmentation (Bogdziewicz & Zwolak, 2014). For these reasons and due to their wide distribution across diverse habitats and numerous trophic niches, small mammals are considered informative models to disentangle environmental effects on individual fitness, demography and, ultimately, population and community dynamics.

The range of abiotic conditions defining the distribution of small mammal species in absence of other species define their *fundamental niche* (*sensu* Hutchinson, 1957), while in presence of interspecific interactions single populations occur in more restricted 'regions' of such environmental space (*realised niche*; see e.g. Peters et al., 2013). Within these regions, population growth rate, expressed by its demographic components such as survival, somatic growth and fecundity (*demographic niche,* Maguire, 1973) depends on the acquisition of food resources, in presence of competitors, predation and diseases (Chase & Leibold, 2003; Sibly & Hone, 2002). In particular, Sinclair and Krebs (2002) suggested that food supply is the primary factor determining rodent population growth rate and only secondarily it is overridden or severely modified by regulatory top-down processes (e.g. predators, intra-specific social interactions and environmental disturbance such as climate severity). This conjecture was then revised by Krebs (2013), proposing instead a multi-factorial explanation of population dynamics changes as an interaction of top-down and bottom-up mechanisms. More recently Flowerdew et al. (2017) supported the earlier model in which food availability, together with density-dependence, play the strongest role in rodent population growth and reproduction, mediated by weather seasonality and only weakly by predation.

A comprehensive analysis of the factors that regulate population dynamics in small rodents is therefore extremely complex because both reproduction and survival are condition-dependent and therefore regulated directly by intrinsic and extrinsic factors. Specifically, intrinsic factors (i.e. density-dependence, behaviour, physiology and life-history traits) cause variations in survival (Johnsen et al., 2018) and reproduction (Aars & Ims, 2002) year-round. Their effect may then interact with environmental extrinsic conditions, such as climatic constraints (Hoset et al., 2009) and food quality and quantity (Boutin, 1990), to shape small rodents demographic patterns.

Several studies evaluating the proximate importance of food in regulating seasonal demography and multiannual population dynamics used supplemental food provisioning as an experimental approach to control food availability (e. g. Boutin, 1990). These studies found that *ad libitum* supplemental food favored breeding (Krebs & DeLong, 1965), increased reproduction (Desy & Thompson, 1983) and promoted survival (Johnsen et al., 2016) leading to an increase in population density (Taitt & Krebs, 1981; Yoccoz et al., 2001). However, in alternate studies food supplementation decreased survival (Hansen & Batzli, 1978), population density (Krebs & DeLong, 1965), and growth rate (Löfgren et al., 1996).

Thus, previous literature was not conclusive in outlining the relationship between food availability and population parameters and patterns, in small mammals. This relationship might be further complicated by interspecific interactions (e.g. Brown & Munger, 1985). Indeed, when ecologically similar species are sympatric, they can either (i) adjust their foraging tactics, habitat selection or activity patterns (i.e. specialist to generalist and opportunistic continuum) to partition the niche in space or time, and thus facilitate coexistence (Leimgruber et al., 2014; Schoener, 1974); or (ii) get involved in competitive mechanisms, such as exploitative competition and behavioural interference that can affect demography parameters of outcompeting subordinate species (e.g., dominant mice aggressiveness *vs* voles, Bartolommei et al., 2018; Grüm & Bujalska, 2000), ultimately leading to the formation of new community assemblies (Chesson & Kuang, 2008; Eccard & Ylönen, 2003; Price, 1986).

A potential way to explore such complex relationship is by integrating experimental food supplementation designs within a niche-based community approach, where the effects of top-down extrinsic factors, such as climatic constraints, intrinsic seasonal variations, and food availability on demographic parameters of sympatric species are assessed *ensemble*. In this study, we aimed to disentangle the effect of food availability and climate severity, as well as seasonality on small rodents' demographic patterns, by contemporarily applying a longitudinal and manipulative experimental approach. Specifically, we manipulated food resources availability (i.e., supplemental feeding in treatment/control designs), while explicitly controlling for seasonal variation, degree of climatic severity and co-occurrence of sympatric species, in wild settings. Small rodents were intensively live-trapped at two latitudinal extremes of the boreal-temperate climatic gradient (South-East Norway and Italian Alps), as proxies for climatic severity. In particular, the Norwegian study area is characterized by a strong seasonality with cold and snowy winters and mild and short summers; conversely, in the Italian study area the seasonal shift is more gradual with short and cool winters and warm and flourishing summers. Our initial hypothesis stated that rodents' population parameters and size would vary with resource availability, seasonal variation, and climatic severity (H1; Table1). In particular, for each detected small mammal species, we expected a general decrease of survival at northern latitudes where conditions are harsher (P1.1; Table 1) whereas, within each study area, survival would depend on species-specific intrinsic factors, such as seasonal physiological cycles (P1.2a; Table 1), and be relatively independent from supplemental food (P1.2b; Table 1). Further, we predicted population size to be positively affected by resources availability at both latitudes, i.e. to be higher in summer (P1.3a; Table 1), especially where supplemental food was available (P1.3b; Table 1). We further hypothesized that rodent assemblage and inter-specific relationships depend on climatic and environmental conditions (H2, Table 1) that when favorable, as at southern latitudes promote a more diversified rodent assemblage (P2.1; Table 1). We also predicted that competitive mechanisms between dominant and subordinate species of the assemblage (e.g. mice *vs* voles) would emerge especially under favorable conditions for dominant species, such as in summer (P2.2a; Table 1) or in presence of supplemental food (P2.2b; Table 1).

Table 1: Hypotheses and corresponding predictions.

Hypotheses	Predictions
H1: Small rodents adjust their population demography according to supplemental food availability, seasonal variation and climatic severity	P1.1: Lower survival at northern latitudes
	P1.2a: Survival depends on species-specific, intrinsic seasonal cycles… 1.2b: …and is independent of supplemental food.
	P1.3a: Higher population size during summer… P1.3b… and when supplemental food was available
H2: Small rodent assemblage and inter-specific relationships depend on climatic and environmental conditions	P2.1: A more diversified rodent assemblage is promoted by favourable climatic and environmental conditions (i.e., at southern latitudes). P2.2a: Inter-specific competition between dominant and subordinate species emerges especially in summer… P2.2b: …or in presence of supplemental food.

2 Materials and Methods

2.1 Study areas

We carried out the study in two areas located at the extremes of the boreal-temperate climatic gradient, namely South-East Norway, and North-Eastern Italian Alps (Figure 1).

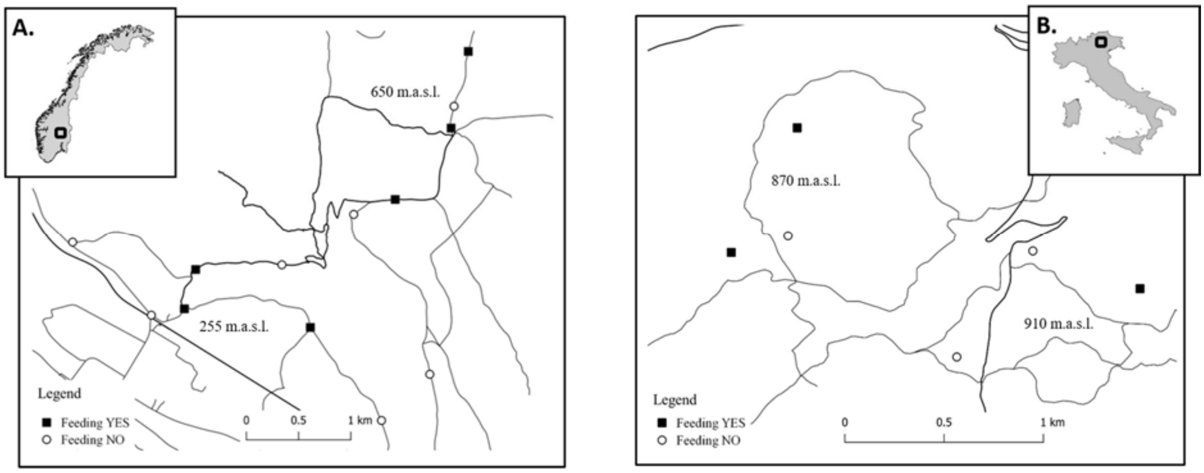

Figure 1: Maps and locations of the study areas. A: Evenstad area, in South-Eastern Norway (61.00N – 11.00E), monitoring period: 2013-2015; and B: Cembra area, in North-Eastern Italian Alps (46.13020N – 11.17843E), monitoring period: 2019-2021. In both study areas, rodents were live-trapped at grids with experimental control-treatment settings regarding food resource availability. Thick lines in the bottom valley indicate the main roads and fine lines the forest roads.

In Norway, Evenstad study area (61.00N – 11.00E; Stor Evdal municipality) includes the forested areas covering the hills and the valley bottom of the Glomma river basin (255 – 700 m a.s.l.). It is characterised by boreal climate (*sensu* Köppen-Geiger classification, Kottek et al., 2006), with long, harsh and snowy winters, with a permanent snow layer that prevents the soil from freezing, and short, cool summers. In particular, mean daily temperature of –8.9 ± 3.2 °C in January and of 14.2 ± 2.1 °C in July, and mean monthly precipitation of 35.2 ± 14.7 mm in January and of 84.1 ± 40.4 mm in July were recorded (data of 2000 – 2020 from the Norwegian Meteorological Institute https://seklima.met.no/, Evenstad, Åkrestrømmen, Drevsjø and Gløtvola weather stations at 260 / 670 m a.s.l.). The landscape is dominated by homogenous boreal coniferous forest of *Picea abies* and *Pinus sylvestris*, bilberry (*Vaccinium myrtillus*) in the understory shrub layer, and mosses (e.g., *Pleurozium schreberi*) on the ground layer (Johnsen et al., 2016).

In Italy, Cembra Valley (46.13020N – 11.17843E; Autonomous Province of Trento, Albiano municipality) lays in a moderate topography region (500 to 1000 m a.s.l.) in orographic continuity with the Lagorai massif. It is characterised by warm-temperate class of the alpine climate, *sensu* Köppen-Geiger classification (Rubel et al., 2017), with moderately cold winters, with occasional snow cover and usually frozen ground, and fresh summers. In particular, mean daily temperature of 0.9 ± 1.3 °C in January and of 20.8 ± 1.4 °C in July, and mean monthly precipitation of 39.0 ± 1.3 mm in January and of 112.9 ± 47.8 mm in July were recorded (2000 – 2020 data obtained from Meteotrentino https://www.meteotrentino.it, Cembra weather station at 652 m a.s.l.). The area is covered with relatively homogeneous secondary growth mixed forest, dominated by *Pinus sylvestris* with abundant shrub undergrowth, as well as mixed stands of *Fagus sylvatica*, *Picea abies, Abies alba* and, to a lower extent, *Quercus petraea,* interspersed with peat bogs and small pastures. In 2020, a mast seeding event of both *Fagus sylvatica* and *Picea abies* occurred in the Italian study area (Ferrari G, pers. comm.).

2.2 Experimental designs

In this study, we assessed rodent demography (i.e. survival and population size) under contrasting abiotic conditions (climatic severity and seasonal variation) and food availability (H1). In particular for the former, we compared (i) study areas at latitudinal extremes, namely Norway and Italy, as a proxy for climatic severity, and (ii) permissive (summer: April-October) vs limiting (winter: November-March) periods, within each year, to evaluate the impact of seasonal variation on rodents demography. To evaluate the demographic implications of food availability, we undertook experimental manipulations by providing *ad libitum* supplemental food in some trapping occasions and/or sites (‘Feeding yes’, or ‘Treatment’ Figure 1, Figure 2), but not in others (‘Feeding no’, or ‘Control’ Figure 1, Figure 2), in both study areas. Additionally, we further investigated rodent adaptability to divergent environmental conditions by comparing the assemblages at the two latitudes (H2). Where sympatric rodent species occurred, we assessed whether they were competing by comparing their demographic parameters under the aforementioned seasonal and supplemental food availability variations (Figure 2).

Specifically, in Norway, we deployed thirteen trapping grids and performed monthly captures between 2013 and 2015 (see Appendix S1, Table S1.1 for a summary of capture sessions). In six of those grids, supplemental food (a mixture of oat and sunflowers) was provided *ad libitum* next to each trap from December 2013 to June 2014, in November 2014, and in May 2015 (state of the sites: 'Feeding yes'). In these six grids from June to August 2015, and in the remaining seven grids from December 2013 to August 2015, no food was provided (state of the sites: 'Feeding no').

In Italy, we deployed six trapping grids and performed monthly captures during winter (from November to March), and bi-monthly captures during spring and summer (i.e., in April, June and August) between February 2019 and April 2021 (see Appendix S1, Table S1.2). Three of these grids were always provided with *ad libitum* corn supply dispensed through ungulate supplemental feeding stations located at the centre of each grid (sites: 'Feeding yes'), while the other three were located at least 500 m from any feeding site (sites: 'Feeding no').

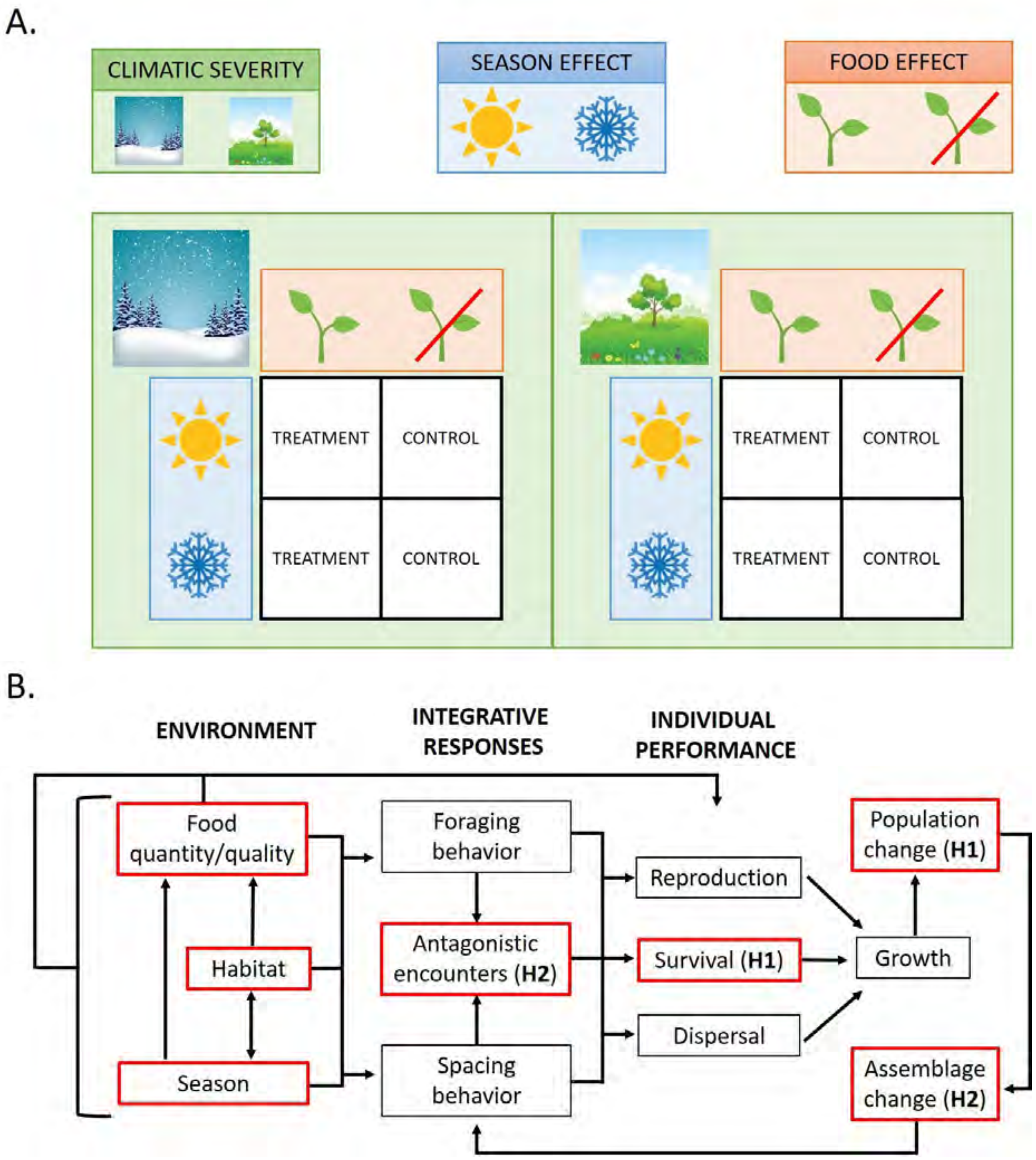

Figure 2: Panel A - In this study, we performed rodent live-trapping at two latitudinal extremes (Norway and Italy) used as a proxy for climatic severity (in green), in a combination of seasonal (in blue) and supplemental food availability (in orange) conditions. Specifically, at both latitudes, we performed experimental control-treatment manipulations in wild settings by providing supplemental food in treatment conditions only, across different seasons. Panel B - These experimental manipulations led to evaluate individual performance and population abundance change in dependence on seasonal variation and food availability, under diverse degree of climatic severity (H1). Additionally, we

evaluated how these relations were affected by competition of sympatric species (H2). Scheme modified from (Batzli, 1992); the red boxes indicate the factors explicitly addressed in the study designs.

2.3 Live-trapping

In Norway, the trapping grids were cross-shaped with 16 trap stations 15 m and 7.5 m apart for the external and internal traps in the grid, respectively (total grid area: 3600 m^2; Appendix S2). Captures lasted 4 days/3 nights per month, and traps were checked every 12 hours (for additional information on the protocol in Norway, see Johnsen et al., 2017).

