Inland Norway
University of
Applied Sciences

Faculty of Applied Ecology, Agricultural Sciences and Biotechnology

**Magne Neby**

# The role of diet in vole population cycles

PhD Applied Ecology and Biotechnology
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*To Harry P. Andreassen (1962-2019)*

# Sammendrag

Smågnagerpopulasjoner med store bestandssvingninger finner vi over hele verden. I denne avhandlingen har jeg inkludert en litteraturstudie om både regelmessige sykluser og mer uregelmessige utbrudd i smågnagerpopulasjoner og som peker på de viktigste problemstillingene å svare på for videre forståelse (**Paper I**). Smågnagere i boreale, alpine og arktiske økosystem er særlig kjent for sine sykliske populasjonsendringer, hvor både sesongvariasjon og/eller endringer i mattilgang er viktige faktorer. I et observasjonsstudium i de boreale skogene på Evenstad, fant vi en klar sammenheng mellom høyde over havet og hvor mye smågnagere det var i museårene. Smågnagerpopulasjonen hadde et syklisk forløp over hele høydegradienten, men syklusen var tydeligst høyt oppe i liene der det var mer mus i toppåret enn det var nede i dalene og bekreftet slik den viktige rollen vintersesongen spiller (**Paper II**). En tydelig tetthetsavhengighet om vinteren er forventet ved begrensede ressurser. En systematisk gjennomgang av litteraturen om forholdet mellom smågnagere og planter (**Paper III**) viste at begrenset mattilgang kan påvirke smågnagernes populasjonstetthet, og at det var best støtte for dette om vinteren. Det var derimot ikke mulig å hverken bekrefte eller avkrefte noen «plantehypothese» på grunn av for få studier med like respons- og forklaringsvariabler. Begge litteraturstudiene (**Paper I** og **Paper III**) poengterer viktigheten av at fremtidige studier utforsker videre om det forekommer systematiske endringer i dietten til smågnagerne som kan påvirke deres populasjonsdynamikk. Jeg har forsøkt å belyse nettopp dette ved å ta i bruk DNA-strekkoding (metabarcoding) for å analysere om diettens komposisjon eller diversitet endrer seg systematisk i forhold til sesong og syklusens faser (**Paper V**). DNA-strekkoding kan dog ikke forventes å reflektere relative proporsjoner av matinntaket nøyaktig (**Paper IV**). Diettstudien fant stor fleksibilitet i dietten, noe som støtter opp under at det forekommer moderate endringer mellom sesong og faser hos både klatremus *Myodes glareolus* og fjellmarkmus *Microtus oeconomus*. Men diettvariasjonen som kan tilskrives syklusenes faser var relativt marginal i forhold til den generelle fleksibiliteten i dietten. Det virker derfor ikke sannsynlig at fasenes diettvariasjon er tilstrekkelig for å drive smågnagerpopulasjonenes særegne dynamikk.

# Abstract

Small rodent populations throughout the world show massive density fluctuations. In this thesis, I have included a review highlighting the most critical issues essential for understanding the generality of small rodent population cycles and outbreaks (**Paper I**). Herbivorous voles in boreal, alpine, and arctic ecosystems are especially renowned for their multi-annual population cycles in which both seasonality and plant-herbivore interaction may play an important role. Using observational data from a boreal ecosystem, the importance of seasonality was confirmed in **Paper II** where winter length was positively associated with the amplitude of vole population cycles. Strong density dependence during winter is expected if resources are limited. Indeed, a systematic literature review focusing on plant-rodent interactions (**Paper III**) found the most robust evidence for food resource dependency during winter. However, the evidence was scattered across study systems to such a degree that only a few specific topics were addressed in a replicated manner. Thus, the hypothesis that interactions with plants cause rodent cycles could not be discarded. Because all hypotheses predicting that plants cause rodent cycles have explicit, yet largely untested, assumptions of diet shifts across population cycles, both review papers (**Paper I, Paper III**) conclude that one of the essential issues to explore further is whether a diet shift occurs that change population dynamics. I pursued this research question with DNA metabarcoding to quantify diets of two functionally important boreal vole species (**Paper V**). However, care should be taken when inferring the diet composition obtained by DNA metabarcoding of vole faeces as a direct mirror of the consumed food's composition (**Paper IV**). Paper V is the first metabarcoding study to assess whether vole diet composition and diversity change systematically according to season and critical phases of a population cycle. We observed large diet flexibility and tendencies for moderate shifts in the proportions of plant taxa in the diets of bank voles *Myodes glareolus* and tundra voles *Microtus oeconomus* both between phases and seasons. Thus, changes do occur through time in vole diet composition, although the temporal change at population level appeared to be minor compared to other sources of diet variation. Overall, this study indicates that the variation in diet that could be attributed to cyclic phases is marginal relative to the overall diet flexibility. Hence, it seems unlikely that temporal variation in diets is driving the transition between increase/peak and crash/low phase of the population cycle.

# List of Papers

Of the included articles in this dissertation, Paper I, II, and IV are published as open access with a Creative Commons Attribution 4.0 which permits use, sharing, adaptation, distribution, and reproduction in any medium or format as long as the correct citation and a link to original source are provided.

List of included articles in this dissertation:

| | |
|---|---|
| Paper I | Andreassen, H. P., Johnsen, K., Joncour, B., **Neby, M.**, & Odden, M. (2020). Seasonality shapes the amplitude of vole population dynamics rather than generalist predators. *Oikos*, *129*(1), 117-123. https://doi.org/10.1111/oik.06351 |
| Paper II | Andreassen, H. P., Sundell, J., Ecke, F., Halle, S., Haapakoski, M., Henttonen, H., Huitu, O., Jacob, J., Johnsen, K., Koskela, E., Luque-Larena, J. J., Lecomte, N., Leirs, H., Marien, J., **Neby, M.**, Ratti, O., Sievert, T., Singleton, G. R., van Cann, J., Vanden Broecke, B., & Ylonen, H. (2021). Population cycles and outbreaks of small rodents: ten essential questions we still need to solve. *Oecologia*, *195*(3), 601-622. https://doi.org/10.1007/s00442-020-04810-w |
| Paper III | Soininen, E. M., **Neby, M.** (2021). *Small rodent population cycles and plants – After 70 years, where do we go?* Unpublished manuscript. |
| Paper IV | **Neby, M.**, Kamenova, S., Devineau, O., Ims, R. A., & Soininen, E. M. (2021). *Issues of under-representation in quantitative DNA metabarcoding weaken the inference about diet of the tundra vole Microtus oeconomus.* PeerJ, *9*, e11936. https://doi.org/10.7717/peerj.11936 |
| Paper V | **Neby, M.**, Kamenova, Ims, R. A., S., Devineau, O., & Soininen, E. M. (2021). *Is diet cyclic phase dependent in boreal vole populations?* Unpublished manuscript. |

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# Introduction

Natural populations are not static, but in constant change. Understanding what governs the changes is key in population ecology. The natural dynamics of populations first became obvious in the seminal works of Charles Elton (1935; 1924), which showed large multiannual variation in animal numbers with data from e.g. Norway (Collett, 1912). In the northern hemisphere, voles and lemmings tend to show regular fluctuations in numbers (i.e. population cycles), while in the southern hemisphere, the periods are often less regular, but can have much larger amplitudes. Population cycles are commonly described as consisting of four phases (Krebs & Myers, 1974), with the maximal animal density occurring during the "peak" phase. Small rodent density fluctuations are often interspecifically and spatially synchronised (Ims & Fuglei, 2005; Krebs et al., 2002), which amplifies their effects on the rest of the ecosystem.

Vole and lemming cycles have pronounced effects on the ecosystem they are a part of (Ims & Fuglei, 2005). In Fennoscandia, voles have key roles in boreal ecosystems (Boonstra et al., 2016). They can reduce plant biomass, change vegetation species composition, and affect flowering frequency (Andersson & Jonasson, 1986; references in Paper III). Furthermore, high vole densities can have negative economic impacts on silviculture (Huitu et al., 2009), public health (Meerburg et al., 2009) and human food security (Singleton et al., 2010). High population densities make voles easier to prey on, and during population peaks voles are thus the first and main choice of many boreal predators (Korpimäki et al., 1991; Sundell & Ylönen, 2008). The numerical and functional responses in the predator community to vole fluctuations lead to cascading effects on alternative prey species (Angelstam et al., 1984) with lower mortality rates and increasing populations of e.g. small game (Lindström et al., 1987). In sum, the small rodent cycles set the rhythm of trophic interactions in the northern ecosystems both for human and nature.

During a century of research on population dynamics, many factors and mechanisms that may generate or change cycles have been suggested – including predation, food, disease, dispersal and sociality (Paper I). The generation of cyclic dynamics in voles seems now best explained by extrinsic factors related to trophic interactions (Paper I; Berryman, 2002), including herbivore-plant interactions (Oksanen et al., 2020; Reynolds et al., 2016; Turchin & Batzli, 2001). Indeed, the role of food plants has repeatedly been confirmed as a contributing factor to cycles (Batzli & Pitelka, 1983; Prevedello et al., 2013), both in experimental (Batzli, 1986; Gilbert & Krebs, 1981; Huitu, Norrdahl, et al., 2003; Johnsen et al., 2017) and in observational studies on boreal and arctic vole populations (Boonstra & Krebs, 2012; Krebs et al., 2010; Laine & Henttonen, 1983). However, contradicting evidence, in terms of failures to show delayed food effects, exists and thus the food plant hypothesis remains debated (Klemola et al., 2003; Myers, 2018).

In boreal ecosystems, rapid vegetation growth and breeding of small rodents characterise the summer season, whereas low temperatures with no or only minor and exceptional reproduction characterise the winter season. Within the boreal region, the length of the winter season varies with latitude and elevation, with more extended snow-cover periods towards both extremes. The snow cover among others, limits regrowth of main herbivore food resources. The stronger seasonality occurring at high latitudes/elevations shows that longer winters are associated with cycles having extended period lengths and larger amplitudes (Hansson & Henttonen, 1985; Lambin et al., 2006; Taylor et al., 2013; Tkadlec & Stenseth, 2001; Paper II). The effect of seasonality may be explained by increased effect of predation during the winter non-reproductive season. However, strong density-dependent mortality during winter due to scramble competition is expected if resources are limited (Hansen et al., 1999). In fact, several studies confirm the significance of food resources for winter survival (Eccard & Ylönen, 2001; Huitu et al., 2007; Huitu, Koivula, et al., 2003; Johnsen et al., 2017; Schweiger & Boutin, 1995). However, Yoccoz et al. (2001) found no evidence for delayed density dependence due to food shortage, the mechanism deemed as the crux for generating multi-annual fluctuations in small rodent populations.

Charles Elton concluded as far back as 1942 that it would be futile to try to explain vole cycles in terms of starvation (Chitty, 1996). Yet, the “food hypothesis” evolved into a collection of different explanations for how vegetation may create rodent population cycles (Paper III). These include both single-factor explanations (Krebs et al., 1973; Pearson, 1966) and multi-factor explanations (Andreassen et al., 2013; Haukioja et al., 1983; Lidicker, 1973).

One variant of the food hypothesis suggests that inherent plant productivity/quality cycles could be a bottom-up driver of rodent cycles (Kalela, 1962). Other variants are based on mutual interactions between rodents and their food plants. High vole densities lead to increased herbivory, which in turn can lead to a decrease of food quantity for the individual voles (Lack, 1954). A similar high feeding pressure can also affect other factors than quantity. Thus, others have hypothesised that high feeding pressure can lead to nutritionally lower quality plants (Batzli & Cole, 1979) or cause plants to induce defences (Haukioja, 1980; Massey et al., 2008). As with quantitative limitation, individuals may become less healthy or be forced to change from their preferred food plants. Yet another variant of the food plant hypothesis argues that low quantitative availability of food leads to a change in diet as less preferred food plants are increasingly exploited. The less preferred food plants may provide fewer nutrients or pass on more defence compounds or toxins to the animal (Freeland, 1974; Jensen & Doncaster, 1999), eventually limiting population growth.

This thesis' three case studies (Papers II, IV and V), concern the tundra voles *Microtus oeconomus* Pallas 1776 and the bank voles *Myodes glareolus* (Schreber, 1780), which are among the most widespread, abundant, and functionally important mammal species in boreal ecosystems in Europe (Boonstra et al., 2016). Bank voles are known to be generalist browsers with an expected broader niche than the grazing tundra voles (Hansson, 1985; Soininen et al., 2013). However, both species exhibit synchronous multi-annual population cycles in the study area, at Evenstad, SE Norway (Figure 1; Ims & Andreassen, 2000). We studied the diets (Paper IV and V) based on DNA metabarcoding of faeces samples. DNA metabarcoding for dietary analysis has become a popular method due to its cost-efficiency and high taxonomic resolution (Taberlet et al., 2018). Thus, it has become a preferred method for resolving the diverse diets also of small rodents (Lopes et al., 2020; Ozaki et al., 2018; Sato et al., 2019; Soininen et al., 2017) and has the potential to describe in detail the temporal variation in vole diets across seasons and phases of the population cycle.

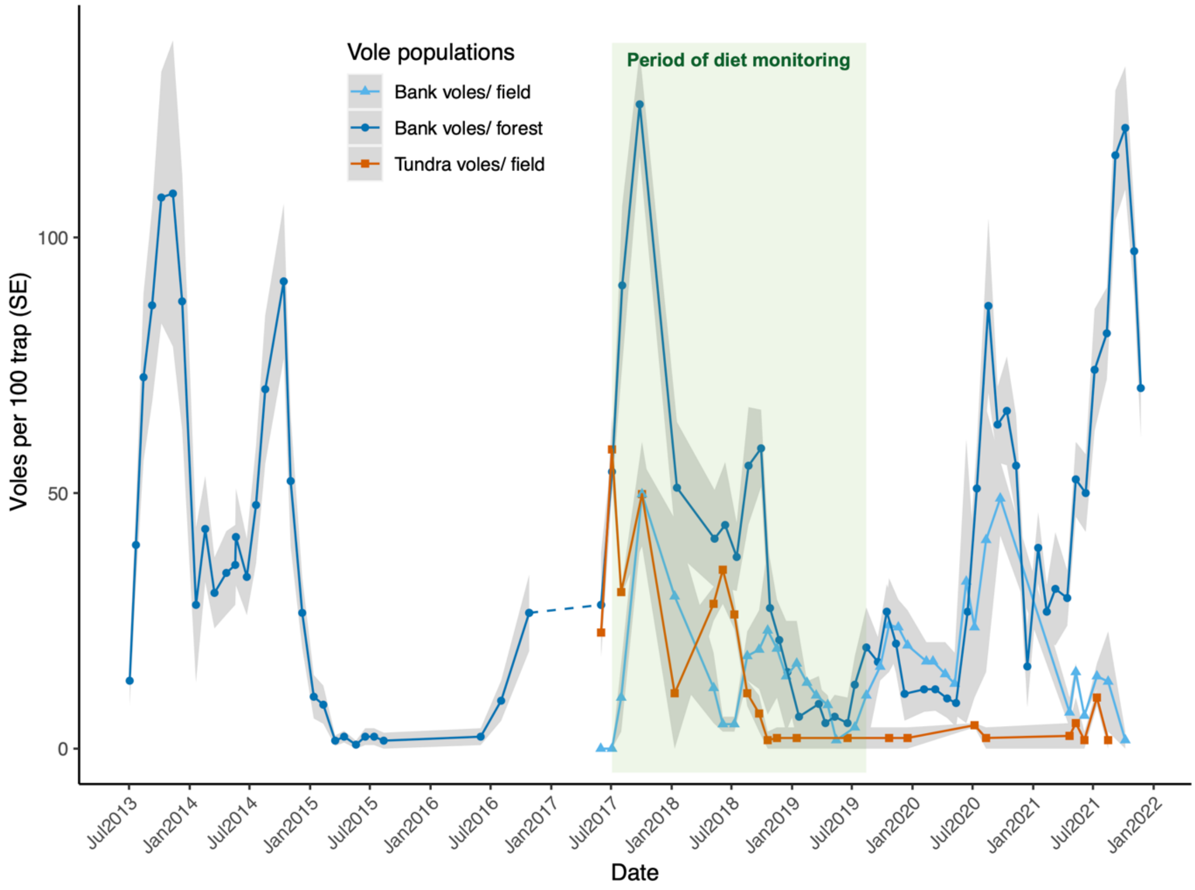

Figure 1. Estimates of vole abundance over time based on capture-mark-recapture data from grids/transects at Evenstad (SE Norway). Here presented as mean per grid/transect ± SE. Data included in Paper V are encompassed the shaded rectangle. Data prior to 2017 was received from Kaja Johnsen and Harry P. Andreassen.

# Objectives

The main aim of this PhD project is to further expand our understanding of the drivers of cyclic population dynamics by focusing on the temporal variation in vole diets. I have pursued this aim with four objectives:

1. To provide an updated state of knowledge based on existing literature and point to research directions to fill in the gaps in knowledge on small rodent population fluctuations in general, and regarding plant-rodent interactions specifically (Paper I and Paper III)
2. To assess how the length of the winter season affects the amplitude of vole population cycles (Paper II)
3. To validate quantitative inference from DNA metabarcoding, commonly relied on in animal diet studies, including Paper V (Paper IV)
4. To explore whether the diet is cyclic phase dependent in two boreal vole species (Paper V).

# Material and methods

### Paper I – Andreassen et al. 2021 *Oecologia*

This paper was initiated on a workshop in February 2018 at Konnevesi Research Station, Finland. Harry P. Andreassen started coordinating the planning and writing of the manuscript. Here, a narrative review approach was used, leaning on literature as well as on the extensive experience from the senior researchers co-authoring this paper. The aim of the review was to synthesise the current state of knowledge on the mechanisms underlying the dynamics of populations, by comparing small rodent cycles and outbreaks. We conclude with the ten most important issues for further understanding the generality of small rodent population dynamics.

### Paper II – Andreassen et al. 2020 *Oikos*

The study was conducted in the boreal forest in southeast Norway (61°N, 11°E). The area has a relatively continental winter climate, with low temperatures. We monitored cyclic bank vole populations during two vole peaks and one low phase along an elevational gradient ranging from 260 m to 801 m a.s.l. We used the elevation gradient as a proxy for winter length since the populations at the highest elevation attained snow cover earlier, and permanent snow cover lasted up almost two months longer at 800 m than at 260 m a.s.l. We estimated the population size of 30 local bank vole populations to study how the length of the winter season correlated with the amplitude of the population cycles. In addition, we estimated track frequencies of red fox *Vulpes vulpes* and pine marten, *Martes martes*, by snow tracking transects to examine if the small mammalian generalist predator community could better explain any of the elevational effects.

### Paper III – Soininen & Neby *Manuscript*

In Paper III, the available literature on plant-rodent interactions relevant for arvicoline population cycles was synthesised, by first describing the rodent-vegetation interactions that have been suggested to potentially cause rodent population cycles, as well as assumptions related to the various hypotheses. In contrast to the approach of Paper I, a systematic and reproducible search/reporting methodology was used. Literature was collected in two steps: First, we searched scientific citation databases for specific search strings. Based on title and abstract, we included publications that fulfilled criteria relevant for the review. Second, we checked for additional publications fulfilling the inclusion criteria among publications citing the already included publications or being cited by the included publications. The filtering was reported following ROSES (RepOrting standards for Systematic Evidence Syntheses) as guide (Haddaway et al., 2018). This was followed by a systematic analysis of the publications using questionnaires written *a priori*, including a quality assessment. The results were summarized through a narrative synthesis, especially highlighting which key studies remain to be done to assess the propensity of plants to drive rodent cycles.

### Paper IV – Neby et al. 2021 *PeerJ*

All DNA metabarcoding studies utilises sequence counts to some degree. In Paper IV, we questioned the potential to infer a quantitative relationship between sequence read proportions and biomass of ingested food. This was especially motivated by the relevance this inference would have for small rodent diet studies, including Paper V. We used an experimental approach to assess the relationship between three plant species' ingested plant biomass and their sequence reads proportions from DNA metabarcoding in the tundra vole.

Prior to the published study, we performed several experiments to validate the protocol with vole faeces at the Department of Biosciences, UiO, Norway. During these validations, we used the DNeasy® PowerSoil® kit (QiaGen, Germany), following the manufacturer's instructions. For the final metabarcoding analysis of Paper IV (and Paper V below), the extraction was performed by Sinsoma GmbH (Innsbruck, Austria) using the Biosprint 96 DNA Blood Kit (Qiagen) on a Biosprint 96 Robotic Platform (Qiagen).

After DNA extraction, all samples were further processed at UiO. We targeted vascular plants and amplified the extractions using G-H primers (Taberlet et al., 2007). The resulting amplicon library was sequenced on an HiSeq 4000 machine and the resulting sequencing data are deposited at the European Nucleotide Archive (accession number PRJEB43470). We carried out bioinformatic analyses using the OBITools bioinformatics pipeline (Boyer et al., 2016) on the Norwegian high-performance computing cluster Sigma2. Subsequently, we created a local reference database from the reference library ‘‘ArctBorBryo’’ (Soininen et al., 2015; Sønstebø et al., 2010; Willerslev et al., 2014) and the European Nucleotide Archive nucleotide library (EMBL) with the ecoPCR program (Bellemain et al., 2010; Ficetola et al., 2010). Finally, we compared the reference database to the sequences in our data, assigning each sequence to a taxon with the ecoTag program (Pegard et al., 2009). Further data filtering, visualisation and analyses were conducted with ROBITools using the R software (R Core Team, 2021). A schematic overview is given in Figure 2.

