JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE
277

Kristian M. Forbes

Ecology of Host-Parasite Relationships in Boreal Europe

Voles, Food and Infectious Diseases

JYVÄSKYLÄN YLIOPISTO

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Esitetään Jyväskylän yliopiston matemaattis-luonnontieteellisen tiedekunnan suostumuksella
julkisesti tarkastettavaksi yliopiston vanhassa juhlasalissa S212
huhtikuun 29. päivänä 2014 kello 12.

Academic dissertation to be publicly discussed, by permission of
the Faculty of Mathematics and Science of the University of Jyväskylä,
in building Seminarium, auditorium S212, on April 29, 2014 at 12 o'clock noon.

UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2014

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URN:ISBN:978-951-39-5655-4
ISBN 978-951-39-5655-4 (PDF)

ISBN 978-951-39-5654-7 (nid.)
ISSN 1456-9701

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Jyväskylä University Printing House, Jyväskylä 2014

“Run mad as often as you chuse; but do not faint”
– Jane Austen

ABSTRACT

Forbes, Kristian M.
Ecology of host-parasite relationships in boreal Europe: voles, food and infectious diseases
Jyväskylä: University of Jyväskylä, 2014, 35 p.
(Jyväskylä Studies in Biological and Environmental Science
ISSN 1456-9701; 277)
ISBN 978-951-39-5654-7 (nid.)
ISBN 978-951-39-5655-4 (PDF)
Yhteenveto: Loisten ja isäntien ekologiset vuorovaikutukset boreaalisessa Euroopassa: myyrät, ravinto ja taudit
Diss.

Populations of many small mammal species exhibit cyclic density fluctuations in boreal Europe. Predation has long been considered a primary regulator of cyclic dynamics, while complementary limiting factors have received far less attention. The purpose of this thesis was to investigate the individual and synergistic effects of food resources and circulating micro and macroparasites on populations of a boreal small mammal species, the field vole (*Microtus agrestis*). Specifically, I wanted to know whether these factors can cause changes in vole population growth, and through which mechanisms their effects occur. Diverse methods were employed, including extensive national vole monitoring and outdoor enclosure experiments. In the first study I assessed spatiotemporal variation in rodent-borne virus prevalence in field voles across Finland. Antibodies against cowpox virus were found localised to vole populations in the southeast of Finland, and their prevalence was positively associated with vole population density. Antibody prevalence to other rodent-borne viruses was low and likely to be due to spillover from sympatric species. I then experimentally demonstrated in the second study that diet quality, specifically protein content, restricted the summer growth of vole populations through effects on reproductive output. In the third study, winter food availability limited vole population growth, while intestinal macroparasites did not. Conversely, vole survival was impaired by infection with the bacterium *Bordetella bronchiseptica* in the final study, such that it negated the positive effects of winter food supplementation on population growth rates. Both of the latter two experiments found that the negative effects of parasites on voles were not enhanced by food deprivation. Overall, I conclude that food resources and parasites are both capable of limiting small mammal population growth in boreal Europe, and that the contribution of these factors to density fluctuations observed in natural populations warrants careful investigation.

Keywords: Enclosure; experiment; food; parasite; population limitation; vole.

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original papers, which are referred to in the text by their Roman numerals I - IV. I am the first author for all papers and substantially contributed to the study design, data collection, statistical analyses and writing of each manuscript.

I Forbes K.M., Voutilainen L., Jääskeläinen A., Sironen T., Kinnunen P.M., Stuart P., Vapalahti O., Henttonen H. & Huitu O. 2014. Serological survey of rodent-borne viruses in Finnish field voles. Vector-borne and Zoonotic Diseases. 14: DOI: 10.1089/vbz.2013.1526.

II Forbes K.M., Stuart P., Mappes T., Hoset K.S., Henttonen H. & Huitu O. 2014. Diet quality limits summer growth of field vole populations. *PLoS One* 9: e91113.

III Forbes K.M., Stuart P., Mappes T., Henttonen H. & Huitu O. 2014. Food resources override intestinal parasitism in winter limitation of boreal vole populations. Submitted manuscript.

IV Forbes K.M., Henttonen H., Hirvelä-Koski V., Kipar A., Mappes T., Stuart P. & Huitu O. 2014. Complex interactions drive pathogen limitation of vole populations. Manuscript.

1 INTRODUCTION

1.1 Population size determinants

Ultimately, four processes determine the size and growth of natural populations: births, deaths, immigration and emigration (Begon *et al*. 2006). Limiting factors are mechanisms that reduce population growth, and can be broadly categorised as intrinsic or extrinsic. Intrinsic factors are self-limiting, such as spacing behaviour caused by territoriality, and population age structure (Caughley and Krebs 1983, Wolff 1997, Tkadlec and Zejda 1998). Extrinsic factors, on the other hand, are environmental stressors and include resources, predation and infectious diseases, amongst other things (Wolff 1997, Sibly and Hone 2002).

Processes behind complex population dynamics can rarely be described or modelled using one or few factors (Lack 1954, Lidicker 1988, Krebs 2013). A primary challenge for ecologists is to disentangle the mosaic of interactions and estimate the relative contribution of its components. Although a multifactorial perspective is often required for the study of animal population dynamics, such techniques are seldom employed, especially experimentally (Mitchell *et al*. 1992).

In this thesis I investigate the extrinsic effects of food and parasites (micro and macroparasites; Anderson and May 1979) on the reproduction and survival of a wildlife small mammal species in boreal Europe.

1.2 Effects of food on animal populations

All wild animal populations are likely to experience food limitation at some time (Sinclair and Krebs 2002). At its most extreme, quantitative restriction can cause starvation-related mortality (Young 1994), which seems to be more common in populations of terrestrial herbivores than carnivores, and is

probably due to the bottom-up sequence of trophic interactions. An increase in available resources, conversely, is likely to enhance reproductive output (White 2005), which may be especially apparent in species with fast reproductive capacity, such as rodents (Ostfeld and Keesing 2000, Singleton *et al*. 2005).

These effects are undoubtedly also related to the quality of available resources (Cole and Batzli 1979, Cameron and Eshelman 1996). Yet the effects of food quality on animal populations are far less studied than the effects of food quantity (Boutin 1990). Nevertheless, essential compounds are known to be in short supply within food webs, especially for primary consumers (Elser *et al*. 2000). Nitrogen, in particular, is suspected to be a primary limiting factor across herbivore species due to its lack of availability in plant tissue (nitrogen limitation hypothesis; Mattson 1980, White 2005). Indeed, the ability of plants to restrict the availability of nutrients, along with the production of toxic compounds, is considered an adaptive response that influences the feeding behaviour of herbivores and thereby protects plants from damage (White 2005).

1.3 Effects of parasites on host populations

Parasitism is a ubiquitous survival strategy whereby one species lives on or in another (the host) and acquires nutrients at its expense (Bush *et al*. 2001). Host disease due to parasite infection is rare (Begon *et al*. 2006). Nevertheless, a number of stochastic die-offs of animal populations due to epizootic disease have been documented (Daszak *et al*. 2000, Dobson and Foufopoulos 2001), which contrary to food limitation, appear to be more prevalent in carnivores than herbivores (Young 1994). The evidence of die-offs can be stark, especially in large or common host species (Dobson and Foufopoulos 2001). A case in point comes from the eastern United States where the spread of West Nile virus is documented through public reports of dead crows and other bird species (Marfin *et al*. 2001).

Epizootic outbreaks occur mainly in host species that are uncommonly infected by a particular parasite. Until relatively recently, it was believed that parasites evolve towards reduced host damage (Anderson and May 1979, Nunn and Altizer 2006). While this still occurs, it is now accepted that evolution towards intermediate, or in rare cases, severe host damage can enhance parasite fitness (Ewald 1994, Nunn and Altizer 2006). In support, a growing body of research has revealed subtle effects of endemic parasites on both the reproduction and survival of wildlife species (Hudson *et al*. 1998, Tompkins and Begon 1999, Bize *et al*. 2004, Hakkarainen *et al*. 2007, Kallio *et al*. 2007, Burthe *et al*. 2008). Nevertheless, experimental evidence of parasite effects on wildlife populations remains rare and valuable (Begon *et al*. 2006).

Individual hosts are usually infected simultaneously by multiple parasite species. Moreover, infection by one parasite species can greatly affect the presence and intensity of others (Lello *et al*. 2004, Cattadori *et al*. 2008, Telfer *et al*. 2010). This occurs either through direct competition between parasites for

resources or indirectly via the host immune system (Pedersen and Fenton 2007). As such, a within-host parasite community approach is advocated for understanding the effects of parasites on host population dynamics (Pedersen and Fenton 2007).