In Italy, animals were caught in square-grids of 8x8 traps placed 10 m apart (total grid area: 4900 m^2; Appendix S2). Captures lasted 4 days/3 nights per month (winter) or bi-month (summer), and traps were checked every 24 hours.

In both the Italian and Norwegian study areas, the traps were left open during non-trapping periods, to habituate animals to their presence. Each trap consisted of a standard Ugglan Multiple Live Trap (model 2, Granhab, Sweden), filled with hay during the cold season, and baited with carrot slices and seeds (oat in Norway, sunflower in Italy). Standard capture-mark-recapture techniques (CMR) were adopted (Amstrup et al., 2005; Pollock et al., 1990). Each animal was individually marked with a Passive Integrated Transponder (PIT) tag (Trovan® Ltd., UK), and standard information (date, time, trap number, grid number, id of the animal) and life-history traits (species, sex, body mass, age, breeding status) were recorded for each capture. In Italy only, blood (from the retro orbital sinus), tissue samples (tail tip and ear biopsy) and fecal pellets were collected, and animals were also screened for the presence of parasites.

We discarded data from 22 animals in total as they were occasional species (shrews, dormice), or individuals that lost their PIT tag (but were identified by the signs of biopsy). Individuals of yellow-necked mouse (*Apodemus flavicollis*) and wood mouse (*Apodemus sylvaticus*) were treated *ensemble* as *Apodemus* spp. as the former was largely prevalent (see below).

All animal handling procedures and ethical issues were approved by the relevant regional or local Wildlife Management Committees.

2.4 Multi-State Open Robust Design models

We analysed capture data separately for Norway and Italy to account for differences in the experimental design (see 2.2 *Experimental designs*).

Our field protocol defined by daily secondary occasions within monthly primary ones corresponds to the so-called robust design framework (Pollock, 1982). Therefore, we used a multistate open robust design (MSORD) approach to estimate demographic parameters (Cooch & White, 2019; G. C. White et al., 2006). This model provides five parameters (Boys et al., 2019; Cooch & White, 2019; Kendall & Nichols, 2002): (i) true survival (S_t), which expresses the probability of surviving from release occasion t to subsequent primary occasion t + 1 occupying a state s; (ii) transition probability (ψ_s), which represents the probability of animals transitioning to different states s between primary occasions t and t + 1; (iii) entry or arrival probability ($pent_j$), which expresses the probability of an animal arriving and being captured in the study area in secondary occasion j; (iv) apparent survival rate (φ_j), which is the probability of an individual to survive and persist in a capture site at secondary occasion j, given it was present in j – 1; (v) capture probability (p_j), the probability of an animal being detected at occasion j, given it was present. The transition probability (ψ) was intended differently for Norway and Italy. For the former, it consisted in the probability of transition between states of food availability at trapping sites, expressed by the covariate 'feeding state', as feeding was treated as a spatio-temporal covariate (i.e., availability of food in certain primary occasions in certain sites; see also 2.2 *Experimental designs*); for the latter, it regarded the probability of transition between state of individual observability, i.e. animals being present in the study area and available for detection (observable state) or otherwise temporary emigrant outside the study area (unobservable state), and it was set as constant. The availability of supplemental food for Italy was expressed instead by a spatial, temporally fixed covariate, ‘feeding site’, corresponding to the trapping sites where food was always provided *ad libitum*, or the alternate sites with no food, that as such did not affect ψ. The MSORD model also provides two derived (i.e. not included in the likelihood formulation) parameters: (vi) population size (N_t), i.e. the number of individuals occupying the study area in state s in that primary occasion t; and (vii) residence time (R_t), which expresses the average number of secondary occasions that an individual spent in the study area for that state s in that primary occasion t (Boys et al., 2019; Chabanne at al., 2017).

We modelled demographic parameters in dependence on temporal, spatial, state, and individual covariates that were biologically meaningful to address our working hypotheses. Based on exploratory analyses, we decided not to consider sex in the CMR models (see Appendix S3 for details). We modelled true (S) and apparent survival (φ) as varying in dependence on primary occasions (‘session’, only for φ) and successive seasonal periods (‘season’) to detect the temporal pattern (H1), supplemental food availability (‘feeding state’ in Norway and ‘feeding site’ in Italy) to identify the food effect (H1), and species (only in Italy) to evaluate interspecific competition (H2). We considered the probabilities of capture (p) and arrival (*pent*) to be dependent on temporal variations (primary occasions i.e. ‘session’ for p, secondary occasions i.e. ‘time’ for *pent*), seasonal periods (‘season’), supplemental food availability (‘feeding state’-Norway and ‘feeding site’-Italy) and species (only in Italy). We modelled ψ in dependence on transition of feeding state for Norway, and kept it constant for Italy (expressing the probability of transition of animal observability in the trapping grid; see above) (Table 1; see also Appendix S4 for description of covariates and models building).

We fitted the models with the RMark package (Laake, 2013) in the R program (R Core Team 2021) and used the AICc (corrected Akaike’s Information Criterion) for model selection (Burnham & Anderson, 2002) with a threshold of ΔAICc ≤ 4 (Appendix S5). Two models for

Norway, and three models for Italy were equivalent in terms of AICc; among these, we chose for prediction of parameters the models which retained the covariates that allowed us to better test our hypotheses (Appendix S5).

3 Results

3.1 Assemblage Description

In Norway, 917 bank voles (*Myodes glareolus*) were captured accounting for 3976 total capture events. Individuals were captured more when supplemental feeding was available (n = 575 for 2880 night traps) than when it was not (n = 455 for 10504 night traps). The sex ratio was similar with and without supplemental food availability (Table S3.1, Appendix S3).

In Italy, 507 animals were captured for three species (P2.1 *supported*): 398 (78.5%) *Apodemus* spp. (specifically, 390 yellow-necked mice *Apodemus flavicollis* and 8 wood mice *Apodemus sylvaticus*, as later determined through genetics) and 109 (21.5%) bank voles (*M. glareolus*), accounting for 1379 capture events in total (926 and 453, respectively). We captured more mice where supplemental food was available (n = 234 for 21888 night traps) than in grids where it was not (n = 164 for 21888 night traps), and the opposite for voles (29 and 80, respectively). Sex ratio was similar with and without supplemental food (see Table S3.1, Appendix S3).

3.2 Population Demography

Probability of survival

Generally, *S* in *M glareolus* was marginally lower in Norway during the winter season compared to Italy (t-test; p-value = 0.051), instead during summer *S* was not statistically different in the two study areas (P1.1, *partially supported*).

For Norway, *S* depended on the additive effect between availability of supplemental food and season (Table S5.1). In particular, *S* was higher during winter with respect to the following summer (for those years where these seasons were comparable) (P1.2a *supported*), and in presence of supplemental food, both in winter and summer (Table S6.1; Figure 3.1; for *p*, *φ* and *pent*, see Appendix S7) (P1.2b *not supported*).

In Italy *S* depended on the additive effect of season with the interaction between species and feeding site (Table S5.2). In particular, both *M. glareolus* and *Apodemus* spp. showed a similar seasonal pattern, with *S* increasing in winter and declining in summer (P1.2a *supported*). In absence of supplemental food, *S* was higher in *M. glareolus* than in *Apodemus* spp.; however, where supplemental food was provided, *S* slightly increased in *Apodemus* spp. but strongly decreased in *M. glareolus* (Table S6.3; Figure 3.2; for *p*, *φ* and *pent*, see Appendix S7) (P1.2b *partially supported* and P2.2b *supported*).

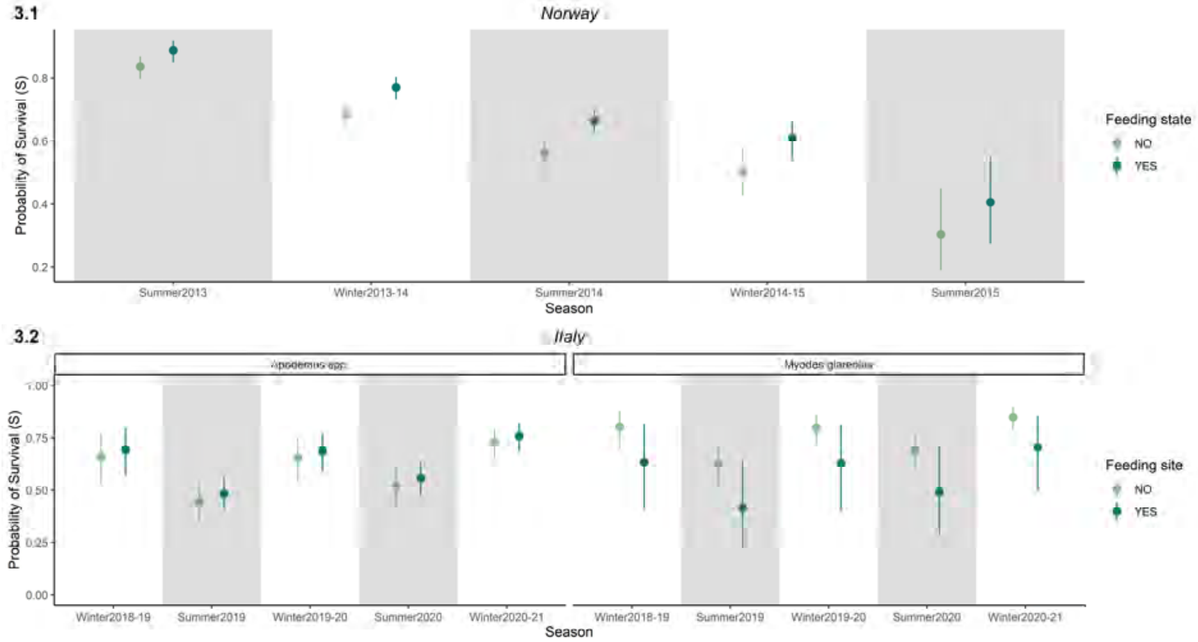

Figure 3: Estimates of survival (S) of rodents in Norway (**Figure 3.1**) and Italy (**Figure 3.2**) by species and seasonal variation, with treatment supplemental food (supplemental food available; dark green) and control (no supplemental food available; light green) conditions ('Feeding state' for Norway and 'Feeding site' for Italy). Grey shaded bars show the summer periods, while white bars show the winter periods. The comparison between successive winter (overwintering animals) and summer (reproductive season) survival estimates is not possible for summer 2013 in Norway and winter 2020-21 in Italy.

Population size

In Norway (Table S6.2; Figure 4.1), when supplemental food was not provided, *M. glareolus* population size was slightly higher in summer than in winter (P1.3a *supported*), although it exhibited a general decreasing trend across years. In the months with availability of supplemental food, the population size was significantly higher (ANOVA, p-value = 0.00013; P1.3b *supported*), but the temporal irregularity of sampling and therefore estimates (Appendix S1, Appendix S6) does not allow to infer any seasonal pattern.

In Italy (Table S6.4; Figure 4.2), when supplemental food was not provided, population size trends of *Apodemus* spp. and *M. glareolus* were asynchronous, reaching population size peaks in different periods. In particular, *Apodemus* spp. showed a summer peak followed by a sharp decrease through winter 2019-20, while in 2020 the population continued to increase after the summer season (P1.3a *partially supported*). On the contrary, *M. glareolus* increased during winter and decreased in summer (P1.3a *not supported*, P2.2a *supported*). With supplemental food, *Apodemus* spp. exhibited the same annual pattern, but with a higher population size (ANOVA, p-value = 0.03) (P1.3b *supported*). Noteworthy, *M. glareolus* was almost absent where food was provided, with the exception of a slight recovery only during the last winter sessions in 2020-21 (ANOVA, p-value = 0.01) (P1.3b *not supported* and P2.2b *supported*).

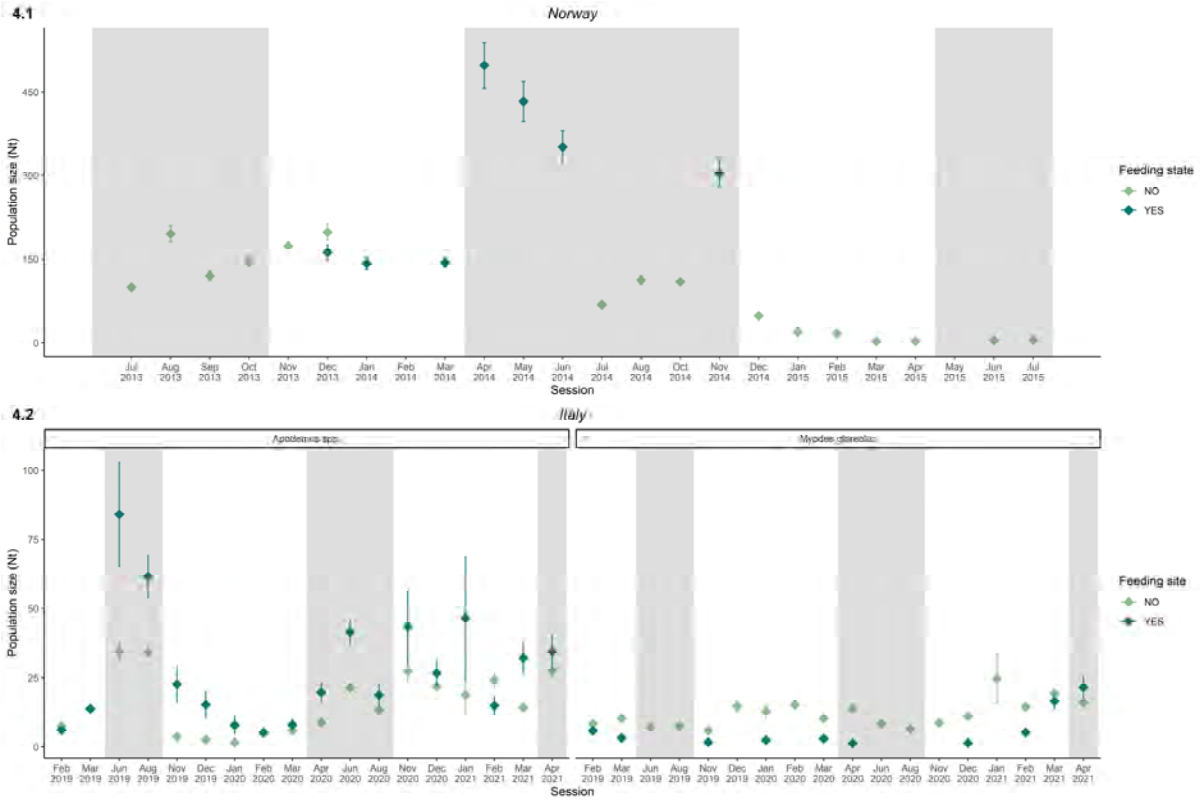

Figure 4: Population size (Nt) of rodents in Norway, 2013-2015 (**Figure 4.1**) and in Italy, 2019-2021 (**Figure 4.2**) with treatment supplemental food (supplemental food available; dark green) and control (no supplemental food available; light green) conditions ('Feeding state' for Norway and 'Feeding site' for Italy). Grey shaded bars show the summer periods, while white bars show the winter periods. The gaps in the series (*M. glareolus* in Norway: Feb 2014 and May 2015; *M. glareolus* in Italy: 8 cases) are due to lack of estimates from the model in those instances.

4 Discussion

In our work, we found evidence that bottom-up extrinsic processes, i.e. food availability, crucially affect woodland rodent demography, and that this effect is mediated by top-down extrinsic drivers, such as climate severity, as well as by intrinsic seasonal cycles and competition between sympatric species. Survival was principally governed by intrinsic seasonal cycles and was affected by food availability only when unfavourable extrinsic conditions occurred (i.e., harsh climate in Norway; exploitative competition in Italy, see below). Conversely, population size was generally enhanced by resources (i.e., with supplemental food in Norway; during masting in Italy), unless *ad libitum* concentrated food availability triggered interactions between sympatric species. In this case, the dominant, opportunistic species (i.e., *Apodemus* spp.) relatively increased its population size both in summer and especially in winter with respect to control conditions, suppressing the temporal niche partitioning with the subordinate generalist species (i.e., *M. glareolus)*. As this resulted in the depression of both population size and survival of the latter, we can infer the emergence of both direct (behavioural interference, i.e. decreased population size) and indirect (exploitative, i.e. depressed survival) competitive mechanisms. Innumerable studies have contributed to describe and explain rodent dynamics by investigating the underpinning demographic processes (Andreassen et al., 2021; Cornulier et al., 2013; Oli, 1999). The niche-based, experimental and longitudinal designs of our work allowed to uncover the interplay between intrinsic and extrinsic drivers of annual population patterns.

Both for bank voles and mice, well-known patterns of seasonal demography for rodents living in temperate and polar climate, i.e. an alternation between breeding (late spring to early autumn) and non-breeding (late autumn to early spring) seasons (Eccard & Herde, 2013) were mainly confirmed. This suggests that intrinsic mechanisms such as physiological cycles represented the leading force in driving survival, which was higher in winter than in summer (P1.2a), and population size that conversely was higher in summer than in winter (P1.3a). Specifically, in late spring, the decreased costs of thermoregulation due to warmer temperatures (Merritt, 2010), together with increased resource availability (Pearse et al., 2016) stimulate reproduction (Steinlechner & Puchalski, 2003), which continues until early autumn producing several generations of new-borns. During the breeding season, the survival is lowered by a combination of (i) high emigration rate of overwintered individuals (i.e. those that successfully survived throughout the previous winter due to their ability to storing food and avoiding predation (Olenev & Grigorkina, 2014) and (ii) deaths of early-born and immature animals

(Flowerdew et al., 1985) that tend to increase their competitiveness for food and mates at the cost of chances of survival (Eccard & Herde, 2013). During the non-breeding season, the survival of late-born animals (i.e., overwintering individuals) is higher, guarantying the bulk for the following year spring population.