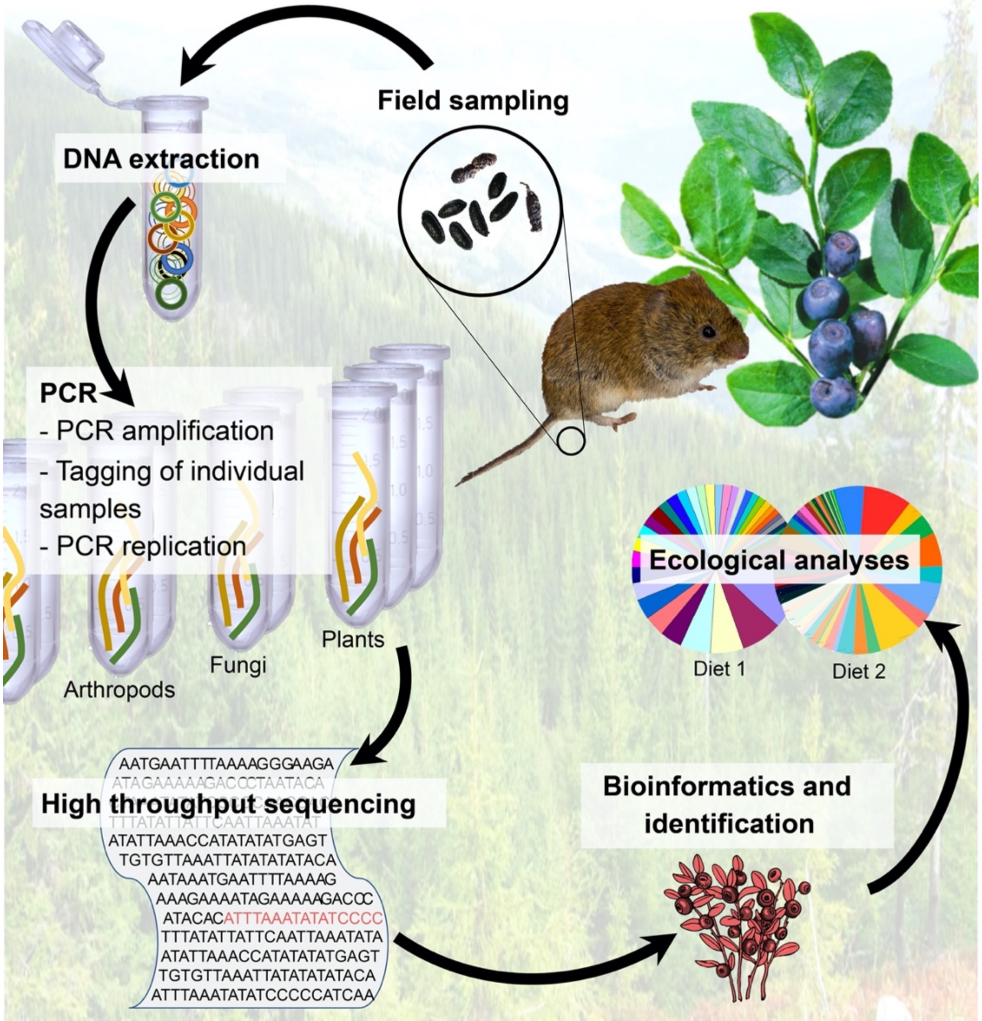

Figure 2. Schematic overview of the diet analysis design employed in Papers VI and V. For paper VI, faeces were sampled from the rodent housing and the meals mixtures offered to the voles. For paper V, faeces were sampled from live traps in the field. For both papers, sampling was followed by DNA extraction, PCR amplification with specific primer pairs for vascular plants, bryophytes, fungi, arthropods, and eukaryotes. This was then followed by next-generation high-throughput sequencing that produced read count data that was identified with reference libraries and filtered.

We assessed the quantitative accuracy of dietary metabarcoding by using a multivariate regression model that establishes a linear function between the multiple compositional outcomes (responses) and compositional predictors (Fiksel et al., 2021). This type of compositional analysis accounts for the fact that an increase in one taxon's proportion will force a decrease in other taxon(s) proportion within the same sample. The model allows, without transformation, for direct interpretation of the relationship between expected and observed compositions.

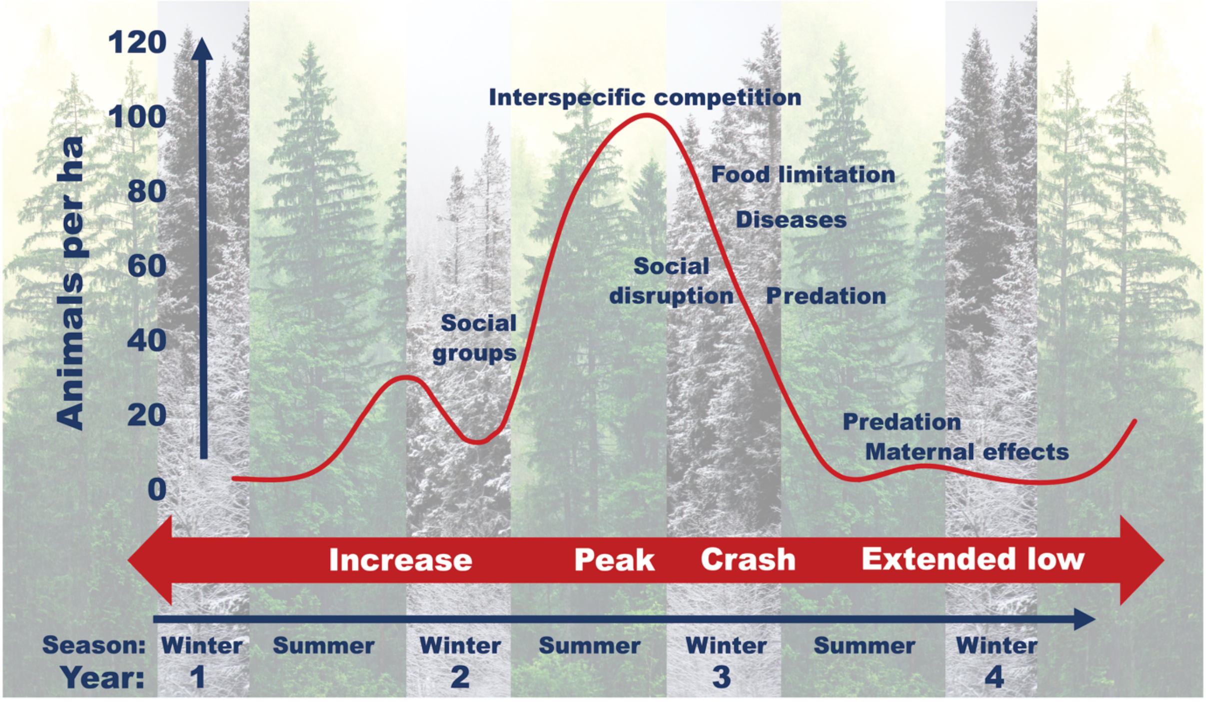

Figure 3. Characteristics of vole population cycles in the northern hemisphere (Figure 2A in Paper I). The different phases have different characteristics, and the seasonality has a strong effect in separating the reproductive period and plant growth period with the non-reproductive period and the non-regenerative plant period.

### Paper V – Neby et al. *Manuscript*

As highlighted in Paper I and Paper III, food related hypotheses make explicit assumptions on how rodent diet taxonomic and nutritional composition changes across the cycle. The increase phase is a good reference where the beginning of the increase phase should be characterised by a surplus of food, healthy individuals, and a steep population growth curve. Similarly, the other phases of a cycle also have characteristics and specific assumptions of their own (Figure 3). Paper V is the first to use DNA metabarcoding to quantify the diets of two functionally important boreal vole species to assess whether their diet changed systematically according to season and critical phases of their population cycle.

For the DNA metabarcoding analysis in Paper V, we used the same methods as described for Paper IV except that we used six metabarcoding primer pairs to cover vascular plants (Taberlet et al., 2007), bryophytes (Epp et al., 2012), eukaryotes (Guardiola et al., 2015), fungi (Epp et al., 2012), arthropods (Zeale et al., 2011), and arvicolinae rodents (Alasaad et al., 2011; Haring et al., 2000). The rationale for selecting these primers were based on the study species and previous studies of diets (Hansson, 1979; Hansson & Larsson, 1978; Smal & Fairley, 1980; Soininen et al., 2013).

We used the six trapping plots described for Paper II that were closest to Evenstad research station to monitor bank vole populations. In addition, we included a field habitat next to two agricultural ditches directly below this forest which were previously known to be inhabited by tundra voles (Ims & Andreassen, 2000). To monitor population dynamics and diets in these two contrasting habitats we used capture-mark-recapture trapping with different trap placement designs for the two species/habitats (Figure 4). Diet data was collected by sampling animal faeces in the traps. Population density and diets were monitored mostly every month (Figure 1) including during the winter (Figure 5).

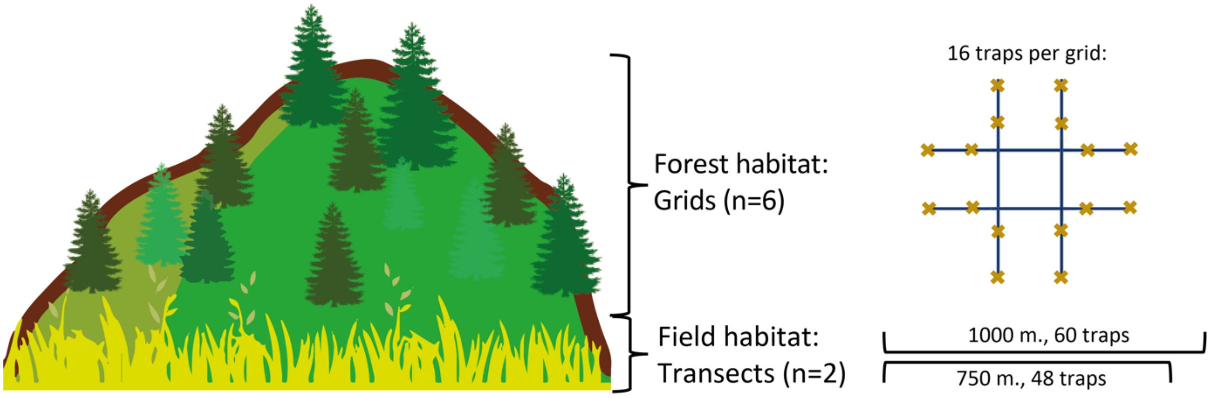

Figure 4. Graphical overview of vole trapping design in Paper V. The field habitat was monitored with transects and the forest habitat in grids.

Despite the advantages of interpreting the diet compositions directly on the compositional space (Fiksel et al., 2021; Hron et al., 2012), the model we used in Paper IV did not allow for multiple covariates of mixed variable types. Thus other statistical approaches was utilised in

Paper V, including Hellinger transformation of the diet compositions, which allowed us to use the principial-component analysis and redundancy analysis (Legendre & Gallagher, 2001).

Figure 5. During snow-cover, we used plastic boxes without floors where the snow could be removed without disturbing the subnivean layer. This photo is from 2017 and shows the top of a box that was covered by more than one meter of snow.

# Results and discussion

## Paper I

The narrative review of this paper shows that large population fluctuation is not a phenomenon constrained to the famous lemming populations in the north. The drivers and mechanisms of small rodent population fluctuations have been studied widely across the world for a century. The maximum small rodent densities (i.e. amplitude) of non-cyclic outbreaks of small rodents in the southern hemisphere often surpasses the northern cyclic peak densities. The southern outbreaks are best explained as responses to more or less stochastic events, often weather-driven, which boost resource availability. In search of generality, it is natural to look for a similar explanation for cyclic dynamics. However, many more factors are suggested to be involved in cyclic dynamics of northern voles and lemmings than in southern species, e.g., predation, diseases, and dispersal. There is also less agreement about the roles that these different factors play for the cyclic dynamics (Myers, 2018). Food resources are repeatedly discussed both in context of cycles and outbreaks. Many of the population outbreaks are observed in agricultural land areas and seem to be directly related to ample changes in the availability of food resources (Belmain et al., 2010). Whether there are temporal shifts in vole diet that could contribute to delayed density-dependent or cyclic phase-dependent population growth (*sensu* Stenseth 1999), remains one of the main knowledge gaps, and is an important question to address. Another remaining question in small rodent population dynamics is how seasonality in the northern ecosystems interacts with density dependent population growth.

## Paper II

A winter dependency in the small rodent population dynamics was evident in the boreal study system reported in Paper II. We observed that winter length was positively associated with the amplitude of cycles in the boreal bank vole populations. A longer winter season resulted in a greater cycle amplitude of the local population fluctuations over the study period. This was mainly due to the higher maximum densities at high elevations in the first peak in the time series. Seasonal effects may essentially shift rodent dynamics from an intrinsically stable regime with irregular fluctuations (generated by density-independent mechanisms) to larger–amplitude and periodic cycles influenced by density-dependent mechanisms (Stenseth et al. 2003).

### Paper III

Out of almost 5000 initial publications, after filtering, the review included 150 publications from the northern hemisphere. We categorised the publications as separate studies (total: 231 studies) when they addressed several hypotheses or considered different methods or study systems. The synthesis of these studies provided a very scattered knowledge base due to it being spread across species of rodents, species of plants, and suggested characteristics. This made it challenging to assess the repeatability of the findings and thus limited the review from rejecting any of the four suggested pathways through which plant-rodent interactions could give rise to rodent population cycles. Indeed, even an assessment of the ecological contexts in which some of the specific interactions may come into play was mostly impossible. However, it did become obvious that studies on herbivory during winter were sometimes reporting higher plant mortality or larger reduction of biomass during winter than other seasons, or even only finding effect during winter, demonstrating that the impact of rodent herbivory on vegetation is likely to be stronger in winter than in summer. The review concludes that the plant-rodent interaction explanations rely heavily on largely untested assumptions of changes in composition and or quality of diets.

### Paper IV

During the last decade, DNA metabarcoding for dietary analysis has become a common approach for resolving the diverse diets of small rodents (Lopes et al., 2020; Sato et al., 2019), but lack insight on the validity in studies inferring relative biomass proportions from this study system. We thus investigated experimentally the relationship between diet composition obtained by DNA metabarcoding of vole faeces and the consumed food's composition. We found that the expected and observed proportions of plant species in vole faeces were correlated for two out of three plant species. The third plant species had consistently low proportion and blurred the overall relationship between expected and observed diet compositions. The findings in this study add to the growing number of assessments of different taxa and show that proportions in diet descriptions should be relied on with care, especially due to possible under-/over-representation of specific taxa (Lamb et al., 2019; Paper IV). Where studies can encompass the target species as mock communities, with known relative proportions, then correction factors may improve validity also in observational field studies (Thomas et al., 2016). However, this is challenging for diverse herbivore diets, especially when exploring previously unknown diets.

### Paper V

Paper V assessed whether the vole diets changed systematically according to season and critical phases of their population cycle. Only tendencies for moderate dietary shifts were observed between seasons in the diets of bank vole and tundra vole. For the tundra vole, the clearest seasonal pattern was a reduction of forbs and an increase of *Salix* shrubs, which is in line with previous findings (Tast, 1966). For the bank vole, the seasonal changes were most apparent in terms of increased proportions of graminoids over forbs during winter (Viro & Sulkava, 1985) and an increase in the use of lichens (Ecke et al., 2018; Hansson, 1985; Hansson & Larsson, 1978; Viro & Sulkava, 1985).

No clear diet shifts – neither in composition, nor in diversity – associated with the transition from increase/peak to the crash/low phase of the population cycle were detected. The modest changes observed in diet composition, such as the reduction in *Salix* proportions in the crash-low phase diets of the tundra vole, cannot readily be interpreted as a change to a less preferable diet as the palatability of the different plant species to the tundra vole is not known. This study as well as previous diet studies (Soininen et al., 2013; Viro & Sulkava, 1985) show that there are large differences between the individuals. The dietary flexibility of the two study species is further underlined by our finding that the spatial differences between local sampling sites were as large as the temporal differences across cyclic phases and seasons. Hence, the dietary flexibility indicates that the consistent phase-dependent tendencies are moderate at best.

# Concluding remarks

Papers I, II and III highlight the importance of the assumption that rodent diets change during the trajectory of the cycle and argue that testing this assumption is one of the best ways to advance. Ideally, insight in diets would help pinpoint where, when, and how plant-herbivore interactions are (or are not) a plausible cause of rodent population dynamics. In ecosystems with cold and long snow-covered winters, the winter seems the most probable period for food limitation to occur and diets shifts to take place.

Seasonality is critical for population cycles to occur (Stenseth et al., 2003). Empirically based modelling studies of cyclic vole population in Fennoscandia have shown the significance of seasonality in terms of strong density-dependence in winter (Bjørnstad et al., 1995; Hansen et al., 1999; Kleiven et al., 2018). These studies describe strong interspecific competition during winter and strong intraspecific competition in both summer and winter. Strong density-dependent mortality during winter due to scramble competition is expected if resources are limited (Hansen et al., 1999). This corroborates with what we found in the systematic review (Paper III) and demonstrates that the impact of rodent herbivory on vegetation is likely to be stronger in winter than in summer. Amount of food is critical in winter for the onset of reproduction and for survival (Eccard & Ylönen, 2001; Huitu et al., 2007; Huitu, Koivula, et al., 2003; Johnsen et al., 2017; Schweiger & Boutin, 1995). However, only few of the studies included data collected during wintertime and we know very little about variation in diet composition and quality between winters.

Rodent winter ecology is poorly known in general (Krebs, 2013) because voles adopt a cryptic, subnivean lifestyle and because methods to study it effectively have been lacking (Ehrich et al., 2020). Recent advances in technology may ameliorate this, for example with the use of below-snow camera trapping (Mölle et al., 2021) or non-invasive methods of diet analyses from faeces. The latter can be used in terms of taxonomic composition of diets (Paper V; Lopes et al., 2020; Soininen et al., 2015) and nutritional quality (Čepelka et al., 2021), e.g. with the use of near infrared reflectance spectroscopy (NIRS) for analysis of the food quality (Janova et al., 2015). Where the use of metabarcoding is appropriate, the advance and refinement of this method (e.g. with validation experiments such as in Paper IV), expanding reference libraries, and costs continue to decrease, metabarcoding will be useful also in future ecological studies. However, dietary metabarcoding cannot give information about what organ of a plant (e.g., root, bark, fruit, seed or leaf) or what life stage of an insect (e.g. adult, larvae or pupa) that was ingested. This challenges some of our interpretation of the diet of both vole species, in particular regarding seasonal variation. Shrubs such as *Vaccinium* produce nutritious berries, while trees such as *Picea* and *Pinus* produce vast amounts of seeds and seedlings, which are much more easily digested than the woody parts of adult trees. It is likely that several of the identified plants are not only ingested as green leaves, but also as roots, bark, seeds, winter buds and berries (Batzli and Henttonen 1990; Canova and Fasola 1993; Hansson 1979; Heroldová 1994; Viro and Sulkava 1985), which may explain finding increased amounts of shrubs in tundra vole winter diets. In addition to the less optimal level of precision from quantitative DNA metabarcoding (Paper IV), other methods (or in combination) that allow for identification of different plant parts in rodent diets, would further increase our understanding of small rodent diets and are arguably necessary when studying food resource dynamics.

In its reply to the calls from the other papers, Paper V found that the two vole species ingest a wide range of taxa and thus were very flexible and diverse in their diets. Though subject to the use of metabarcoding, this indicates that the consistent phase-dependent tendencies are moderate at best and in contrast to the large changes in population density occurring during the vole cycle. This is also supported by a recent study that found that plant-vole abundance relations were not consistent over two consecutive population cycles (Soininen et al., 2018). Hence, our *a priori* categorizations of the tundra vole as a specialized grazer and the bank vole as a more generalist browser is hardly warranted in light of the results of the diet analysis. Indeed, both species can be regarded as generalist herbivores within their respective habitats.

Several previous studies of plant-rodent interactions have presumed that the quality dynamics of single plant species could drive population cycles, e.g., *Vaccinium myrtillus* for *Myodes* spp. (Dahlgren et al., 2007; Selås et al., 2002), *Carex bigelowii* for *Lemmus lemmus* (Seldal et al., 1994), or *Deschampsia caespitosa* for *Microtus* spp. (Massey et al., 2008). One reason for this is that food quality analyses of single plant species are obviously easier performed than of foodscape quality (Petit Bon et al., 2021; Vonthron et al., 2020). Implicitly, it is assumed, but rarely confirmed, that the diet is dominated by the plant species in question. However, the present and other DNA metabarcoding studies (e.g., Soininen et al., 2013;

Soininen et al., 2009) have shown that some boreal and arctic vole species renowned for their cyclic dynamics do have diverse and flexible diets. In that case, we argue that interactions with a single plant species is not likely to underlie their population cycles.

According to general theory on consumer-resource interactions (e.g., Murdoch et al., 2013; Turchin, 2003) profound cyclic oscillations are expected only when the consumer is specialized on a specific resource (i.e. stenotopic consumers). Consumers with flexible diets will not be expected to have the kind of tight coupling with the dynamics of a single resource that acts to destabilize their dynamics. While this conjecture has been guiding empirical studies of predator-prey and host-parasitoid interactions (e.g. Klemola et al., 2002), it appears less influential to the study of herbivore-plant interactions in rodents. Indeed, determining whether a herbivore is a generalist with diverse and flexible diet or a specialist with a narrow and inflexible diet ought to be the first step towards an understanding of the role of herbivore-plant interactions in cyclic vole populations.

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# Dissertation articles

# I

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CONCEPTS, REVIEWS AND SYNTHESES

# Population cycles and outbreaks of small rodents: ten essential questions we still need to solve

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## Abstract

Most small rodent populations in the world have fascinating population dynamics. In the northern hemisphere, voles and lemmings tend to show population cycles with regular fluctuations in numbers. In the southern hemisphere, small rodents tend to have large amplitude outbreaks with less regular intervals. In the light of vast research and debate over almost a century, we here discuss the driving forces of these different rodent population dynamics. We highlight ten questions directly related to the various characteristics of relevant populations and ecosystems that still need to be answered. This overview is not intended as a complete list of questions but rather focuses on the most important issues that are essential for understanding the generality of small rodent population dynamics.