1.4 Interactions between food and parasites upon hosts

The outcome of interactions between parasite and host often depend, at least partially, on the host immune response (Beldomenico and Begon 2010). Individual trade-offs are believed to occur in the allocation of finite nutrient reserves between immune defences and other nutrient-costly processes such as homeostasis, growth and reproduction (Sheldon and Verhulst 1996, Lochmiller and Deerenberg 2000, Zuk and Stoehr 2002, Mills *et al*. 2010). Malnutrition has been associated with lowered immune competence and increased infection prevalence and intensity (Katona and Katona-Apte 2008, Beldomenico and Begon 2010), although difficulty in distinguishing cause from effect is often acknowledged. Moreover, these relationships can translate into population level outcomes (Moret and Schmid-Hempel 2000, Pedersen and Greives 2008). Moret and Schmid-Hempel (2000), for example, demonstrated that non-fatal starvation of bumblebee workers increased the likelihood of mortality following an immune challenge. Since parasites extract resources that would otherwise be available to the host, a vicious circle may develop between physiological condition and infection, whereby poor condition increases parasite susceptibility, which further reduces condition and so on (Beldomenico *et al*. 2008, Beldomenico and Begon 2010).

1.5 Small mammal cycles

In boreal Europe, populations of small mammal are renowned for their high-amplitude cyclic density fluctuations, which occur in many species in 3 - 5 year periods (Elton 1924, 1942, Hansson and Henttonen 1985, Krebs 2013). A population cycle usually entails 2 - 3 successive years of positive summer population growth, with connecting periods of negative or stable winter growth (increase phase). Peak density is attained in late summer to autumn of the last increase year, and is followed by a severe winter decline (known as a population crash) that continues into the following summer (Myllymäki 1977, Hansson and Henttonen 1985, Boonstra *et al*. 1998). A latitudinal gradient is seen in cycle amplitude and period in Fennoscandia, whereby both are greater in northernmost than southernmost areas. In southernmost areas of northern Europe, small mammal populations are non-cyclic and display seasonal patterns of growth (Hansson and Henttonen 1985, Hanski and Henttonen 2002). The search for mechanisms responsible for cyclic dynamics has stimulated

considerable research and debate for several decades, and consensus has not yet been reached (Hanski and Henttonen 2002, Krebs 2013).

Together, specialist and generalist predation appears the most likely candidate to explain the cyclic pattern of vole abundance in Fennoscandia (Hanski *et al*. 1991, Klemola *et al*. 2000). According to this, a delayed numerical response by predators causes the oscillations in small mammal density (Hanski *et al*. 1991, Hanski and Henttonen 2002). Specifically, the dependency of least weasels (*Mustela nivalis*) on field voles (*Microtus agrestis*) is hypothesised to initiate the pattern throughout much of northern Europe (Henttonen 1987). Meanwhile, generalist predation (by foxes, owls etc.) stabilises population dynamics (Andersson and Erlinge 1977, Korpimäki 1985). A latitudinal gradient in the diversity of generalist predators positively corresponds to the gradient in small mammal cycle amplitude and period (Hanski *et al*. 1991, Hanski and Henttonen 2002), and a switch to alternative prey as populations of preferred species are exhausted is likely to explain the interspecific and proximate spatial synchrony of cycles (Andersson and Erlinge 1977, Ims and Andreassen 2000, Korpimäki *et al*. 2005).

Within this framework of cyclic density fluctuations, numerous limiting factors may operate, which are currently obscured by the emphasis placed on discovering ultimate regulatory mechanisms. In particular, winter food resources in boreal Europe are insufficient to support high-density vole populations (Huitu *et al*. 2003, 2007, Korslund and Steen 2006), and limitation via this mechanism may initiate the cyclic decline phase. Habitat quality has been demonstrated to influence vole cycle amplitude in summer (Cole and Batzli 1979). Meanwhile, several parasites, identified in declining populations, are suspected to inhibit vole survival and potentially contribute to population crashes (Elton 1924, Soveri *et al*. 2000).

Widespread dampening of small mammal cycle amplitude has recently been identified across Europe (Cornulier *et al*. 2013). If this trend continues, the relative importance of seasonally and spatially variable limiting factors to population abundance patterns is likely to increase.

1.6 Implications for human health

Zoonoses are infectious diseases transmitted between animals and people (Taylor *et al*. 2001). Most emerging infectious diseases of humans are zoonotic in origin, and many of these come from wildlife species (Taylor *et al*. 2001). Recent decades have seen a spike in the number of emerging human diseases (Daszak *et al*. 2004), which is undoubtedly related to increased contact between wildlife and humans (Mahy and Brown 2000, Wolfe *et al*. 2007), at least in part due to habitat destruction associated with land clearing and climate change (Daszak *et al*. 2001, 2004).

In the same way, high abundance of small mammals during cyclic peaks increases contact between rodent hosts and humans (Vidal *et al*. 2009). This is

evidenced by the incidence of human Puumala hantavirus infection in northern Europe, which can be predicted from the density of the bank vole host (Kallio *et al*. 2009). Human knowledge of wildlife parasites has been described as the 'tip of the iceberg' (Wobesser 2006), and it is the unknown parasites to which we may potentially be exposed that pose one of the greatest risks to human health in the future.

1.7 Aims of the thesis

The purpose of my thesis is to investigate the individual and synergistic effects of food resources and parasite infection on small mammal populations in boreal Europe. Due to their high abundance and potential role in driving population cycles (Henttonen 1987), field voles are an ideal model system.

The simultaneous effects of food resources, individual health and parasitic infection on animal population dynamics have only rarely been studied, let alone experimentally. Therefore a primary aim of my thesis is to use the boreal vole context to empirically test whether resource limitation enhances the negative effects of parasites on vole population growth. An understanding of the mechanisms that contribute to population limitation has practical implications in animal conservation and pest control. It is hoped that scholars in these fields will also find the information presented in this thesis useful.

My thesis is based on four distinct studies, which address gaps in existing knowledge. Very little is known of the viruses that infect field voles in Finland. I therefore used a large survey of natural vole populations to first establish which virus species circulate in field voles and whether their prevalence varies in space and time. In the second study I addressed the lack of knowledge regarding diet quality on herbivore population growth. For this, I randomised a series of large outdoor enclosures to high protein content food supplementation, low protein content food supplementation or no food supplementation, and monitored the vole populations contained therein throughout the summer reproductive season. Thus far my studies have dealt with parasites and food as independent entities. In the final two studies I sought to investigate the relationship between food and parasite infection on vole populations over the resource limited boreal winter (Huitu *et al*. 2003, Fey *et al*. 2008). In the first winter experiment, I used the same enclosures and applied a two-factor experiment design with *ad libitum* food supplementation and antihelminthic treatment of intestinal parasites of voles. The following winter I employed similar methods in my final study, this time to investigate the effects of food supplementation and introduction of the bacterium, *Bordetella bronchiseptica*.

2 METHODS

2.1 Study system

Field voles (Fig. 1) are one of the most abundant and widely distributed of small mammal species in northern Europe (Hansson and Henttonen 1985). These herbivorous rodents live primarily in heterogeneous field, clear-cut or marsh habitat (Hansson 1977, Myllymäki 1977), where they display a folivorous diet (Faber and Ma 1986; Butet and Delettre 2011). The primary breeding season lasts from spring until the end of summer, and the female usually gives birth to 4 - 7 pups per litter (Myllymäki 1977). Gestation lasts just under three weeks and reproductive output is maximised by postpartum estrus; that is, females mate within 1 - 2 days of giving birth (Myllymäki 1977, Gilbert 1984). Sexual maturity occurs approximately three weeks post-partum, although young born late in the summer tend to delay sexual maturity until the following spring (Myllymäki 1977). During winter, voles of boreal Europe primarily inhabit the subnivean cavity, a fragmented space between the ground and bottom layer of snow (Pruitt 1984). It is unusual for a wild vole to survive long enough to overwinter twice (Myllymäki 1977).

2.2 Monitoring of natural populations (I)

Field voles were trapped from 14 sites throughout the southern half of Finland in spring (late April - May) and autumn (late September to October) for three consecutive years (see Korpela *et al*. 2013). For each trapping occasion, 100 metal standard mouse kill-traps were set in clusters of three at 10 – 20 m intervals. Traps were baited with a small piece of bread and left for one night. The following morning, captured voles were weighed and measured, sex, age and reproductive status were recorded, and vole carcasses were frozen (< −20° C).

Carcasses were later thawed and organ samples collected. The heart of each vole was placed into an individual tube with 150 µl of phosphate buffered saline (PBS) and refrozen until serology tests.