Our integrated niche-based approach allowed to experimentally evaluate how extrinsic environmental factors, especially trophic resources, modulate these relatively conserved demographic patterns. Specifically, the latitudinal comparison highlighted that *M. glareolus* survival was slightly lower in Norway than in Italy, but only in winter, likely due to prolonged harsh seasons, scarcity of food resources, and delayed phenology (P1.1; Haapakoski & Ylönen, 2013; Korslund & Steen, 2006). Consistently, the survival in *M. glareolus* increased following the manipulation of food availability in Norway (on the contrary of what expected; P1.2b), providing a further evidence of the importance of tropich resources in modulating survival at northern latitudes (Johnsen et al., 2016; Rémy et al., 2013). Indeed, at those latitudes harsh conditions increase the energetic costs for thermoregulation and reproduction (Ylönen & Eccard, 2004). On the opposite, short winters, milder temperatures and more predictable precipitations might favour stability in survival annual trends at southern latitudes (Yoccoz & Ims, 1999), as observed for *Apodemus* spp. in Italy, also when supplemental food was provided (P1.2b).

Conversely, trophic resources were the major driver of population abundance at both latitudes, with higher values in summer (P1.3a), and when supplemental food was provided (P1.3b). This might be linked to the restriction of home ranges when resources are abundant (see e.g. Stradiotto et al., 2009; Taitt & Krebs, 1981), which our models support by showing that individual residency time increased in proximity of food resources, when these were provided (Ferrari G., pers. comm.). As a consequence, the creation of vacant areas due to home range contraction might discourage emigration and favor immigration (Le Galliard et al., 2012;

Rémy et al., 2013), ultimately increasing population size. Remarkable exceptions to this general pattern were recorded: in Norway, *M. glareolus* showed an overall decreasing trend in population size, due to the upcoming crash phase of multi-annual cycles (as detected in Johnsen et al., 2019) that typically govern rodents dynamics at northern latitudes (Andreassen et al., 2021); in Italy, population abundance of both *Apodemus* spp. and *M. glareolus* kept increasing from summer 2020 to winter 2020-2021, following a beech and spruce mast seeding, which produced overabundant spatially dispersed food resources (Bogdziewicz at al., 2016); further, population size of *M. glareolus* in Italian alpine woodlands exhibited an asynchronous seasonal pattern with respect to that of *Apodemus* spp., with peaks in winter and low phases in summer (P2.2a).

At southern latitudes, milder conditions in forestry environments promoted the co-occurrence of multiple species, in accordance with latidudinal biodiversity gradients (Araújo & Costa-Pereira, 2013; Mannion et al., 2014) (P2.1). While spring decline has been recorded in voles in other habitats (see Flowerdew et al., 1985 for a review), we intepret the asynchrony of annual population trends within the rodent community as an evidence of temporal niche partitioning. In sympatry, *Apodemus* spp. are generally dominant over *M. glareolus* (Amori et al., 2015; Casula et al., 2019), with the latter thus being forced to shift its niche to avoid competition. Benefiting by its wide dietary spectrum (Butet & Delettre, 2011), the generalist *M. glareolus* may exploit harsher seasonal conditions to temporally shift its niche, thus partitioning it with respect to the diet-specialist *Apodemus* spp. (Viviano et al., 2022), not able to cope with winter resource depletion. The overabundance of concentrated food of our experiment altered these processes, consistently enhancing the population demography of *Apodemus* spp., so buffering its population decline in winter (P1.3b). As a consequence, the ecological niches of *Apodemus* spp. and *M. glareolus* should have overlapped, and the niche partitioning failed. This eventually resulted in interspecific competition (P2.2b), with *M.*

glareolus survival decreasing and population size collapsing. In nature, behavioral interference and exploitative competition (Wallace & Benbow, 2019) often co-exist, and their effects are difficult to separate (Schmidt et al., 2005; Shenbrot & Krasnov, 2002). Our experimental setting of food manipulation helps speculate the underpinning process to the observed demographic patterns. The presence of clumped food resources might have triggered behavioral interference, attracting both species (higher residency time, Ferrari G. pers. comm.), but favoring the dominant one. When food is confined, it becomes a defensible resource for species that cache food in larger hoards such as *Apodemus* spp. (Zwolak, Bogdziewicz, Wróbel, et al., 2016), potentially leading to aggressive interactions. These interactions might have chased *M. glareolus* away from the area surrounding the sites, eventually causing the observed collapse of population size (see e.g. Eccard & Ylönen, 2007). The concurrent decrease of survival experienced by *M. glareolus* might be instead imputed to exploitative competition (Gilad, 2008). As concentrated food resources enhance it abundance, *Apodemus* spp. might have consumed other resources than those provided at feeding sites, depriving *M. glareolus* also of other food sources. The lack of competition in presence of overabundant and spatially dispersed food resources i.e. masting, supports this explanation.

Under climate change. we expect more permissive environmental conditions for the rodent community in the boreal-temperate gradient, with a likely northward expansion of species' distributional limits (Parmesan, 2006). The colonization of new habitats by opportunistic species e.g. *Apodemus* spp. would expand the spatial overlap with species such as *M. glareolus* which is well-adapted to cold environments (Butet and Delettre 2011, Markova et al. 2018). This scenario is supported by our latitudinal comparison, which suggests a tendency to niche partitioning as sympatry establishes (Bartolommei et al., 2018), or species competition to the disadvantage of adaptable but subordinate species.

Our assessment of the role of extrinsic and intrinsic factors, in interplay with biotic interactions, in shaping rodent demography, highlights the complexity of such relationships. Only by simultaneously controlling for several components of the ecological niche of the studied species (extrinsic top-down factors, i.e. climate severity, through latitudinal multi-population comparison; extrinsic bottom-up factors, i.e. resource availability, through food manipulation; intrinsic cycles, through seasonal variation; competition, through assessment of interactions between sympatric species) we have been able to evaluate their relative impact on the demography of each species. We suggest adopting an analogous approach when investigating other underpinning determinants of rodent demography patterns, such as parasitism and emergence of related diseases (Deter et al., 2008; Telfer et al., 2005), e.g. by comparing host-parasite-pathogens associations across environmental gradients or under different intensity of human disturbance.

Acknowledgements

G. Ferrari was supported by Inland Norway of Applied Sciences Doctoral Program in Applied ecology and biotechnology and Fondazione Edmund Mach International Doctoral Program. F.C. contributed to this work partly under the IRD Fellowship 2021/2022 at Fondation IMéRA, Institute for Advanced Studies at Aix-Marseille Université. We are grateful to all the people involved in the rodent trapping and data collection, and especially Arnoldi D., Dal Farra S., Inama E. and Savazza S. We thank all the members of Applied Ecology and Animal Ecology Research Units and researchers from Evenstad Campus for their valuable suggestions.

Conflict of Interest

The authors declare no competing interests.

Authors' contributions

G.F., O.D., V.T. and F.C. conceived the ideas and designed methodology; F.C., V.T. and O.D. equally supervised the work; G.F., V.T. and F.O. collected the data in Italy and K.J. collected the data in Norway; G.F. analysed the data with the support of O.D.. F.C., V.T.; G.F. led the writing of the manuscript, together with O.D., V.T., F.O., and F.C. All authors contributed critically to the drafts and gave final approval for publication.

Data Availability Statement

Data are available from the Eurosmallmammals database (https://eurosmallmammals.fmach.it).

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Supporting Information

Appendix S1: Trapping sessions

In Norway the dataset included 26 primary occasions with two to seven secondary occasions from 2013 to 2015 (Table S1.1), while Italian trapping data consisted of 18 primary occasions with 3 secondary occasions each from 2019 to 2021 (see Table S1.2). Since the analysis used calendar months as temporal categorical variable, we decided to unify the secondary occasions of captures occurring in different sessions but within the same month (e.g. March 2019 in Italy and July 2013 in Norway).

Table S1: Primary and secondary capture occasions in Norway (**Table S1.1**) and Italy (**Table S1.2**). Grey-shaded occasions in Norway showed when food was provided at treatment grids (see main text).

Season	Summer 2013					Winter 2013/2014				
Primary occasion	1		2	3	4	5	6	7	8	9
Secondary occ 1	03 Jul	22 Jul	10 Aug	09 Sep	09 Oct	09 Nov	09 Dec	20 Jan	17 Feb	17 Mar
Secondary occ 2	04 Jul	23 Jul	14 Aug	10 Sep	10 Oct	11 Nov	10 Dec	21 Jan	18 Feb	18 Mar
Secondary occ 3	05 Jul	24 Jul	15 Aug			12 Nov	11 Dec		19 Feb	19 Mar
Secondary occ 4	10 Jul		16 Aug			13 Nov	12 Dec		20 Feb	20 Mar
Secondary occ 5			17 Aug			14 Nov				

	Summer 2014						Winter 2014/2015				
Primary occasion	10	11	12	13	14	16	17	18	19	20	21
Secondary occ 1	22 Apr	19 May	23 Jun	21 Jul	18 Aug	13 Oct	03 Nov	08 Dec	12 Jan	10 Feb	18 Mar
Secondary occ 2	23 Apr	20 May	24 Jun	22 Jul	19 Aug	14 Oct	04 Nov	09 Dec	13 Jan	11 Feb	19 Mar
Secondary occ 3	24 Apr	21 May	25 Jun	23 Jul	20 Aug	15 Oct	05 Nov	10 Dec	14 Jan	12 Feb	20 Mar
Secondary occ 4	25 Apr	22 May	26 Jun	24 Jul	21 Aug	16 Oct	06 Nov	11 Dec	15 Jan	13 Feb	

	Summer 2015				
Primary occasion	22	23	24	25	26
Secondary occ 1	14 Apr	18 May	16 Jun	13 Jul	11 Aug
Secondary occ 2	15 Apr	19 May	17 Jun	14 Jul	12 Aug
Secondary occ 3	16 Apr	20 May	18 Jun	15 Jul	13 Aug
Secondary occ 4		21 May	19 Jun	16 Jul	14 Aug

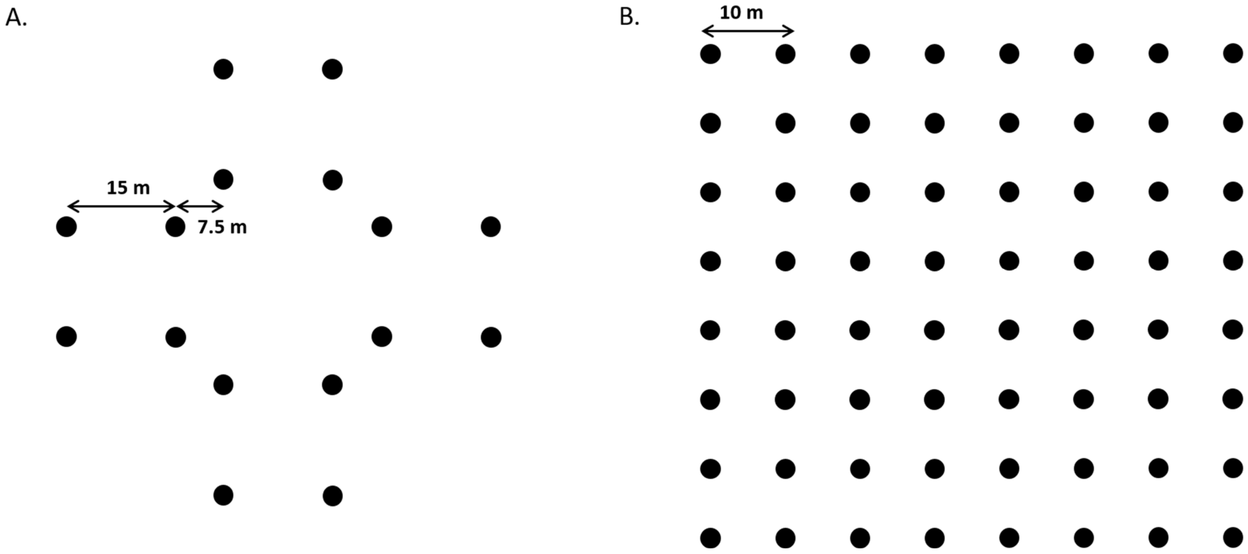
Table S1.1

Season			Winter 2018/2019			Summer 2019		
Primary occasion			1	2		3	4	
Secondary occ 1			19 Feb	05 Mar	19 Mar	25 Jun	27 Aug	
Secondary occ 2			20 Feb	06 Mar	20 Mar	26 Jun	28 Aug	
Secondary occ 3			21 Feb	07 Mar	21 Mar	27 Jun	29 Aug	
	Winter 2019/2020					Summer 2020		
Primary occasion	5	6	7	8	9	10	11	12
Secondary occ 1	12 Nov	10 Dec	14 Jan	11 Feb	04 Mar	21 Apr	30 Jun	25 Aug
Secondary occ 2	13 Nov	11 Dec	15 Jan	12 Feb	05 Mar	22 Apr	01 Jul	26 Aug
Secondary occ 3	14 Nov	12 Dec	16 Jan	13 Feb	06 Mar	23 Apr	02 Jul	27 Aug
	Winter 2020/2021					Summer 2021		
Primary occasion	13	14	15	16	17	18		
Secondary occ 1	17 Nov	15 Dec	12 Jan	23 Feb	9 Mar	7 Apr		
Secondary occ 2	18 Nov	16 Dec	13 Jan	24 Feb	10 Mar	8 Apr		
Secondary occ 3	19 Nov	17 Dec	14 Jan	25 Feb	11 Mar	9 Apr		

Table S1.2

Appendix S2: Trapping grid design

Trapping grid design. The panel A shows the cross-shaped design with 16 traps used in Norway, while in the panel B the square design of 64 traps applied in Italy is represented.



Appendix S3: Preliminary exploratory analyses

Norway – Evenstad site

In Norway, as expected from the trapping protocol performed, only one species was encountered (*Myodes glareolus*). The density of captures had a similar pattern in females and males with two annual peaks during early summer and autumn (Figure S3.1; Table S3.1).

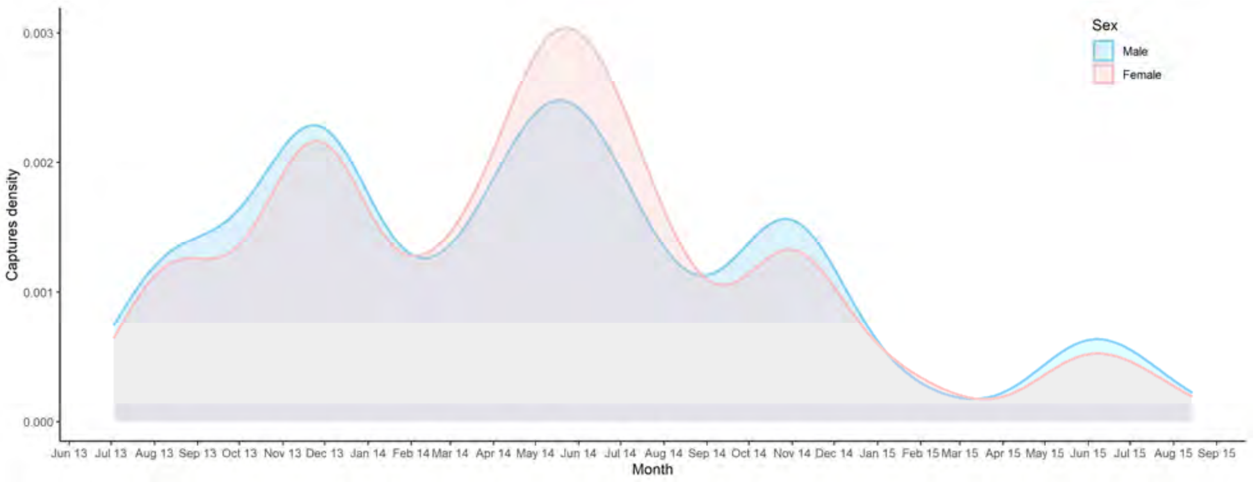


Figure S3.1: Captures density of *M. glareolus* in Norway across seasons in female (rose) and male (light blue).

Italy – Cembra site

When we compared the density of captures across trapping occasions, we detected a cyclic asynchronous pattern between the three detected species *(Apodemus* spp. and *Myodes glareolus*) (Figure S3.2). In particular, *Apodemus* spp. were captured more during summer compared to winter, with the exception of winter 2020-21. On the contrary, *M. glareolus* was captured more in winter than in summer.

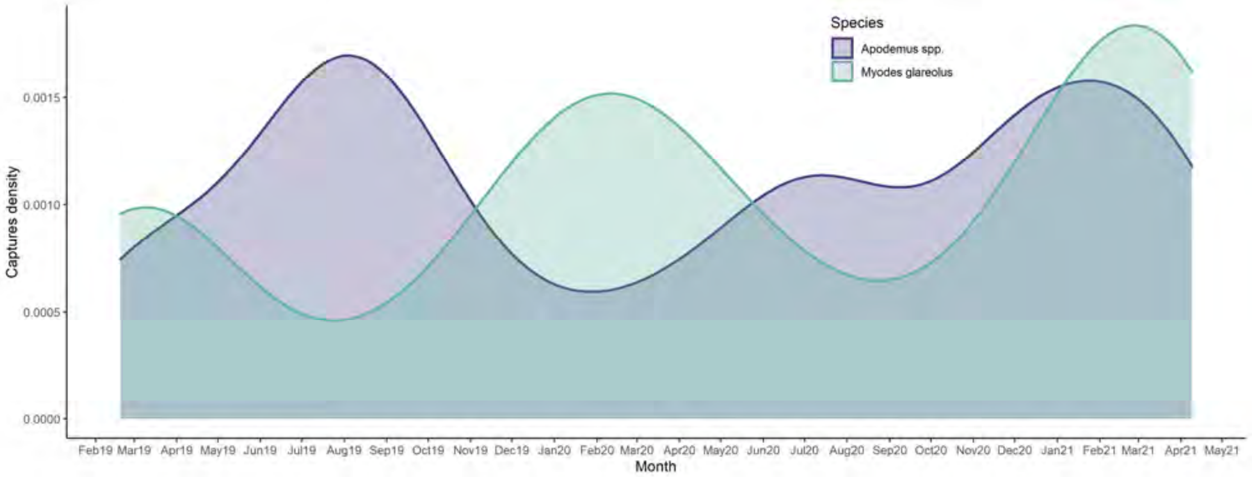


Figure S3.2: Capture density in Italy across seasons for *Apodemus* spp. (in purple) and *Myodes glareolus* (in green).