**Keywords** Density dependence · Phase dependence · Voles · Mice · Lemmings

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## Introduction

Populations of small rodents have fascinated ecologists all over the world due to their extreme eruptive dynamics, or regular periodic fluctuations known as multiannual population cycles. Population cycles have fuelled decades of research since Charles Elton (1924, 1942), who described this phenomenon based on historical data in northwest Europe and Canada (Lindström et al. 2001; Myers 2018).

Many small rodent populations have erratic dynamics. However, voles and lemmings in the northern hemisphere, and particularly in Northern Europe, tend to have regular population fluctuations manifesting as cycles with a peak every 3–5 years (peak densities may attain 100–600 ind./ha, or 0.3–1.8 tons/km$^2$). Elsewhere, small rodents can have larger outbreaks (1000–3000 ind./ha, or 1–5 tons/km$^2$; Saunders 1986; Singleton et al. 2005, 2007; Leirs et al. 2010) with irregular intervals, usually, but not necessarily, exceeding 5 years (Singleton et al. 2007). Outbreaks occur both in the northern (Ostfeld et al. 1996; Jacob and Tkadlec 2010) and southern hemispheres, having major economic (Meerburg et al. 2009b; Singleton et al. 2010), conservation (Holland et al. 2015) and health impacts (Ostfeld et al. 1996; Meerburg et al. 2009a) both in developed and developing countries. In addition to the economic and health impacts of rodent outbreaks, population fluctuations in voles and lemmings are key for the functioning and structuring of boreal and arctic ecosystems (Ims and Fuglei 2005; Krebs 2011; Boonstra et al. 2016).

In this review, to improve our understanding of the mechanisms underlying the dynamics of populations, we compare small rodent cycles and outbreaks. There is a tradition in studies of population cycles to investigate the mechanisms driving the remarkably regular variation in density, which has resulted in a multitude of hypotheses explaining population dynamics (literature starting from Elton 1924, 1942, over Krebs 2013 and continuing). Ecologists studying outbreaks of small rodents have, however, often focused on the management of rodents due to their enormous impacts on humans through crop losses and disease transmission (Singleton et al. 2010).

The focus of the review is on population ecology. We have thus combined intellectual inputs from ecologists studying both population cycles and outbreaks in an attempt to achieve a synthesis. In our discussion, we highlight ten questions, the answers to which are essential for improving our perception of the various phases of the cycle or outbreaks. We do not provide a complete or specific list of open questions, but rather a selection of those major questions that require answers to better understand the generality of small rodent population dynamics.

## Outbreaks and cycles

### An overview

Population cycles have been well described as periodic multiannual density fluctuations characterized by delayed density dependence in population growth rates (Stenseth 1999). The periodicity may be statistically derived from e.g. simple autocorrelations of abundance in time series data (Begon et al. 1996), autoregressive models (Stenseth 1999), or spectral and wavelet analyses (Elmhagen et al. 2011) and nonlinear time series analyses (Hsieh et al. 2008). In addition to periodicity, population cycles are often characterised by their astonishing amplitude, i.e. the difference between the maximum and minimum densities. During cycles, rodent densities typically increase by 2–3 orders of magnitude from the low phase, often with $< 1$ ind./ha, to the peak. Furthermore, the four phases of a population cycle, i.e. increase, peak, crash and low phase (e.g. Krebs and Myers 1974), are accompanied by various distinct phase-dependent features (Fig. 1).

Rodent outbreaks are less strictly analysed statistically as they occur largely at irregular intervals (Fig. 1). Nonetheless, their magnitude in both agricultural and forest landscapes can be so impressive that they have been described in the literature as early as the time of Aristotle (384–322 BC; Jacob and Tkadlec 2010). Rodent outbreaks have had dramatic economic, ecological, societal, and even political ramifications (Singleton et al. 2010). In recent times, rodent population outbreaks triggered by bamboo flowering and fruiting have been closely associated with changes in governments because of their devastating effects on vulnerable human communities of upland habitats in Asia (Aplin and Lalsiamliana 2010). Economically, global annual losses caused by rodents are consistently reported to be around 10–15% when pre-harvest (Meerburg et al. 2009b) and post-harvest losses (Belmain et al. 2015) are combined. Occasional outbreaks of rodent populations in developing countries have important implications for food and economic security from local to regional scales (Singleton et al. 2010). In developed countries, consequences are less drastic, but given that high rodent, density is often prevalent in specific regions and crops, the effect on businesses and supply chains can be dramatic (Jacob et al. 2014).

Defining a rodent outbreak is challenging because of the broad range of species and environments involved. Species that undergo outbreaks vary considerably in their population densities between non-outbreak and outbreak years. Long-term studies of house mice *Mus domesticus* indicate extremely low densities in most non-outbreak years ($< 1$ ind./ha), yet during outbreaks, population

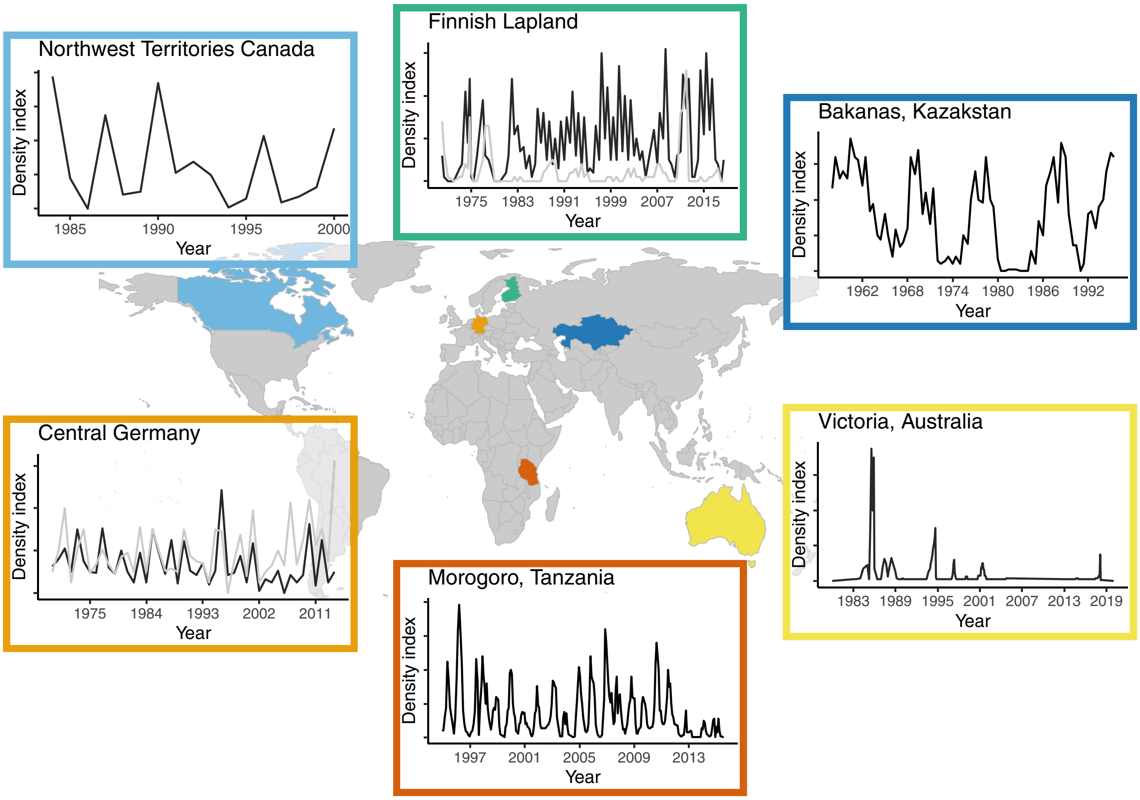

**Fig. 1** Small rodent population dynamics examples from representative long-term time series in different regions of the world: collared lemmings *Dicrostonyx torquatus* and brown lemmings *Lemmus trimucronatus* from northern Canada (top left; data sent by C.J. Krebs; Krebs 2011), two common vole species the bank vole (*Myodes glareolus*) = black line, and the field vole (*Microtus agrestis*) = grey line, from northern Finland (top centre) and Germany (below left), Great gerbils (*Rhombomys opimus*) in Kazakhstan (below right), Multi-mammate rat (*Mastomys natalensis*) in Tanzania, and house mouse (*Mus musculus*) in Australian grain-growing region. Discussion of the different population dynamics and references are found in the main text

densities can be more than 3 orders of magnitude higher (Singleton et al. 2005). Saunders (1986) reported densities of > 3500 ind./ha and this is likely to be the norm for hundreds of thousands of square kilometres of wheat fields in Australia during a mouse plague. By contrast, other outbreaking species have moderate densities in non-outbreak years, while an outbreak entails increases in population densities of only 1–2 orders of magnitude. For example, African multi-mammate mice in Tanzania typically have seasonal peaks of about 150 ind./ha (Fig. 1), whereas in outbreak years densities can be tenfold (Leirs et al. 2010). A review of the bio-economics of five agricultural rodent pest species drawn from four continents highlights these differences in baseline densities and consequent outbreak trajectories (Stenseth et al. 2003).

Interestingly, the population dynamics of the same rodent species can have regular cyclic dynamics in some parts of their distribution, and irregular outbreak dynamics in other parts. This is true for the arctic lemming species, which appear to have a mix of cyclic and irregular outbreak dynamics within and between species (Ehrich et al. 2020) and over time (Henden et al. 2009). Field and bank voles (*Microtus agrestis and Myodes glareolus*) tend to exhibit population cycles in Fennoscandia (Hansson and Henttonen 1985), but less regular outbreaks in Central European deciduous forests. The exceptionally high population densities of especially forest-dwelling bank voles are related to bottom-up regulation by weather-driven beech mast (Imholt et al. 2015 Fig. 1). Common vole populations *Microtus arvalis* in Central Europe have also been shown to alternate between cyclic and non-cyclic dynamics, likely due to changes in habitat structure and land use (e.g. van Wijngaarden 1957). Both cycles and outbreaks in these *Myodes* and *Microtus* species are spatially synchronous across large regions and at least some features of their fluctuations are similar. According to Lambin et al. (2006), there may be no fundamental causal differences between cycles and outbreaks in Northern and Southern Europe.

## Phase dependent variation in population demography and behaviour

Phase dependent variation in the physiology and demography of cyclic vole and lemming populations has been well described. The most characteristic feature is the so-called Chitty effect, which involves vole body mass changes through a cycle with adults being 20–30% heavier in the peak phase than in the low-density phase (Chitty 1967; Boonstra 1994; Oli 1999, 2019; Sundell and Norrdahl 2002; Lambin et al. 2006). The smaller voles in the low phase tend to show delayed reproductive maturity. This phenomenon seems to be universal for cyclic vole populations. Animals are heaviest in the peak phase and produce the largest litters in the increase phase, while they are lightest and produce small litters in the decline and low phases. These demographic characteristics contribute to the asymmetric time series where both outbreaks and cycles show that the low phase may last up to several years, and the increased phase tends to be longer than the sudden crash and decline (Ginzburg and Inchausti 1997).

Chitty (1960) and later Boonstra (1994) proposed systematic changes in demographic population structure as the driving force of vole population cycles. Consequently, not only the quantity but also the quality of individuals may change during a cycle. Changes in the quality of individuals are likely to manifest as behavioural changes. Spacing is dramatically different at peak densities with more than 1000 ind./ha compared to the low phase with 1 ind./ha and less. During low density, it has been proposed that family groups in separated colonies may survive "by accident" and they would form the kernels to build up a local or area-wide increase again (Stenseth 1978; Glorvigen et al. 2013a, b). This well-documented phenomenon was even discussed as a possible driving force of population cycles (e.g. social fence hypothesis; Hestbeck 1982). The senescence hypothesis by Boonstra (1994) states that density-dependent social inhibition of breeding during the peak summer forces young to delay maturation until the next breeding season. Such density-dependent inhibition of maturation is quite common in territorial arvicoline rodents (e.g. Andreassen and Ims 2001).

Non-cyclic rodent outbreaks are predominantly driven by an elevation of reproductive rates some 6–9 months preceding a population outbreak. The conditions that trigger this atypical breeding pattern vary depending on the rodent species and the ecosystem. Nevertheless, species that have population outbreaks exceeding > 1000 ind./ha are typically characterised by an ability to extend their breeding season and/or to increase their production of young in response to climatic conditions and human agriculture that increase food supply. Such patterns have been reported in Australia (Singleton et al. 2001), Africa (Leirs et al. 1996), South America (Lima et al. 2003), Asia (Htwe and Singleton 2014), Europe (Jacob et al. 2014) and New Zealand (Ruscoe and Pech 2010) across many species. Apart from the breeding patterns, there are few generalities associated with the density-dependent and independent factors that influence the growth rates of species with erratic outbreaks (Stenseth et al. 2003).

## The seasonal structure of population dynamics

We refer to seasonality as the sequence of a breeding and a non-breeding season yearly. In high latitudes, seasons are defined by summer with vegetation growth and breeding of small rodents, and winter as a cold season with no, or only minor and exceptional, reproduction except for arctic lemmings where winter is the primary reproductive season. The length of the winter season varies with latitude and altitude with longer snow–covered periods polewards and upwards. Mediterranean climates in both the northern and southern hemispheres provide a comparable response, with usually more intense breeding of small rodents in spring and early summer, and low or absent breeding in the hot dry late summer and colder winter. In both cases, the non-breeding season is characterised by almost no photosynthesis, and thus practically no vegetation growth and no replenishment of food resources.

Stronger seasonality in high latitudes of the North shows that longer winters are associated with extended period lengths and larger amplitudes of the population cycles (Hansson and Henttonen 1985; Tkadlec and Stenseth 2001; Lambin et al. 2006; Taylor et al. 2013, but see Korpela et al. 2013). One piece of evidence for the importance of seasonality is the opposite geographical pattern in common vole fluctuations (Tkadlec and Stenseth 2001) as compared to the North–South gradient of Fennoscandian vole cycles (Hansson and Henttonen 1988). In northern Central Europe close to the Baltic Sea, common vole populations were more stable and increasingly cyclic towards southern Central Europe.

Also empirically-based modelling studies support the significance of seasonality as a determinant of the dynamics of cyclic populations (Bjørnstad et al. 1995; Stenseth et al. 2003; Kleiven et al. 2018). In arctic lemmings, the winters are key to reproduction while population densities often decline in summer (Ims and Fuglei 2005; Therrien et al. 2014). Due to the lack of reproduction during winter in voles, the strong, direct density dependence during winters necessarily involves winter survival. Seasonal and direct density-dependent mortality, together with direct and delayed density-dependent processes causing summer declines of populations, are necessary factors promoting multiannual cycles (Korpela et al. 2014). Examples are the population cycles of grey-sided voles *Myodes rufocanus* in Hokkaido, northern Japan (Batzli 1999; Stenseth et al. 2003), the cycle gradient of a whole vole community from northern to southern Fennoscandia (Hansson and Henttonen 1988; Hörnfeldt 2004), and cycles of the bank vole *Myodes glareolus* (Tkadlec and Zejda 1998) and the common vole *Microtus arvalis* in Central Europe (Tkadlec and Stenseth 2001; Pinot et al. 2016). The underlying process in this seasonal variation may be connected to predation or a limited amount of food produced during the preceding summer. Indeed, several studies confirm the significance of food resources for winter survival (Ylönen and Viitala 1991; Schweiger and Boutin 1995; Eccard and Ylönen 2001; Huitu et al. 2003, 2007; Boonstra and Krebs 2006; Johnsen et al. 2017; Soininen et al. 2018, but see Yoccoz et al. 2001).

Besides the significance of food resources, other resources related to the winter habitat may emerge as limiting factors. Larger territories will give access to a multitude of resources, such as food, nest sites, and mates. Korslund and Steen (2006) found that survival of tundra voles *Microtus oeconomus* increased with the increasing availability of the subnivean space. Similar results have been found for collared lemmings *Dicrostonyx kilangmiutak* and brown lemmings *Lemmus trimucronatus*. In arctic regions where snow is a strong limiting factor in the population growth of lemmings, amongst others the density of winter nests increased with snow depth (Reid and Krebs 1996; Reid et al. 2012; Bilodeau et al. 2013). Finally, Ylönen and Viitala (1985) found that bank voles aggregated in areas with brush vegetation before winter, which were also the areas with the thickest snow cover during winter. Winter aggregations benefit from a high level of social interactions (Ylönen and Viitala 1991), which promotes thermoregulation, i.e. heat and energy saving during mid–winter (Vickery and Millar 1984), and high reproduction at the onset of the breeding season in spring (Rémy et al. 2013; Andreassen et al. 2013; Radchuk et al. 2016). This may give rise to large growth rates in summer.

The picture of population fluctuations in non-seasonal environments in the tropics or in dry–temperate areas in the southern hemisphere is far fuzzier, as factors promoting resource availability and population growth are more stochastic (Leirs et al. 1997). Initiation of an outbreak seems to require the enhancement of food resources, which most often depends on e.g. rainfall and agricultural practices. In these environments, there are often distinct wet and dry seasons, which clearly determine the breeding seasons of rodents (Leirs et al. 1989; Massawe et al. 2011; Bâ et al. 2013). Unusually, wet periods or a prolonged rainy season result in longer or off-season breeding periods, with additional generations and therefore a multiplicative effect on abundance (Leirs et al. 1993).

However, extreme weather events with heavy rain and storms (Singleton et al. 2010) are not necessarily occurring regularly timed in the annual cycle. Thus, long-lasting droughts may maintain low population densities, while unpredictable rainfall periods boost irregular outbreaks of small mammals, like the house mice in dry-temperate Australia (Singleton et al. 2010).

This kind of irregularity is typical to tropical rats and other rodent outbreaks following bamboo masts in Southeast Asia, but nevertheless, outbreaks may also occur as not related to specific climatic events (Aplin and Lalsiamliana 2010; Belmain et al. 2010). Such climatic uncoupling has also been reported in beech mast-driven outbreaks of Central European rodent species (Reil et al. 2015). If extreme weather events like cyclones are followed by rapid plant growth in natural habitats and asynchronous, non-seasonal planting of rice in managed agricultural habitats, rodent densities and following agricultural damage may escalate rapidly. Outbreaks may also be favoured by the high mortality of predators due to cyclone hazards, leading to lower predation pressure (Singleton et al. 2010), but this idea has not been fully documented yet.

To conclude, seasonal effects may essentially shift rodent dynamics from an intrinsically stable regime with irregular fluctuations (generated by density-independent mechanisms) to larger–amplitude and periodic cycles influenced by density-dependent mechanisms (Stenseth et al. 2003). Irregular outbreaks, on the other hand, seem to be primarily linked to stochastic weather events.

## Phase dependent effects and related questions

We acknowledge a recent statement by Oli (2019) that "Solving the enigma of population cycles may necessitate identifying factors and processes that cause phase-dependent demographic changes and performing conclusive experiments to ascertain the mechanisms that generate multiannual density fluctuations". Hence, in the following we discuss the mechanisms shaping population dynamics of voles and lemmings, for which four cycle phases, i.e. increase, peak, crash and low phase, can typically be identified. We will, however, also consider population outbreaks whenever this is feasible, and comparison may provide relevant insight.

### The increase phase

The literature regarding small rodent population cycles mostly focuses on the crash phase and the ensuing low phase, and on the factors that may cause these (e.g. Boonstra et al. 1998). Surprisingly, much less effort has been devoted to studying processes of populations escaping regulation from low densities and transitioning into extended periods of increasing density (Hein and Jacob 2015).

The transition of a stable, low-density population into one with density independent population growth is facilitated by a shift in population demography, such that reproductive rates and/or immigration become greater than mortality and/or emigration. Increasing population densities of small rodents in favourable environmental conditions and low intraspecific competition can be easily explained by the intrinsically high rates of sexual maturation and reproduction (Turchin and Ostfeld 1997). The challenge is, however, to identify the factors that define good environmental conditions which allow the increase. This is particularly relevant, as both the rates of increase and the duration of the increase phase vary substantially from one peak to the next, suggesting that also environmental conditions vary (see e.g. Boonstra et al. 1998).

Firstly, adequate food resources are a necessity for population growth. Food resource availability is, by and large, governed by abiotic conditions. In low and early increase phases, densities are often very low. Therefore, competition for high-quality food is likely to be negligible. At high latitudes, reproduction in cyclic small rodent populations commences at the onset of plant growing season in spring after several months of winter (e.g. Prévot-Julliard et al. 1999), except for the arctic lemmings mostly breeding under sub-niveal protection (e.g. Ims and Fuglei 2005). At lower latitudes, rainfall determines the condition of vegetation, and hence acts as a pivotal limiting factor for small rodent population growth. This is especially true for arid regions (see Bennison et al. 2018) and for semi-arid regions with seasonal rainfall (Tann et al. 1991; Leirs et al. 1994; Luque-Larena et al. 2013). In desert environments, patterns of precipitation are often highly unpredictable, and often affected by large–scale climatic anomalies such as the El Niño Southern Oscillation (Lima et al. 1999). Small rodent reproduction can also be strongly impacted by pulsed variation in food availability (so-called mass occurrences) in more productive areas, such as in the case of European beech *Fagus sylvatica* (Jensen 1982; Wolff 1996) or several bamboo species (Belmain et al. 2010; Htwe et al. 2010).

Secondly, small rodent population growth cannot be achieved in environments in which the mortality effects of predation override rates of reproduction. According to the specialist predator hypothesis (Andersson and Erlinge 1977), cyclic vole populations can sustain many predators during the peak and crash phases. However, after vole densities remain low for a sufficient time, predator numbers dwindle due to either starvation or emigration (Norrdahl and Korpimäki 2002), providing small rodents with enemy–free conditions in which to procreate. Such settings are typical for Northern Europe.