2.3 Field enclosures (II, III, IV)

Experiments for papers II, III, and IV were conducted in naturally vegetated field enclosures (Fig. 2) near the town of Suonenjoki in central Finland (lat 62° 45.672', lon 27° 6.015'). A total of 32 adjoining enclosures, each 20 × 25 m, were constructed of sheet metal that was buried to a depth of approximately 50 cm and rose 1 m aboveground. As such, voles were contained within enclosures and access by mustelid predators was greatly, though not entirely (see III), restricted. Avian predation was uninhibited, and signs of owl strikes were occasionally observed on the snow surface during the winter experiments.

Each enclosure comprised eight lidded shelter boxes, positioned approximately 10 m apart. Shelter boxes had two entrances holes at the base for vole access, and contained a multiple-capture Ugglan live trap (Grahnab, Sweden), which was unset when vole monitoring was not being conducted. Supplementary food resources for experimental treatments were distributed from feeders placed in each shelter box.

FIGURE 1 Field voles, *Microtus agrestis* (Image by Erkki Oksanen, Metla).

2.4 Enclosure monitoring (II, III, IV)

Vole populations within enclosures were monitored at 2 – 8 week intervals (depending on the particular experiment and its stage) using capture-mark-recapture methodology (Amstrup *et al*. 2005). For each trapping occasion, traps were baited with oats, set and checked each day at 7 am, 2 pm and 9 pm for

three consecutive days. A passive integrated transponder (PIT; EID Aalten BV, Aalten, the Netherlands) was subcutaneously injected into every vole when it was first captured, and the unique identification number was recorded at each encounter. Voles were taken to an onsite field laboratory where blood was collected from the retro-orbital sinus with heparinised capillary tubes, head width and weight were measured, and sex and reproductive status were evaluated based on external appearance. Voles were then returned to the enclosure from which they were captured (except during the final trapping occasions of II and IV). If blood was not required for a particular vole or trapping occasion (see IV), vole indices (weight, sex etc.) were recorded at the point of capture and the vole was immediately released.

FIGURE 2 Field enclosure site near the town of Suonenjoki in central Finland. Summer is represented in the top panel, and winter in the bottom panel (Images by Kristian Forbes).

2.5 Antihelminthic treatment (III)

Ivermectin antihelminthic medication (Ivomec Vet 10 mg/ml, Merial Animal Health, Lyon, France) was used to treat the intestinal helminths of voles in the first winter experiment (III). This medication is effective against nematodes, but not coccidian protozoa, which are also found abundant in rodent species (Ferrari *et al*. 2004, Pedersen and Antonovics 2013). Antihelminthic medication

(diluted with linseed oil) was orally administered to voles concurrent with sampling. Captured voles were treated once per trapping occasion, and juvenile voles weighing under 20 g were excluded from treatment. Voles from non-antihelminthic assigned enclosures were treated with linseed oil alone. To evaluate the presence of intestinal parasites, faeces were collected from transportation containers, which were wiped clean and sterilised between voles.

2.6 Bacteria introduction (IV)

The bacterium *Bordetella bronchiseptica* was introduced to assigned enclosure populations at the end of winter in the final study (IV). Bacteria were originally isolated from the lung of a wild field vole captured in the area. Two voles, infected by pipetting bacteria suspension into their nose and mouth, were introduced to each *B. bronchiseptica* assigned enclosure. Experimentally infected voles were released into the two shelter boxes from which the highest number of voles were captured during the prior trapping occasion. Additionally, one vole from each existing enclosure population was experimentally infected in the field. Untreated voles were introduced to non-*B. bronchiseptica* assigned enclosures via the same methods.

2.7 Laboratory diagnostics

2.7.1 Antibody detection (I)

Heart extracts were tested for antibodies against hantavirus, lymphocytic choriomeningitis virus (LCMV), Ljungan virus (LV) and orthopoxviruses (OPV) using previously described indirect fluorescent antibody tests (IFAT; Kallio-Kokko *et al*. 2006, Jääskeläinen *et al*. 2013, Kinnunen *et al*. 2011).

2.7.2 PCR (I)

RT-PCRs were conducted on the lungs of voles that were seropositive to hantavirus and LCMV, and on the livers of voles seropositive to LV. The methods for each test have also been described previously (Klempa *et al*. 2003, Donoso Mantke *et al*. 2007, Vieth *et al*. 2007).

2.7.3 Haematological indices (II, IV)

Enzyme-linked immunosorbent assays (ELISA) were used to measure the concentration of albumin and total Immunoglobulin-G (IgG) in plasma samples collected during the enclosure experiments (II, IV). Albumin concentrations were measured using commercially purchased mouse-albumin kits (Alpha

Diagnostics International, Texas, USA), based on instructions provided by the manufacturer. A detailed description of the process to measure total IgG is provided in paper II.

Blood smears were May-Grünwald-Giemsa –stained for white blood cell counts (IV). Three counts of leucocytes in a ×10 magnification field-of-view were averaged to determine the total number of leucocytes. Differential counts were conducted on 200 leucocytes using ×50/ ×100 magnification under oil immersion.

2.7.4 Macroparasite identification (III)

The eggs and oocysts of intestinal macroparasites were separated from faecal samples using salt flotations and transferred onto microscope slides for visual inspection. Morphology-based parasite identification to the genus or family level was achieved with a light microscope. Infection intensity was quantified by counting the number of eggs or oocysts of each parasite group observed in a slide transect, and standardised by the faeces sample weight.

2.7.5 Bacteria cultures (IV)

Vole lung samples were cultured to determine the presence or absence of *B. bronchiseptica* at the experiment conclusion. Briefly, after thawing, lung specimens were inoculated onto blood agar and bromthymol blue lactose agar plates and incubated for six days at 37 ± 1° C. Suspected *B.bronchiseptica* colonies were then sub-cultured onto blood agar, MacConkey agar (Merck) and bromthymol. The criteria used for positive identification of *B. bronchiseptica*, as well as the methods used to prepare bacterial suspensions for experimental introduction into the enclosure populations, are described in detail in the paper IV.

2.7.6 Histopathology (IV)

Tissue specimens were trimmed and routinely paraffin wax embedded. Sections (3-5μm) were prepared and stained with hematoxylin-eosin, before examination.

2.8 Data analyses

An abundance index was calculated for wild field vole populations (I) by dividing the number of trapped voles by the number of traps set. Enclosure population abundance (II, III and IV) was estimated for each trapping occasion with program CAPTURE (Otis *et al*. 1978), using either M_h (heterogeneity) or M_{bh} (removal) models. Enclosure population growth rates between trapping occasions were calculated using the formula:

$$R_t = \ln(N_t -1/N_t),$$

where N_t is the population abundance at time t. Meanwhile, enclosure population survival for trapping intervals was estimated from the most parsimonious recapture rate model in program MARK (White and Burnham 1999). The exception to this occurred after the introduction of *Bordetella bronchiseptica* (IV), when individual survival was monitored until the experiment conclusion.

The studentized residuals of vole head width regressed by body mass were used to express an individual body condition index (II, IV). General and generalized linear models were used in the statistical analyses of data (I – IV). For data derived from the enclosure experiments (II – IV), mixed models were used to account for potential variation between enclosures, and at times, repeated measures from the same vole.

3 RESULTS AND DISCUSSION

3.1 Geographical variation in viruses (I)

Rodent species harbour numerous parasites that may negatively affect their population growth and potentially be transmitted to humans (Luis *et al*. 2013). Yet very little is known of the viruses that infect field voles in boreal Europe. I therefore conducted a large survey of natural vole populations in Finland to evaluate spatiotemporal variation in the prevalence of antibodies against common European rodent-borne viruses or virus groups. These included hantaviruses, lymphocytic choriomeningitis virus (LCMV), Ljungan virus (LV) and orthopoxviruses (OPV).

Antibodies against orthopoxviruses (probably cowpox; Kinnunen 2011) were highly prevalent, but consigned to field vole populations in the southeast of Finland (I). Evidence of cowpox virus in wildlife (Kinnunen *et al*. 2011), as well as a human case (Pelkonen *et al*. 2003), has previously been reported in southern Finland. However, due to the sampling scope, this research is the first indication that the spatial distribution of the virus may be localised. Interestingly, bank voles were also captured in the trapping sites with highest field vole antibody prevalence. This species is considered an important reservoir of cowpox virus (Kinnunen 2011), and may have enhanced field vole exposure to the virus. As such, the finding that cowpox virus prevalence is geographically restricted in Finland must be validated in other host species, and the relative ability of different species within a community to support the virus delineated.

Antibody prevalence against cowpox virus was density dependent and higher in spring than autumn. Akin to the 'juvenile dilution effect' described for chronic rodent viruses (Mills *et al*. 1999), this seasonal difference in antibody prevalence is likely to be due to recruitment of naïve voles during the summer reproductive period. Cowpox virus has been previously demonstrated to inhibit the survival of field voles (Burthe et al. 2008). However, its localised

distribution does not render it a strong candidate as a widespread limiting factor for vole populations in Finland.