When breaking down the captures by sex, we found slightly shifted peaks for females and males in the three species, although the proportion of captures were similar (Figure S3.3; Table S3.1).

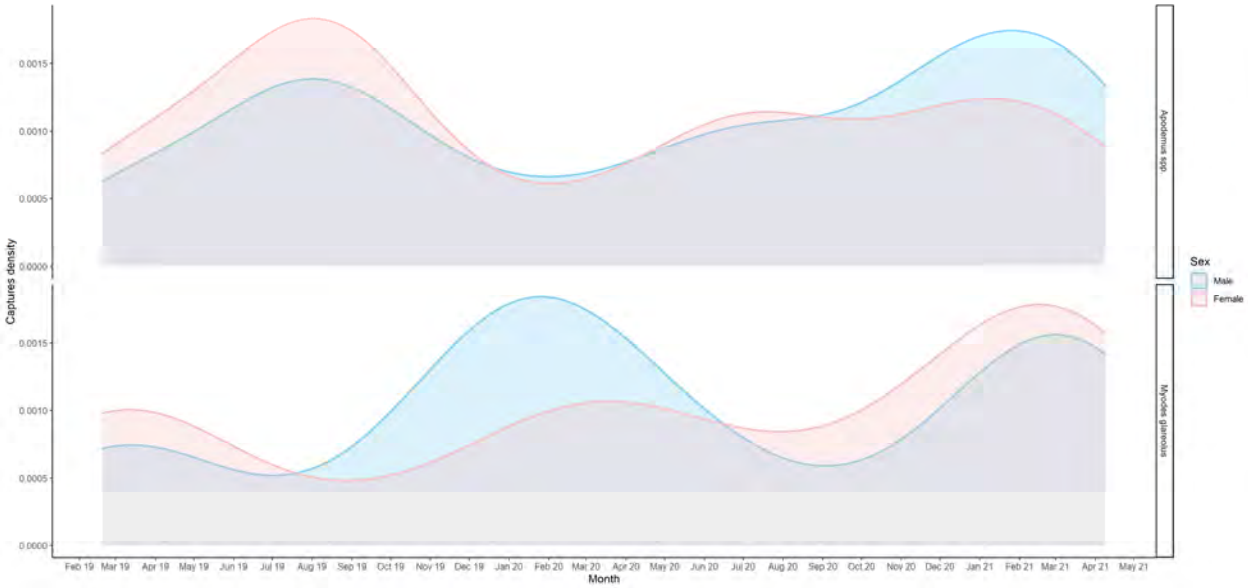


Figure S3.3: Captures density of *Apodemus* spp. and *Myodes glareolus* for female (rose) and male (light blue) individuals.

Table S3.1: Summary of the number of captures by sex in presence and absence of supplemental food for *M. glareolus* (Norway and Italy), and *Apodemus* spp. (Italy only).

	Myodes glareolus		*Apodemus spp.*	
Site	Feeding no	Feeding yes	Feeding no	Feeding yes
Norway				
Individuals	455	462		
Females	212	213		
Males	243	249		
Total captures	1927	2049		
Italy				
Individuals	80	29	164	234
Females	34	10	84	117
Males	46	19	80	117
Total captures	391	62	440	486

Appendix S4: Models building

We identified a set of potentially biological meaningful covariates to disentangle seasonal, supplemental food availability, and inter-specific competition effect on rodent demography, and specifically:

(i) temporal covariates:

- **season (acting as an ordinary variable)**: successive binned time intervals based on seasonal periods throughout the study duration: winter (from November to March included) and summer (from April to October included);
- **time**: temporal effect within secondary occasions, i.e. daily **secondary trapping occasions;**
- **session**: temporal effect across primary occasions, i.e. monthly **primary trapping occasions**.

(ii) state/spatial covariate:

- **feeding state** of the site (Norway: spatio-temporally defined) and **feeding site** (Italy: spatially defined): presence (treatment) *vs* absence (control) of *ad libitum* supplemental feeding **(binary variables: yes/no)**.

(iii) individual covariate:

- **species (categorical variable;** Italy only**)**: *Apodemus* spp. and *Myodes glareolus*.

In CMR modelling, a main model is described by sub-models estimating each parameter, or response variables, in dependence on several covariates, or explanatory variables (Laake and Rexstad 2008). In particular, multistate open robust design (MSORD) is defined by five parameters (true survival rate (S), arrival probability ($pent$), apparent survival rate (φ), capture probability (p) and transition probability (ψ)).

For modelling each parameter, we chose those covariates that were biologically meaningful in our study systems, while being constrained by a relatively small sample size (i.e., few recaptures). In particular, we modelled true (*S*) and apparent survival (φ) as varying in dependence on primary occasions ('session', only for φ) and successive seasonal periods ('season') to detect the temporal pattern (H1), supplemental food availability ('feeding state' in Norway and 'feeding site' in Italy) to identify the food effect (H1), and species (only in Italy) to evaluate interspecific competition (H2). We considered the probabilities of capture (*p*) and arrival (*pent*) to be dependent on temporal variations (primary occasions i.e. 'session' for *p*, secondary occasions i.e. 'time' for *pent*), seasonal periods ('season'), supplemental food availability ('feeding state'-Norway and 'feeding site'-Italy) and species (only in Italy). We modelled *ψ* in dependence on transition of feeding state for Norway, and kept it constant for Italy (expressing the probability of transition of animal observability in the trapping grid). Table S4 summarizes the list of covariates or combination of covariates used to build the sets of sub-models (univariate, additive, or with two-way interactions among covariates) that were subsequently combined into main models.

Then we used a model selection based on AICc scores to rank the main models composed by sub-models and we retained the models with ΔAICc ≤ 4 as equally plausible (Burnham and Anderson 2002). Among these models, we chose the best model as the more biologically meaningful (Appendix S5).

Table S4: Covariates and combination of covariates used to build the set of sub-models for each demographic parameter in Norway (**Table S4.1**) and Italy (**Table S4.2**). Legend: 'S' = true survival; 'pent' = entry probability; 'φ' = apparent survival; 'p' = capture probability; 'ψ' = transition probability, set as constant in Italy and dependent on feeding states in Norway; 'session' = primary trapping occasions; 'time' = secondary trapping occasions; 'feeding site' = sites with supplemental food (only in Italy); 'feeding state' = supplemental food at sites (only in Norway); 'feeding state : to feeding state'

= transition between feeding states (only in Norway); ‘species’ = rodent species (only for Italy); ‘season’ = seasonal variation across years.

Table S4.1	S	pent	φ	p	ψ
feeding state : to feeding state					X
state : session					
feeding state : season	X	X	X	X	
feeding state + session		X	X	X	
feeding state + season	X	X	X	X	
feeding state	X	X	X	X	
season	X	X	X	X	
session	X	X	X	X	

Table S4.2	S	pent	φ	p	ψ
feeding site: season : species	X				
feeding site : season		X		X	
feeding site : season + species	X				
feeding site : species + season	X				
feeding site + season + species	X				
feeding site + season	X	X		X	
feeding site + session				X	
feeding site + species	X				
season + species	X				
feeding site	X	X	X	X	
season	X	X	X	X	
session	X			X	
time		X			
species	X	X	X	X	
1	X	X	X	X	X

Appendix S5: Model selection

In Norway, we found only one model with ΔAICc ≤ 4 (Model1). In the rest of the models, *S* and *pent* were dependent on 'feeding state', 'session' and 'season'. Moreover, for *pent*, most of the models were defined by 'feeding state : session' and 'session' effect; while φ seemed equally affected by 'feeding state', and equally by 'session' and 'season'. Finally, in mostly models, *p* was dependent on 'feeding state' and equally by 'session' and 'season' (Table S5.1). In conclusion, we decided to select Model1, because it showed the lowest AIC score and was the most biologically meaningful. Model1 retained 'feeding state : session' effect on *S*, 'season + state' on *pent* and finally 'season' on φ and 'feeding state' on *p*.

In Italy, the model selection returned 3 models with ΔAICc ≤ 4 which showed that 'species', 'feeding site', and 'season had a stronger effect on *S*. In the three models, *pent* was always affected by 'time'; φ by 'species' and *p* by 'feeding site + session' (Table S5.2). Although Model1 was the best parsimonious model with less parameters and lower AICc, we selected Model3 as best model because it allowed us to test our hypotheses. Specifically, Model 3 retained the interaction between 'feeding site' and 'species' added to 'period' on *S*; 'time' on *pent*; 'species' on φ and finally, 'feeding + session' on *p*.

Table S5: Model selection of Norwegian (**Table S5.1**) and Italian (**Table S5.2**) rodent population and capture parameters, reporting the best models (i.e. ΔAICc<4; in bold the one used for model predictions as most biologically meaningful with respect to the tested hypotheses), and the first model below the AICc threshold (in italic). Legend: ‘S’ = true survival; ‘pent’ = entry probability; ‘φ’ = apparent survival; ‘p’ = capture probability; ‘ψ’ = transition probability, set as constant in Italy and dependent on feeding states in Norway; ‘session’ = primary trapping occasions; ‘time’ = secondary trapping occasions; ‘feeding site’ = sites with supplemental food (only in Italy); ‘feeding state’ = supplemental food at sites (only in Norway); ‘feeding state : to feeding state’ = transition between feeding states (only in Norway); ‘species’ = rodent species (only for Italy); ‘season’ = seasonal variation across years; ‘npar’ = number of parameters, ‘AICc’ = AIC with a correction for small sample sizes; ‘ΔAICc’ = relative differences between the fitted model and the Akaike 'best-ranked' model with the smallest AICc value; ‘AICc weight’ = relative likelihood of a model; ‘Deviance’ = difference between null deviance and model deviance.

Table 5.1	S			pent			Φ			p		ψ					
Model Norway	feeding state	season	session	feeding state	season	session	season	session	feeding state	season	feeding state	feeding state : to feeding state	npar	AICc	ΔAICc	AICc weight	Deviance
1	**X**	**X**		**X**		**X**	**X**				**X**	**X**	**45**	**8795.79**	**0.00**	**0.74**	**8704.61**
2			X	X	X			X		X	X	X	65	8797.86	2.07	0.26	8665.40
3	*X*	*X*		*X*		*X*		*X*	*X*		*X*	*X*	*69*	*9631.72*	*835.93*	*0.00*	*9490.94*

Table 5.2	S				pent	φ		p		ψ					
Model Italy	feeding site	species	season	feeding site : species	time	species	feeding site	feeding site	session	.	npar	AICc	ΔAICc	AICc weight	Deviance
1		X	X		X	X		X	X	X	33	11415.11	0	0.56	11347.41
2	X	X	X		X	X		X	X	X	34	11417.15	2.04	0.20	11347.35
3			**X**	**X**	**X**	**X**		**X**	**X**	**X**	**36**	**11417.69**	**2.58**	**0.15**	**11343.67**
4		*X*	*X*		*X*		*X*	*X*	*X*	*X*	*33*	*11421.11*	*6.00*	*0.02*	*11353.42*

Appendix S6: Selected models' output: parameters' estimates

Norway – Evenstad site

Table S6.1: Parameter estimates of the best model (Model1, Table S5.1), with standard error (SE) and confidence intervals (Lower, Lcl, and Upper, Ucl). Legend: 'S' = true survival; 'pent' = entry probability; 'φ' = apparent survival; 'p' = capture probability; 'ψ' = transition probability depending on feeding states; 'session' = primary trapping occasions; 'feeding state' = supplemental food at sites; 'season' = seasonal variation across years. For some sessions, the model fitting did not converge.

Parameter	Estimate	SE	Lcl	Ucl	Feeding state	Session	Season
S	0.84	0.02	0.80	0.87	NO	1	Summer2013
S	0.68	0.02	0.64	0.73	NO	5	Winter2013-14
S	0.56	0.03	0.51	0.61	NO	10	Summer2014
S	0.50	0.04	0.43	0.58	NO	17	Winter2014-15
S	0.31	0.07	0.19	0.45	NO	22	Summer2015
S	0.89	0.02	0.85	0.92	YES	1	Summer2013
S	0.77	0.02	0.73	0.80	YES	5	Winter2013-14
S	0.67	0.02	0.63	0.70	YES	10	Summer2014
S	0.61	0.03	0.55	0.67	YES	17	Winter2014-15
S	0.41	0.07	0.27	0.55	YES	22	Summer2015
S	NA	NA	NA	NA	Un-observable	1	Summer2013
pent	0.15	0.01	0.14	0.17	NO	1	Summer2013
pent	NA	NA	NA	NA	NO	2	Summer2013
pent	0.06	0.03	0.02	0.14	NO	3	Summer2013
pent	0.56	0.06	0.44	0.67	NO	4	Summer2013
pent	0.24	0.01	0.23	0.26	NO	5	Winter2013-14
pent	NA	NA	NA	NA	NO	7	Winter2013-14
pent	NA	NA	NA	NA	NO	16	Summer2014
pent	0.17	0.16	0.02	0.64	NO	21	Winter2014-15
pent	NA	NA	NA	NA	NO	22	Summer2015
pent	0.17	0.10	0.05	0.44	NO	25	Summer2015

pent	NA	NA	NA	NA	YES	3	Summer2013
pent	0.23	0.11	0.08	0.51	YES	7	Winter2013-14
pent	NA	NA	NA	NA	YES	8	Winter2013-14
pent	0.33	0.00	0.33	0.33	YES	9	Winter2013-14
φ	0.60	0.05	0.50	0.68	NO	1	Summer2013
φ	0.78	0.02	0.73	0.83	NO	5	Winter2013-14
φ	0.85	0.05	0.73	0.92	NO	10	Summer2014
φ	NA	NA	NA	NA	NO	17	Winter2014-15
φ	0.98	0.04	0.48	1.00	NO	22	Summer2015
p	0.66	0.02	0.61	0.71	NO	1	Summer2013
p	0.40	0.02	0.37	0.44	YES	1	Summer2013
ψ	0.16	0.02	0.13	0.19	NO; YES	1	Summer2013
ψ	0.03	0.02	0.01	0.10	NO; Un-observable	1	Summer2013
ψ	0.06	0.01	0.05	0.08	YES; NO	1	Summer2013
ψ	NA	NA	NA	NA	YES; Un-observable	1	Summer2013
ψ	NA	NA	NA	NA	Un-observable; NO	1	Summer2013
ψ	NA	NA	NA	NA	Un-observable; YES	1	Summer2013

Table S6.2: Derived estimates of population size (N_t) retrieved by the best model (Model 1, Table S5.1), with confidence intervals (Lower, Lcl, and Upper, Ucl). Legend: ‘session’ = primary trapping occasions; ‘feeding state’ = supplemental food at sites. For some sessions, the model fitting did not converge.

Estimate	Lcl	Ucl	Session	Feeding site
98.96	94.16	103.76	1	NO
195.09	180.85	209.34	2	NO
119.56	111.01	128.11	3	NO
145.37	136.93	153.82	4	NO
172.97	166.84	179.10	5	NO
198.12	183.65	212.58	6	NO
68.06	63.09	73.03	13	NO
111.91	103.74	120.09	14	NO
108.67	104.72	112.63	15	NO
48.40	44.86	51.93	17	NO
19.66	18.23	21.10	18	NO
16.64	15.42	17.85	19	NO
2.67	2.08	3.26	20	NO
3.27	3.15	3.39	21	NO
4.54	4.21	4.87	23	NO
4.98	3.98	5.98	24	NO
161.82	148.45	175.19	6	YES
141.76	131.05	152.48	7	YES
143.33	135.52	151.14	9	YES
497.91	456.78	539.04	10	YES
433.18	397.40	468.97	11	YES
351.03	322.03	380.02	12	YES
303.72	278.63	328.82	16	YES

Italy – Cembra site

Table S6.3: Parameter estimates of the best model (Model3, Table S5.2), with standard error (SE) and confidence intervals (Lower, Lcl, and Upper, Ucl). Legend: 'S' = true survival; 'pent' = entry probability; 'φ' = apparent survival; 'p' = capture probability; 'ψ' = transition probability set as constant; 'session' = primary trapping occasions; 'feeding site' = sites with supplemental food; 'species' = rodent species; 'season' = seasonal variation across years. For some sessions, the model fitting did not converge.