In temperate areas vertebrate communities are more complex, containing more of both alternative prey species and generalist predators that prey on them. The latter has been shown to have a stabilizing effect on vole population dynamics (Hansson and Henttonen 1985; Hanski et al. 1991), partly by a considerable shortening of the time window with enemy-free conditions during which rodent population growth is expected to take off. In small mammals exhibiting irregular population outbreaks in arid regions, the periods between peaks are often too long for predator populations to subsist in moderate densities (Sinclair et al. 1990), thus restricting their impact to the proximity of the peak itself (Meserve et al. 2003). However, not all species behave in the same way: Lima et al. (2003) showed that in the leaf-eared mouse (*Phyllotis darwini*) in Chile, population growth rate throughout the year is dependent on survival (for which predation is thought to be important), while for the multi-mammate mouse (*Mastomys natalensis*) in Tanzania, changes in reproductive output are much more important for population growth.

Thirdly, the intrinsic behavioural and social processes operating within populations of both cyclic and eruptive species vary considerably during different phases of their dynamics. Several of these processes may be beneficial during increasing population densities. For example, the increase phase is initiated by the demes of animals distributed in high-quality patches of the landscape (Sundell et al. 2012). Resource patchiness may promote social behaviour in females and enhance their reproductive success compared to solitary territorial females (Ylönen et al. 1988; Ylönen and Viitala 1991; Lambin and Yoccoz 1998; Sutherland et al. 2005; Rémy 2011). The benefits may manifest through communal breeding and thermoregulation, particularly during winter (Hayes 2000; Gilbert et al. 2010), and shared protection against infanticide (Wolff 1993; Ylönen et al. 1997). The early phases of the increase will be associated with dispersal and rapid colonisation of vacant habitat patches (Glorvigen et al. 2013a, b), as dispersal is inversely density-dependent in voles (Andreassen and Ims 2001). The correlation between amicable social behaviour and population growth rates have been described for several species of rodents, such as house mouse (*Mus* spp.; Krebs et al. 1995; Sutherland et al. 2005), yellow-necked field mouse (*Apodemus flavicollis*; Bogdziewicz et al. 2016), and *Myodes* and *Microtus* voles (Ylönen et al. 1990; Andreassen et al. 2013 and references therein). These species inhabit various biomes in the world and vary in population dynamics from occasional outbreaks to population cycles.

In conclusion, it seems to be obvious that small rodent population increases are associated with abundant food resources, enemy-free conditions, and certain types of social behaviour. However, there are details regarding the increase phase that is currently poorly understood, which can be broadly summarized into the essential question related to the increase phase:

1. *What factors determine the rate and the timing at which rodent populations increase, and what defines the length of the increase phase?*

The rate of population growth, assuming closed populations and minimal mortality, is a function of reproductive output. Small herbivorous mammals subsist primarily on a relatively poor quality diet, particularly regarding the intake of nitrogenous compounds, and especially essential amino acids (Mattson 1980) that are crucial for maintenance and reproduction. Certain amino acids are a limiting factor for per capita reproductive output in cotton rats *Sigmodon hispidus* (Webb et al. 2005). This indicates that diet quality may well affect population growth rates of both cyclic and eruptive species during the increase phase. However, this association has received virtually no research attention, let alone how diet quality varies in response to the weather. In arid areas, the quantity of food clearly influences the population growth rates of small mammals. Such an association may also affect cyclic small mammal populations in more predictable growing regimes, e.g. in Northern Europe, where dry and hot summers often appear to inhibit vole population growth.

The quantity and quality of food resources are likely to have major effects also on the duration of population increase. In general, multivoltine small mammals with several litters in one—and often the only—breeding season of their lifetime, require long growing seasons or need to breed in several consecutive summers (Prévot-Julliard et al. 1999), to reach the absolute carrying capacity of the population. As a seasonal effect, it is obvious that environments that exhibit long winters also have a short growing season.

Furthermore, rodents depend heavily on intestinal microbes for the digestion of their bulky and cellulose-rich food (Ley et al. 2008). The composition of the rodent intestinal microbiota is greatly affected not only by their diet (Kohl et al. 2014), but also by pathogens and parasites (Guarner and Malagelada 2003), and this, in turn, may reflect upon the immune system (Guarner and Malagelada 2003). The role of such changes in the intestinal microbiota on phase-related changes in rodent demography has, to our knowledge, never been investigated until the work of Li et al. (2019).

## The peak phase

Population peaks largely determine the attained density amplitude of the population. They are reached when mortality first equals then exceeds reproduction, to prevent a further increase in density. Immigration and emigration are supposed to be in balance during the peak phase, which is a reasonable assumption as small rodent fluctuations are commonly spatially synchronous over vast areas (e.g. Sundell et al. 2004).

Peak densities typically vary substantially from one cyclic peak or outbreak to the next, also for the same population in the same area (Fig. 1). Variation in the limiting factors is associated with changes in the carrying capacity of the environment, as determined by either abiotic conditions (e.g. weather or habitat availability), or biotic factors such as food resources or predation. Variation in weather may affect primary production and biomass accumulation, which in turn affects the amount of available food resources or the extent of foraging and breeding habitats. For example, a warm and dry summer may greatly limit the growth of rodent food plants, resulting in a low amount of accumulated food resources with which to overwinter (Korpela et al. 2013) (Fig. 2).

Most of the factors proposed to cause cyclic dynamics in small rodents can also influence peak density levels and even cause cessation of population growth. These include competition, predation, reduced food availability and quality, pathogens and parasites, stress, and quality of individuals, as well as social factors such as infanticide (e.g. Stenseth and Ims 1993; Oli 2019). These factors potentially limit population growth, but they do not necessarily regulate populations, i.e. they do not cause the cyclic dynamics per se. This problem may be exemplified by the multiannual fluctuations of northern voles, which are thought to be mainly caused by delayed density-dependent factors such as predation by specialist predators (e.g. Korpimäki and Norrdahi 1998; Hanski et al. 2001; Korpimäki et al. 2002). However, even the classic predator–prey models inherently require some direct density-dependent process to slow the prey's population growth, so that predators with their much lower reproductive potential can "catch" the prey population and cause the subsequent crash (e.g. Hanski et al. 2001). Yet, it is important to note that, in seasonal environments, the predator functional response alone can generate direct dependence even when predator species express various functional responses (e.g. Gilg et al. 2003). Huitu et al. (2003) identified winter food resources as such as a direct density-dependent limiting factor in a two-factor experiment manipulating both predation and winter food supply. The great gerbil *Rhombomys opimus* in the Central-Asian steppe in Kazakhstan exhibits cyclic population fluctuations that are linked with the flea burden on these rodents and epizootics of *Yersinia pestis* plague (Reijniers et al. 2014). Meanwhile, Kausrud et al. (2007) showed that climate forcing synchronizes the dynamics of these gerbils over large geographical areas. In ecological population models, many of these factors can co-occur, and their relative strength is almost impossible to gauge or even parameterise. Hence, this "untouchable clump of factors" is often incorporated as a black-box in the models (Stenseth 1999).

There are many additional direct density-dependent factors that may contribute to population fluctuation patterns. These may be related to predation, for example, selective predation on the reproductive part of the prey population (Cushing 1985), changes in the predator spectrum due to shifts in prey activity patterns (Halle and Lehmann 1987;

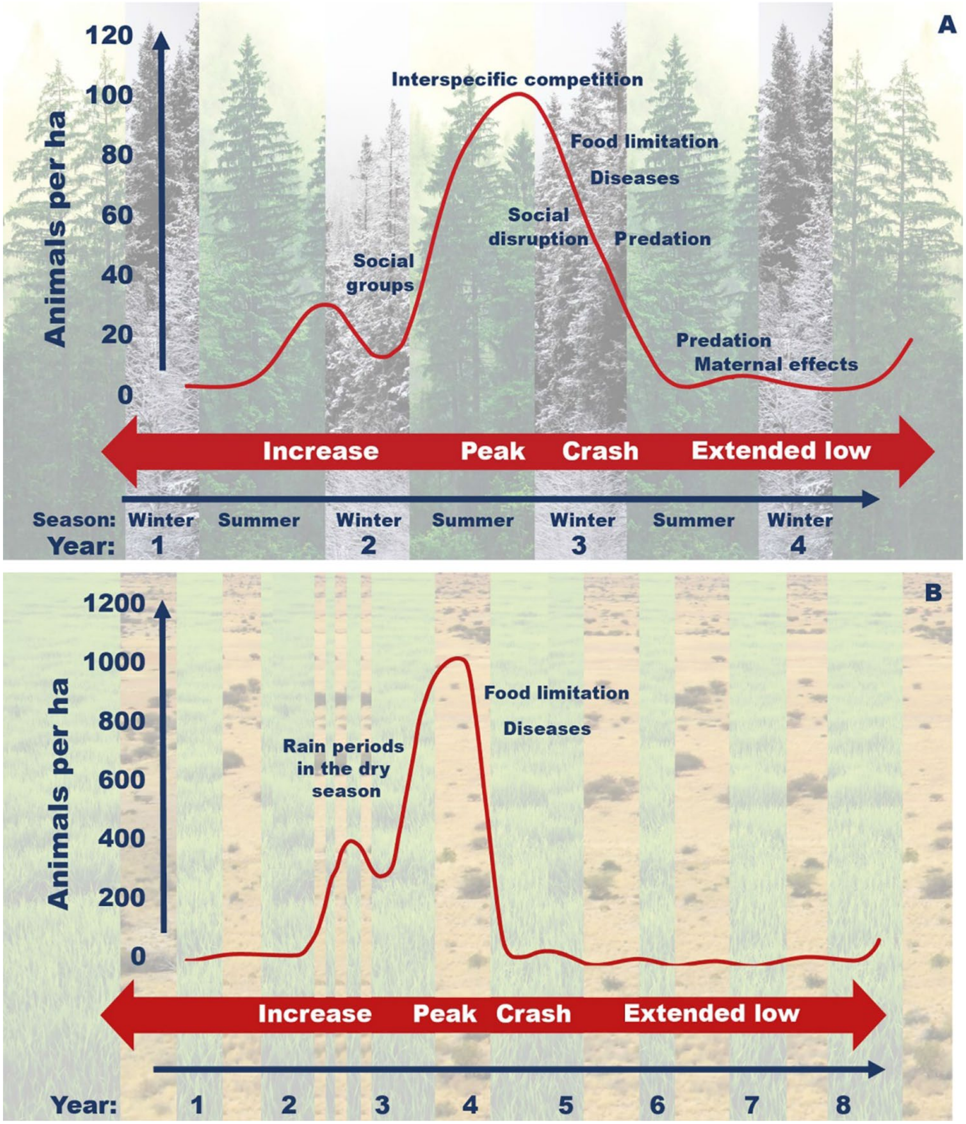

**Fig. 2** Characteristics of vole population cycles in the northern hemisphere (**a**) and mice outbreaks in the southern hemisphere (**b**). Seasonality connected to reproductive and non-reproductive periods. Winter is the non-reproductive season in the northern hemisphere and the dry season in the south

Halle 1993), indirect predation effects (Ylönen 1994; Ylönen and Ronkainen 1994; Sheriff et al. 2009), fast functional response of the generalist predators (Hanski et al. 1991), and fast numerical response of nomadic avian predators (Sundell et al. 2004). Little is known about the many potential interactions of the multiple factors, as this kind of network is hard to control in experimental studies. Food and predation/parasite—interactions are the most studied of such interactive effects (e.g. Pedersen and Greives 2008; Haapakoski et al. 2012; Forbes et al. 2015), but other or multifactorial interactions are hardly touched.

The shape and magnitude of the peak phase of population cycles vary considerably between species (Turchin et al. 2000; Turchin and Batzli 2001), for example between the sympatric northern species *Myodes rufocanus* and *Lemmus lemmus* (Ims et al. 2011). *Myodes* populations, as many vole species in general, have cycles with blunt, often two-year peaks (Ylönen 1988) compared to *Lemmus* populations that have more angular, saw-toothed cycles with higher maximum densities (Turchin et al. 2000; Ehrich et al. 2020). These differences are suggested to be due either to different causal trophic interactions (predator–prey in *Myodes* and plant–herbivore in *Lemmus*; Turchin et al. 2000), or to winter breeding (most prevalent in *Lemmus*; Ims et al. 2011). Andreassen et al. (2013) suggested that different social organisations between the species or genera might be linked to the shape of cycles, with sharp, high–amplitude cycles being typical for species with male territoriality and female sociality. Thus, *Microtus* species tend to have sharper cycles than *Myodes* species, where the social system is characterised by female territoriality (Kalela 1957; Viitala 1977; Ylönen 1988). The social system of lemmings is more flexible and may rather depend on territorial males (Heske and Jensen 1993). Moreover, the extreme shifts in dispersal and social behaviour observed in *Lemmus* (specifically in *L. lemmus*; Stenseth and Ims 1993) fit well to the suggestion that the shape of the cycle may be linked to behaviour and social organisation.

The absolute height of the peak in animal numbers is determined to a large extent by food resources. As an example, the numbers of house mice in Australia, breeding in grain fields, reach even thousand(s) of individuals per hectare during outbreaks (Singleton et al. 2005). In Europe, the herbivorous *Microtus* voles inhabiting agricultural landscapes reach two- to three-fold higher densities than the granivorous *Myodes* voles (Henttonen 2000). Exceptionally good food supply may promote disruption of the social system and territorial behaviour, which normally controls the numbers of breeding females as observed by Ylönen et al. (1988).

To compile this section, a rather simple essential question arises in connection with the peak phase:

2. *What are the factors that determine the height of the density peak, and how do they interact?*

A thorough understanding of the continuous variation in density amplitude for each cyclic or eruptive population in any geographical region and habitat productivity could bring new insights into population dynamics. More specifically, it is important to recognize those direct density-dependent factors that hinder population growth near the peak densities. An additional question is whether the social structure of peak density vole populations remains the same as in increasing populations, or whether some kind of change or disruption of the social structure occurs, which would enable more females to breed in a stressing high–density environment. This kind of loss of social control in breeding during very high densities was observed by Eccard et al. (2011).

## The crash phase

The decline of the population after a peak or outbreak is often abrupt and dramatic, and therefore it is called a crash. The crash phase has received the most attention in the literature and it is indeed critical for the understanding of small rodent population dynamics (Tkadlec and Zejda 1998). In cyclic small rodent populations, the crash often starts in late summer or fall and extends into winter and the following breeding season (Krebs and Myers 1974; Hansson and Henttonen 1988; Huitu et al. 2003; Pinot et al. 2016; Johnsen et al. 2017). In many cyclic populations, the initial autumn/winter crash is followed by summer declines strongly affected by specialist predation (Henttonen et al. 1987; Hanski et al. 1991). However, summer declines during the population crash are also observed in cyclic populations of the field vole in Kielder Forest in UK (Lambin et al. 2000), where virtually no strictly specialist predators are present, and in other non–cyclic rodent populations in Central Europe (Giraudoux et al. 2019).

The crash itself also has most often been connected to specialist predators, especially to small mustelids that can enter the holes and cavities of small mammals, their nests and the subnivean space in winter (Norrdahl and Korpimäki 1995; Boonstra et al. 2016; Ylönen et al. 2019). The predator hypothesis is supported by mathematical models (e.g. Hanski et al. 2001) as well as by experimental studies (Korpimäki 1993; Klemola et al. 1997; Korpimäki and Norrdahi 1998). Although no one denies that (specialist) predators contribute greatly to the crash of small rodent populations, some authors have combined predation, or other extrinsic factors, with intrinsic factors as potential enforcers of the decline. For instance, Andreassen et al. (2013) suggest that predation disrupts the social system, followed by intraspecifically induced mortality such as infanticide (see e.g. Ylönen et al. 1997; Andreassen and Gundersen 2006; Opperbeck et al. 2012). The problem with this framework is, however, that it considers a typical scenario for a crash during the breeding season with strong social interactions between territorially breeding animals (e.g. Ylönen et al. 1990). Nevertheless, in most crashes, the steepest decline in numbers is observed during the winter when the territorial behaviour of voles is expected to be relaxed and animals rather aggregate for thermoregulation (e.g. Ylönen and Viitala 1985, 1991; Sipari et al. 2016). Whether the mortality rate throughout winter is constant is yet to be assessed since most studies do not measure population changes throughout the winter but compare before and after winter numbers.

Intraspecific competition during winter for food resources has repeatedly been suggested as a factor limiting the growth of vole populations at peak phases. Obviously, as food resources are not being renewed during the winter, food depletion and deterioration of its quality can easily be regarded as a contributing factor also to the crash phase, as suggested by Boonstra and Krebs (2006) for red-backed voles *Myodes rutilus*. Several experimental studies also show that supplemental feeding during winter can create high autumn densities in local patches in red-backed voles (Schweiger and Boutin 1995), advance breeding of bank voles *Myodes glareolus* in spring (Eccard and Ylönen 2001; Ylönen and Eccard 2004), and reduce territorial behaviour in bank voles (Ylönen and Viitala 1991). It may even prevent winter crashes of bank voles (Johnsen et al. 2017) and, when supplementary feeding was combined with the elimination of predation, in the field vole *Microtus agrestis* (Huitu et al. 2003).

In support of winter food limitation, Huitu et al. (2007) found evidence for deterioration in the physiological condition of field voles in the winter of the decline phase compared to the winter of the increase phase. The poor condition of these voles may expose them to diseases, parasites and/or predators, leading to a feedback loop of increasing mortality (Beldomenico et al. 2008). Intraspecific competition for food resources may also explain why larger activity ranges are beneficial for survival during winter (Johnsen et al. 2018).

Related to this is the effect of the larger animals in peak years as described by the Chitty effect (see above; Chitty 1967). It has been shown that small rodents have a physiological optimal winter body mass that is species-specific (Iverson and Turner 1974; Wiger 1979; Aars and Ims 2002). The characteristic of larger animals during the peak may be age-related, as younger cohorts have been inhibited from maturation to the adult subdivision of the population (Andreassen and Ims 2001). The large peak animals may struggle to survive the winter because they are physiologically “too big” and energetically sub-optimal for the limited food resources. This may, together with social intolerance in males, be one reason why the survival of males is generally lower over winter as compared to females (e.g. Klemme et al. 2008; Haapakoski et al. 2012; Sipari et al. 2016).

Depletion of food resources or some specific food items needed in only small amounts (Aulak 1973; Andreassen and Bondrup-Nielsen 1991) may also explain the continuing decline into the following summer, although this has been refuted experimentally by Klemola et al. (2000b). Furthermore, the challenge with the food hypothesis is, however, to understand how this can affect the whole small rodent community consisting of species with markedly different diet requirements, like seeds in *Myodes*, graminoids in *Microtus* and mosses in *Lemmus* (Hansson and Henttonen 1985); but see (Soininen et al. 2017b).

Limited food resources may also interact with predation and/or pathogens and diseases to further reduce population numbers (Huitu et al. 2003). Studies focusing on the mortality causes in cyclic vole populations support the strong effect of predation, as Steen (1995) observed in cyclic tundra voles *Microtus oeconomus*, and Norrdahl and Korpimäki (1998) for radio–collared *Microtus* voles. In studies where predation rates were precisely estimated, the population growth of arctic lemmings in summer was limited by predation pressure, e.g. by predatory birds (Therrien et al. 2014). Nevertheless, during the crash phase, predators are likely to act compensatively, i.e., kill starving or diseased individuals that would die anyway. Relatively few animals are found dead during a crash except for Norwegian lemming *Lemmus lemmus* where surplus killing can translate into many carcasses (Steen et al. 1997); for voles, however, with crashes mostly occurring during winter, scavenging by many predators can be a simple reason behind the absence of dead animals found in spring. So, the essential question related to the crash phase can be framed as:

3. *How does the population demographic structure affect the crash phase?*

The population crashes of eruptive species in the southern hemisphere are more rapid and impressive than the decline in vole and lemming populations. House mouse populations literally crash synchronously within weeks over thousands of square kilometres (occasionally as fast as 90% reduction of animals within a week; Singleton et al. 2007), including around grain stores where there is still ample food.

Changes in spacing behaviour of house mice during the development of high population densities and during the rapid population crash in wheat fields in Australia indicate that they are highly territorial during the breeding season of an increase phase. These changes in spacing behaviour also signal that there is a complete breakdown of social and anti-predatory control mechanisms once populations are high and during the rapid decline in population numbers (Chambers et al. 2000; Ylönen et al. 2002; Jacob et al. 2004; Sutherland and Singleton 2006). This resembles the breakdown of social breeding control in the bank vole during high densities of mature females (Ylönen 1988; Eccard et al. 2011) and may indicate that population growth to very high densities is a combined effect of resource availability and changes in population social structure. Following a crash, eruptive species like house mice may be under pressure by a combination of caloric and disease stress (Singleton et al. 2007), and finally doomed by predation on sick and weak individuals.

Although the scientific literature and experimentation regarding both cyclic and eruptive small rodent populations have focused on the crash phase, we have still not reached a consensus on conclusive explanatory factors. The Chitty effect characterising individuals in peak phases has received little attention for decades, and we know even less about changes in the population demographic structures in the more eruptive tropical populations. In the latter populations, however, the increase is often very fast as well, in immediate response to stochastic climatic events, and both increase and crash often happen within the same year, not allowing for a shift in demography. Population demography and the structure in the population (e.g. sex ratio, age structure and body mass) shift through the population cycles. It is about time to solve eventual mechanisms for the effect of population demography structure during the crash phase, for instance through physiological constraints in body mass, or senescence (Boonstra 1994).

## The low phase

A feature as remarkable as the density peaks in the cyclic population is that densities do not start to grow immediately after a crash despite ample food resources and low intraspecific competition. This so-called extended low phase has initiated much research on population dynamics. For populations to stay stable at low densities over a longer period, mortality and reproduction, as well as immigration and emigration, should be in balance. Hence, since immigration and emigration are primarily considered to compensate for local density disparities (see above), there has to be a factor that lowers reproduction and/or increases mortality, preventing the population from increasing.

In cyclic populations, the low phase may last 2–3 years before the populations start to increase again (Boonstra et al. 1998). The same phenomenon is observed in eruptive populations that have unpredictably long low phases (most often 5–10 years; Singleton et al. 2007). We need, however, to distinguish between rodent outbreaks in arid areas like Australia vs. temperate Europe where low phases may also be long, but not necessarily so (Jacob and Tkadlec 2010). Multi-mammate mice revert to "normal" seasonal fluctuations after the end of an outbreak and that condition may then last for up to several years until a new outbreak is triggered (Leirs et al. 1996).