The prevalence of voles with antibodies against hantavirus, LCMV and LV was low. In the case of hantavirus and LV, the irregular pattern of low prevalence is indicative of virus spillover from sympatric species (the evidence is less clear for LCMV). This assumption is supported by negative hantavirus PCR identification in the tissues of all seropositive voles (hantavirus infections are chronic in rodent hosts; Easterbrook and Klein 2008). Tula and Tatenale virus are the only known hantaviruses to infect *Microtus* voles in northern Europe. Tatenale virus was recently identified in field voles in the UK (Pounder *et al*. 2013). Meanwhile, Tula virus has been previously found in field voles in Croatia and central Europe (Scharinghausen *et al*. 2002; Schmidt-Chanasit *et al*. 2010), and it is therefore not (yet) likely to occur in Finland.

LV is present in Finland (Jääskeläinen *et al*. 2013), and seropositive individuals demonstrate that field voles have been exposed to the virus. However, despite high prevalence in this species in the UK (Salisbury *et al*. 2013), field voles in Finland do not seem to harbour the virus. Very little is known of LV, and a plausible explanation that warrants evaluation is that virus strain-host combinations vary geographically.

3.2 Limitation by food resources

3.2.1 During summer (II)

During the summer reproductive period, food resources consumed by folivorous voles are quantitatively abundant in boreal Europe (see Fig. 2). Habitat vegetation type has been identified as an important determinant of vole population dynamics (Cole and Batzli 1979), and the purpose of this study was to evaluate the effects of qualitative components, specifically proteins and the nitrogen they contain, on vole population growth during the summer reproductive period.

I found vole population growth was constrained by food quality during summer (II). By the end of the summer breeding season, vole densities were clearly greater in populations that received high protein content food supplementation than populations that received food supplementation with equivalent energy, but lower protein content. Interestingly, and while not statistically significant, control populations (no treatment) displayed intermediate densities that roughly correspond with the protein content of natural vegetation (Suleiman *et al*. 1999). The finding that low protein supplemented populations attained lower density than control populations is perhaps due to the protective aspect of shelter boxes, from where food supplementation was distributed, exceeding the temptation of outside resources.

Differences between treatment groups were attributable to reproduction. Diet quality is of high importance to the breeding success of herbivores (Boutin 1990), and the nitrogen contained in proteins is believed to be a primary limiting factor across the taxon (Mattson 1980, White 2005). In terms of population dynamics, this study demonstrates that diet quality limitation is a highly plausible explanation for the variation observed in the amplitude of cycle peaks (Korpela *et al*. 2013). Further, since the nitrogen content of vegetation is altered by environmental conditions (Kummerow 1980, Laine and Henttonen 1987), these findings provide a mechanistic link for a recent association between summer weather conditions and vole population growth (Korpela *et al*. 2013).

A key reason to investigate mechanisms that influence population dynamics in a controlled setting is to facilitate their identification under natural conditions. To assess the relative contribution of diet quality to natural population dynamics, it is important to measure these components in natural vegetation and compare them to the abundance patterns of vole populations in surrounding areas. In the same vein, qualitative traits of vole food plants, which restrict access to proteins, such as silica (Massey *et al*. 2007, Hunt *et al*. 2008), require evaluation in natural settings.

3.2.2 During winter (III, IV)

Winter food limitation was addressed in two separate studies, within the context of factorial experiments involving parasite manipulation. The rationale was that food resources consumed by voles are highly likely to be limited during the boreal winter, and may enhance negative parasitic effects on host populations.

I found that vole population density declined during the two winter enclosure experiments. However, this decline was mitigated in both years by food supplementation. This effect was again mediated by reproduction, which although marginal, was clearly more prevalent in food supplemented than non-supplemented populations. This finding is consistent with previous research that has shown high quality food resources to facilitate winter reproduction in voles (Kaikusalo and Tast 1984), as well as advance the onset of spring reproduction (Taitt and Krebs 1981, Haapakoski *et al*. 2012, Helle *et al*. 2012).

The concept of food limitation of high-density small mammal populations during the boreal winter is well established (Huitu *et al*. 2003, Korslund and Steen 2006, Fey *et al*. 2008). However, this effect has been found to be attributable primarily to variation in survival (Huitu *et al*. 2003, 2007, Korslund and Steen 2006). In the current winter experiments, food resources did not influence vole survival, despite high vole density in enclosures and the fact that food supplemented voles exhibited better physiological condition than non-supplemented voles (IV; see also Haapakoski *et al*. 2012). As such, these results challenge the generality of food limitation acting primarily through survival in high density small mammal populations during the boreal winter.

The amount of natural vegetation is highly contingent on the length of the growing season in boreal Europe. In the current experiments, increased resources were probably due to extended autumns. This assumption is supported by similar findings in a separate experiment conducted nearby with bank voles (Haapakoski *et al*. 2012). Whether the years in question were anomalous, or reflections of a changing climate upon the dynamics of small mammals, will need to be determined over time.

3.3 Limitation by infectious disease (III, IV)

Nearly a century ago Charles Elton proposed that epidemic disease caused the periodic crash in cyclic small mammal populations (Elton 1924). However, experimental investigation of candidate parasites was rarely done. To evaluate the ability of parasites to limit the growth of cyclic small mammal populations, I conducted two separate winter experiments on high density field vole populations using manipulation of parasites that are known to infect voles in boreal Europe.

Consistent with previous research (Haukisalmi *et al*. 1994, Laakkonen *et al*. 1998), the eggs of Heligmosomidae nematodes and oocysts of *Eimeria* coccidians were regularly identified in vole faeces (III). The antihelminthic medication effectively reduced the prevalence of Heligmosomoidae nematodes, as expected. However, the prevalence of neither parasite group was found to influence vole population growth rates during the boreal winter. Intestinal nematodes have been shown to contribute to seasonal crashes and restrict reproductive output in *Peromyscus* mouse populations in North America (Pedersen and Greives 2008, Vandergrift *et al*. 2008). These contrasting results suggest that characteristics of the geographical area (including the parasite species) or host system are prime determinants of the role of intestinal parasites as a limiting factor.

The manipulation of Heligmosomoidae nematode prevalence did not indirectly affect the prevalence of eimerians through competitive release (Pedersen and Antonovics 2013). Thus strong conclusions regarding their effect on vole population growth cannot be formed. Nevertheless, according to earlier findings, the spatial and temporal patterns of eimerian prevalence and intensity do not support a limiting effect on vole populations in Finland (Laakkonen *et al*. 1998; but see Hakkarainen *et al*. 2007).

Although intestinal parasites were found not to influence the survival of voles during the boreal winter, their effects on reproduction could not be properly evaluated due to the low prevalence of reproducing voles. Previous research has found that these parasite groups can impair reproduction in rodent species (Vandegrift *et al*. 2008), and comparative research on cyclic vole populations is required during the summer breeding period. Moreover, negative effects induced by parasites may be more apparent during summer

when juvenile voles, which can harbour greater parasite loads (Ball and Lewis 1984, Laakkonen *et al*. 1998), are likely to increase population-wide infection pressure.

In the second parasite experiment, *Bordetella bronchiseptica* introduction did limit vole population growth during the boreal winter (IV). The negative effects of *B. bronchiseptica* manifested through reduced survival and were seen solely in food-supplemented populations (discussed in the next section). Interestingly, *B. bronchiseptica* infected voles were often co-infected by an apicomplexan protozoan, morphologically identified as *Hepatozoon erhardovae* (Laakkonen *et al*. 2001). This protozoan commonly infects bank voles in northern Europe, but rarely field voles (Laakkonen *et al*. 2001). This finding thus supports previous research, which has identified that one parasite species in a host, can greatly affect the presence of another (Telfer *et al*. 2010). Together these parasite species were seen in the majority of vole lungs with severe inflammation, implying that their association contributed to the severity of disease.

This research experimentally demonstrates that the bacterium, *B. bronchiseptica*, can limit the growth of vole populations under certain circumstances. However, very little is known of the distribution or spatiotemporal variation in prevalence of this bacterium in natural vole populations. Both seasonal and inter-annual variation in *B. bronchiseptica* seroprevalence was identified in a rabbit population (Pathak *et al*. 2011), and further research is required to gauge the influence of this bacterium on the dynamics of natural vole populations.

3.4 Interactions between food and parasites (III, IV)

A primary theme of my thesis is the relationship between food resources and infectious disease. Immune defences are nutrient demanding, and theory indicates that trade-offs can occur in the partitioning of finite reserves between pertinent processes, such as homeostasis, growth and immune defence (Sheldon and Verhulst 1996, Lochmiller and Deerenberg 2000, Zuk and Stoehr 2002).