Parameter	Estimate	SE	Lcl	Ucl	Session	Feeding site	Species	Season
S	0.66	0.06	0.53	0.77	0	NO	Apodemus spp.	Winter2018-19
S	0.44	0.05	0.36	0.54	4	NO	Apodemus spp.	Summer2019
S	0.65	0.05	0.54	0.75	9	NO	Apodemus spp.	Summer2019
S	0.52	0.05	0.43	0.61	14	NO	Apodemus spp.	Winter2019-20
S	0.73	0.04	0.65	0.79	21	NO	Apodemus spp.	Summer2020
S	0.69	0.06	0.57	0.80	0	YES	Apodemus spp.	Winter2018-19
S	0.48	0.04	0.40	0.56	4	YES	Apodemus spp.	Summer2019
S	0.69	0.05	0.59	0.77	9	YES	Apodemus spp.	Summer2019
S	0.56	0.04	0.47	0.64	14	YES	Apodemus spp.	Winter2019-20
S	0.76	0.03	0.68	0.82	21	YES	Apodemus spp.	Summer2020
S	0.80	0.05	0.69	0.88	0	NO	Myodes glareolus	Winter2018-19
S	0.63	0.05	0.52	0.72	4	NO	Myodes glareolus	Summer2019
S	0.80	0.04	0.72	0.86	9	NO	Myodes glareolus	Summer2019
S	0.69	0.04	0.60	0.77	14	NO	Myodes glareolus	Winter2019-20
S	0.85	0.03	0.78	0.90	21	NO	Myodes glareolus	Summer2020
S	0.63	0.11	0.41	0.82	0	YES	Myodes glareolus	Winter2018-19
S	0.42	0.11	0.22	0.64	4	YES	Myodes glareolus	Summer2019
S	0.63	0.11	0.40	0.81	9	YES	Myodes glareolus	Summer2019
S	0.49	0.12	0.28	0.71	14	YES	Myodes glareolus	Winter2019-20
S	0.71	0.09	0.50	0.85	21	YES	Myodes glareolus	Summer2020
pent	0.78	0.04	0.70	0.84	1	NO	Apodemus spp.	Winter2018-19
pent	0.10	0.04	0.05	0.20	1	NO	Apodemus spp.	Winter2018-19
pent	0.61	0.07	0.47	0.73	2	NO	Apodemus spp.	Winter2018-19

pent	0.08	0.03	0.04	0.16	2	NO	Apodemus spp.	Winter2018-19
pent	0.10	0.07	0.02	0.35	2	NO	Apodemus spp.	Winter2018-19
pent	0.10	0.07	0.02	0.34	2	NO	Apodemus spp.	Winter2018-19
pent	0.02	0.04	0.00	0.65	2	NO	Apodemus spp.	Winter2018-19
φ	0.87	0.03	0.81	0.92	1	NO	Apodemus spp.	Winter2018-19
φ	0.98	0.02	0.86	1.00	1	NO	Myodes glareolus	Winter2018-19
p	0.77	0.06	0.63	0.87	1	NO	Apodemus spp.	Winter2018-19
p	0.78	0.05	0.68	0.86	2	NO	Apodemus spp.	Winter2018-19
p	0.56	0.06	0.43	0.67	5	NO	Apodemus spp.	Summer2019
p	0.71	0.05	0.61	0.79	7	NO	Apodemus spp.	Summer2019
p	0.56	0.07	0.41	0.69	10	NO	Apodemus spp.	Winter2019-20
p	0.51	0.07	0.38	0.64	11	NO	Apodemus spp.	Winter2019-20
p	0.39	0.07	0.26	0.53	12	NO	Apodemus spp.	Winter2019-20
p	0.56	0.07	0.43	0.68	13	NO	Apodemus spp.	Winter2019-20
p	0.61	0.07	0.46	0.73	14	NO	Apodemus spp.	Winter2019-20
p	0.73	0.06	0.59	0.83	15	NO	Apodemus spp.	Summer2020
p	0.78	0.04	0.68	0.86	17	NO	Apodemus spp.	Summer2020
p	0.71	0.07	0.56	0.83	19	NO	Apodemus spp.	Summer2020
p	0.50	0.07	0.37	0.63	22	NO	Apodemus spp.	Winter2020-21
p	0.65	0.05	0.54	0.74	23	NO	Apodemus spp.	Winter2020-21
p	0.18	0.04	0.12	0.28	24	NO	Apodemus spp.	Winter2020-21
p	0.53	0.05	0.42	0.63	25	NO	Apodemus spp.	Winter2020-21
p	0.60	0.05	0.50	0.69	26	NO	Apodemus spp.	Winter2020-21
p	0.59	0.05	0.48	0.69	27	NO	Apodemus spp.	Summer2021
p	0.56	0.09	0.39	0.72	1	YES	Apodemus spp.	Winter2018-19
p	0.57	0.07	0.43	0.70	2	YES	Apodemus spp.	Winter2018-19
p	0.32	0.05	0.22	0.43	5	YES	Apodemus spp.	Summer2019
p	0.48	0.06	0.37	0.59	7	YES	Apodemus spp.	Summer2019
p	0.32	0.06	0.21	0.45	10	YES	Apodemus spp.	Winter2019-20
p	0.28	0.06	0.18	0.41	11	YES	Apodemus spp.	Winter2019-20
p	0.19	0.05	0.11	0.30	12	YES	Apodemus spp.	Winter2019-20
p	0.32	0.06	0.21	0.45	13	YES	Apodemus spp.	Winter2019-20
p	0.36	0.07	0.24	0.50	14	YES	Apodemus spp.	Winter2019-20

p	0.49	0.08	0.34	0.65	15	YES	Apodemus spp.	Summer2020
p	0.57	0.06	0.45	0.68	17	YES	Apodemus spp.	Summer2020
p	0.48	0.08	0.32	0.64	19	YES	Apodemus spp.	Summer2020
p	0.27	0.06	0.17	0.39	22	YES	Apodemus spp.	Winter2020-21
p	0.40	0.06	0.30	0.52	23	YES	Apodemus spp.	Winter2020-21
p	0.08	0.02	0.05	0.13	24	YES	Apodemus spp.	Winter2020-21
p	0.29	0.05	0.21	0.39	25	YES	Apodemus spp.	Winter2020-21
p	0.36	0.05	0.27	0.46	26	YES	Apodemus spp.	Winter2020-21
p	0.35	0.05	0.26	0.45	27	YES	Apodemus spp.	Summer2021
ψ	NA	NA	NA	NA	1	NO	Apodemus spp.	Winter2018-19

Table S6.4: Derived estimates of population size (N_t) retrieved by the best model (Model3, Table S5.2) with confidence intervals (Lower, Lcl, and Upper, Ucl). Legend: ‘S’ = true survival; ‘pent’ = entry probability; ‘φ’ = apparent survival; ‘p’ = capture probability; ‘ψ’ = transition probability set as constant; ‘session’ = primary trapping occasions; ‘feeding site’ = sites with supplemental food; ‘species’ = rodent species. For some sessions, the model fitting did not converge.

Estimate	Lcl	Ucl	Session	Feeding site	Species
7.51	7.12	7.89	1	NO	Apodemus spp.
13.71	13.32	14.11	2	NO	Apodemus spp.
34.52	30.84	38.20	3	NO	Apodemus spp.
34.25	32.64	35.85	4	NO	Apodemus spp.
3.69	3.21	4.17	5	NO	Apodemus spp.
2.57	2.20	2.94	6	NO	Apodemus spp.
1.52	1.17	1.87	7	NO	Apodemus spp.
4.91	4.33	5.49	8	NO	Apodemus spp.
5.91	5.30	6.52	9	NO	Apodemus spp.
8.78	8.24	9.31	10	NO	Apodemus spp.
21.38	20.61	22.16	11	NO	Apodemus spp.
13.26	12.34	14.19	12	NO	Apodemus spp.
27.43	23.36	31.51	13	NO	Apodemus spp.

21.82	20.34	23.31	14	NO	Apodemus spp.
18.71	11.94	25.47	15	NO	Apodemus spp.
24.11	21.56	26.66	16	NO	Apodemus spp.
14.25	13.18	15.33	17	NO	Apodemus spp.
27.57	25.31	29.82	18	NO	Apodemus spp.
NA	NA	NA	1	NO	Apodemus spp.
6.14	5.21	7.07	1	YES	Apodemus spp.
13.77	12.75	14.79	2	YES	Apodemus spp.
84.05	65.12	102.99	3	YES	Apodemus spp.
61.51	53.79	69.24	4	YES	Apodemus spp.
22.69	16.27	29.11	5	YES	Apodemus spp.
15.31	10.66	19.96	6	YES	Apodemus spp.
7.82	4.67	10.96	7	YES	Apodemus spp.
5.21	3.78	6.63	8	YES	Apodemus spp.
7.95	5.96	9.94	9	YES	Apodemus spp.
19.68	16.29	23.06	10	YES	Apodemus spp.
41.40	37.20	45.60	11	YES	Apodemus spp.
18.75	15.14	22.35	12	YES	Apodemus spp.
43.48	30.21	56.76	13	YES	Apodemus spp.
26.69	22.04	31.33	14	YES	Apodemus spp.
46.52	24.34	68.70	15	YES	Apodemus spp.
14.90	11.44	18.36	16	YES	Apodemus spp.
32.17	26.46	37.88	17	YES	Apodemus spp.
34.50	28.09	40.91	18	YES	Apodemus spp.
8.38	8.05	8.71	1	NO	Myodes glareolus
10.29	10.11	10.46	2	NO	Myodes glareolus
7.06	6.38	7.74	3	NO	Myodes glareolus
7.51	7.22	7.79	4	NO	Myodes glareolus
5.88	5.20	6.55	5	NO	Myodes glareolus
14.66	12.80	16.53	6	NO	Myodes glareolus
12.84	10.08	15.59	7	NO	Myodes glareolus
15.25	13.66	16.83	8	NO	Myodes glareolus
10.21	9.29	11.12	9	NO	Myodes glareolus

13.87	13.19	14.54	10	NO	Myodes glareolus
8.37	8.13	8.60	11	NO	Myodes glareolus
6.44	6.07	6.80	12	NO	Myodes glareolus
8.68	7.50	9.85	13	NO	Myodes glareolus
11.07	10.43	11.72	14	NO	Myodes glareolus
24.63	15.87	33.39	15	NO	Myodes glareolus
14.49	13.09	15.89	16	NO	Myodes glareolus
19.36	18.10	20.63	17	NO	Myodes glareolus
16.07	14.92	17.23	18	NO	Myodes glareolus
5.86	5.07	6.65	1	YES	Myodes glareolus
3.23	3.08	3.39	2	YES	Myodes glareolus
1.63	1.18	2.07	5	YES	Myodes glareolus
2.40	1.45	3.35	7	YES	Myodes glareolus
2.98	2.27	3.69	9	YES	Myodes glareolus
1.24	1.05	1.44	10	YES	Myodes glareolus
1.39	1.16	1.63	14	YES	Myodes glareolus
5.20	4.01	6.39	16	YES	Myodes glareolus
16.56	13.73	19.38	17	YES	Myodes glareolus
21.50	17.63	25.37	18	YES	Myodes glareolus

Appendix S7: Ancillary demographic parameters

Apparent survival

Apparent survival (φ) showed differences across the two latitudes. In particular, in Norway φ in *M. glareolus* varied through seasons with slight differences between winter and summer, but no feeding effect was detected (Figure S7.1).

In Italy, on the contrary, φ was affected by species and it was higher in *M. glareolus* than in *Apodemus spp.* (Figure S7.2).

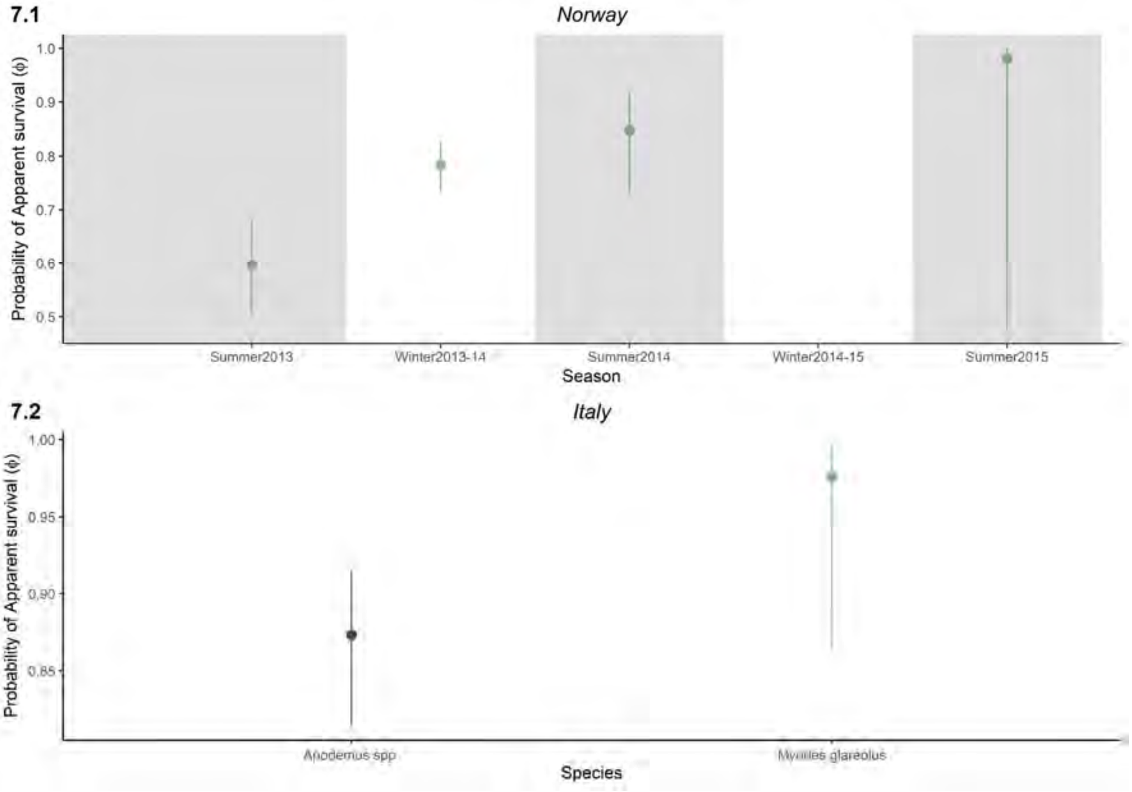


Figure S7.1 and S7.2: Real estimates of apparent survival (φ) in Norway (**Figure S7.1**, above) depending on seasonal periods and in Italy (**Figure S7.2**, below) for each detected species (*M. glareolus* and *Apodemus spp.*). Grey-shaded boxes show the summer periods, while white boxes show the winter periods.

Arrival probability

Arrival probability (*pent*) was time-dependent both in Norway and in Italy. In Norway *pent* retained a temporal effect, but depended on primary occasions rather than on secondary occasions (Figure S7.3). In particular, *pent* was high during autumn when food was not provided, and then during winter with supplemental food.

In Italy *pent* was affected by secondary trapping occasions, with higher values at the first secondary occasion, which decreased with the subsequent fives (Figure S7.4).

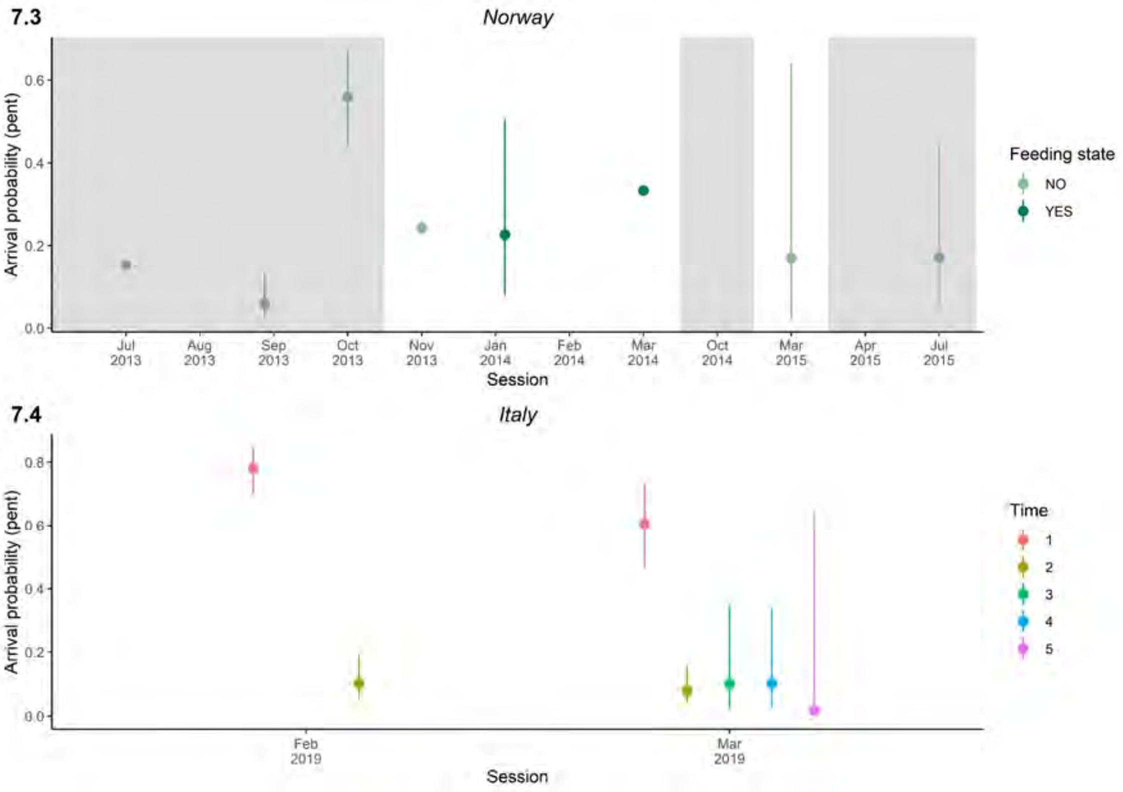


Figure S7.3 and S7.4: Real estimates of arrival probability (pent) in Norway (**Figure S7.3**) for each seasonal periods and under treatment (supplemental food availability; dark green) and control (no supplemental food; light green) conditions, and in Italy (**Figure S7.4**) for each secondary trapping occasions. Grey-shaded boxes show the summer periods, while white boxes show the winter periods.

Capture probability

In Norway, *p* depended only on feeding state and in particular, when supplemental food was provided, animals were less captured than without food (Figure S7.5).

Similarly, in Italy *p* changed in relation with supplemental food and across primary occasions. In particular, *p* was lower where food was provided during the entire sampling period for both species. In general, *p* declined from summer to winter, with some exceptions (December 2020 and January 2021) (Figure S7.6).