Food availability was rejected as an explanation for the extended low phase by Boonstra et al. (1998); and experiments have not found delayed effects of food availability on population growth (Turchin and Batzli 2001). Food becomes a limiting factor only at higher densities (Huitu et al. 2003), and previous overgrazing does not prevent vole populations from increasing (Klemola et al. 2000b). Several food plants of cyclic voles induce phytochemical defences in response to intensive vole grazing (Massey and Hartley 2006; Reynolds et al. 2012; Huitu et al. 2014), and some of these responses are delayed. However, no evidence exists for any universal induced defence substance, applicable across cyclic small rodent taxa (Soininen et al. 2017a).

The lack of universality also applies to rodent pathogens. Although a growing number of studies are reporting significant negative effects of pathogens on the survival of its host (e.g. Soveri et al. 2000; Kallio et al. 2007; Burthe et al. 2008), no pathogen can be common and widespread enough to be responsible for the delay in host population growth at low densities. Pathogen prevalence is generally highest when their hosts reach large densities (e.g. Singleton et al. 1993, 2000), but whether density alone or a combined effect with the cycle phase drives pathogen prevalence remains unsolved. A recent study using cyclic populations by Forbes et al. (2014) identified delayed density-dependent patterns of orthopoxvirus (likely cowpox) prevalence in field voles in Finland, implying that this pathogen may contribute to the low phase of the cycle.

For cyclic populations, Boonstra et al. (1998) concluded that predation and maternal effects are the most likely explanations for the extended low phase. A delayed numerical response of the predators continues to inflict mortality on the population in the low-density phase. A density increase of the prey is impossible until the predation pressure subsides (Henttonen 1985; Korpimäki 1986; Sonerud 1988), which happens when predator numbers are reduced due to mortality, cessation of reproduction and emigration, or if the predators switch to other prey species.

Reduction or removal of predators in the low phase should shorten its duration. Predator removal experiments have been conducted, but they have seldom covered the period between decline and increase of prey populations. One comprehensive experiment exists (Korpimäki et al. 2002) which showed clear effects of predator removal on the abundance of voles in all studied cycle phases, albeit without a marked impact on the length of the low phase. The reason for this might have been that the reduction of all vole predators was conducted only during the breeding season. In two studies (Klemola et al. 2000a; Huitu et al. 2003), all vole predators were excluded from fenced areas during the low phase, with a similar result—fenced populations increased while unfenced control populations remained at a low level.

Several attempts have been made to employ predators as biocontrol agents, e.g. to prevent crop damage associated with outbreaks (e.g. Mahlaba et al. 2017). These attempts often generate high predator densities, but generally fail to keep rodent numbers down. However, most of these rodent populations were not cyclic. Apart from Duckett (1991) and Kay et al. (1994), there is no convincing empirical field data to suggest that promoting the presence of avian predators (by nest boxes and perches) leads to lower rodent abundance or reduced damage to crops (Labuschagne et al. 2016).

Predation may also have indirect, delayed effects on voles through maternal effects, involving e.g. stress. This is likely to affect in particular individuals of the low phase of cycles, as has recently been shown for snowshoe hares *Lepus americanus* (Sheriff et al. 2009; Krebs et al. 2018). Furthermore, it is important to note that some 10 years ago classical Mendelian heritability of individual traits was assumed a prerequisite for intrinsic effects to be relevant for population regulation. The recent advent of epigenetics has dramatically changed this view (e.g. Bossdorf et al. 2008).

We largely share the views presented by Boonstra et al. (1998) over two decades ago, and conclude that the extended low phase of cyclic small mammal populations is indeed most likely caused partly by extrinsic predation, but partly also by delayed intrinsic, inter-generational effects of predation pressure that modify the quality of individuals living at low densities. Future studies should aim to determine the relative importance of delayed effects of predation, and other density-induced stressors such as social and nutritional stress, through direct and indirect pathways on the demography of small rodent populations. As the evidence on intergenerational effects of early-life environment on survival and reproductive success is accumulating also from voles (Bian et al. 2015, van Cann et al. 2019a, b), more emphasis should be placed on quantifying the phenotypic and (epi-) genetic characteristics of individuals in different phases of the population cycle.

Hence, an essential question for future research on the low phase is:

4. *Why do populations not begin to grow immediately after a crash, and are pathogens or maternal effects relevant ingredients for the extended low phase of cyclic populations?*

For populations with eruptive dynamics, the periods between outbreaks likely represent a normal, more or less stable state of populations, in which densities are not particularly low, compared to most cyclic populations in the North.

## Small rodent populations in a changing world

The world is changing rapidly, also for small rodent populations. Besides the obvious changes of global warming and the increased occurrence of extreme weather events, land-use change is another important factor that could influence the dynamics, health and resilience of small mammal populations. Below, we discuss some related aspects that potentially are important future research issues for ecologists of small rodent population dynamics.

### Climate change

In eruptive populations of the southern hemisphere and temperate Europe, rodent population increases to abnormally high densities are often associated with stochastic climate events or irregular resource changes. In Tanzania, unusually abundant rainfall early in the wet season triggers early reproductive maturation in multi-mammate mice *Mastomys natalensis*. This produces an additional generation within a year, resulting in a tenfold production of young and outbreak densities (Leirs et al. 1993). In Southeast Asia from 1996 to 1999, unusual rainfall patterns led to asynchronous planting of rice crops, followed by rodent population outbreaks each year (Huan et al. 2010). Similarly, the high degree of asynchronous planting of new rice crops over a large area in Myanmar after the cyclone Nargis in 2008 has been suggested to be the most likely contributing factor to the massive population outbreak of *Bandicota* species some 15–18 months later, in areas where outbreaks had never been experienced before (Htwe et al. 2013).

In Central Europe, beech mast triggers bank vole population outbreaks in the following year (Tersago et al. 2009; Reil et al. 2015). The weather conditions favourable for beech mast are likely to occur at higher frequencies in the future due to climate warming. In New Zealand, mouse populations erupt during a beech mast and it has been reported that the magnitude of change in mean summer temperature between consecutive years can predict mast events. Therefore, the frequency of outbreaks of mouse populations in New Zealand forests, and perhaps also of bank voles in beech forests in Europe, may rise with increased variance predicted in climatic events (Imholt et al. 2015; Holland et al. 2015).

There are two major scenarios of how small mammals in the North are affected by climate change. Enhancement in habitat productivity due to warming and increased precipitation may result in agricultural intensification and related land-use changes (cf. Cornulier et al. 2013; see below). On the other hand, winters are predicted to become more unstable and the duration of permanent snow cover shorter, which affects the life of ground-dwelling small mammals and food webs in many ways (Penczykowski et al. 2017). Currently, approximately one-third of the world's land surface is covered by snow during winter (Lemke et al. 2007). Snow cover provides thermoregulatory advantages in the insulated subnivean space, shelter for nest sites, and physical and visual refuge from predators. Hence, shorter periods with snow cover are most likely to affect winter survival of voles and lemmings negatively.

Predation by specialist predators, especially the least weasel *Mustela nivalis* and the stoat *Mustela erminea*, is suggested to be a key factor promoting the population crash and causing extended low phases (see above). Both species belong to the group of vertebrates in the North changing from dark or brownish summer pelage to a white winter coat. Late and unpredictable onset of snow cover and its earlier melting could increase the vulnerability of individuals with a mismatched white coat colour due to intra–guild predation by larger mammalian predators and resident owls. This, in turn, may have dramatic effects on vole dynamics (Ylönen et al. 2019) and further cascading trophic effects at the ecosystem level (Terraube et al. 2015).

Empirical studies on the interaction between climate and predation are scarce. There are two northern-boreal examples of severe changes in vole dynamics, the temporal disappearance and return of vole cycles together with weasel disappearance in Finnish Lapland (Fig. 1, Henttonen et al. 1987, Magnusson et al. 2015), and the low densities of grey-sided voles *Myodes rufocanus* and field voles *M. agrestis* in Sweden (Hörnfeldt 2004; Hörnfeldt et al. 2005). Dampening of the Swedish grey-sided vole cycle is more clearly attributed to changes in forest landscape structure (Hörnfeldt 2004; Ecke et al. 2006; Magnusson et al. 2013, 2015), while dampening of the cycles of the field vole along with their recent recovery, are more likely related to a climatic driver (Magnusson et al. 2015). In contrast, the disappearance and subsequent return of vole cycles in Finnish Lapland seem to be due to a more complex network of changing seasonality and predator–prey interactions in a whole rodent community (Henttonen 2000; Ylönen et al. 2019). Several arctic lemming populations showed perhaps the most compelling examples of collapsing cycles in recent years (Ims et al. 2008), e.g. in North-Eastern Greenland (Schmidt et al. 2012). It is possible that several observed collapses are actually transitions to non-stationary population dynamics as detected by analysing hundred-year long time series (e.g. Henden et al. 2009). Such transitions between stationary and non-stationary can be triggered by several factors, such as non-linear trophic dynamics (Hastings et al. 2018; Clark and Luis 2020; Blasius et al. 2020).

The examples above show how global warming and more variation in extreme weather may change the dynamics of small rodent populations. In the northern hemisphere, a warmer climate may improve habitat quality, while a drier, and more unfavourable climate is expected in the southern hemisphere. How this will affect population dynamics is not obvious. One possibility is that with a warming climate, northern populations would begin to exhibit similar types of erratic outbreak dynamics as currently observed in southern populations. For the South, we already know that rainfall is one of the most important determinants of outbreaks today because it increases primary productivity and food availability, as exemplified for instance by the *Mastomys* rats in Africa (Leirs et al. 1996). As a response to a drier climate, outbreaks may occur more rarely in the southern hemisphere. Yet, those outbreaks may be more dramatic than before, since a long dry spell of several years, broken by an unexpected wet period, may result in uncontrolled growth of the rodent population; all supported by abundant vegetation growth due to the build-up of a rich seed bank and soil nutrients and the absence of predators as suggested by Fiedler (1988).

The above assumptions are reasonable but speculative, since they are projections into possible future complex developments while evidence is missing up to now. An essential question related to global climate changes to follow up is, therefore:

5. *How will climate and land-use change affect small rodent dynamics in both cyclic and eruptive populations?*

The most regularly cyclic populations are found in the northern hemisphere, while outbreaks are more typical from Central Europe to the tropics and Australia. However, there are examples from temperate or arid Europe on eruptive types of fluctuations, resembling a hybrid between cycles and outbreaks (Luque-Larena et al. 2013; Reil et al. 2015). Whether this is a result of cyclic population dynamics eventually turning into more eruptive dynamics, possibly induced by climate change, is plausible but remains to be verified. However, not all rodent species respond in the same way or at a similar pace to climate change (e.g. Gilg et al. 2009). Furthermore, changes in temperature affect other climatic components such as precipitation differently in different parts of the globe, which affects the intervals and intensity of outbreaks. It is therefore too early to cast global predictions regarding the potential effects of climate change on small rodent dynamics.

Of note, climate change is also a major driver of changes in rodent-borne disease patterns (Kausrud et al. 2010; Voutilainen et al. 2012; Altizer et al. 2013; Khalil et al. 2014). Increased trafficking and human encroachment into wildlife habitats will probably accelerate the spread of parasites around the world, also increasing the incidence of rodent-borne zoonotic outbreaks.

Land-use change may influence the presence and absence of small mammal species, or influence their temporal and spatial dynamics directly or indirectly. Populations of *Microtus agrestis* in the UK, in Sweden and in Finland are cyclic. In forested areas, the successional stage affects the dynamics of small mammals and especially that of *Microtus* voles, which largely disappear where grassy clear-cut areas become unsuitable habitats when afforested (Savola et al. 2013). In Sweden, the cyclicity of *Myodes* voles remained despite natural succession or land-use change from e.g. old-growth forest to clear-cuts (Ecke et al. 2002). In Finland, a high degree of agricultural landscape fragmentation is associated with increased spatial variation in *Microtus* population growth rates, as compared to unfragmented agricultural landscapes (Huitu et al. 2004). Cyclic dynamics of common vole *Microtus arvalis* populations emerged overtime on expanding meadows in reclaimed areas in The Netherlands (van Wijngaarden 1957). All these observations imply that land-use change is capable of influencing small rodent population dynamics.

Land cover changes in combination with precipitation may well be an important predictor of rodent outbreaks in agricultural systems (Stenseth et al. 2003). Also, clear-cutting has been reported as an important driver of outbreaks in deer mice *Peromyscus maniculatus* in Canada (Sullivan and Krebs 1981). Land cover changes, the spatial structure of landscape elements, the quantity and quality of food, and general habitat availability may all promote population outbreaks, but the causality and relevance of these factors still need further research.

Nevertheless, a prominent effect of land-use change seems to be the increase in agricultural or grassland areas. This effect on landscape structure may permanently induce chronic high vole densities and outbreaks (Delattre et al. 1996; Fichet-Calvet et al. 2008), as has been observed for many species all over the world. For instance, land cover change from cropped fields to interconnected hay meadows facilitated population outbreaks of water voles *Arvicola amphibius* (Halliez et al. 2015). Agricultural irrigation increased the area of grassy crops, resulting in invasions and following eruptive dynamics of common voles *Microtus arvalis* in Spain (Luque-Larena et al. 2013). In Mongolia, outbreaks of Brandt's vole *Microtus brandti* occurred more frequently due to increases in livestock populations (Zhang et al. 2003).

In Southeast Asia, an important factor for outbreaks of rodents in agricultural landscapes, dominated by rice, is the intensity and timing of land use. In Vietnam, the rice field rat *Rattus argentiventer* causes chronic problems, but their population dynamics have changed markedly when the agricultural management shifted from two to three rice crops per year in the Mekong delta. Breeding of the rice field rat is synchronized with the pre-booting stage of rice, with more crops per year resulting in more breeding seasons (Lam 1983; Brown et al. 2005). This effect is further exacerbated if there are conditions that lead to higher asynchrony of cropping (Brown et al. 2011).

Land-use change can occur over large spatial scales (e.g. clear-cutting of boreal forest, the succession of arable land after the collapse of the Soviet Union, forest fires in North America), but they are not temporally synchronized and hardly recur with a specific time interval. Rather than inducing cyclicity *per se*, changes in land use and landscape structure may create conditions suitable for cyclic population dynamics, e.g. through changes in trophic interactions. Hence, the study of land-use change may give new insight into the dynamics of small rodent populations. In particular, the repeated observation that the prevalence of outbreaks generally increases due to more homogeneous land cover on large spatial scales may be of interest, especially for rodents that are well adapted to these modified habitats. An essential question related to land-use changes is:

6. *What are the possible pathways of how changes in land use and landscape structure affect small mammal population dynamics?*

## Community processes, conclusions and further questions

The fundamental basis for understanding small rodent population dynamics lies in its inherent annual density variation—a peak in the late breeding season, and low numbers at the end of the non-breeding season. The demographic machinery that generates this pattern is fairly well understood. If the annual density fluctuation exceeds the year-to-year variation in peak and low numbers, respectively, this hints to intrinsic regulating mechanisms, together with limiting food resources, that keep the populations within an envelope of regular density fluctuation only. However, populations can escape from these mechanisms, either occasionally as in the case of outbreaks, or following a systematic temporal pattern with a persistent sequence of the four-cycle phases. The comparison of both eruptive and cyclic populations that we follow in this review will, therefore, enable the identification of the driving force, or forces, that cause the dramatic and still enigmatic bursts in rodent numbers.

Modern ecology is based on the experimental testing of hypotheses. Thus, also population ecologists of small rodents have leaned towards single–species processes and population dynamics, as this allows simpler experimental designs. Manipulating whole communities and defining the causality of responses and population processes in different species of the community is difficult if not impossible. In the northern hemisphere, we have three main rodent genera: *Myodes*, *Microtus* and lemmings. They have three different habitat preferences, three different diets, i.e. seeds, buds, lichens for *Myodes*, graminoids for *Microtus* and mosses for lemmings (Hansson and Henttonen 1985) but see Soininen et al. (2017b), and probably three different social systems—but still, they have synchronised dynamics over large areas. Despite contrasting diets and social systems, the species are exposed to common predators and share the same abiotic factors, environmental change and climate. The community ecological approaches applied by Hansson and Henttonen (1988) and Henttonen (2000) should encourage us to develop other comparative studies on community levels (e.g. Sundell et al. 2012, Ecke et al. 2017) and even experiments monitoring concurrently the responses of several species to community-level manipulations in environmental variables, including food, predation or the social environment (Eccard and Ylönen 2007, Sundell et al. 2008, Eccard et al. 2011).

Further, we should try to understand why the dynamics of some rodent species deviate from the dynamics of other members of the rodent guild in a certain area. In particular, it may be worth searching for a temporal factor that first causes outbreaks in some species, which in turn releases other species from predation pressure so that they can start growing. A potential study system may involve the large European water vole *Arvicola amphibius* in Northern Europe, and *Apodemus* mice species and the tiny harvest mouse *Micromys minutus* in Central Europe.

Two essential questions on the community level would be:

7. *How does the temporal synchrony in the dynamics within the small rodent community shape population cycles and outbreaks?*

and

8. *How does the small rodent community affect the whole ecosystem dynamics?*

The small rodent communities are good models for these kinds of questions as they are logistically easy to work with in natural populations. There is, however, an inherent problem in studying the low phase in the wild, as it is difficult or even impossible to obtain large enough samples to reveal which of the vital population parameters are affected. Just on that account, we urgently need to intensify studies on the low phase of the cycles.

Although we discussed food resources repeatedly throughout the paper, there is still a lack of data specifying rodent diet (but see e.g. Hansson 1971; Hansson and Larsson 1978; Soininen et al. 2018) and potential shifts in the diet through an outbreak or a cycle. Many of the eruptive populations in homogenous agricultural land seem to be directly connected to ample food resources during the outbreaks. However, studying diet changes is challenging because the mechanisms may involve both the quantity and quality of food resources, and their interactions with other factors, for instance predation and/or pathogens and diseases.

An essential question for future research may be:

9. *Is there a systematic shift in small rodent diet through a population cycle or season that is important for shaping the dynamics?*

We have not discussed new insights from studies of how small rodent behaviour may affect population dynamics (Sih et al. 2012). During certain phases of the cycles, different individual behavioural strategies—now often called animal personalities—could be advantageous (Boonstra and Krebs 1979; Eccard and Herde 2013; Nicolaus et al. 2016). The concept combines different aspects such as dispersal, physiology and life history characteristics of individuals into a composite syndrome (Réale et al. 2010; Carere and Maestripieri 2013; Dammhahn et al. 2018). With cycles likely resulting from community-level interactions, a novel approach would be to look at how cycle phases affect in turn individual differences in immunological responses, survival and reproductive investment.

Most studies on population dynamics in small rodents search for one factor shaping population dynamics, possibly even confined to one particular cycle phase. Recurrently, however, we are almost inevitably faced with questions about how various factors interact. For some of these, we assume multifactorial frameworks, but hard data are largely missing. For instance, there is experimental evidence that food supply during winter increases survival (Johnsen et al. 2017), because all animals without access to supplemental food die and the population crashes. But it remains an open question whether nourishment as such is the only factor, or whether food availability secures healthy animals that can better escape predation or diseases (but see Huitu et al. 2003). This novel multifactorial approach raises the last and probably most central question:

10. *How do different factors such as seasonality, predation, behaviour, food and diseases interact?*

We probably know much more about the mechanisms causing outbreaks than about the driving forces of population cycles, even though population cycles have been under long and intensive research. Outbreaks occur as a response to a more or less stochastic pulse of resource availability. Compared to that, many more factors are suggested to be involved in cyclic dynamics (Fig. 1). Such networks of mutual interactions are complex and difficult to disentangle in practice, but seen in this light the cycles are not at all a mystic phenomenon. Cyclic vole populations and their pathogens have overall received relatively little research attention, and more research in this field is sorely needed. We already know many of the mechanisms involved in population dynamics, hence new questions of how they act together seem to be a most promising direction for a better understanding of outbreaks as well as population cycles. Obviously, the study of small rodent population dynamics will give new insight into general population ecological theory also in the future.

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# II

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Research

# Seasonality shapes the amplitude of vole population dynamics rather than generalist predators

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Seasonality has been suggested as a necessary factor for the initiation of vole population cycles in Fennoscandia. This has been well described for a latitudinal gradient. Here, we used an elevational gradient as a proxy for winter length to study how the length of the winter season correlates with the amplitude of bank vole *Myodes glareolus* population cycles. In addition, we studied whether the small mammalian generalist predator community present locally could explain any elevational effects. We estimated the population size of 30 local bank vole populations. We found a strong effect of elevation on the amplitude of the population cycle with local populations at around 800 m elevation having 1.5 times greater densities than populations in the valley (ca 300 m elevation). A univariate model with elevation as predictor for amplitude was twice as likely to be the best model than models including generalist predators. Our results fit well with the theories of a positive effect of winter length on the amplitude of vole population cycles in Fennoscandia, irrespective of whether the seasonal effect corresponds to a latitudinal or elevational gradient. The mechanisms may be limited resources during winter rather than generalist predators.

Keywords: density dependence, myodes, population cycles, predator community, red fox

## Introduction

Periodic fluctuations in the size of small mammal populations (i.e. population cycles) are common in northern ecosystems (Finerty 1980, Krebs 1996, Myers 2018). These fluctuations tend to have a greater amplitude with increasing latitude. Such a relation is even found in other organisms such as grouse, hares and forest insects (Ims et al. 2008, Myers 2018). In small mammals, increasing amplitude of population fluctuations are associated with a longer winter season (Hanson and Henttonen 1985, 1988) and stronger delayed density-dependence related to winter predation by specialist predators (Stenseth et al. 2003). At the other end of the latitudinal gradient, stronger direct density-dependence occurs due to the presence of a larger number of generalist predators further south (Hanson and Henttonen 1985, Bjørnstad et al. 1995, Ims et al. 2008, Henden et al. 2009).