For the current experiments (III, IV), a natural setting of resource limitation was established by the boreal winter, when grasses consumed by folivorous voles do not grow (Myllymäki 1977). Although food limitation did not cause excess vole mortality in the experimental populations, food supplemented voles generally displayed higher physiological condition than non-supplemented voles (IV). In contrast, no difference occurred in physiological condition between food supplemented and non-supplemented voles during summer when resources were quantitatively abundant (II).

In the first winter experiment (III), antihelminthic medication reduced the prevalence of Heligmosomoidae nematodes over time. Moreover, their prevalence was also lower with food supplementation, suggesting that immune function was enhanced by the quantity or quality of supplemental food. In any

case, intestinal parasites and their interaction with food resources, did not translate into demographic or survival outcomes in voles. Again these results contrast *Peromyscus* mouse populations in North America where the interaction between resource limitation and intestinal nematodes exacerbated seasonal abundance crashes (Pedersen and Greives 2008).

In the second winter experiment (IV), *B. bronchiseptica* introduction limited vole population growth through negative effects on survival (IV). However, contrary to expectations, neither immune response nor survival was enhanced by food supplementation in the presence of *B. bronchiseptica*. In fact, mortality associated with *B. bronchiseptica* infection occurred predominantly in food supplemented populations. Both the prevalence of *B. bronchiseptica* infected voles and associated lung damage were also higher in food supplemented populations, indicating that vole aggregation around feeding stations increased transmission of the bacterium. Increased social encounters may have promoted reinfection and higher bacterial loads, both of which are expected to increase the pathogenicity of *B. bronchiseptica* (Bemis *et al*. 2003). These findings validate concerns related to disease transmission at backyard bird feeding stations (Brittingham and Temple 1986; Robinson *et al*. 2000).

Broadly speaking, both winter enclosure experiments failed to support the hypothesis that the effects of parasites on animal population demography, and hence dynamics, are alleviated by abundant food resources, as previously identified (Pedersen and Greives 2008). Few studies have evaluated the relationship between resources and parasite infection on wildlife populations, and here I demonstrate that the negative effects of parasites may in fact also supress the positive effects of food. This research addressed two parasite groups (intestinal macroparasites and the bacterium, *B. bronchiseptica*) in one host system. Further empirical research in different settings and host systems is clearly required to enable generalisations regarding the nature of the relationship between food, individual condition and infectious disease on host dynamics.

4 CONCLUSIONS

In boreal Europe, populations of many small mammal species display cyclic density fluctuations. This oscillatory pattern has largely been attributed to predation, while complementary limiting factors have received far less attention. The purpose of this thesis was therefore to investigate whether food and parasite infection could limit the growth of vole populations.

A particular emphasis of my work was to address the interactive effects of food and parasites on voles. The theory being that individuals are less able to respond immunologically to parasite infection in a resource limited environment such as the boreal winter, and that these effects would translate into population level outcomes. However, the studies presented in this thesis found no clear evidence that resource limitation enhanced the negative effects of parasite infection. In fact, it was identified that parasites may supress the positive effects of food resources in this seasonal environment.

Several other important findings were revealed through this research. I found that the distribution of cowpox virus is restricted to field vole populations in southeast Finland, and that rodent-borne viruses known to infect field voles in other areas of Europe do not do so in Finland. I experimentally demonstrated that diet quality limits vole population growth through effects on fecundity during the summer reproductive season, and that food resources also facilitate reproduction during the winter period. Further, I showed that winter food resources were more important to vole population growth rates than intestinal macroparasites. Lastly, I identified that the bacterium *B. bronchiseptica* inhibited vole survival, and in turn population growth, through a series of complex interactions, likely involving vole aggregation and co-infecting parasites. I therefore conclude that food resources and parasites can indeed limit the growth of vole populations, and that their effects are likely to vary seasonally. As such, the potential contribution of these factors to density fluctuations observed in natural vole populations warrants careful attention.

More broadly, while both food and infectious diseases are considered important causes of abundance fluctuations in vertebrate animal populations, high quality evidence of their effects is sometimes lacking. In particular,

experimental evidence to demonstrate animal population limitation by diet quality or pathogenic disease is rare and this thesis, using field voles in boreal Europe, significantly contributes to this wider body of research.

Acknowledgements

It is an uncomfortable feeling to take credit for a thesis that so many people have contributed to, both directly through research input and indirectly by helping me with other tasks. It brings me great pleasure to now be able to thank you all.

First and foremost, I wish to express my deepest gratitude to my primary supervisor Otso Huitu. You had the courage (or folly!) to take on a PhD student without ecology training, and then taught me how to 'do' ecology. Your expertise, patience and commitment to my development have made this thesis possible! The skills that I have learnt from you will be invaluable in my career moving forward. To my other supervisor, Tapio Mappes, although we didn't see each other too often, you were my link to the university and I appreciate your help with organising the coursework aspects of my degree. Your contributions to fieldwork and laboratory analyses were also important to achieving this work.

Two other people deserve special mention here. Firstly, I want to thank Peter Stuart for all that you have done for the different studies. I especially appreciate that you were never too busy to answer my many questions. Secondly, I want to thank the leader of the Metla vole research group, Heikki Henttonen. Your expertise is immense and has helped me to think differently about aspects of the research. I have very much enjoyed your tales from years past, moose feasts and the occasional whisky. Lemming trapping at Kilpisjärvi will certainly go down as a highlight of my time working with rodents in northern Europe.

I have been extremely fortunate to collaborate with a very talented and diverse group of scientists. A big thank you Varpu Hirvelä-Koski, Katrine Hoset, Anne Jääskeläinen, Paula Kinnunen, Anja Kipar, Jukka Niemimaa, Heidi Rossow, Tarja Sironen and Liina Voutilainen. Anja, you have shown me what pathology can add to disease ecology research. Behind the scenes, Sami Kyröläinen and Sisko Juutinen provided invaluable laboratory support, Ilkka Taponen maintained the voles, Voitto Haukisalmi was always available to lend his expertise to parasite related queries, and Hanna Ruhanen kindly helped me with just about everything laboratory related at one time or another. Thank you all very much!

On the theme of collaborators, I wish to thank my thesis support group members, Tero Klemola and Olli Vapalahti. You both went beyond your thesis support group responsibilities to actively contribute to my experiments and scientific development.

Over the duration of my PhD, our Suonenjoki research group has been fortunate to attract a very capable collection of university trainees. Thank you Aitor Barbero, Nuria Blanco, Jean-Emmanuel Fournier, Geert Meen, Chelsea Powell, Elisabet Martínez Sancho, Stephen Ryan and Anaïs Zimmer. You all contributed so much for so little in return. I hope that you enjoyed the experience!

Metla Suonenjoki and Vantaa each provided me with an office space, and I am especially grateful to Liisa Kylmälä and Leena Vallinkoski for the many hours you devoted to help me with administrative tasks that were often, though not always, in Finnish. I don't know what I would have done without you! I also want to thank the technical staff at Metla Suonenjoki - Mervi Ahonpää, Esko Jalkanen, Juha Kaipainen, Jukka Laitinen, Auli Lehtinen, Esa Mölkänen, Martti Udd and Pekka Voipio - for your assistance with various tasks over the journey. And of course my friends (see last paragraph also) and colleagues at Metla Suonenjoki who all contributed to a great working environment and helped with the transition to living and working in a foreign country.

I wish to acknowledge the Academy of Finland, University of Jyväskylä and European Union for financial support during my degree, and the BIOINT graduate school and Finnish Mammalogical Society for grants to attend international conferences.

Lastly, to my family and friends - old and new, near and far - each of you in your own way help make life the great adventure that it is. I especially want to recognise my mum, Marilyn Forbes. For you have instilled in me the confidence and determination to travel the world and follow my aspirations.

Peace out!