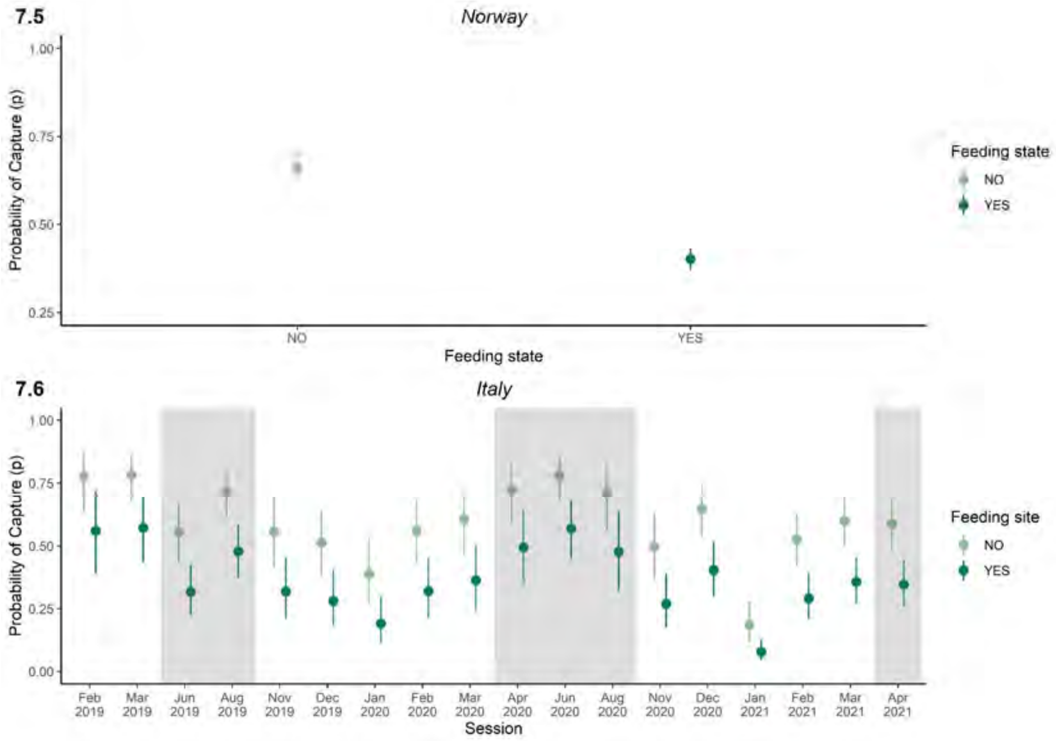


Figure S7.5 and S7.6: Estimates of capture probability (*p*) in rodents in Norway (**Figure S7.5**) and in Italy (**Figure S7.6**) for each primary trapping occasion, with treatment supplemental food (supplemental food available; dark green) and control (no supplemental food available; light green) conditions ('Feeding state' for Norway and 'Feeding site' for Italy). Grey-shaded boxes show the summer periods, while white boxes show the winter periods.

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II

III

IV

microorganisms

Communication

First Record of *Hepatozoon* spp. in Alpine Wild Rodents: Implications and Perspectives for Transmission Dynamics across the Food Web

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Abstract: Among the Apicomplexa parasites, *Hepatozoon* spp. have been mainly studied in domestic animals and peri-urban areas. The epidemiology of *Hepatozoon* spp. is poorly investigated in natural systems and wild hosts because of their scarce veterinary and economic relevance. For most habitats, the occurrence of these parasites is unknown, despite their high ecosystemic role. To fill this gap for alpine small mammals, we applied molecular PCR-based methods and sequencing to determine the *Hepatozoon* spp. in 830 ear samples from 11 small mammal species (i.e., *Apodemus*, *Myodes*, *Chionomys*, *Microtus*, *Crocidura* and *Sorex* genera) live-trapped during a cross-sectional study along an altitudinal gradient in the North-Eastern Italian Alps. We detected *Hepatozoon* spp. with an overall prevalence of 35.9%. Two species ranging from 500 m a.s.l. to 2500 m a.s.l. were the most infected: *My. glareolus*, followed by *Apodemus* spp. Additionally, we detected the parasite for the first time in another alpine species: *C. nivalis* at 2000–2500 m a.s.l. Our findings suggest that several rodent species maintain *Hepatozoon* spp. along the alpine altitudinal gradient of habitats. The transmission pathway of this group of parasites and their role within the alpine mammal community need further investigation, especially in consideration of the rapidly occurring environmental and climatic changes.

Keywords: *Hepatozoon* spp.; *Chionomys nivalis*; *Apodemus* spp.; *Myodes glareolus*; Italian Alps

Citation: Ferrari, G.; Girardi, M.; Cagnacci, F.; Devineau, O.; Tagliapietra, V. First Record of *Hepatozoon* spp. in Alpine Wild Rodents: Implications and Perspectives for Transmission Dynamics across the Food Web. *Microorganisms* **2022**, *10*, 712. https://doi.org/10.3390/microorganisms10040712

Academic Editor: Sonia Almeria

Received: 14 February 2022
Accepted: 18 March 2022
Published: 25 March 2022

1. Introduction

Protozoa parasites are a highly diverse group of successful organisms globally distributed via a wide range of hosts [1] and whose role is crucial for ecosystem functioning [2]. Among the blood protozoa parasites, the phylum Apicomplexa has received much attention for its zoonotic relevance, for example, the vector borne diseases due to *Plasmodium* spp. or the *Toxoplasma* spp. transmitted in urban cycles that include domestic species and humans. Other protozoan parasites, such as the *Hepatozoon* genus represented by 340 species, have also been commonly recorded in peri-urban cycles sustained by domestic species (e.g., cats and dogs) [3–8], although no zoonotic relevance has been reported so far [9,10]. Further, *Hepatozoon* spp. have been detected in amphibians, reptiles, birds that act as intermediate hosts, and mammals, which can be both paratenic and reservoir hosts [11].

The transmission of *Hepatozoon* spp. can occur through different modalities that most often involve the ingestion of infected vectors or hosts. In mammals, for example, animals can become infected through grooming behavior [12] by eating infected arthropod ectoparasites attached to another individual, such as ticks [13], fleas [14], and mosquitoes [15]. Further, predator–prey food web transmission routes can occur through the ingestion of prey either containing infective meronts or infested by infected vectors [16]. Additionally, vertical transplacental transmission has also been reported [17].

Hepatozoon spp. have been found in a diversity of habitats across the globe, such as temperate broad-leaved forests [18,19], conifer forests [20], Mediterranean scrubland [21], subtropical forests and savannah [22], and taiga [23], likely indicating complex transmission cycles that are still largely unknown. However, the occurrence and prevalence of *Hepatozoon* spp. in some crucial ecosystems remain unexplored, for example, across altitudinal gradients where the rapid succession of habitats often corresponds to the high biodiversity of partially sympatric hosts. Under global and climate changes, such mountain habitats are particularly exposed to abiotic and biotic variations that favor upward distributional shifts of host mammal species, with local modifications of the communities [24,25]. This may result in parasite expansion in terms of geographic distribution and diversity of the host species involved [26,27]. The monitoring of the occurrence of a parasite with a very plastic transmission cycle, such as *Hepatozoon* spp., is therefore of crucial importance in these habitats undergoing intense dynamics. This is the case for Alpine ecosystems, where *Hepatozoon* spp. were identified in wild carnivores [18,20] but have never been recorded in alpine small mammals [28]. The occurrence of the parasite in the small mammal community is of particular interest, as it prevalently depends on arthropods acting as vectors, the vertical transmission route being secondary. The main arthropods transmitting *Hepatozoon* spp. to rodents are blood ectoparasites, such as ticks, fleas, mites, and mosquitoes [29], the occurrence of which is shifting upward in the Alps as the temperature and humidity conditions become milder with the climate change [30].

In small mammals, *Hepatozoon* spp. life cycles involve schizogony, which occurs in various organs, such as muscles, lymph nodes, the spleen, and generally in the liver, while gametogony occurs in lymphocytes, monocytes, or occasionally, in granulocytes [29]. Further development requires an arthropod vector in which fertilization and sporogony take place [29]. Several species of the *Hepatozoon* genus were detected in European rodents, e.g., *H. lavieri* [31], *H. erhardovae* [23,32], *H. sylvatici* [33,34], *H. griseisciuri* [33], and sporadically *H. muris* (formerly known as *H. perniciosum*) [33,35]. In rodents, the infection of *Hepatozoon* spp. may be pathogenic [36–40], although generally mild and asymptomatic [41]. Conversely, in carnivores (i.e., secondary hosts), such as canids [42], felids [43], mustelids [44], and in snakes [45], *Hepatozoon* spp. may compromise the immune status, thus predisposing it toward coinfection by other pathogens.

In this work, we conducted a cross-sectional study, i.e., we assessed the parasite prevalence without specifically considering the temporal pattern from 2019 to 2021 in the Italian Alps by intensively live-trapping small mammals from forested habitats up to above the tree line. Our main aim was to evaluate the occurrence of *Hepatozoon* spp. across the altitudinal gradient of the Alpine habitat also in consideration of the anthropic pressure on such habitat. To the best of our knowledge, this is the first study investigating *Hepatozoon* spp. occurrence in small mammals, and more generally in rodents in Italy, with a special focus on the Alpine habitat.

2. Materials and Methods

2.1. Study Area and Animal Sampling

The study was carried out in two sites located in the Province of Trento (Italy): Cembra Valley (CEV; 46.13020 N–11.17843 E, altitude 1000 m a.s.l.) and Calamento Valley (CAV; 46.12092 N–11.48842 E, altitude from 500 to 2500 m a.s.l.) (Figure 1). CEV comprises peri-urban areas with an alternation of small villages and areas of anthropic employment (quarries and forest harvest) and semi-naturalized forests. CAV is instead characterized by the full vegetation succession, from broad-leaf woodland and mixed forest to conifer and stands forest, shrubs, and alpine prairies. The area is relatively undisturbed, with few anthropic activities (traditional cattle husbandry and seasonal tourism). In CEV, small mammal monitoring was performed from 2019 to 2021 at monthly or bimonthly intervals during the winter (November–March) and summer (April, June, and August), respectively, in a mixed broad-leaved and coniferous forest at 1000 m a.s.l. In CAV, live trapping was performed in 2019 and 2020 at monthly intervals during the summer (May–October) along an altitudinal gradient (from 500 m a.s.l. to 2500 m a.s.l.), corresponding to the succession of alpine habitats (i.e., from broad-leaf woodland down the valley up to grasslands above the tree line).

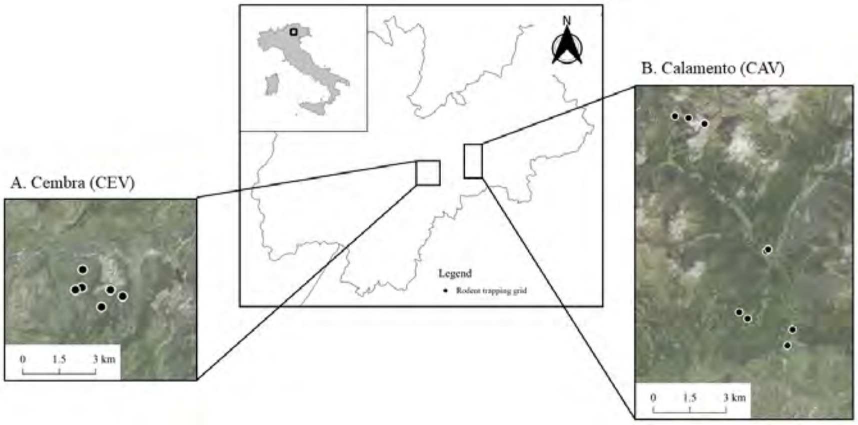


Figure 1. Map of the study sites located in the Province of Trento (Italy). (**A**) Cembra Valley (CEV), where square grids (8 × 8 traps) were placed at 1000 m a.s.l. (**B**) Calamento Valley (CAV), where cross-shaped grids (16 traps) were placed from 500 to 2500 m a.s.l. Black circles show trapping grid locations.

Small mammals were captured using standard Ugglan Multiple Live Traps (model 2, Granhab, Sweden) set in a grid array, following a protocol that aimed at capturing especially rodents to compare assemblages across an altitudinal gradient. Capture–mark–recapture (CMR) techniques were adopted, and each animal was individually tagged with a subcutaneous Passive Integrated Transponder (PIT) tag (Trovan® Ltd., Douglas, UK). At each capture event, we recorded information on individuals, such as species, PIT tag code, sex, body mass, breeding status, and ectoparasites load. Additionally, at first capture, an ear biopsy (using sterile disposable ear punch needles, Ø 3 mm; 20 mg) was collected for each animal and individually placed in tubes stored at −80 °C.

All animal handling procedures and ethical issues were approved by the Provincial Wildlife Management Committee (Prot. n. S044-5/2015/277268/2.4).

2.2. DNA Extraction, Amplification, and Sequencing

For DNA extraction, the ear samples (506 from CEV and 326 from CAV) were incubated overnight at 56 °C and isolated using the DNeasy 96 Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

This study is part of a wider project investigating tick-borne pathogen circulation in small mammals. Therefore, the identification of *Hepatozoon* spp. was a bycatch while assessing the protocol for *Babesia* spp. detection on ear tissue samples. We applied a conventional polymerase chain reaction (PCR) method using the *Babesia* BJ1 5′–GTC TTG TAA TTG GAA TGA TGG–3′ and BN2 5′–TAG TTT ATG GTT AGG ACT ACG–3′ primers [46]. Indeed, beyond *Babesia* spp., BJ1 and BN2 primers can amplify the 18S RNA gene of some other protozoans [47], such as the 600-bp fragment of *Hepatozoon* spp. [48–50]. To discriminate the two parasites and detect coinfections, we analyzed all the samples separately (protocol of *Babesia* spp. not described) by specifically adapting the PCR reactions and temperature cycling profiles for each protozoan [47].

In particular, for *Hepatozoon* spp. detection, the PCR reactions were composed of 3-mM $MgCl_2$, 0.1-mg/mL BSA, 0.2-mM each dNTP, 0.3-μM of each primer, 5× Flexi buffer, and 1.25-U/μL Go Taq Hot Start Polymerase (Promega) in 50 μL of total volume, including 1 μL of extracted DNA. After the initial denaturation at 95 °C for 2 min, 40 cycles of 95 °C for 20 s, 46 °C for 15 s, and 72 °C for 50 s were performed before a final incubation at 72 °C for 5 min. Negative controls, i.e., samples that did not contain DNA but only reagents, were included in each molecular essay to cross-check the quality for DNA extraction and PCR amplification. The success of amplification was confirmed using the Qiagen QIAxcel® capillary electrophoresis system. Positive PCR products were purified using ExoSAP-IT (USB, Cleveland, OH, USA) according to the manufacturer's instructions and then sequenced using Sanger sequencing. The DNA sequences were analyzed and aligned using Sequencher software version 5.4.6 (Gene Codes Corp., Ann Arbor, MI, USA) and Clustal X software version 2.0 [51] and subsequently compared with the data stored in the GenBank database with the Basic Local Alignment Search Tool (Blast; online version).

To verify the robustness of the PCR method, we also analyzed and sequenced double ear samples on a subset of individuals (N = 20, 2 samples from 10 individuals).

2.3. Statistical Analyses

The prevalence of *Hepatozoon* spp. was calculated for each rodent species with a 95% confidence interval (CI) using the EpiR package [52] in the R program [53]. We applied the Two Proportion Z-test to analyze the differences of the prevalence rates among rodent species. The differences were considered statistically significant if the p-values were <0.05.

Moreover, we investigated the role of the host species and environmental context on the probability of rodent infection with *Hepatozoon* spp., controlling for the life history traits. Specifically, we applied two analytical designs: (i) we evaluated the effect of anthropogenic pressure on rodent infection by comparing the study areas (CEV: high pressure; CAV: low pressure), only for a homogeneous altitude; (ii) we assessed how the probability of infection would vary across species along the alpine altitudinal gradient in a particularly wild context (CAV: 5 belts of 500 m, from 500 m a.s.l. to 2500 m a.s.l.). To this end, we fitted Generalized Linear Mixed Models (GLMMs; [54]) with binomial distribution of errors to the probability of infection of all samples with anthropogenic pressure and altitudinal belts, respectively, for the two designs, as the main covariates together with the species. We controlled for the sex and breeding status of the captured rodents (juveniles, subadults, and adult classes for both males and females). Finally, to account for spatial autocorrelation between captures, we included a grid as the most parsimonious random effect (Appendix A). Model selection was performed on the basis of the AICc score [55] (see Appendix A for details). We fitted the models using R software 4.1.2 [53] and packages *tidyverse* [56], *ppsr* [57], *MuMIn* [58], and *glmmTMB* [59].

In both proportion tests and in GLMMs, we discarded those sporadic species that were captured only a few times (<20 capture events).

3. Results

We analyzed 830 individual ear samples from 11 species (genetically determined through cytochrome-b [60]): 562 yellow-necked mice (*Apodemus flavicollis*), 10 wood mice (*Apodemus sylvaticus*), 24 snow voles (*Chionomys nivalis*), 2 common voles (*Microtus arvalis*), 2 pine voles (*Microtus subterraneus*), 1 short-tailed field vole (*Microtus agrestis*), 199 bank voles (*Myodes glareolus*), 25 common shrews (*Sorex araneus*), 3 alpine shrews (*Sorex alpinus*), 1 pygmy shrew (*Sorex minutus*), and 1 bicolored-shrew (*Crocidura leucodon*) (see Table S1). Additionally, 1790 ticks (1745 larvae and 45 nymphs) and 3782 ticks (3718 larvae and 64 nymphs) were counted on captured rodents in CEV and CAV, respectively. All sampled ticks belonged to the genus *Ixodes*. We also recorded the occurrence of other ectoparasites, i.e., fleas, and mites.