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Empirically-based modelling studies have confirmed that seasonality is a driver of the dynamics of cyclic populations, with density-dependent regulation occurring in winter (Stenseth et al. 2003). Generally, these results show that longer winters are associated with longer cycles and greater amplitudes (Stenseth et al. 2003, Lambin et al. 2006). These studies describe stronger interspecific competition during winter and greater intraspecific competition in both summer and winter at higher latitudes. While seasonal effects have primarily been used to explain the latitudinal gradient in the dynamics of small rodents, Strann et al. (2002) suggested similar effects for variations in the dynamics of the cycles along a coastal–continental gradient.

The mechanisms underlying the seasonality hypothesis remain unclear, but a high density-dependence during winter is expected if resources are limited (Hansen et al. 1999a). Another obvious mechanism is the connection between seasonality and community processes such as predation along the north–south gradient. Similar mechanisms may also be expected along an elevational gradient as generalist predator densities (especially the red fox *Vulpes vulpes*) tend to be higher in valleys close to human activities (Salek et al. 2014, Walton et al. 2017). Snow cover and condition also vary with elevation, with less stable winters in the valleys causing periodic melting and icing on the ground which limits access to resources such as nests and conspecifics for thermoregulation, due to reduced mobility under the snow. Predation from generalist predators like the red fox is also expected to decrease with increasing snow cover (Hansson and Henttonen 1988, Lindström and Hörnfeldt 1994).

In a recent review, Myers (2018) concluded that one of the remaining mysteries of population cycles is the variation in amplitudes. Here we used an elevational gradient as a proxy for variations in the winter season to study how the amplitude of bank vole *Myodes glareolus* populations correlate with seasonality. We monitored 30 local vole populations during two peaks and one low phase along an elevational gradient ranging from 260 m to 801 m a.s.l. Populations at the highest elevation attained snow cover earlier, and permanent snow cover lasted longer than at low elevations. The difference was approximately two months longer snow cover at 800 m than at 260 m.

We tested the seasonality hypothesis empirically, expecting greater amplitudes in the population cycles at high than at low elevation. We also tested whether seasonality, described by the elevation proxy, or the small predator community best described the amplitude variations.

## Methods

### Study area

The study was conducted in the boreal forest of Stor-Elvdal municipality, southeast Norway (61°N, 11°E, Fig. 1). The area has a relatively continental winter climate (Boonstra et al. 2016) with vegetation dominated by Norway spruce *Picea abies* and Scots pine *Pinus sylvestris* at low and medium elevations, and by mountain birch *Betula pubescens* and Norway spruce at higher elevations. The forest ground vegetation layer is dominated by bilberry *Vaccinium myrtillus*, mosses e.g. *Pleurozium schreberi* and lichens, e.g. *Cladonia rangiferina*. The average winter length (number of days with mean temperature below 0°C) during the study period was at the nearest weather stations 2.5 times longer at 930 m a.s.l. (Venebu), and 1.5 times longer at 672 m a.s.l. (Drevsjø) compared to 257 m a.s.l. (Evenstad) (eKlima 2019).

### Trapping plots

The bank vole is a small arvicoline rodent, found primarily in the mature forests of Europe (Myllymäki 1977). We haphazardly selected 30 bilberry–spruce forest sites suitable for bank voles along forest roads to establish an elevation gradient in presumed good habitat for bank voles. The mean distance

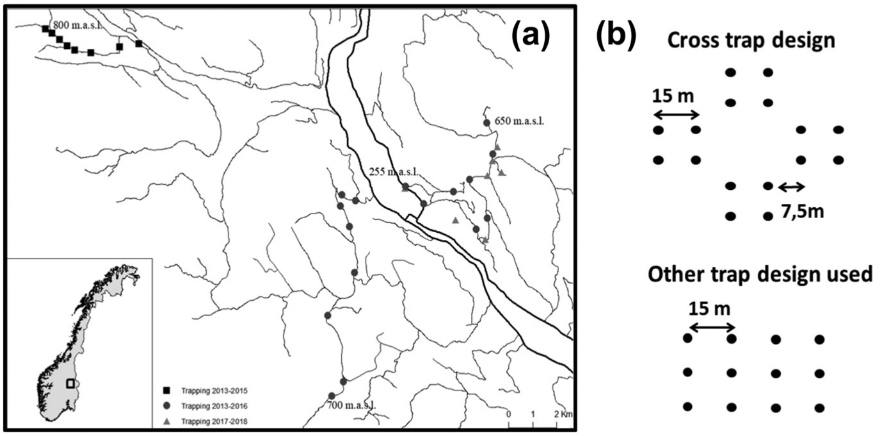

Figure 1. (a) The location of the trapping plots in the total study area. The black thick lines surrounding the label '255 m a.s.l.' indicate main roads in the valley bottom. Elevation is expressed as m above sea level. (b) Trapping plot design. The top panel shows the main, cross-shaped design with 16 traps, and the bottom panel the alternative design used when the main design did not encompass any suitable vole habitat, with 12 traps.

between plots was 1037 m, and minimum 500 m. At each of the 30 sites one trapping plots was established at 5–10 m from the forest roads (see Johnsen et al. 2017 for more details about field procedures). For logistical reasons, all plots could not be trapped every year and the number of trapped plots ranged from 24 to 6 (Table 1).

Trapping plots ranged from 250 to 801 m a.s.l. in a total area of ca 200 km$^2$. However, due to lower trapping effort in 2017 and 2018 (Table 1), the highest elevation was then 650 m a.s.l. Traps were allocated within the plots in a cross-shaped design of 60 × 60 m (Fig. 1; Ehrich et al. 2009). Each plot consisted of 16 Ugglan Special live traps. The cross design was chosen to maximise the area covered with a limited number of traps. If the cross design did not fit within the suitable habitat in a plot, we used trapping lines with a total 9, 10 or 12 traps, each 15 m apart. This adjusted trapping design covered approximately the same total trapping area (ca 0.6 ha) as the cross design. Traps were placed close to vole runways, dead trees or potential holes to increase the probability of catching voles. We avoided ant-hills, ant paths or areas with potential exposure to sun or water, to increase the survival probability of trapped individuals.

## Field procedures

We monitored the plots every June and August (primary session; see Table 1 for details about trapping frequencies) during the years 2013–2018. Trap checking took place over three days with two checks per day, morning and evening (secondary trapping occasions). Traps were baited with carrots and oats, and activated 12 h before the first trap check. Traps were checked four to six times per session (Table 1). On the final check, we removed any remaining bait to avoid the supplementation of food and we left the traps open, i.e. deactivated, until the next trapping session.

We marked each new individual weighing more than 10 g by injecting a small passive integrated transponder (PIT) tag (7 mm length) into the subcutis. For each capture, we recorded identity, trap location, sex, sexual maturity and we weighed the trapped animals to the nearest gram.

Table 1. Trapping history. Number of trapping plots used, the number of times the traps were checked per session/month (secondary occasions) and total number of captures per month.

| Year | Month | No. trapping plots | No. secondary trap occasions | Total number of captures |
|---|---|---|---|---|
| 2013 | June | 24 | 5 | 72 |
| | August | 24 | 6 | 707 |
| 2014 | June | 24 | 6 | 497 |
| | August | 24 | 6 | 961 |
| 2015 | June | 24 | 6 | 11 |
| | August | 24 | 6 | 11 |
| 2016 | June | 16 | 6 | 3 |
| | August | 16 | 6 | 19 |
| 2017 | June | 6 | 5 | 23 |
| | August | 6 | 5 | 81 |
| 2018 | June | 6 | 5 | 42 |
| | August | 7 | 5 | 57 |

## Density estimates

Each plot was assumed to have an independent local population. Population size was estimated as the minimum number of animals known to be alive (MNA; Krebs 1966) from trapping data. An individual was defined as present in the study area at a primary trapping session if it was caught at least once during the secondary occasions. In addition, an individual was assumed to be alive and present in the trapping plot at primary session $t$ if it had been caught before during primary session $t-1$, and subsequently during primary session $t+1$. MNA of the local populations was calculated over time from the estimated individual capture histories. When an animal was found dead in the trap it was counted as present in the trapping station, but the life history ended at that point, with no possibility of surviving and affecting the number of individuals in the next trapping session.

The trapping plots covered ca 0.6 ha. We obtained an approximate estimate of local density by multiplying the population-specific MNA by $1/(0.6\times0.6)$ ha. We did not correct population size estimated by the MNA for differences in capture probabilities as this was impossible for populations with very low densities. Mean capture probability, estimated from the number of individuals observed at a primary trapping session $t$ divided by the MNA at session $t$, is normally high during the summer season (0.94 ± 0.01 SE) (Aars and Ims 2002).

Season was defined as summer from June to August trapping, and winter from August to June. Trapping twice a year (spring and fall), is a common way to create time series of voles and lemmings. Hence, the population density was estimated at the end of the winter in June, and at the end of summer in August. Amplitude was estimated per plot by using the maximum difference between maximum and minimum monthly estimate of MNA.

We applied generalised linear mixed models with population ID as a random factor in all models and carried out all statistical analyses using the package glmmTMBR in R ver. 3.5.2 software (Brooks et al. 2017, <www.r-project.org>).

## Small predator community

We estimated track frequencies of generalist predators red fox and pine marten, *Martes martes*, by snow tracking along 2.95 km (SD = 0.5) transect lines in January for the period from 2003 to 2014. The transect lines were part of a nationwide monitoring program for Eurasian lynx *Lynx lynx* and were based on voluntarily work from members of the Hedmark Chapter of the Norwegian Association of Hunters and Anglers (Tovmo and Brøseth 2011). The transect line density was three (SE = 4) lines per 100 km$^2$. Of a total of 621 different lines, 281–484 lines were surveyed annually during favourable snow conditions, i.e. 2 ± 5 days after snowfall (see Breisjøberget et al. 2018 for more details). We used estimates of track frequencies, i.e. the number of tracks per km divided by the number of days since last snowfall, from 19 transect lines allocated between 300 m and 800 m a.s.l. and within 20 km south and north of the vole trapping plots.

Table 2. The most parsimonious Poisson regression models selected for the response variable minimum number of animals known to be alive per ha. All other models ΔAIC >50.

| Model | AIC | ΔAIC |
|---|---|---|
| Elevation × Season + Elevation × Year + Season × Year | 1008.5 | 0.0 |
| Elevation × Season × Year | 1009.4 | 0.9 |
| Elevation × Year + Season × Year | 1010.4 | 1.9 |

We used two estimates of predator abundance: fox tracks, and the sum of fox and marten tracks.

## Data deposition

Data are available through Figshare digital repository <www.figshare.com>: doi: 10.6084/m9.figshare.8293535, doi: 10.6084/m9.figshare.8293937 and on request.

## Results

The population trajectories showed that yearly and seasonal variations in the minimum number of animals alive in each population correlated with elevation as a continuous variable (Table 2). We categorised plots into two: 15 low elevation plots < 550 m and 15 high elevation plots > 350 m (Fig. 2). The highest peak population densities occurred in fall 2014, with up to 125 animals per ha in some of the high elevation populations. Low elevation populations had less than 100 animals per ha at the peak of 2014. The minimum densities in both low and high elevation populations were <2.2 animals per ha and did not differ between high and low elevations (Table 3). Peak population densities were 1.5 times greater in high elevation than low elevation populations (Table 3).

Table 3. Mean amplitude (animals $ha^{-1} \pm SE$) of population cycles estimated as the difference between the maximum and minimum monthly estimates of population size for each population. We have split the populations into 15 at low (250–532 m a.s.l.) and 15 at high (564–801 m a.s.l.) elevation. Statistics (F, p) compare high and low elevation populations.

| | Number of individuals | | | |
|---|---|---|---|---|
| | High elevation | Low elevation | $F_{1,28}$ | p |
| Amplitude | 68.3 ± 7.9 | 44.9 ± 6.2 | 10.76 | 0.003 |
| Minimum | 1.7 ± 1.0 | 2.2 ± 1.4 | 0.1 | 0.754 |
| Maximum | 70.0 ± 7.6 | 46.1 ± 6.2 | 5.89 | 0.022 |

There was a strong positive correlation between the amplitude and elevation ($r^2 = 0.28$, slope = 0.09 ± 0.03 (SE), $F_{1,28} = 10.76$, $p = 0.003$; Fig. 3). The indices of fox and generalist predators were highly correlated ($r > 0.99$), and both predator indices were also correlated negatively with elevation ($r = -0.98$). Hence, elevation and generalist predators were highly confounded. According to AIC weights, the univariate model with elevation as the predictor of amplitude was twice as likely to be the best model than the models including generalist predators (Table 4, Fig. 4).

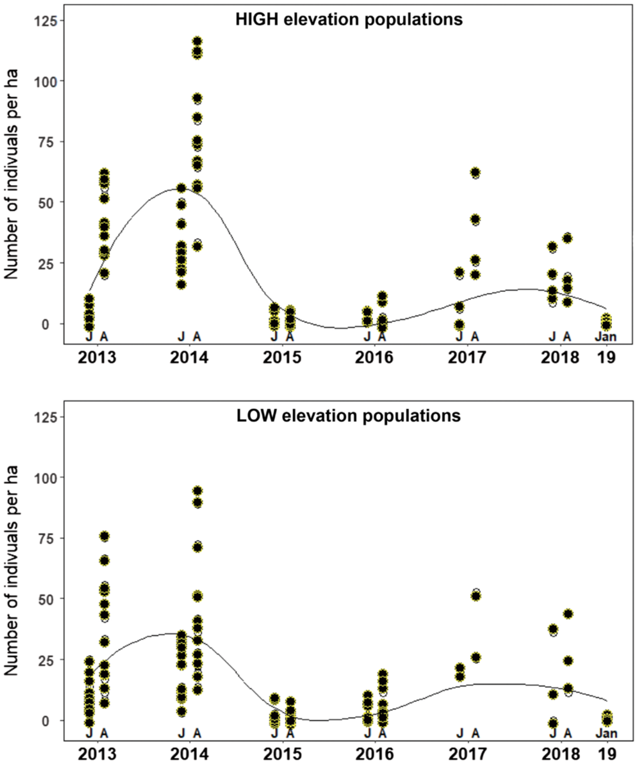

Figure 2. Trajectories of bank vole densities of each population (animals $ha^{-1}$), based on the minimum number of individuals known to be alive per ha. We have split the populations into 15 at low (250–532 m a.s.l.) and 15 at high (564–801 m a.s.l.) elevations. The smoothed line is based on the means for each month. We added January 2019 for illustrative purposes.

## Discussion

Our results confirmed our expectations that winter length was positively associated with the amplitude of vole population cycles. A two-month longer winter season resulted in

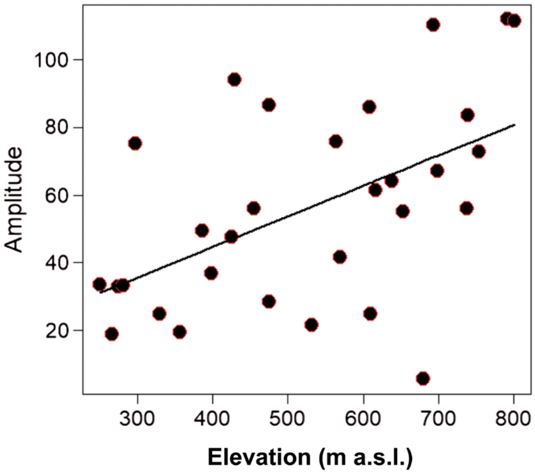

Figure 3. The regression between amplitude (no. of animals $ha^{-1}$) and elevation. Each symbol is one population.

Table 4. AIC information criteria for various models including elevation and generalist predators as predictors for amplitude (wAIC = AIC weight).

| Predictor | Slope ± SE | AIC | ΔAIC | wAIC |
|---|---|---|---|---|
| Elevation | 0.09 ± 0.03 | 283.9 | 0 | 0.41 |
| Elevation + Generalists | 0.20 ± 0.15<br>95.98 ± 125.6 | 285.3 | 1.4 | 0.20 |
| Generalists | −71.44 ± 23.72 | 285.3 | 1.4 | 0.20 |
| Fox | −87.31 ± 29.28 | 285.4 | 1.5 | 0.19 |

a 1.5-fold greater cycle amplitude of the local population fluctuations over the six-year study period. This was mainly due to the higher maximum densities at high elevations in the first peak (year 2014) in the time series. Profound temporal variation in cycle amplitude is a common characteristic in rodent populations (Kleiven et al. 2018, Soininen et al. 2018). Also, the fact that fewer plots with a lower altitude range were trapped in 2018 may have contributed to the lower amplitude of the second peak of the time series. Hence, the seasonality effect is elevational as well as latitudinal. As far as we know, we have for the first time tested directly whether the community of small generalist predators is part of the seasonality effect. Obviously, the small mammal community is completely confounded with elevation and season and it may be impossible to falsify the predation hypothesis completely. Furthermore, we cannot exclude the possibility of an elevational variation in predation impact due to a functional response rather than a numerical response of the predators. However, our results demonstrate that seasonality alone as a predictor of amplitude is twice as likely to be the best model, compared to models including generalist predators.

Seasonality is important for population cycles to occur (Stenseth et al. 2003), although this assumption is not supported by cyclic vole populations in agricultural areas in southwest France (Lambin et al. 2006), it seems robust at least for more natural boreal and arctic ecosystems. Empirically-based modelling studies based on time series from Fennoscandia have shown the significance of seasonality as a driver of the dynamics of cyclic populations showing strong density-dependence in winter (Bjørnstad et al. 1995, Hansen et al. 1999a, b, Kleiven et al. 2018). These studies describe strong interspecific competition during winter and high intraspecific competition in both summer and winter.

High density-dependence during winter is expected if resources are limited (Hansen et al. 1999a). Indeed, several studies of the winter ecology of cyclic vole populations confirm the significance of food resources during winter for winter survival (Ylönen and Viitala 1991, Schweiger and Boutin 1995, Eccard and Ylönen 2001, Huitu et al. 2003, 2007, Boonstra and Krebs 2006, Johnsen et al. 2017, Soininen et al. 2018, but see also Yoccoz et al. 2001). Food availability influences the bank vole social system (Ostfeld 1990), and overwintering groups have been reported to operate on high-quality patches with potentially high survival (Ylönen and Viitala 1991, Sundell et al. 2012).

Other resources than food may be limited during winter. For instance, Korslund and Steen (2006) found that survival of tundra voles *Microtus oeconomus* increased with increasing space availability in the subnivean area. Similar results show that snow depth could increase the summer density of brown lemmings *Lemmus trimucronatus*, affecting the amplitude and possibly the periodicity of the cycle (Bilodeau et al. 2013). Reid and Krebs (1996) found that shallow winter snow appeared to be a strong limiting factor in the population growth of collared lemmings *Dicrostonyx kilangmiutak*, and that the density of winter nests in voles and lemmings increased with snow depth (Reid et al. 2012). Finally, Ylönen and Viitala (1985) found that bank voles aggregated in areas with brush-vegetation

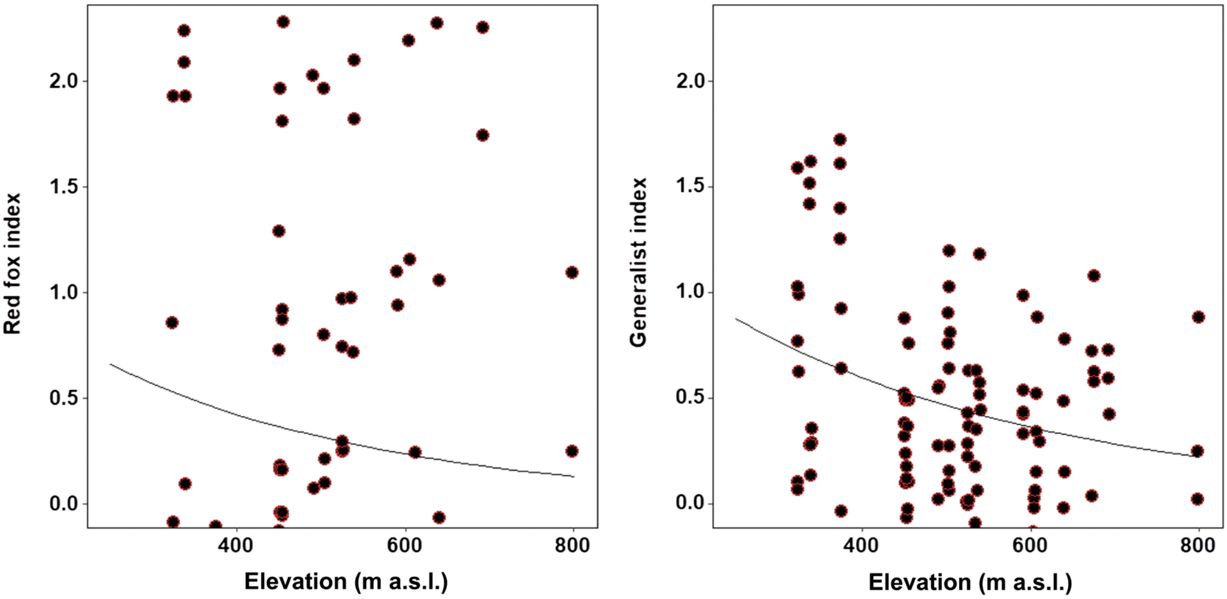

Figure 4. Correlations between indexed of generalist predators and amplitude.

before winter, which were also the areas with thickest snow cover during winter (see also Hambäck et al. 2002).

Winter aggregations benefit from a high level of social interactions (Ylönen and Viitala 1991) which allow high reproduction at the onset of the breeding season in spring (Andreassen et al. 2013, Hansen et al. 2013, Rémy 2013, Radchuk et al. 2016). This may give rise to high population growth rates in summer and consequently greater cycle amplitudes. It is to be noted that the described resources could be interacting with other factors and be used to minimise the direct causes of mortality such as predation or diseases during winter.