YHTEENVETO (RÉSUMÉ IN FINNISH)

Monella Pohjois-Euroopan boreaalisen havumetsävyöhykkeen pikkunisäkäslajilla esiintyy säännöllistä eli syklistä kannanvaihtelua. Saalistuksen on jo pitkään ajateltu olevan tärkein syklistä kannanvaihtelua aikaansaava tekijä, minkä seurauksena muiden ilmiöön vaikuttavien tekijöiden tutkiminen on ollut vähäistä. Tämän väitöskirjan tarkoituksena on tutkia ravintovarantojen sekä loisten ja tautien erillis- ja yhteisvaikutuksia peltomyyrän (*Microtus agrestis*) kantoihin. Tarkemmin sanoen, halusin tietää vaikuttavatko yllä mainitut tekijät peltomyyräkantojen kasvuun ja minkä mekanismien aikaansaamina vaikutukset ilmenevät. Tutkimuksessa käytettiin monipuolisia menetelmiä, muun muassa laajamittaista kansallista myyräseuranta-aineistoa sekä ulkoaitauskokeita. Ensimmäisessä osatutkimuksessa selvitin jyrsijävälitteisten virusten ajallista ja alueellista esiintyvyyttä peltomyyrissä eri puolilla Suomea. Lehmärokkoviruksen vasta-aineita tavattiin Kaakkois-Suomessa ja niiden esiintyvyys oli suorassa yhteydessä peltomyyrien kannan suuruuteen. Muiden virusten vasta-aineiden esiintyvyys oli alhaista, aiheutuen todennäköisesti virusten satunnaisesta leviämisestä peltomyyriin muista myyrälajeista. Toisessa osatutkimuksessa osoitin kokeellisesti, että ravinnon laatu, erityisesti sen proteiinipitoisuus, rajoittaa peltomyyrien kannan kasvua kesällä lisääntymisen kautta. Kolmannessa tutkimuksessa havaittiin, että ravinto rajoittaa peltomyyrien kannankasvua talvella mutta suolistoloiset eivät. Neljännessä tutkimuksessa *Bordetella bronchiseptica* -bakteeri-infektio alensi peltomyyrien elossasäilyvyyttä talvella siinä määrin, että se kumosi lisäravinnon aikaansaaman kannankasvua edistävän vaikutuksen. Kahden viimeksi mainitun kokeen tulokset osoittivat, että alhainen ravinnon saanti ei voimistanut loisten ja tautien haitallista vaikutusta myyriin. Johtopäätökseni on että sekä ravintovarat että loiset ja taudit voivat rajoittaa boreaalisen Euroopan pikkunisäkäskantojen kasvua. Näiden tekijöiden vaikutukset on otettava tarkoin huomioon luonnollisten eläinkantojen vaihtelua selvittävissä tutkimuksissa.

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ORIGINAL PAPERS

I

SEROLOGICAL SURVEY OF RODENT-BORNE VIRUSES IN FINNISH FIELD VOLES

by

Kristian M. Forbes, Liina Voutilainen, Anne Jääskeläinen, Tarja Sironen, Paula M. Kinnunen, Peter Stuart, Olli Vapalahti, Heikki Henttonen & Otso Huitu 2014

Vector-borne and Zoonotic Diseases. 14: DOI: 10.1089/vbz.2013.1526.

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VECTOR-BORNE AND ZOONOTIC DISEASES
Volume 14, Number 4, 2014
© Mary Ann Liebert, Inc.
DOI: 10.1089/vbz.2013.1526

ORIGINAL ARTICLE

Serological Survey of Rodent-Borne Viruses in Finnish Field Voles

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Abstract

In northern Europe, rodent populations display cyclic density fluctuations that can be correlated with the human incidence of zoonotic diseases they spread. During density peaks, field voles (*Microtus agrestis*) become one of the most abundant rodent species in northern Europe, yet little is known of the viruses they host. We screened 709 field voles, trapped from 14 sites over 3 years, for antibodies against four rodent-borne, potentially zoonotic viruses or virus groups—hantaviruses, lymphocytic choriomeningitis virus (LCMV), Ljungan virus (LV), and orthopoxviruses (OPV). Antibodies against all four viruses were detected. However, seroprevalence of hantaviruses, LV, and LCMV was low. OPV antibodies (most likely cowpox) were more common but restricted geographically to southeastern Finland. Within these sites, antibody prevalence showed delayed density dependence in spring and direct density dependence in fall. Higher seroprevalence was found in spring than fall. These results substantially increase knowledge of the presence and distribution of viruses of field voles in Finland, as well as CPXV infection dynamics.

Key Words: Cowpox virus—Finland—Hantavirus—Ljungan virus—Lymphocytic choriomeningitis virus—Orthopoxvirus—Rodent—Vole—Zoonotic.

Introduction

To control zoonotic diseases, the identification of reservoir hosts and study of transmission dynamics in their populations, is essential (Mills and Childs 1998, Luis et al. 2013). Of taxonomic groups, rodents are considered one of the largest sources of zoonotic agents (Luis et al. 2013). In northern Europe, rodent populations display multiannual, high-amplitude, cyclic density fluctuations (Hansson and Henttonen 1985, Norrdahl 1995, Korpela et al. 2013), which can be correlated with the human incidence of zoonotic diseases they spread (for example, Kallio et al. 2009, Olsson et al. 2009). During density peaks, field voles (*Microtus agrestis*) become one of the most abundant rodent species in northern Europe (Hanski and Henttonen 1996), yet very little is known of the viruses they carry.

The purpose of this study was to evaluate zoonotic and potentially zoonotic viruses circulating in field vole populations in Finland. The best-known rodent-borne zoonotic viruses in Europe include hantaviruses, lymphocytic choriomeningitis virus (LCMV), Ljungan virus (LV), and cowpox virus (CPXV) (Kallio-Kokko et al. 2005, Kinnunen et al. 2011, Jääskeläinen et al. 2013, Vaheri et al. 2013). No human-to-human transmission has been identified for these viruses (except for LCMV infections associated with organ transplantation; Fischer et al. 2006). European hantaviruses and LCMV are naturally transmitted solely from rodents (Vapalahti et al. 2003, Charrel and de Lamballerie 2010). Human CPXV infections have emerged from pet rats and cats (Ninove et al. 2009), with wild rodents as the reservoir. The ultimately important role of rodents makes infection epidemiology in rodent populations especially germane to human risk assessment.

In Europe, several hantavirus species circulate in populations of their rodent and insectivore hosts (Olsson et al. 2010, Vaheri et al. 2013). Puumala virus (PUUV) is widely

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distributed in bank vole (*Myodes glareolus*) populations (Brummer-Korvenkontio et al. 1982, Vapalahti et al. 2003, Olsson et al. 2010), and Tula (TULV) and Tatenale (TATV, proposed name) viruses in *Microtus* voles. TULV has been mainly associated with common and sibling voles (*Microtus arvalis* and *Microtus levis*, respectively) (Plyusnin et al. 1994). However, more recent detection of TULV in other *Microtus* species (Scharninghausen et al. 2002, Plyusnina et al. 2008, Schmidt-Chanasit et al. 2010), including field voles regionally separate from other carrier species in Germany (Schmidt-Chanasit et al. 2010), indicates a wider host range. TULV is not currently considered pathogenic to humans, although a suspected case has emerged (Schultze et al. 2002, Klempa et al. 2003). TATV was recently isolated from field voles in the UK (Pounder et al. 2013).

LCMV was thought to be the only arenavirus in Europe and to reside primarily in the house mouse (*Mus musculus*) (Blasdell et al. 2008). However, high seroprevalence in other mice and vole species (including field voles) (Kallio-Kokko et al. 2006, Laakkonen et al. 2006, Blasdell et al. 2008, Tagliapietra et al. 2009), and the identification of an independent genetic lineage in wood mice (Ledesma et al. 2009), has led to the suggestion of spillover and/or the circulation of multiple related and cross-reactive arenaviruses.

LV was first isolated from bank voles in Sweden (Niklasson et al. 1999), and has since been detected in several mouse and vole species (Hauffe et al. 2010, Jääskeläinen et al. 2013), including most recently, field voles in the United Kingdom (Salisbury et al. 2014). This parechovirus has attracted research interest due to its alleged, although highly debated, association with severe human conditions (Niklasson et al. 2007, Nilsson et al. 2009). Notably, high seroprevalence to LV or LV-like virus has been detected in humans in Finland (Jääskeläinen et al. 2013).

High CPXV seroprevalence has been found in field and bank voles, and wood mice (Crouch et al. 1995, Chantrey et al. 1999, Kinnunen et al. 2011). Human infection with this orthopoxvirus (OPV) is uncommon, although suggested to be increasing following the cessation of cross-reactive smallpox vaccinations (Vorou et al. 2008). Because all OPV antibodies are cross-reactive, OPVs other than CPXV may induce some of the serological findings. CPXV is, nevertheless, the only known wildlife-borne OPV in Europe (Kinnunen et al. 2011) and is therefore used in this article to describe OPV antibody presence in field voles.

Although all of the described viruses have been reported in multiple rodent species, comprehensive surveys are required to understand occurrence patterns and draw inferences regarding the host role in virus maintenance. Here we use widespread sampling of field vole populations in Finland to evaluate the spatial and temporal distribution of antibodies to selected rodent-borne viruses, as well as factors that influence infection dynamics within their populations.

Methods

Vole trapping and abundance

Field voles were trapped from 14 open grassland fields, each more than 1 ha, across central-eastern Finland (Fig. 1). Trapping was conducted at each site in spring (late April–May) and fall (late September–October) for 3 consecutive years to include each phase of a vole cycle (fall 2008 to spring 2011). The fall 2010 trapping occasion at Tohmajärvi was not possible due to unavailability of the site. These sites are included in the long-term national vole monitoring program (see Korpela et al. 2013), and past abundance data are available for most.