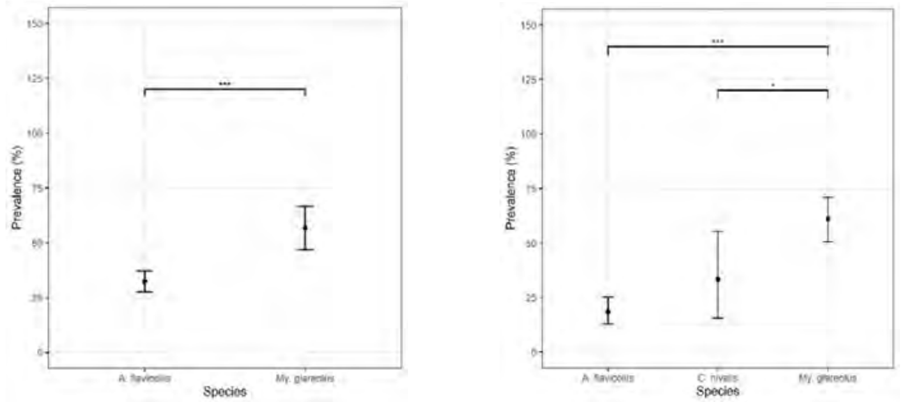
All accidentally caught shrews tested negative for *Hepatozoon* spp. and were therefore not included in the following analysis (see Table 1). The results from the 10 double samples were identical and confirmed the robustness of the PCR method.

Table 1. Prevalence of *Hepatozoon spp*. in Italy (2019–2021) from rodent and shrew specimens in two study sites (CEV = Cembra Valley; CAV = Calamento Valley) and across altitudes. CI = 95% Confidence Interval.

Family	Genus	Species	Study Site	PCR Positive/Total	% Prevalence (CI)	Altitude of Positives (m a.s.l.)
Muridae	*Apodemus*	*flavicollis*	CEV	127/394	32.23 (27.64–37.1)	1000
		flavicollis	CAV	31/168	18.45 (12.89–25.16)	500, 1000, 1500
		sylvaticus	CEV	3/8	37.5 (8.52–75.51)	1000
		sylvaticus	CAV	0/2	-	-
Arvicolidae	*Chionomys*	*nivalis*	CAV	8/24	33.33 (15.63–55.32)	2000, 2500
	Microtus	*agrestis*	CAV	0/1	-	-
		arvalis	CAV	1/2	50.00 (1.26–98.74)	2000
		subterraneus	CAV	0/2	-	-
	Myodes	*glareolus*	CEV	59/104	56.73 (46.65–66.41)	1000
		glareolus	CAV	58/95	61.05 (50.50–70.89)	1000, 1500, 2000
Soricidae	*Crocidura*	*leucodon*	CAV	0/1	-	-
	Sorex	*alpinus*	CAV	0/3	-	-
		araneus	CAV	0/25	-	-
		minutus	CAV	0/1	-	-
Total positive				287/830	34.58 (31.34–37.92)	
Total positive rodents				287/800	35.87 (32.55–39.30)	

Among rodents, for all samples, five species tested positive for *Hepatozoon* spp.: *A. flavicollis* (28.1%), *A. sylvaticus* (30.0%), *C. nivalis* (33.3%), *M. arvalis* (50%), and *My. glareolus* (58.8%) (see Table 1 for details), with an overall prevalence of 35.9% (see Appendix B for sequences).

In Cembra Valley (CEV; 506 samples), *Hepatozoon* spp. was detected in all three rodent species captured (Table 1). *Hepatozoon* spp. prevalence was higher in *My. glareolus* if compared with *A. flavicollis* (Z-test, *p*-value = 6.855×10^{-6}) (Figure 2a).



(**a**) (**b**)

Figure 2. Difference of the prevalence rates of *Hepatozoon* spp. among rodent species from the Province of Trento, Italy (2019–2021): (**a**) in Cembra Valley (CEV) and (**b**) in Calamento Valley (CAV). Vertical bars represent the 95% confidence interval. Horizontal bars refer to significant differences, with *** = significance level < 0.001 and * = significance level < 0.05.

In Calamento Valley (CAV; 294 samples), four rodent species tested positive (Table 1). *My. glareolus* showed a statistically significant higher prevalence compared to *A. flavicollis* and *C. nivalis* (Z-test, *p*-value = 6.075×10^{-12} and *p*-value = 0.027, respectively) (Figure 2b). Among the species with too few captures to compute prevalence, *Hepatozoon* spp. was recorded in *M. arvalis*, in one individual out of the two captures and *A. sylvaticus*, in three out of eight captures.

The results of the GLMMs showed that rodent species have a crucial role in driving the infection of *Hepatozoon* spp. Conversely, both human pressure and the altitudinal gradient were not selected in the best models, indicating their scarce relevance in influencing the probability of becoming infected by *Hepatozoon* spp. (Appendix A). In particular, in the first analysis between study areas at 1000 m a.s.l., the infection probability depended on the species, with *My. glareolus* more likely to be infected than *A. flavicollis* ($\beta = 1.07 \pm 0.21$; *p*-value = 3.27×10^{-7}; reference category: *A. flavicollis*). In the second analysis, the infection of *Hepatozoon* spp. depended on the additive effect of the species and breeding status, confirming that *My. glareolus* was more prone to becoming infected if compared to *A. flavicollis* ($\beta = 1.97 \pm 0.30$; *p*-value = 5.98×10^{-11}; reference category: *A. flavicollis*), while no significant difference emerged between *A. flavicollis* and *C. nivalis* (Appendix A). In addition, a minor effect of the breeding status emerged, in which juveniles seemed to be marginally more infected compared to adults ($\beta = 1.29 \pm 0.70$; *p*-value = 0.06; reference category: Adults), while we did not detect any significant difference between adults and subadults (Appendix A).

It is relevant to observe that, after sequencing, two samples (one from *S. araneus* and one from *My. glareolus*) previously assigned to *Hepatozoon* spp. were instead confirmed as *Babesia microti* (see Appendix B).

4. Discussion

This study reports for the first time the occurrence and prevalence of *Hepatozoon* spp. in wild rodents in the Italian Alps. In particular, we found a high prevalence in the most common woodland rodent hosts, i.e., *My. glareolus* and *A. flavicollis*, as well as in two other alpine species: the sympatric *A. sylvaticus* and *C. nivalis* at high altitudes.

This study is part of a wider project investigating tick-borne pathogen circulation in small mammals in the Italian Alps. In this context, the detection of rodent infection by *Hepatozoon* spp. represented a bycatch of the assessment of *Babesia* spp. For this reason, *Babesia*-specific primers were used for the screening, and the positive samples were further identified through sequencing. The identification of two positive samples of *Babesia microti* underlines the risk of mismatch when *Babesia*-specific primers are used for detecting *Hepatozoon* spp., as already underlined by other studies [47]. This finding suggests that follow-up sequencing is a compulsory step to discriminate this parasite by other protozoa when their detection is due to nonspecific protocols. For these reasons, we were not able to further identify *Hepatozoon* spp. at the species level due to the low quality of the obtained sequences. The identification could be refined by using *Hepatozoon*-specific primers (HEPF/HEPR [47]).

European studies on *Hepatozoon* spp. in wild rodents have been generally based on blood [32,47,61,62], organs [14,22,23], and, more rarely, on skin [13,63] samples, which are often collected through invasive sampling methods that, in many cases, require the suppression of the animals. In live rodents, *Hepatozoon* spp. can be identified only through blood sampling, to the best of our knowledge [31,32]. This comes with the drawback that traditional microscopy based on a blood smear can underestimate *Hepatozoon* spp. prevalence, especially with low intensity of infection [47]. On the contrary, PCR-based assays, such as those that we implemented using ear biopsy samples collected from live-trapped rodents, are considered more sensitive and robust. Coupling molecular screening approaches with less invasive sampling methods may accomplish conservation issues in cases of endangered and vulnerable species, e.g., *C. nivalis* [64], limiting the impact on the studied species. Within this framework, we believe that our contribution may provide a useful methodological approach. Although the comparison of past studies based on different protocols or matrices might not be feasible, the comparison between different procedures to assess *Hepatozoon* spp., e.g., by comparing ear tissue samples with skin ones as those collected in studies [13,63], could be an interesting direction of research.

The number of species of the small mammal community in which *Hepatozoon* spp. was detected, its first identification in *C. nivalis* (33.3%), and its occurrence at 2000 and 2500 m a.s.l., the highest elevation at which these parasites have ever been recorded, represent key findings to speculate on the transmission pathways of *Hepatozoon* spp. in the Alpine range. Additionally, this is the first record of *Hepatozoon* spp. in *A. sylvaticus* (30.0%) using molecular approaches [34,65]. Since small mammals can become infected from *Hepatozoon* spp. mainly though the ingestion of infected vectors, it is reasonable to consider *Hepatozoon* spp. as a vector-borne infectious agent for small mammal hosts and specifically in our case, although we did not assess the infective status and transmission dynamics of arthropod vectors. Our results suggest that *Hepatozoon* spp. are common in alpine environments, showing a high prevalence in a broad spectrum of alpine small mammal species. In particular, we did not detect any relevant difference in *Hepatozoon* spp. prevalence between anthropic and wild systems, implying a stable and ubiquitous presence of *Hepatozoon* spp. in the analyzed small mammal species. This is interesting, as *Hepatozoon* spp. have been previously studied mainly in domestic species [3–8]. The low host specificity displayed by *Hepatozoon* spp. [23,29] may indicate that these parasites persist in the environment sustained by competent and reservoir hosts, such as small mammals [66], both in wild and more anthropic settings. These conditions may increase the risk of spillover events among wildlife and domestic animals [67,68]. For example, *A. sylvaticus* generally shares food resources, part of the habitats and vectors, with other rodent competitor species, especially in case of high rodent and vector density [69], as it may occur in permissive environmental conditions (low elevations, in our case). The high density of reservoir hosts and of potentially infected vectors may amplify parasite circulation and spreading within the ecosystem, facilitating also the infection of less abundant host species [70]. This mechanism may have also favored the transition of the parasite to high altitudes, especially under increasingly milder climatic conditions. The less-limiting abiotic conditions along the altitudinal gradient occurring under climate change may promote an upward distributional shift of opportunistic small mammal host species (e.g., *My. glareolus* and *Apodemus* spp.) and, in turn, the survival and development of arthropod vectors (e.g., mites, fleas, and ticks) [27,71,72]. The combination of abiotic (i.e., climate) and biotic (i.e., interspecific interactions) conditions that permit host and vector persistence, together with the ability of a species to colonize favorable habitats, may therefore alter the local alpine communities, leading to novel host–vector species pairings and, thus, to the emergence of vector-borne diseases in new environments [73–76]. The occurrence of *Hepatozoon* spp. in *C. nivalis* and high altitudes that we recorded is compatible with these dynamics, although there is no track record of previous investigations at the same altitudes in small mammals (see Reference [28]).

The high rate of infection that we observed in different rodent species across a diversity of Alpine habitats may induce relevant cascading effects across the food web. In particular, being carnivore-competent hosts for *Hepatozoon* spp., a broad spectrum of both domestic (e.g., shepherd dogs [77]) and wild animals (e.g., snakes [78], meso- and large carnivores [20,79], and birds of prey [80]) may become infected through the ingestion of parasitized rodent preys. Specifically, small mammals are paratenic hosts for *Hepatozoon* spp., meaning that, despite not being necessary for parasite development, they help in maintaining its life cycle in the environment by supporting the cystozoite stages [40] infective for predators via ingestion [81]. The parasite can affect the immune response of predator secondary hosts, leading to population declines [39]. However, at the same time, since vector competence in becoming infected, replicating and transmitting the parasite varies across hosts [81], predators may represent dead end hosts for *Hepatozoon* spp. so indirectly reducing parasites transmission across ecosystem (i.e., dilution effect). This aspect of the transmission pathway of *Hepatozoon* spp. needs further investigation.

The prevalence of *Hepatozoon* spp. that we recorded in the most common rodent hosts, i.e., *My. glareolus* and *A. flavicollis*, is in partial accordance with other studies in Europe. In particular, we found that *My. glareolus* showed the highest prevalence of *Hepatozoon* spp. among all captured rodent species (58.8%), as was also observed in other studies [13,32,47,63], although with very variable levels (from 3.7% to 87.5% [13,23,47,48,62,82]). Conversely, the prevalence we found for *A. flavicollis* (28.1%) is higher than all previously published estimates that were below 10% [13,47,48,63] and may support a high prevalence of *Hepatozoon* spp. infection at lower elevations. The small sample size of *Microtus* spp. voles does not allow conclusive indications on the rate of infection of this genus (one positive *M. arvalis* out of five in total), as was the case also for other studies [23,31,32,47].

In conclusion, Apicomplexa remains one of the most poorly investigated groups among protozoa, so that accurate identification (via PCR-based methods coupled with sequencing) of *Hepatozoon* spp. in new wild hosts and habitats importantly contributes to the understanding of the ecological role of these parasites, especially under the current global change. Collectively, by detecting *Hepatozoon* spp. in a broad range of rodent hosts throughout the altitudinal gradient of Alpine habitats, we indicated the widespread occurrence of this parasite as likely supported by an expanding availability of arthropod vectors, due to variations in the temperature and moisture linked to climate change [75]. Therefore, this study represents a crucial starting point for future research combining ecological, epidemiological, and molecular analysis to evaluate the trophic transmission route of *Hepatozoon* spp. across the food chain in habitats particularly exposed to climate change, such as the Alpine range. In particular, studies investigating vector competence and distribution, as well as host ecology and their role in maintaining and transmitting *Hepatozoon* spp., are needed to clarify the function of this group of parasites in such changing ecosystems.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/microorganisms10040712/s1: Table S1: List of animals captured per site at Cembra and Calamento Valleys (Trento, Italy) and PCR results.

Author Contributions: Conceptualization: G.F., F.C., O.D. and V.T.; Methodology: G.F., M.G., F.C., O.D. and V.T.; Data curation: G.F. and V.T.; Investigation: G.F., M.G. and V.T.; Formal analysis: G.F. and O.D.; Writing—Original Draft: G.F.; Writing—Review and Editing: F.C., O.D., M.G. and V.T.; and Supervision: F.C., O.D. and V.T. All authors have read and agreed to the published version of the manuscript.

Funding: G.F. was supported by the Inland Norway of Applied Sciences Doctoral Program in Applied ecology and biotechnology 2019/2022 and Fondazione Edmund Mach International Doctoral Program. F.C. contributed to this work partly under the IRD Fellowship 2021/2022 at Fondation IMéRA, Institute for Advanced Studies at Aix-Marseille Université.

Institutional Review Board Statement: The animal study protocol was approved by the Provincial Wildlife Management Committee (Authorization n. 663; date: 23 April 2015).

Data Availability Statement: The data presented in this study are available in the Supplementary Materials section and from the EuroSmallMammals database (https://eurosmallmammals.fmach.it (accessed on 6 March 2022)).

Acknowledgments: We are grateful to three anonymous reviewers for their comments that greatly improved the clarity and outcomes of this article. We are also grateful to all the people involved in the rodent trapping and data collection, and especially Arnoldi D., Dal Farra S., Inama E. and Savazza S., as well as Casari S. and Rosso F., for their suggestions in molecular analysis. We thank all the members of the Applied Ecology research unit, Animal Ecology research unit, and Conservation Genomics research unit of Fondazione Edmund Mach.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

To investigate the role of host species and environmental context (explanatory variables) on the probability of rodent infection with *Hepatozoon* spp. (response variable), a set of potentially biological meaningful covariates was considered, also controlling for life history traits, and specifically:

(i). life-history covariates
   1. Sex (**categorical variable**): Female or Male;
   2. Breeding status (**categorical variable**): Juvenile, Sub-adult, Adult;
   3. Species (**categorical variable**): Small mammal species captured ad least 20 times (i.e., *Apodemus flavicollis*, *Myodes glareolus*, *Chionomys nivalis* and *Sorex araneus*).

(ii). environmental covariates
   1. Study area (**categorical variable**): based on the anthropic pressure, Cembra Valley (CEV) is characterized as 'high anthropic' and Calamento Valley (CAV) as 'low anthropic'.
   2. Altitude (**categorical variable**, only for CAV): the altitudinal gradient defined by 5 classes: 500 m, 1000 m, 1500 m, 2000 m and 2500 m a.s.l.

Specifically, we applied two analytical designs: (i), we evaluated the effect of anthropogenic pressure on rodent infection by comparing the study areas (Cembra: high pressure; Calamento: low pressure), only for altitudes homogeneous across the sites; (ii), we assessed how the probability of infection would vary across species along the alpine altitudinal gradient in a particularly wild context (Calamento Valley: 5 belts of 500 m from 500 m a.s.l. to 2500 m a.s.l.). To this end, we fitted Generalized Linear Mixed Models (GLMMs; [54]) with binomial distribution of errors to the probability of infection. To account for spatial autocorrelation of captures, we performed a first model selection on random effects based on second-order Akaike Information Criterion (AICc), by maximizing the fixed effects (i.e., considering the full model). In particular, we fitted model with random effect on trap nested within grid ('Plot/Trap'), only on trapping grid ('Plot') and only on trap ('Trap') (Table A1, Table A4). After the selection of the most appropriate random effect [83], we performed a model selection on fixed effect starting from the full model, by ranking all possible model combinations derived from the full model on the basis of the AICc (Table A2, Table A5). The explanatory variables included in the full model were: small mammal species ('Species'), breeding status ('Status'), sex ('Sex'), anthropogenic pressure characterizing the area ('Area'; used only for first analysis) and altitudinal belts ('Altitude'; used only for second analysis). The model with the lower AICc was selected as the best model [55] (Table A3, Table A6).

Appendix A1. Models across Study Areas with Different Anthropic Pressure

The most parsimonious model included 'Species' as fixed effect, and trapping grid as random effects (Table A1 and Table A2). The model coefficients revealed a strong positive effect of species and specifically, *Myodes glareolus* was more prone to becoming infected if compared with *Apodemus flavicollis* (Table A3; Figure A1). Conversely, the area and therefore the degree of anthropic pressure was not retained in the best model.