In contrast, populations at low elevation may be more vulnerable to unstable mild winter weather that reduces habitat availability and quality, with variable snow cover, due to repeated thawing and freezing phases at ground level (Aars and Ims 2002, Stien et al. 2012, Bilodeau et al. 2013, Hansen et al. 2013). This may limit access to food resources and shelter while increasing exposure to mammalian and avian predators and to lower critical temperatures (Hansson and Henttonen 1985, Aars and Ims 2002, Hoset et al. 2009, Haapakoski and Ylönen 2013). Breeding during winter is a characteristic of increasing vole populations (Krebs and Myers 1974). This may be restricted by weather conditions and some studies suggest that the timing of ice and snow melt in spring might be the most critical factor, as it is the spring population that initiates the peak phase of the population cycles (Aars and Ims 2002, Korslund and Steen 2006, Kausrud et al. 2008, Hoset et al. 2009, Ims et al. 2011, Cornulier et al. 2013, Haapakoski and Ylönen 2013, Fauteaux et al. 2015). Still, more knowledge is needed on small rodent winter ecology, and winter breeding is poorly understood (Krebs 1993, Aars and Ims 2002).

In conclusion, seasonality is an important factor in shaping the dynamics of cyclic vole and lemming populations. The effect may be seen as an intrinsic characteristic of cyclic vole populations. However, more probable the effect is connected to winter resource availability and inter- and/or intraspecific density-dependence. It may be empirically impossible to untangle the many confounding factors of the small mammal community, elevation and season. In a theoretical modelling approach Tyson and Lutscher (2016) found that even a small change in season length would have large effects on a simple predator–prey system. In this study the modelled predator behaved as specialist predator during the winter and a generalist during the summer. Thus, to elucidate to what extent predators act as a decisive mechanism connected to seasonality may thus require more detailed data than were available in the present study. Still we have been able to show that the abundance of generalist predators were not the best predictor of variation in cycle amplitude of a rodent species in boreal forest.

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*Author contributions* – All authors contributed developing the design of the study and commented on the various versions of the manuscript. HPA analysed the data and wrote the first draft of the manuscript. KJ, BJ and MN did all fieldwork. MO was responsible for the data on generalist predators.

*Permits* – The fieldwork was conducted in accordance with the regulations for animal experiments at the university with permission from landowners, the Norwegian Environment Agency and Norwegian Food Safety Authority (FOTS ID 5525 and ID 13908).

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# III

# IV

# Issues of under-representation in quantitative DNA metabarcoding weaken the inference about diet of the tundra vole *Microtus oeconomus*

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## ABSTRACT

During the last decade, methods based on high-throughput sequencing such as DNA metabarcoding have opened up for a range of new questions in animal dietary studies. One of the major advantages of dietary metabarcoding resides in the potential to infer a quantitative relationship between sequence read proportions and biomass of ingested food. However, this relationship's robustness is highly dependent on the system under study, calling for case-specific assessments. Herbivorous small rodents often play important roles in the ecosystem, and the use of DNA metabarcoding for analyses of rodent diets is increasing. However, there has been no direct validation of the quantitative reliability of DNA metabarcoding for small rodents. Therefore, we used an experimental approach to assess the relationship between input plant biomass and sequence reads proportions from DNA metabarcoding in the tundra vole *Microtus oeconomus*. We found a weakly positive relationship between the number of high-throughput DNA sequences and the expected biomass proportions of food plants. The weak relationship was possibly caused by a systematic under-amplification of one of the three plant taxa fed. Generally, our results add to the growing evidence that case-specific validation studies are required to reliably make use of sequence read abundance as a proxy of relative food proportions in the diet.

**Subjects** Ecology, Molecular Biology, Plant Science, Zoology
**Keywords** DNA diet analysis, High-throughput sequencing, Feeding trial, Rodent, Herbivore, Dietary metabarcoding, Food proportions

## INTRODUCTION

Knowledge about animals' fundamental needs, such as food choice, is central in ecology. Knowing how many different food taxa a species consumes, what these food taxa are, and their quantitative contribution to the overall diet are important questions. Indeed, overall diet composition and the relative contribution of food items with different nutritional content have repercussions to individuals health and growth (*Boutin, 1990*), which in turn affect population dynamics (*Huitu et al., 2007*), food web dynamics (*Ims et al., 2013*), and ecosystem functioning (*Schaus, Vanni & Wissing, 2002*). Increased knowledge

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about diet can improve our understanding of the ecological and conservation needs of a particular species (*Balmford, Green & Murray, 1996*; *Bohmann et al., 2014*; *Elfström et al., 2014*; *Kowalczyk et al., 2011*). However, characterising species diets at a scale that reflects the complexity of diets (*i.e.,* spatio-temporal variations in diet composition) is challenging, and especially when relying on traditional methods such as direct observations or microhistology method (*Hansson, 1970*). Consequently, improving our capacity to access unbiased and taxonomically resolved diet data in a cost- and time-efficient way is crucial for accelerating basic knowledge about the trophic ecology of animals and conservation management.

Molecular analyses offer a new set of tools for accurately describing diet. DNA metabarcoding (*Taberlet et al., 2018*; *Taberlet et al., 2012b*) has quickly gained popularity thanks to its efficient and precise identification of food items based on their DNA sequences (*Pompanon et al., 2012*; *Soininen et al., 2009*; *Valentini et al., 2009a*). This method relies on the extraction of DNA from digested food remains in a dietary sample (*i.e.,* stomach content, regurgitate or faeces), its amplification with universal primers (*Taberlet et al., 2012a*; *Valentini, Pompanon & Taberlet, 2009b*), and then the sequencing of individual DNA molecules, identified by matching them to a sequence reference database. Metabarcoding is especially advantageous for complex and cryptic diets consisting of many taxonomically diverse taxa, as the method requires little prior knowledge about the system under study (*De Barba et al., 2014*). From a qualitative point of view, the capacity of dietary metabarcoding to detect even highly degraded, low abundant DNA, while providing high taxonomic resolution is particularly valuable, as it allows for accessing rare or taxonomically cryptic dietary taxa (*Soininen et al., 2015*; *Sullins et al., 2018*). DNA metabarcoding also has the potential to be quantitative—*i.e.,* to inform about the relative biomass proportions of ingested food (*Newmaster et al., 2013*; *Willerslev et al., 2014*). If realised, such a potential implies an essential breakthrough, as traditional methods such as microhistology are known to overestimate the proportion of taxa such as grasses compared to forbs (*Anthony & Smith, 1974*).

There are two approaches to achieve quantitative estimates of diet from metabarcoding data. One approach is to count the number of individuals/samples with recorded presence/occurrence of a given food item in the population of samples (*Biffi et al., 2017*; *Xiong et al., 2016*). The higher the count within the population, the larger the food item's ecological contribution. In this way, the frequency of occurrence can provide quantitative information at the population level. However, this approach requires large sample sizes, especially if the diet is diverse. Moreover, even though an item may occur frequently in the populations, it may still be ecologically unimportant if most individuals consume it in relatively low quantities. The other approach is based on calculating the relative frequencies of sequence reads (*i.e.,* relative read abundance, RRA), where the number of reads is assumed to be proportional to the relative biomass of the corresponding food items (*Deagle et al., 2010*). In a recent review and meta-analysis, *Lamb et al. (2019)* show that relative read abundance and ingested food biomass correlate positively in some model systems (*e.g.*, *Kartzinel et al., 2015*; *Newmaster et al., 2013*; *Nichols, Akesson & Kjellander, 2016*; *Thomas et al., 2014*), but not in others (*e.g.*, *Deagle et al., 2013*; *Elbrecht, Peinert &*

Leese, 2017; Hatzenbuhler et al., 2017; Piñol, Senar & Symondson, 2019). The highly variable correlation suggests that the proportion of reads should not be used as a proxy for diet proportions *a priori*, and that biases can arise from *e.g.*, DNA extraction (Majaneva et al., 2018) and DNA amplification (Bellemain et al., 2010). Also, bias from differential digestion of plants with different functional characteristics or digestibility can further increase variation in the quantitative output (Deagle et al., 2013; Leal et al., 2014; Nakahara et al., 2015; Thomas et al., 2014). Thus, validations specific to different animal groups are required for measuring DNA metabarcoding's potential for the quantitative assessment of diets. However, food-item specific validations with information on ingested biomass proportions remain rare.

Small rodents are commonly used in ecological research because they make convenient model species (Hickman et al., 2017), and because of the fluctuating dynamics of many populations and important roles in food webs (Boonstra et al., 2016; Ehrich et al., 2017). Despite the increasing use of DNA metabarcoding for analysing their diets (Ozaki et al., 2018; Sato et al., 2018b; Soininen et al., 2015), no quantitative validations are available for small rodent systems, with earlier methodological studies mainly focusing on the comparison with alternative methods (Khanam et al., 2016; Soininen et al., 2009) or a molecular mock community (Iwanowicz et al., 2016). Here, we use experimental feeding trials to test the hypothesis that relative read abundance from rodent faecal samples closely reflects the ingested food biomass.

## MATERIALS & METHODS

We used the tundra vole *Microtus oeconomus*—a commonly studied herbivorous small rodent species with a circumpolar distribution—as our model species. Captive tundra voles were offered three experimental meal mixtures, each containing three plant species representing 60%, 30% and 10% of the total diet biomass. We collected vole faecal samples in each feeding trial and analysed them with a DNA metabarcoding approach using the universal *gh* plant primers of the *trnL* P6 loop region (Taberlet et al., 2007). Finally, we compared the plant biomass proportions from meal mixtures to the relative read abundance estimated by DNA metabarcoding.

### Feeding experiment

The feeding trials were conducted in accordance with Norwegian laws and regulations concerning experiments with live animals, which are overseen by the Norwegian Food Safety Authority (FOTS 15309, 15585). We obtained our experimental units, the tundra vole individuals ($n = 9$) from Håkøya, northern Norway (69.7°N, 18.5°E). The sample size was decided based on similar previous experiments (Deagle et al., 2013; Willerslev et al., 2014). All animals were juveniles trapped in July 2019 within their natural boreal meadow habitat, characterised by the frequent occurrence of the plant species we used as food in the experiment. Once trapped, individuals were kept close together in the same room, but in separate 40 × 30 × 25 cm cages. The room was naturally ventilated through large open windows, without heating or an artificial light scheme. We observed the animals intensively during the start of each experimental trial, and subsequently every

2 h throughout the experiment to refill food and to inspect animal health and welfare. Until the experiment started, we fed the animals *ad libitum* with fresh food items known to be eaten by small rodents in previous studies, including the plant species used in the meal mixtures. At this stage, regular observations showed that *Trifolium* was the most preferred item although no systematic measures were performed on the exact amount eaten. Voles were also offered small portions of the experimental meal mixtures for familiarisation.

We selected three plant species to compose the artificial meal mixtures offered to the animals –the white clover (*Trifolium repens* L., Fabaceae), the wavy hairgrass *(Avenella flexuosa* (L.) Drejer, Poaceae), and the pussy willow (*Salix caprea* L., Salicaceae). We selected these species because they (i) represent different functional groups (*i.e.*, forb, graminoid and shrub), (ii) are known to be preferred food items (*Soininen et al., 2013*); and (iii) are readily available in natural tundra vole habitats. We collected the plant material in separate bags from natural habitats. We cut the plants, ground them and stored them temporarily at 3 °C immediately after collection. We then extracted one subsample of ground plant biomass from each plant species individually ($n = 3$) to be used as reference. The remainder of the ground plant biomass was used for composing three meal mixtures (mock communities of fresh plant material) of the three plant species, to yield three dry weight biomass proportions, *i.e.*, 60%, 30% and 10% for each species (Fig. S1, Table S1). To make the mixtures, we used the dry weight ratios of plant subsamples that have been dried for 24 h at 80 °C. Plant biomass from the three plant species was mixed, homogenised into a porridge-like substance, and stored at 3 °C until use. This resulted in three meal mixtures named after their taxonomic contribution (*i.e.*, T10_S60_A30, T30_S10_A60 and T60_S30_A10, where T, S and A are abbreviations of the plant genus names). We also withdrew one sub-sample from each meal mixture before starting the feeding trials and stored it apart prior molecular analyses to disentangle biases arising from digestion from those arising from molecular analysis.

The three meal mixtures were offered *ad libitum* to all nine animals in three separate trials, though not all 9 × 3 samples were retrieved for analysis (see below). Arvicoline rodents have a fast metabolism, with 50% of the green plant particles passing through the alimentary tract in only 3–3.5 h, and with complete passage in 20 (*Kostelecka-Myrcha & Myrcha, 1964*) to 30 h (*Lee & Houston, 1993*). For hardly digestible items such as seeds, it may take up to twice this time to completely pass through the arvicoline alimentary tract (*Kostelecka-Myrcha & Myrcha, 1964*). Therefore, we decided to use only green plant material and allow the animals to feed on the same meal for 48 h before collecting faecal samples and starting a new trial using the same animals. Thus, the total length of the active experiment was six days. We collected an equal quantity of ten faecal pellets per animal and trial using forceps that has been sterilised with chlorine solution prior to each individual sampling. Faecal pellets were placed in filter paper bags and stored in plastic zip-lock bags, pre-filled with silica gel. We cleaned the cages with a chlorine solution prior to the experiment and between each trial in order to reduce the risk of cross-contamination. In a preceding pilot study, we also evaluated the risk of cross-contamination with environmental DNA coming from previous use of the same cages *via* the animals or the air by rubbing cages' floor with sterile cotton tips. These analyses showed that contamination risk from the experimental setting was negligible (see Table S5). Consequently, we did not further consider this aspect.

We offered the meals to voles as a thoroughly mixed homogenous substance. Although the consistency of this mixture was unfamiliar to the animals, they ate it in all trials, except two of the individuals that ate very little of the second meal mixture T30_S10_A60. To prevent any animal welfare issues, these individuals were relieved from the trial with this meal. After their quick recovery they were included in the subsequent trial. In the end, all animals were euthanised by cervical dislocation. Since some of the samples were discarded after post-sequencing bioinformatic processing and data filtering, the final sample size was $n = 23$, including samples from meal mixtures ($n = 3$, one per meal mixture), individual plant subsamples ($n = 3$, one per plant species), and faecal samples ($n = 17$). Faecal samples were distributed between the meal mixtures so that mixtures T30_S10_A60 and T60_S30_A10 had $n = 5$, while meal mixture T10_S60_A30 had $n = 7$. We marked the samples with codes to process the samples and sequences blindly.

## Molecular analysis

DNA extractions from faecal pellets, individual plants and meal mixtures were performed by Sinsoma GmbH (Innsbruck, Austria) using the Biosprint 96 DNA Blood Kit (Qiagen) on a Biosprint 96 Robotic Platform (Qiagen). DNA extractions were carried out according to the manufacturer's instructions, except that (1) the lysis step consisted in adding 250 µl lysis buffer (TES buffer: Proteinase K (20 mg/ml) 19:1) in each sample before vortexing and overnight lysis at 58 °C; and (2) DNA was eluted in 200 µl 1× TE buffer. DNA extraction negative controls (water instead of DNA) were systematically included. As part of the standard procedure for quality control at Sinsoma, a subset of samples (all DNA negative controls and a random subset of DNA extracts from samples) were used to control for both possible cross-contaminations and the successful extraction of DNA. The general mitochondrial cytochrome oxidase I gene, COI (*Folmer et al., 1994*) was used for detection of animal DNA, while the nuclear internal transcribed spacer rDNA regions, ITS (*Taberlet et al., 2007*; *Taberlet et al., 1991*), was used for plant detection. As expected, the extraction negative controls were negative, and a positive band was observed for the extraction positive control, and thus these control samples were not included further.

As part of our feeding experiment, all samples were amplified with the *g* and *h* primers (*Taberlet et al., 2007*), targeting a highly variable length region (10–220 bp) from the P6 loop of the chloroplast *trnL* (UAA) intron in vascular plants. This primer set is particularly suitable for the analysis of highly degraded DNA (*Clarke et al., 2020*; *Hollingsworth, Graham & Little, 2011*; *Särkinen et al., 2012*; *Schneider et al., 2021*; *Willerslev et al., 2014*) due to its short amplicon size, highly variable gene region and conserved priming sites (*Deagle et al., 2014*; *Taberlet et al., 2012a*). The primer sequences are 5′-GGGCAATCCTGAGCCAA-3′and 5′-CCATTGAGTCTCTGCACCTATC-3′, respectively. We labelled the forward and reverse primers with unique 8–9 nucleotides sequence tags modified from *Taberlet et al. (2018)*, allowing to distinguish individual samples following high-throughput sequencing. All PCR reactions were carried out in a total volume of 15 µL using the AmpliTaq Gold 360 PCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA), 0.4 µl/15 ml of bovine serum albumin (BSA; Sigma-Aldrich, USA), 0.5 μM of each primer and 2 μl of undiluted DNA. We initiated the PCR reaction by a denaturation step at 95 °C for 10 min, followed by 40 cycles consisting of denaturation at 95 °C for 30 s, annealing at 52 °C for 30 s, elongation at 72 °C for 1 min, and finally elongation at 72 °C for 7 min. We conducted three PCR replicates per sample. For each PCR-plate ($n = 3$), we included one PCR negative control (ultra-pure Milli-Q water instead of DNA) and one PCR positive control (*i.e.,* a mixture of six synthetic standard sequences with varying GC content, homopolymers, sequence length and concentrations, see Table S2). We visualised PCR products on a 1.5% gel electrophoresis before pooling and purifying PCR products using the QIAquick PCR Purification Kit (Qiagen). DNA concentration from purified amplicon pools was then quantified using a Qubit 2.0 fluorometer and the dsDNA HS Assay kit (Invitrogen, Life Technologies, USA). Purified pools were used for libraries preparation using the KAPA HyperPlus kit (Kapa Biosystems, USA), and sequenced (2 × 150 bp paired-end reads) on a HiSeq 4000 machine (Illumina, USA) following manufacturer's instructions at the Norwegian Sequencing Centre. The sequencing was carried out in two separate runs, and we merged the sequence reads data from both runs during the bioinformatic filtering process.

## Bioinformatics

We carried out bioinformatic analyses using the OBITools bioinformatics pipeline (*Boyer et al., 2016*) on the Norwegian high-performance computing cluster Sigma2. All commands referred to in this paragraph are from the OBITools python package (http://metabarcoding.org/obitools). We processed the raw data in the following order: (i) merging of the forward and reverse reads (with minimum quality score threshold of 40) with the *illuminapairedend* command, (ii) removing low quality reads (with alignment score less than 50) with the *obigrep* command, (iii) assigning sequences to samples based on identification tags with the *ngsfilter* command (*i.e.,* demultiplexing, which also required perfect match between the tag and the target sequence, and a maximum of 2 bp mismatch between the primers and the target sequence), (iv) merging strictly identical sequences into single molecular operational taxonomic units (*i.e.,* MOTUs) with the *obiuniq* command, (v) removing short (less than 10 bp) and rare (occurring with less than 10 copies in the entire dataset) sequences with the *obigrep* command, and (vi) flagging erroneous sequences owing to PCR and/or sequencing with the *obiclean* command.

We created a local reference database from the reference library "ArctBorBryo" (*Soininen et al., 2015*, *Sønstebøet al., 2010*; *Willerslev et al., 2014*) and the European Nucleotide Archive nucleotide library (EMBL, release 143, accessed in April 2020) with the *ecoPCR* program (*Bellemain et al., 2010*; *Ficetola et al., 2010*). Finally, we compared the reference database to the sequences in our data, assigning each sequence to a taxon with the *ecoTag* program (*Pegard et al., 2009*).

Further data filtering, visualisation and analyses were conducted with the R software version 4.0.3 (*R Core Team, 2020*) using ROBITools package (http://metabarcoding.org/obitools). To start with, all MOTUs flagged as erroneous by *obiclean* (OBITools, *Boyer et al. (2016)*) were removed. Afterwards, we filtered out PCR outliers based on the comparison of Euclidean distances of PCR replicates with their average, and with the distribution of pairwise dissimilarities between all average samples. PCR replicates flagged as outliers were iteratively removed from the dataset. We averaged the number of reads per MOTU in the remaining PCR replicates for each sample. At this stage, all remaining MOTUs whose relative frequency in a PCR was inferior to 1% were filtered out. Next, only MOTUs with identity match ≥85% to sequences in the reference library were kept for further analyses. Due to the known and taxonomically very restricted diet, we only kept relevant MOTUs to estimate proportions were the best identified taxonomic level. Finally, we normalised the sequence read abundances by dividing the number of reads for each MOTU by the total number of reads within each sample.

### Statistical analyses

We assessed the quantitative accuracy of dietary metabarcoding by using a multivariate regression model that establishes a linear function between the multiple compositional outcomes (responses) and compositional predictors (*Fiksel, Zeger & Datta, 2021*). Here we used the composition of relative read abundance of each of the three plant species (RRA from faeces or meal mixtures) as response variables and the expected plant species composition (*i.e.,* known biomass composition) as predictor variables. This type of compositional analysis accounts for the fact that an increase in one taxon's proportion will force a decrease in other taxon(s) proportion within the same sample (*Alenazi, 2019*; *Chen, Zhang & Li, 2017*; *Fiksel, Zeger & Datta, 2021*). The model allows, without transformation, for direct interpretation of the relationship between expected and observed compositions through a Markov transition matrix "B" based on the estimated regression coefficients. Since both the predictor and the response variables are compositions, the regression coefficients (*i.e.,* the matrix B) is constrained to non-negative values in the range of 0 to 1, and each row of the matrix sums to 1. These coefficients describe how the outcome composition would change in relation to a change in the predictor composition. Values close to one along the diagonal indicate a high correlation between the outcome and the predictor compositions.

We computed the regression coefficient (B matrices) and tested for overall linear independence *via* permutation tests (using an $\alpha$-level of 0.05) with the codalm package (*Fiksel & Datta, 2020*; *Fiksel, Zeger & Datta, 2021*) in the R software (R version 4.0.3). To assess the model's goodness of fit, we plotted the predicted values *versus* the observed RRA of the faecal samples, using leave-one-out cross-validation (LOOCV) (*Fiksel, Zeger & Datta, 2021*, see Fig. S2).