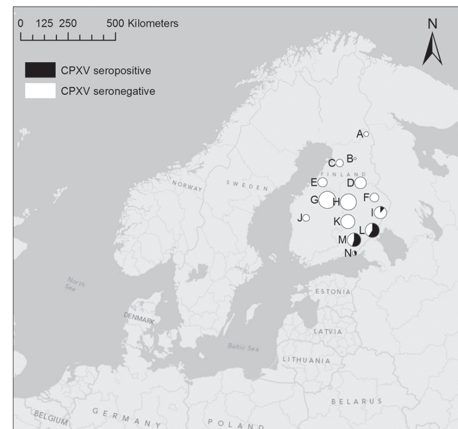

FIG. 1. Trapping sites in Finland. Circle size denotes the total number tested [3 (B) to 110 (G) individuals] for antibodies against the four viruses. Black and white colors refer to CPXV-seropositive and -seronegative individuals, respectively.

For each sampling occasion, 100 standard metal mouse snap-traps were set in clusters of three along a line with an intercluster distance of 10–20 meters. Traps were baited with a small piece of bread, and left for one night. The following morning captured voles were measured, sexed, aged (overwintered or not overwintered), and frozen at less than $-20°C$.

Dissection and serology

Voles were thawed, and the heart was removed and placed into a tube with phosphate-buffered saline (Sironen et al. 2002). Lung and liver samples were also collected from each individual and refrozen for potential PCR analyses. Occasionally voles were damaged during the trapping process or by scavengers, thereby preventing the ascertainment of organ samples. Antibodies reactive to PUUV/hantaviruses, LCMV, LV, and CPXV/OPV were detected from heart extracts using immunofluorescent antibody tests (IFAT), as described previously (Kallio-Kokko et al. 2006, Kinnunen et al. 2011, Jääskeläinen et al. 2013).

Statistical analyses

Only CPXV prevalence was sufficient to permit further enquiry. Variation in CPXV seroprevalence was studied using data from sites where CPXV antibodies were detected on at least one trapping occasion. Throughout the sampling period, only four early spring–born juvenile voles were captured in spring and no overwintered voles in fall. Juveniles were therefore removed from spring data, and generalized

linear models with binomial error distributions and a logit link function were used to separately evaluate CPXV seroprevalence in spring and fall. The full models for each season included site, year, current density, density on the previous trapping occasion, weight, sex, and the interaction of weight and sex. Weight and densities were centered by mean. A model set constituting 95% of Akaike weights of all models nested within the full model was then averaged (Grueber et al. 2011) using the MuMIn package (Barton 2011) in R software (R Development Core Team 2012). A generalized linear model, including the main effects of site, year and season, was used to compare seroprevalence between spring and fall.

Results

A total of 715 field voles were captured from fall 2008 to spring 2010, of which 709 were tested for antibodies against the four rodent-borne viruses. Ten (1.4%), 17 (2.4%), and four (0.6%) voles were seropositive to hantaviruses, LCMV, and LV, respectively (results are summarized in Table 1). RT-PCRs specific for respective viruses were conducted on lung samples from individuals seropositive to hantavirus and LCMV (also some samples from seronegative voles within the same sites) (Klempa et al. 2006, Vieth et al. 2007), and on liver samples from individuals seropositive to LV (Donoso Mantke et al. 2007). All results were negative (data not shown).

A total of 117 voles were seropositive to CPXV. All seropositive individuals were captured from four sites (Fig. 1), where seroprevalence on a sampling occasion ranged from 0% to 93% (Fig. 2). The likelihood of an individual field vole to be seropositive in spring was positively associated with vole density in the previous fall (Table 2, Fig. 3), and in fall, positively associated with current density. Seroprevalence to CPXV was higher in spring than fall (estimate = 1.27 ± 0.47, $Z = 2.7$, $p = 0.007$).

TABLE 1. SITE-BASED SEROPREVALENCE TO HANTAVIRUSES, LYMPHOCYTIC CHORIOMENINGITIS VIRUS, AND LJUNGAN VIRUS

Code	*Site*	*No. sampled*	*Hantavirus*	*LCMV*	*LV*
E	Kannus	38	1 (2.6%)	1 (2.6%)	0
J	Karvia	23	0	1 (4.3%)	0
F	Koli	35	0	2 (5.7%)	0
A	Kuusamo	13	1 (7.7%)	0	0
M	Luumäki	73	1 (1.4%)	1 (1.4%)	0
K	Mikkeli	78	0	0	2 (2.6%)
C	Muhos	27	0	1 (3.7%)	0
L	Punkaharju	79	0	1 (1.3%)	1 (1.3%)
B	Puolanka	3	0	0	0
D	Sotkamo	57	1 (1.8%)	1 (1.8%)	0
H	Suonenjoki	100	1 (1.0%)	2 (2.0%)	0
I	Tohmajärvi	62	2 (3.2%)	2 (3.2%)	0
G	Viitasaari	110	3 (2.7%)	5 (4.5%)	1 (0.9%)
N	Virolahti	11	0	0	0
	Total	709	10 (1.4%)	17 (2.4%)	4 (0.6%)

The number of sampled voles and seropositive individuals is summed for the six trapping occasions (five for Tohmajärvi). The percentage of seropositive voles is shown in brackets alongside the number seropositive. Codes correspond to the map in Fig.1.

LCMV, lymphocytic choriomeningitis virus; LV, Ljungan virus.

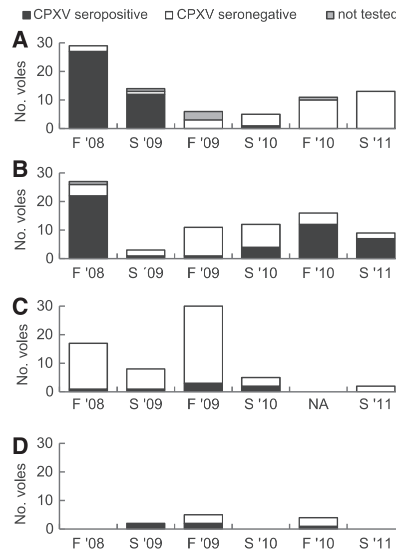

FIG. 2. Summary information of CPXV seropositive sites (S, spring; F, fall). (**A**) Luumäki (M in Fig. 1); (**B**) Punkaharju (L); (**C**) Tohmajärvi (I); and (**D**) Virolahti (N).

Discussion

This is the first study to evaluate the occurrence of zoonotic viruses in this highly abundant and widely fluctuating rodent species, the field vole, in northern Europe. Research on viruses of field voles has been neglected, largely due to emphasis on Puumala hantavirus in bank voles (*M. glareolus*), the causative agent of nephropathia epidemica (hemorrhagic fever with renal syndrome) and a common zoonosis in Finland (Vaheri et al. 2013). As such, we present new results on the distribution of zoonotic viruses and the first description of cowpox virus dynamics in field vole populations of northern Europe.

Hantaviruses in arvicoline rodents (voles and lemmings) are highly cross-reactive (Vaheri et al. 2008). In our earlier unpublished smaller surveys, hantavirus antibodies were regularly detected in field voles in Finland (prevalence 3–5%), but no antigen was found. Therefore, spillover of PUUV from sympatric bank voles was considered the source. TULV has been found in field voles in central Europe (Scharninghausen et al. 2002, Schmidt-Chanasit et al. 2010), and TATV virus in England (Pounder et al. 2013). The RT-PCR employed detects all hantavisuses (PUUV, TULV, and TATV). Due to the lack of PCR-positive field vole samples (hantavirus infections are chronic with presence of RNA and antigen in tissues of reservoir hosts; Easterbrook and Klein 2008), PUUV spillover remains the probable cause of antibodies in the sampled field voles.