Table A1. Model selection according to AICc to identify the random effect. In bold the model selected. Legend: 'Area': anthropic pressure depending on study area; 'Sex': sex; 'Status': breeding status; 'Species': rodent species, 'Plot': trapping grid; 'Trap': trap station; Fixed effect' = fixed effect modelled; 'Random effect' = random effects modelled; 'AICc' = AIC with a correction for small sample sizes; 'ΔAICc' = relative differences between the fitted model and the Akaike 'best-ranked' model with the smallest AICc value; 'weight' = relative likelihood of a model.

Fixed Effect	Random Effect	AICc	ΔAICc	Weight
Area + Sex + Status + Species	**1∣Plot**	**766.2**	**0.00**	**0.71**
Area + Sex + Status + Species	1∣Plot/Trap	768.3	2.06	0.25
Area + Sex + Status + Species	1∣Trap	772.2	5.98	0.03

Table A2. Model selection to predict probability of infection of *Hepatozoon* spp., reporting the best model (in bold), the models retained within a ΔAICc ≤ 4, and the first model with ΔAICc > 4 (in italic). Legend: 'Area': anthropic pressure depending on study area; 'Sex': sex; 'Status': breeding status; 'Species': rodent species, 'Plot': trapping grid; 'Trap': trap station; Fixed effect' = fixed effect modelled; 'Random effect' = random effects modelled; 'AICc' = AIC with a correction for small sample sizes; 'ΔAICc' = relative differences between the fitted model and the Akaike 'best-ranked' model with the smallest AICc value; 'weight' = relative likelihood of a model.

Fixed Effect	Random Effect	AICc	ΔAICc	Weight
Species	**1|Plot**	**752.72**	**0.00**	**0.41**
Area + Species	1|Plot	754.68	1.96	0.15
Sex + Species	1|Plot	754.74	2.03	0.15
Status + Species	1|Plot	755.12	2.41	0.12
Area + Species + Sex	1|Plot	756.71	4.00	0.05
Area + Species + Status	*1|Plot*	*757.05*	*4.33*	*0.05*

Table A3. Coefficients of the best model (Model 1, Table A2) fitting the probability of infection with *Hepatozoon* spp. The reference category for the covariate 'Species' is *A. flavicollis*. Legend: 'Species': rodent species; 'Plot': trapping grid; 'τ00' = method heterogeneity.

	Probability of Infection			
Predictors	***Estimate***	***Std. Error***	*Z value*	*Pr(>|z|)*
Intercept	−0.77 ***	0.16	−4.86	1.15×10^{-6}
Species *My. glareolus*	1.07 ***	0.21	5.11	3.27×10^{-7}
Random effects				
τ00 Plot	0.12			
N trap	10			
Observations	590			
Marginal R²/Conditional R²	0.06/0.09			

*** $p < 0.01$.

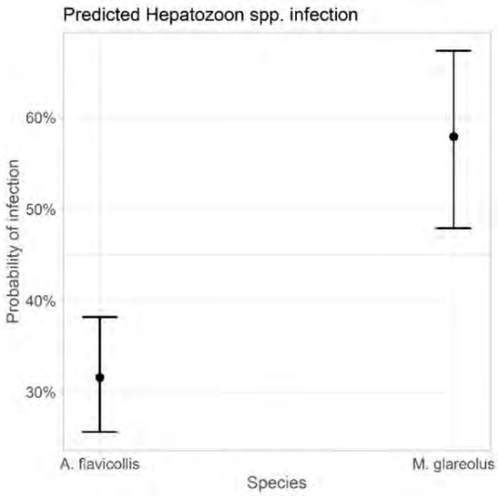


Figure A1. Predictive plot showing the effect of rodent species on probability of infection of *Hepatozoon* spp., after controlling for the effects of other covariates in the Generalized Linear Mixed Models (GLMMs; best model Table A2).

Appendix A2. Models across Altitudinal Gradient in Calamento Valley

The most parsimonious model included 'Species' and 'Status' as fixed effects, and trapping grid as random effects (Table A4, Table A5). The model coefficients revealed a strong positive effect of species and a slightly positive effect of breeding status. Specifically, we confirmed that *Myodes glareolus* positively drove the infection of *Hepatozoon* spp. rather than *Apodemus flavicollis* and *Chionomys nivalis* (Table A6; Figure A2). Moreover, juvenile animals tended to be more infected compared to adults and sub-adults (Table A6; Figure A3). Conversely, elevation was not selected in the best model.

Table A4. Model selection according to AICc to identify the random effect. In bold the model selected. Legend: 'Altitude': elevation a.s.l.; 'Sex': sex; 'Status': breeding status; 'Species': rodent species, 'Plot': trapping grid; 'Trap': trap station; Fixed effect' = fixed effect modelled; 'Random effect' = random effects modelled; 'AICc' = AIC with a correction for small sample sizes; 'ΔAICc' = relative differences between the fitted model and the Akaike 'best-ranked' model with the smallest AICc value; 'weight' = relative likelihood of a model.

Fixed Effect	Random Effect	AICc	ΔAICc	Weight
Altitude + Sex + Status + Species	**1 ∣ Plot**	**314.4**	**0.00**	**0.43**
Altitude + Sex + Status + Species	1 ∣ Plot/Trap	314.4	0.00	0.43
Altitude + Sex + Status + Species	1 ∣ Trap	316.6	2.19	0.14

Table A5. Model selection to predict probability of infection of *Hepatozoon* spp., reporting the best model (in bold), the models retained within a ΔAICc ≤ 4, and the first model with ΔAICc > 4 (in italic). Legend: 'Altitude': elevation a.s.l.; 'Sex': sex; 'Status': breeding status; 'Species': rodent species, 'Plot': trapping grid; 'Trap': trap station; Fixed effect' = fixed effect modelled; 'Random effect' = random effects modelled; 'AICc' = AIC with a correction for small sample sizes; 'ΔAICc' = relative differences between the fitted model and the Akaike 'best-ranked' model with the smallest AICc value; 'weight' = relative likelihood of a model.

Fixed Effect	Random Effect	AICc	ΔAICc	Weight
Species + Status	**1 ∣ Plot**	**306.96**	**0.00**	**0.38**
Species	1 ∣ Plot	307.35	0.39	0.31
Sex + Species + Status	1 ∣ Plot	309.05	2.09	0.13
Sex + Species	1 ∣ Plot	309.41	2.45	0.11
Altitude + Species	*1 ∣ Plot*	*312.30*	*5.34*	*0.03*

Table A6. Coefficients of the best model (Model 1, Table A5) fitting the probability of infection with *Hepatozoon* spp. The reference category for the covariate 'Species' is *A. flavicollis*; the reference category for the covariate 'Status' is 'Adult'. Legend: 'Species': rodent species; 'Status': breeding status; 'Plot': trapping grid; 'τ00' = method heterogeneity.

	Probability of Infection			
Predictors	***Estimate***	***Std. Error***	***Z value***	***Pr(>∣z∣)***
Intercept	−1.69 ***	0.25	−6.75	1.47×10^{-11}
Species *C. nivalis*	0.76	0.54	1.39	0.16
Species *My. glareolus*	1.97 ***	0.30	6.54	5.98×10^{-11}
Status Juvenile	1.29 *	0.70	1.84	0.06
Status Sub-adult	0.39	0.30	1.31	0.19
Random effects				
τ00 Plot	1.15×10^{-9}			
N trap	9			
Observations	272			
Marginal R^2/Conditional R^2	0.22/0.22			

* $p < 0.1$, *** $p < 0.01$.

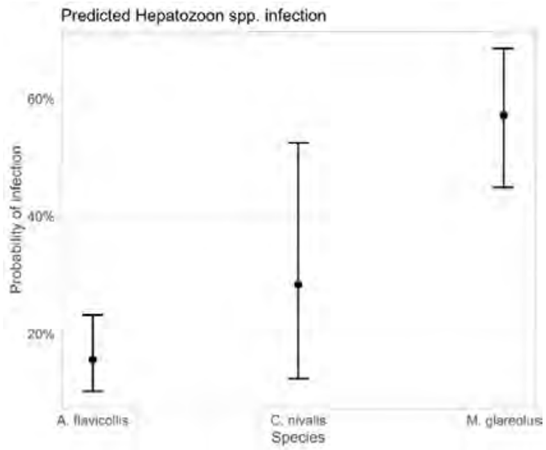


Figure A2. Predictive plot showing the effect of rodent species on probability of infection by *Hepatozoon* spp., after controlling for the effects of other covariates in the Generalized Linear Mixed Models (GLMMs; best model Table A5).

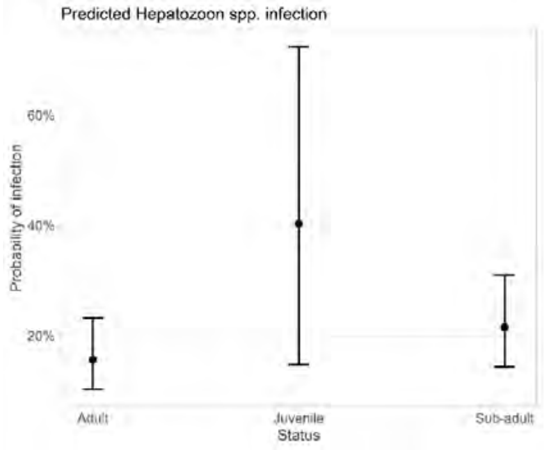


Figure A3. Predictive plot showing the effect of rodent status on probability of infection by *Hepatozoon* spp., after controlling for the effects of other covariates in the Generalized Linear Mixed Models (GLMMs; best model Table A5).

Appendix B

Among the 287 positive samples for *Hepatozoon* spp., we identified 151 entire high-quality sequences. After checking the mutations, four main different sequences emerged (TN-1–TN-4), which are repeated in all positive samples. The four different sequences were listed below. We also added the two sequences of *Babesia microti* (F-TN-5, F-TN-6) detected through *Hepatozoon* spp. protocol.

>TN-1-Hep

ACGGTATCTGATCGTCTTCGATCCCCTAACTTTCGTTCTTGATTAATGAA

AACATCTTTGGCAAATGCTTTCGCAGTAGTGTGTCTTTAACAAATCTAAG

AATTTCACCTCTGACAGTTAAATACAAATGCCCCCAACTGCTCCTATCAA

TCATTAATTTAGTTCTTAAAACCAATAACGTAGAACTAAAATCCTATTTT

ATTATTCCATGCTGCAGTATTCAAAACGTTAGCCTGCTTGAAACACTCTA

ATTTTCTCAAAGTAAAAATCCTCAAAATTGCATTCTACAATAAAGTAAAA

AACATTCCAAAGGACATTATTGCTAAAAACACACCAAGATACCACTCTTA

TAAATAAAAGCAGACCGGTTATTTCTAGCAAAAATTCAACTACGAGCTTT

TTAACTGCAACAATTTTAATATACGCTATTGGAGCTGGAATTACCGCGGC
TGCTGGCACCAGACTTGCCCTCCAATTGATACTTTAAAAAGTATTTAAAT
TT

>TN-2-Hep
ACGGTATCTGATCGTCTTCGATCCCCTAACTTTCGTTCTTGATTAATGAA
AACATCTTTGGCAAATGCTTTCGCAGTAGTGTGTCTTTAACAAATCTAAG
AATTTCACCTCTGACAGTTAAATACAAATGCCCCCAACTGCTCCTATCAA
TCATTAATTTAGTTCTTAAAACCAATAACGTAGAACTAAAATCCTATTTT
ATTATTCCATGCTGCAGTATTCAAAACGTTAGCCTGCTTGAAACACTCTA
ATTTTCTCAAAGTAAAAATCCTCAAAATTGCTTTTTACAATAAAGTAAAA
AACATTCCAAAGGATATTATTGCTAAAAGCACACCAAGATACCACTCTTA
TAAATAAAAGCAGACCGGTTATTTCTAGCAAAAATTCAACTACGAGCTTT
TTAACTGCAACAATTTTAATATACGCTATTGGAGCTGGAATTACCGCGGC
TGCTGGCACCAGACTTGCCCTCCAATTGATACTTTAAAAAGTATTTAAAT
TT

>TN-3-Hep
ACGGTATCTGATCGTCTTCGATCCCCTAACTTTCGTTCTTGATTAATGAA
AACATCTTTGGCAAATGCTTTCGCAGTAGTGTGTCTTTAACAAATCTAAG
AATTTCACCTCTGACAGTTAAATACAAATGCCCCCAACTGCTCCTATCAA
TCATTAATTTAGTTCTTAAAACCAATAACGTAGAACTAAAATCCTATTTT
ATTATTCCATGCTGCAGTATTCAAAACGTTAGCCTGCTTGAAACACTCTA
ATTTTCTCAAAGTAAAAATCCTGAAAATTGCTTCTCACAATAAAGTAAAA
AACATTTCAAAGGACATTATTGCTAAAAACACACCAAGATGCCACTTTTA
ATAATAAAAGCAGACCGGTTATTTTTAGCAAAAATTCAACTACGAGCTTT
TTAACTGCAACAATTTTAATATACGCTATTGGAGCTGGAATTACCGCGGC
TGCTGGCACCAGACTTGCCCTCCAATTGATACTTTAAAAAGTATTTAAAT
TT

>TN-4-Hep
ACGGTATCTGATCGTCTTCGATCCCCTAACTTTCGTTCTTGATTAATGAA
AACATCTTTGGCAAATGCTTTCGCAGTAGTGTGTCTTTAACAAATCTAAG
AATTTCACCTCTGACAGTTAAATACAAATGCCCCCAACTGCTCCTATCAA
TCATTAATTTAGTTCTTAAAACCAATAACGTAGAACTAAAATCCTATTTT
ATTATTCCATGCTGCAGTATTCAAAACGTTAGCCTGCTTGAAACACTCTA
ATTTTCTCAAAGTAAAAATCCTGAAAATTGCTTTTTACAATAAAGTAAAA
AACATTTCAAAGGACATTATTGCTAAAAACACACCAAGATACCACTTTTA
TTAATAAAAGCAGACCGGTTATTTTTAGCAAAAATTCAACTACGAGCTTT
TTAACTGCAACAATTTTAATATACGCTATTGGAGCTGGAATTACCGCGGC
TGCTGGCACCAGACTTGCCCTCCAATTGATACTTTAAAAAGTATTTAAAT
TT

>F-TN-5_Babesia
ACTACGACGGTATCTGATCGTCTTCGATCCCCTAACTTTCGTTCTTGATT
AATGAAAACATCCTTGGCAAATGCTTTCGCAGTAGTTCGTCTTTAACAAA
TCTAAGAATTTCACCTCTGACAGTTAAATACGAATGCCCCCAACTGCTCC
TATTAACCATTACTCTGGCTCAATAACCAACAAAATAGAACCAAAGTCCT
ACTTCATTATTCCATGCTGTAGTATTCAAGGCAAATGGCCTGTTTGAAAC
ACTCTAGTTTTCTCAAAGTAAATACTGGAAGATAGTAACCAATTAAGGAT
ACCAAATCCAGAAGATGCCAAGTCAATAAAATAACGCTCGAAAGCGAGAT
TAATGACAAGGCAGAAATTCAACTACGAGCTTCTTAACTGCAACAACTTT
AATATACGCTATTGGAGCTGGAATTACCGCGGCTGCTGGCACCAGACTTG
CCCTCCAATTGATACTCTGGGAAGGGTTTAGATTC

>F-TN-6_Babesia
ACTACGACGGTATCTGATCGTCTTCGATCCCCTAACTTTCGTTCTTGATT
AATGAAAACATCCTTGGCAAATGCTTTCGCAGTAGTTCGTCTTTAACAAA
TCTAAGAATTTCACCTCTGACAGTTAAATACGAATGCCCCCAACTGCTCC
TATTAACCATTACTCTGGCTCAATAACCAACAAAATAGAACCAAAGTCCT
ACTTCATTATTCCATGCTGTAGTATTCAAGGCAAATGGCCTGTTTGAAAC
ACTCTAGTTTTCTCAAAGTAAATACTGGAAGATAGTAACCAATTAAGGAT
ACCAAATCCAGAAGATGCCAAGTCAATAAAATAACGCTCGAAAGCGAGAT
TAATGACAAGGCAGAAATTCAACTACGAGCTTCTTAACTGCAACAACTTT
AATATACGCTATTGGAGCTGGAATTACCGCGGCTGCTGGCACCAGACTTG
CCCTCCAATTGATACTCTGGGAAGGGTTTAGATTC

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The increasing human footprint is rapidly modifying the environmental gradients, altering animal populations and community composition, with consequences on disease transmission. However, in an eco-epidemiological framework, some links between ecological underpinning processes and the host-parasite-pathogen patterns have not been disentangled, yet their understanding is becoming urgent under the current climate and global change. This thesis includes a collection of papers that assess the drivers of small mammal community composition and demography, and the cascading effects on the parasitic load and the circulation of common and emergent pathogens. This was pursued by applying a multi-factorial, niche-based approach using field experimental designs based on manipulation of anthropogenic food availability, and longitudinal transects across heterogeneous habitats. We observed that climate severity, seasonal variation and food availability strongly interplay in shaping small mammal demography and their space use patterns, although also triggering interspecific competition. These mechanisms also enhance tick-burden amplification in anthropic, fragmented landscapes, while determining geographic expansion of generalist species, and vectors alongside, in mountain habitats. This upward distributional shift is also accompanied by circulation of common rodent- and vector-borne pathogens, as well as of emergent protozoans that may hold crucial epidemiological role along the food web. Hence, we experimentally demonstrated the consequences of climate and anthropic disturbances on small mammal communities and populations, and their implications for the health of humans and ecosystems.