## RESULTS

### Sequencing output

This experiment's samples were multiplexed with samples from another project, so the number of sequences is only known after identifying the sequences with their sample tags during the *ngsfilter* step, resulting in 2,080,764 sequences (Table S3 gives step-by-step details of read/sequence counts during the bioinformatics workflow). After data cleaning and merging of the PCR replicates, 1,477,342 reads were assigned to faecal samples. Of the nine MOTUs in the final dataset, one was only assigned to the family level, one to the subfamily/tribe, and the remaining seven were assigned to the species/genera (Table S4). Four of these MOTUs were identified at a lower taxonomic level using BLAST search (*Altschul et al., 1990*). For details on the filtering steps, see Table S3. The final filtered dataset, as well as the raw high-throughput sequences, are available in Dataverse (https://doi.org/10.18710/HJAVSN).

## Taxonomic assignments

We assigned most of the cleaned sequence reads (>97%) to the three expected plant species. We retrieved all three positive PCR control replicates after sequencing, and we only detected the synthetic sequences in their corresponding sample. None of the negative control samples (*i.e.,* samples without DNA) passed the data filtering steps. The synthetic sequences added as a positive PCR control had varying amplicon length (30-60 bp) and varying GC content (20–40%), and close to the expected log-linear relationship (Fig. S3). The MOTUs retrieved from the faecal samples, individual plants and meal mixtures (Table S4) had similar GC-content composition (13–21%) and amplicon length (45–56 bp) as the amplified positive control sequences with little variation among MOTUs.

## Analyses of plant items and meal mixtures

In the subsampled plant material from the single plant taxa, the mean number of reads per sample ranged between 10,166 and 29,438, from Fabaceae being the least amplified to Salicaceae with almost three times more reads retrieved (note that these plants were sequenced in separate PCR replicates). From each single plant sample, we only detected MOTUs corresponding to the respective taxonomic family of the plant sequenced—*i.e.,* one MOTU per plant sample. In the *Avenella flexuosa* sample, we also detected a second MOTU best identified as *Festuca* sp., but which was amplified in much smaller proportion. As this plant material was collected in the field, a non-targeted species might thus have been accidentally included.

In the meal mixtures, we detected only the three expected MOTUs, with the exception of one sample (T10_S30_A60), from which *Trifolium* MOTU was missing. All three samples had high RRA of *Salix* and low RRA of *Trifolium* (Fig. S1, filtered dataset at Dataverse). We found no evidence for a relationship between the RRA and expected composition (permutation test for linear independence $p = 0.49$, Figs. 1 and 2, Fig. S4). The estimated B-matrix

$$\begin{array}{cc} & \begin{array}{ccc} {}_{\text{meal}}T & {}_{\text{meal}}S & {}_{\text{meal}}A \end{array} \\ B = & \left(\begin{array}{ccc} \sim 0.0 & 0.75 & 0.24 \\ \sim 0.0 & 0.92 & 0.08 \\ 0.13 & 0.79 & 0.08 \end{array}\right) \begin{array}{c} {}_{\text{exp}}T \\ {}_{\text{exp}}S \\ {}_{\text{exp}}A \end{array} \end{array}$$

showed that the outcome composition of Salix was well predicted by its proportion in the predictor composition (corresponding to 0.92 on the diagonal). However, the proportions of Trifolium and Avenella in the outcome compositions were little related to their proportions in the predictor composition (corresponding to 0.0 and 0.08 on the diagonal).

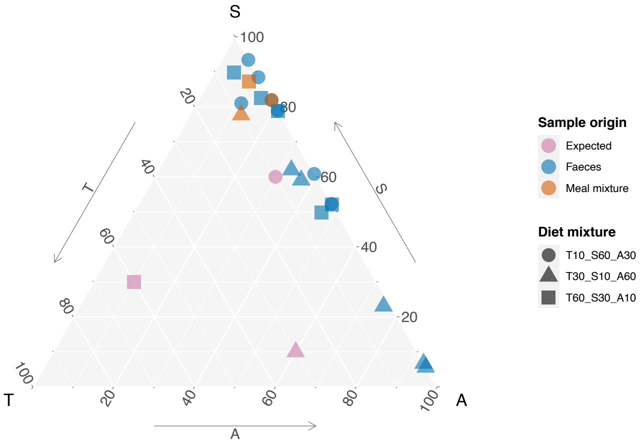

**Figure 1** **Expected composition of diet mixtures and relative read abundance (RRA) acquired from meal mixtures and rodent faeces.** Edges of the triangle represent the three species proportions, T, S, and A short for the plant species *Trifolium repens, Salix cabrea,* and *Avenella flexuosa*, respectively. Each tip of the triangle represents 100% for the given species and 0% for the other species. Symbols for expected composition are based on known biomass proportions, whereas symbols for meal mixture and faeces RRA represent one sample each (*i.e.,* mean across three PCR replicates). Note that the symbols are plotted transparency, and stronger colours thus indicate several overlapping data points.

Full-size DOI: 10.7717/peerj.11936/fig-1

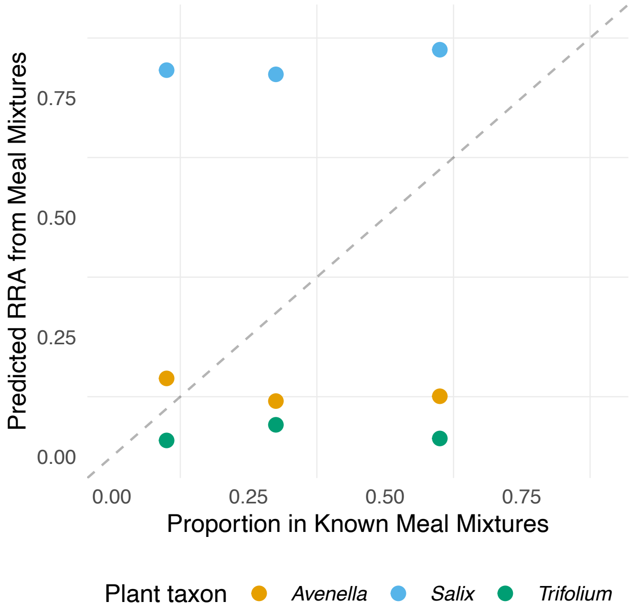

**Figure 2** **Relationship between expected proportions of the known diet and predicted proportions of food items in meal mixtures.** Each point is based on model predictions from the compositional regression. The dashed line shows 1:1 relationship.

Full-size DOI: 10.7717/peerj.11936/fig-2

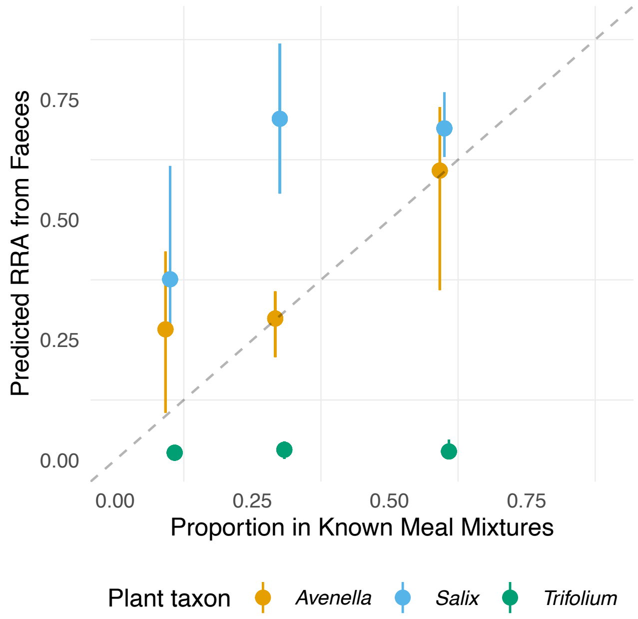

**Figure 3 Relationship between expected proportions of the known diet and predicted proportions of food items in vole diets.** Each point is based on model predictions from the compositional regression, with the bootstrapped upper/lower confidence intervals boundaries around each prediction. The dashed line shows 1:1 relationship.

Full-size DOI: 10.7717/peerj.11936/fig-3

## Dietary analyses

All faecal samples contained MOTUs belonging to two of the expected plant species, *Salix caprea* and *Avenella flexuosa*, but several samples did not retain reads from *Trifolium* after data processing. We identified five unexpected MOTUs that were seemingly contaminants (Table S4). Two of these potentially originate from the food given to the voles before the experiment (*Maleae* sp. found in three samples, total 0.2% of the reads, and *Avena* sp. found in 1 sample, total 0.1% of the reads). Additionally, we identified the MOTU best representing *Festuca* in six of the faeces samples. Due to the possibility for field sampling error, we therefore merged *Festuca* (4% of the total composition) with *Avenella flexuosa* in the quantitative assessment (see above).

The compositions of RRA from faecal samples had a weak or moderate relationship with the expected composition (Fig. 3). In particular, all faecal samples had meagre *Trifolium* proportions compared to the expected proportions (Figs. 1, 3 and Fig. S4). However, we found evidence for a positive linear relationship between expected and observed values for two of the species. The estimated B-matrix

$$\mathrm{B}=\begin{array}{cccc} {}_{\text{faeces}}\mathrm{T} & {}_{\text{faeces}}\mathrm{S} & {}_{\text{faeces}}\mathrm{A} & \\ 0.02 & 0.69 & 0.28 & {}_{\text{exp}}\mathrm{T} \\ 0.01 & 0.99 & \sim 0.0 & {}_{\text{exp}}\mathrm{S} \\ 0.03 & 0.08 & 0.89 & {}_{\text{exp}}\mathrm{A} \end{array}$$

**Table 1 Confidence intervals of the parameter estimates.** Values in the B-matrices below represent 95% confidence intervals obtained from bootstrapping around each prediction of the compositional regression.

**(A) Meal ~ Expected (95% confidence intervals)**

$$B = \begin{array}{cccc} {}_{\text{meal}}T & {}_{\text{meal}}S & {}_{\text{meal}}A & \\ \sim 0.0 - 0.32 & 0.58 - 0.87 & 0.10 - 0.24 & {}_{\text{exp}}T \\ \sim 0.0 - 0.10 & 0.78 - 0.93 & 0.08 - 0.20 & {}_{\text{exp}}S \\ \sim 0.0 - 0.15 & 0.76 - 0.87 & 0.07 - 0.18 & {}_{\text{exp}}A \end{array}$$

**(B) Faeces ~ Expected (95% confidence intervals)**

$$B = \begin{array}{cccc} {}_{\text{faeces}}T & {}_{\text{faeces}}S & {}_{\text{faeces}}A & \\ \sim 0.0 - 0.08 & 0.38 - 0.92 & 0.03 - 0.62 & {}_{\text{exp}}T \\ \sim 0.0 - 0.03 & 0.81 - \sim 1 & \sim 0.0 - 0.18 & {}_{\text{exp}}S \\ \sim 0.0 - 0.06 & \sim 0.0 - 0.52 & 0.42 - \sim 1 & {}_{\text{exp}}A \end{array}$$

showed that the proportions of *Salix* and *Avenella* in the outcome compositions were well predicted by their proportions in the predictor compositions (*i.e.,* 0.99 and 0.89 on the diagonal). The same was not true for *Trifolium* (0.02 on the diagonal). See Table 1 for confidence intervals on parameter estimates. The permutation test ($p = 0.009$) yielded evidence for a linear dependence between the expected compositions and RRA. The predicted values obtained through the leave-one-out cross-validation procedure indicated that the model fit was reasonably good (Fig. S2).

## DISCUSSION

We investigated the relationship between diet composition obtained by DNA metabarcoding of vole faeces and the consumed food's composition. This is one of few studies relating ingested biomass to quantitative metabarcoding analysis of animal faeces, and as far as we know, the only one on herbivorous small mammals. We found that the expected and observed proportions of plant species in vole faeces were correlated for two out of three plant species. However, the third plant species had consistently low proportion and blurred the overall relationship between expected and observed diet compositions. Our results suggest that a certain degree of caution is necessary when making conclusions on the species' ecology based on relative read abundances (RRA) estimated from DNA metabarcoding data.

We found an overall poor consistency between the RRA of meal mixture samples and the actual composition of the meals. We find potential biases with food item-specific DNA retrieval (*Deagle & Tollit, 2007*), as the main issue was that one of the three species in our study (*Trifolium*) performed consistently poor. It had a very low recovery in the meal mixtures, with several samples containing no reads at all. Furthermore, the number of retrieved *Trifolium* read abundance from the samples containing only this species were considerably lower than for corresponding samples for other species. This pattern in *Trifolium* detectability is puzzling since previous studies did not report bias related to this species (*Willerslev et al., 2014*). One plausible explanation for our results is the small sample size, as we analysed a single sample of each meal mixture. Therefore, we recommend a more substantial sub-sampling of meal mixtures used in such feeding trials. An additional advantage with a higher number of sub-samples is the possibility to use RRA from meal mixtures to generate correction factors (*sensu Thomas et al. (2014)*) to control for potential differences in digestion of food items. However, the practicality of such correction factors in generalist herbivores such as voles is to be further demonstrated, as they often include a large number of food plant species in their diet (*Soininen et al., 2013*). Also, as ecological studies of diets most often are based on several individuals averaged together (as opposed to a single sampling or sample site), some of the benefits of using correction factors are already incorporated (*Thomas et al., 2016*). Rather, in such study systems, it may be better to aim at understanding the underlying processes that inflict food-item specific biases.

A similar poor consistency between RRA of meal mixture samples and the actual composition of the meals has previously been described (*Deagle & Tollit, 2007*; *Deagle et al., 2010*). Unfortunately, our study design do not allow us to pinpoint the exact mechanisms behind the observed limited retrieval of *Trifolium*. However, the consistently low detection of *Trifolium*, irrespective of the sample type, could indicate a systematic bias, potentially due to lower chloroplast DNA content (*Soltis, Soltis & Milligan, 1992*), although not much information is available about the chloroplast numbers variation in *Trifolium* comparatively to other plant taxa such as *Poaceae* or *Salicaceae* for example. However, the nuclear DNA content in *Trifolium* has been reported to have large variation (*Vižintin & Bohanec, 2008*; *Vizintin, Javornik & Bohanec, 2006*), *T. repens* differing up to 21% between lineages/varieties (*Campbell, Caradus & Hunt, 1999*; *Vižintin & Bohanec, 2008*). Nevertheless, and regardless of possible variations in chloroplast content, one of the advantages of DNA metabarcoding is its ability to retrieve even very small proportions of DNA, as exemplified by other feeding experiments using *Trifolium* (*Willerslev et al., 2014*).

Another potential source of bias can arise from DNA amplification. The *gh* gene marker used in our study offers well-conserved priming sites across lineages and should be well adapted for amplifying our target species (*Baksay et al., 2020*; *Taberlet et al., 2018*). Based on earlier DNA metabarcoding studies using the *gh* primers, there are seemingly no issues with differential DNA extraction or amplification of *Trifolium* (*Nichols, Akesson & Kjellander, 2016*; *Pornon et al., 2016*; *Willerslev et al., 2014*). Our analyses of single-species samples indicate that the low amplification of *Trifolium* did not depend on which other plants were present in the samples. Furthermore, as positive control standards showed close to the expected log-linear relationship, we have no indication of issues related to amplicon length and GC content. Finally, the *Trifolium* reads abundance falls within the range of variation for the (successfully amplified) positive control standards. Moreover, the priming sites of the *Trifolium* MOTUs we retrieved had no mismatches as compared to the *gh* primer pair. Yet, these exploratory results are based on low sample sizes, and our study do not allow for a more precise assessment of the mechanisms behind this species' low amplification success. Using quantitative PCR (qPCR) to calculate amplification efficiency would have avoided speculation on this issue, and we recommend considering this in future feeding experiments.

The weak relationship between diet inferred from faeces and the expected plant composition could also be explained by differential digestion of plants (Deagle et al., 2010). The amplification of *Trifolium* was, however, problematic independent of whether samples had been digested by voles or not. We also controlled for some influential sources of variation in digestion (*i.e.,* we offered only plant leaves and kept the animals on the same meal mixture for an extended period). While DNA traces of the previous meals might still be present in the voles' digestive system, we did our best to minimize their effect in our design. In the absence of studies on the DNA decay of food in the digestive tracts of rodents (but see Schattanek et al. 2021), we selected a conservative time-frame for the different feeding trials that is compatible with the digestion of diet's hard remains. Furthermore, the RRA of the two other plants (*Salix* and *Avenella*) seemed to correlate well in meal mixtures and faecal samples. We thus conclude that the impact of digestion on our results must be rather small.

Differences in the sequence reads number can to some extent be corrected for in the bioinformatic processing by using proportions or rarefying to normalize data, and contrasting methods are currently a topic of discussion (McKnight et al., 2019; McMurdie & Holmes, 2014). During data processing, we also attempted a stricter filtering of the faecal samples compared to what is currently presented in the main results. Stricter filtering resulted in discarding more samples, but interestingly, the reduction was not equal between meal compositions. Faecal samples resulting from diets with high proportions of *Trifolium* were filtered more strongly than samples from diets with low proportions of *Trifolium*. This resulted in an over-representation of samples where proportion of *Trifolium* was both expected and observed to be low, thus strengthening the correlation estimates. This shows the importance for consistent *a priori* decisions for the bioinformatic processing.

Despite the growing use of DNA metabarcoding for analysing small rodent diets (Ozaki et al., 2018; Sato et al., 2018; Soininen et al., 2015), our study was the first to compare the quantitative reliability of the method using known meals fed to rodents. We found a correlation between observed and expected diets for only some of the plant species, and the overall observed diet composition did not reflect the expected composition well. Even moderate but systematic deviations in retrieval greatly reduce a correlation between observed and expected compositions in a mixture containing only few taxa (like our three-species meals). Thomas et al. (2016) suggest that diets with a higher number of species are less biased as different DNA molecules are more equally represented during the PCR, consequently reducing biases such as self-annealing (for the very abundant molecules). The correlation between observed and expected compositions may thus be weaker for meal mixtures containing only few taxa compared to meal mixtures containing more taxa. This also gives hope that sampling of more complex mixtures and natural diets of generalist herbivores in general provide more robust output than the one observed here. Moreover, independently of the number of species, the composition itself (*i.e.,* having extreme proportions) influences the correlation (Deagle et al., 2019). This is exemplified in Willerslev et al. (2014) with proportions of both 0% and 100% in their composition, showing a strong correlation in a two-species system. Consequently, previous studies of complex diets give relatively consistent compositions across different methods for rodents

(*Khanam et al., 2016*; *Soininen et al., 2009*) and other herbivorous mammals (*Newmaster et al., 2013*), or for mock communities (*Iwanowicz et al., 2016*). However, we find it likely that DNA-based analyses of also diets composed on many food items will be hampered if any dominant food item has the same problematic issues as we have identified for *Trifolium*.

During the last 10 years, DNA metabarcoding has proved valuable to expand the understanding of trophic interactions. Our findings for small rodents add to the growing number of assessments of different taxa showing that caution is necessary when drawing ecological conclusions from sequence reads count data. Based on our experience with these analyses, we have two main messages for future developments of DNA metabarcoding diet studies. *First*, comparisons between observed and expected diets will benefit from using the direct regression approach of *Fiksel, Zeger & Datta (2021)*, where both response and predictor variables are compositional. Although this method does not require transformation and is easier to interpret, it does not allow for further covariates. Most compositional analysis methods currently available have similar or other shortcomings, which would require further developments to overcome. *Second*, while DNA metabarcoding can give quantitative results, they are unlikely to be perfect. The metabarcoding process involves many steps, each of which is susceptible to errors and biases (cf. *Alberdi et al. (2018)*). Our study is only the first step aiming at testing whether a positive correlation between observed and expected diet compositions exists for rodents. Currently, DNA metabarcoding represents the most accessible and cheap DNA-based option for diet analysis. We therefore see the advantage of studies that aim to pinpoint and better understand the mechanisms of potential biases, as no such study exists on the matter, in neither rodents and herbivores.

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## ADDITIONAL INFORMATION AND DECLARATIONS

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## Competing Interests

The authors declare there are no competing interests.

## Author Contributions

- Magne Neby conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, funding acquisition, project administration, writing original draft, and approved the final draft.
- Stefaniya Kamenova, Olivier Devineau, Rolf A. Ims and Eeva M. Soininen conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.

## Animal Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

All experiments were performed by certified personnel and approved by the Norwegian Animal Research Authority, i.e., Norwegian Food Safety Authority (FOTS 15309, 15585).

## Data Availability

The following information was supplied regarding data availability:

Raw read data are available at the European Nucleotide Archive (ENA): PRJEB43213.

Raw read data and processed data are available at Dataverse:

Magne Neby, 2021, "Replication Data for: Issues of under-amplification in quantitative DNA metabarcoding weaken the inference about diet of the tundra vole Microtus oeconomus", https://doi.org/10.18710/HJAVSN, DataverseNO, V1.

## Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.11936#supplemental-information.

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# V

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Small rodent populations throughout the world show massive density fluctuations. This thesis includes a collection of papers that highlight the persistent gaps in knowledge limiting our understanding of the mechanisms driving population dynamics. Herbivorous voles in boreal, alpine, and arctic ecosystems are especially renowned for their multi-annual population cycles in which both seasonality and plant-herbivore interaction may play an important role. Because all hypotheses predicting that plants cause rodent cycles have explicit, yet largely untested, assumptions of diet shifts across population cycles, one of the essential issues to explore further is whether a diet shift occurs that change population dynamics. This was pursued with DNA metabarcoding to quantify diets of two functionally important boreal vole species. We observed large diet flexibility and tendencies for moderate shifts in the proportions of plant taxa in the diets of bank voles and tundra voles both between phases and seasons. Thus, changes do occur through time in vole diet composition, although this study indicates that the variation in diet that could be attributed to cyclic phases is marginal relative to the overall diet flexibility. Hence, based on this study it seems unlikely that temporal variation in diets is driving the transition between the phases of the population cycle.