Antibodies against LV (or a LV-like virus) were recently identified in Finland for the first time, in both bank voles and humans (Jääskeläinen et al. 2013). Although the virus

Table 2. Averaged Coefficients of Generalized Linear Model Sets with Binomial Error Distributions Used to Examine the Likelihood of a Field Vole Being Cowpox Antibody Positive on a Site Where Cowpox Antibodies Were Detected at Least Once

Parameter	*Spring estimate (SE)*	Z	p	*Fall estimate (SE)*	Z	p
Intercept	−0.70 (0.70)	1.0	0.326	0.51 (0.69)	0.7	0.469
Current abundance	0.12 (0.16)	0.7	0.460	**0.19 (0.08)**	**2.6**	**0.011**
Past abundance	**0.16 (0.05)**	**2.8**	**0.004**	0.25 (0.15)	1.7	0.095
Male sex	0.06 (0.81)	0.1	0.943	0.17 (0.53)	0.3	0.752
Body mass	−0.12 (0.10)	1.2	0.234	0.06 (0.05)	1.3	0.180
Male sex × body mass	−0.24 (0.16)	1.5	0.142	0.02 (0.09)	0.2	0.867
Site (Punkaharju)	1.37 (0.91)	1.5	0.142	1.04 (0.76)	1.4	0.175
Site (Tohmajärvi)	−1.26 (1.24)	1.0	0.320	−3.15 (1.74)	1.8	0.070
Site (Virolahti)	19.51 (1696.28)	0.0	0.991	4.14 (1.64)	2.5	0.012
Year (2010/2009)	−0.29 (1.13)	0.3	0.800	−2.97 (1.52)	1.9	0.052
Year (2011/2011)	−0.19 (1.06)	0.2	0.861	−2.32 (1.71)	1.4	0.177

Intercepts represent a female of average body mass at site M (Luumäki) in year 2008 (spring) or 2009 (fall) in a population of average current and past densities. Estimates are given on a logit scale; statistically significant coefficients are in boldface.

presence is now known, the search for animal reservoirs, and identification of the circulating strain(s), remains. In this study, LV seroprevalence was low and viral RNA non-detectable, which is indicative of spillover from sympatric species, probably bank voles (Hauffe et al. 2010, Jääskeläinen et al. 2013). Our finding contrasts those from the United Kingdom where high prevalence was reported in field vole populations through PCR (Salisbury et al. 2013).

The relationship between LCMV and field voles is unclear. Although seroprevalence was mostly low, at some sites it was within the range reported for house mouse populations (Childs et al. 1992). In particular, four of 45 (8.9%) voles tested from Viitasaari (G in Fig. 1) in fall 2008 were seropositive. Our results are in line with the earlier findings of LCMV-like antibodies in a number of rodent species in Europe without sympatric *Mus* species (Laakkonen et al. 2006, Tagliapietra et al. 2009), and thus support the circulation of multiple LCMV-like strains (see Ledesma et al. 2009).

Contrary to the other viruses, seroprevalence to CPXV was high in certain populations. A localized distribution was identified in southeastern Finland (Fig. 1). Moreover, this geographical area corresponds to OPV antibody findings in other rodent species, cats, dogs, horses, and lynxes (Pelkonen et al 2003, Kinnunen 2011), and importantly, to a severe human cowpox case in a 4-year-old girl from 2000 (Pelkonen et al. 2003). Of note, bank voles were also captured in the two sites with highest field vole CPXV seroprevalence (Fig. 1), suggesting potential interspecific transmission. Pelkonen et al. (2003) found high seroprevalence in bank voles in Southern Finland.

At CPXV-positive sites, antibody prevalence showed delayed density dependence in spring and direct density dependence in fall. Density dependence, along with the finding that CPXV reduces field vole survival (Burthe et al. 2008), indicates that CPXV may contribute to the cyclic regulation of vole populations. Although the identified spatial distribution precludes any widespread regulatory effect in Finland,

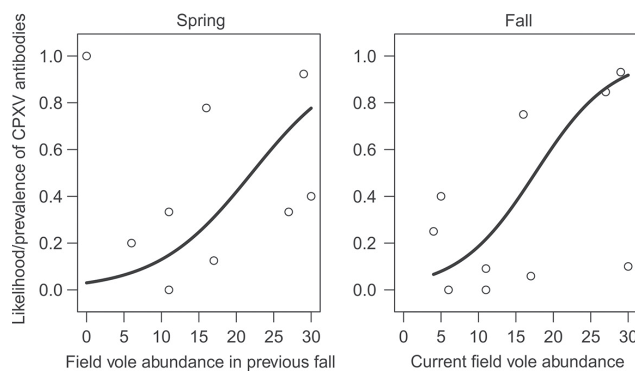

FIG. 3. The predicted probability of a field vole being CPXV antibody positive in spring in relation to field vole density the previous fall and in fall in relation to current density based on averaged model coefficients in Table 2. Circles denote observed prevalence.

elsewhere and at local scales the potential contribution of cowpox virus to vole density fluctuations warrants further investigation.

CPXV seroprevalence was higher in spring than fall. The relatively high proportion of seropositive voles in spring is probably diluted toward fall by recruitment of naïve juvenile voles during spring and summer. Reproduction essentially ceases during winter (Myllymäki 1977), while transmission continues to occur. It is worth noting that CPXV is a DNA virus with a short viremia of 2–3 weeks, whereas hantaviruses and arenaviruses cause chronic infection. Therefore, the transmission dynamics of these viruses differ. For the same reason, PCR identification of CPXV-positive individuals is more difficult (Kinnunen et al. 2011).

In summary, serological evidence of hantavirus, LCMV, LV, and CPXV was found in field vole populations of Finland. These are the first published results on viral pathogens based on comprehensive field vole sampling. Although seropositivity to hantavirus was shown, no PCR-positive field voles were found, supporting the idea of spillover from sympatric species. LV has been associated with bank voles (Hauffe et al. 2010, Jääskeläinen et al. 2013), and the host role of field voles may be minor. The evidence is less clear for LCMV. CPXV antibodies were locally common, and antibody prevalence was most influenced by population density and season. However, the influence of sympatric species, particularly bank voles, deserves further attention.

Acknowledgments

We thank Elizabet Martinez, Irina Suomalainen, and Kirsi Aaltonen for assistance with serology tests. The study was financially supported by the Academy of Finland (grant 133495 to O.H.) and partially funded by European Union grant FP7-261504 EDENext and is catalogued by the EDENext Steering Committee as EDENext179 (www.edenext.eu).

Author Disclosure Statement

No competing financial interests exist.

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II

DIET QUALITY LIMITS SUMMER GROWTH OF FIELD VOLE POPULATIONS

by

Kristian M. Forbes, Peter Stuart, Tapio Mappes, Katrine S. Hoset, Heikki Henttonen & Otso Huitu 2014

PloS One 9: e91113.

OPEN ACCESS Freely available online

PLOS ONE

Diet Quality Limits Summer Growth of Field Vole Populations

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Abstract

Marked variation occurs in both seasonal and multiannual population density peaks of northern European small mammal species, including voles. The availability of dietary proteins is a key factor limiting the population growth of herbivore species. The objective of this study is to investigate the degree to which protein availability influences the growth of increasing vole populations. We hypothesise that the summer growth of folivorous vole populations is positively associated with dietary protein availability. A field experiment was conducted over a summer reproductive period in 18 vegetated enclosures. Populations of field voles (*Microtus agrestis*) were randomised amongst three treatment groups: 1) food supplementation with *ad libitum* high protein (30% dry weight) pellets, 2) food supplementation with *ad libitum* low protein (1% dry weight; both supplemented foods had equivalent energy content) pellets, and 3) control (no food supplementation), n = 6 per treatment. Vole density, survival, demographic attributes and condition indicators were monitored with live-trapping and blood sampling. Highest final vole densities were attained in populations that received high protein supplementation and lowest in low protein populations. Control populations displayed intermediate densities. The survival rate of voles was similar in all treatment groups. The proportion of females, and of those that were pregnant or lactating, was highest in the high protein supplemented populations. This suggests that variation in reproductive, rather than survival rates of voles, accounted for density differences between the treatment groups. We found no clear association between population demography and individual physiological condition. Our results demonstrate that dietary protein availability limits vole population growth during the summer growing season. This suggests that the nutritional quality of forage may be an underestimated source of interannual variation in the density and growth rates of widely fluctuating populations of herbivorous small mammals.

Citation: Forbes KM, Stuart P, Mappes T, Hoset KS, Henttonen H, et al. (2014) Diet Quality Limits Summer Growth of Field Vole Populations. PLoS ONE 9(3): e91113. doi:10.1371/journal.pone.0091113

Editor: Gabriele Sorci, CNRS, Université de Bourgogne, France

Received November 4, 2013; **Accepted** February 7, 2014; **Published** March 12, 2014

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Funding: The experiment was funded by the Academy of Finland (www.aka.fi/eng: grant 133495 to OH, grant 132190 to TM). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: Tapio Mappes is a PLOS ONE editorial board member. This does not alter the authors' adherence to PLOS ONE editorial policies and criteria.

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Introduction

Populations of northern small mammals are renowned for their high-amplitude density cycles, with peaks every 3–5 years [1–5]. Although delayed density-dependent predation is often considered the principle driver of cyclic dynamics [6–10], regulatory processes are likely to be multifactorial and geographically variable [1,11]. Hence, consensus on causal factors behind cyclicity has not been reached despite several decades of research [1,10–14].

Boreal vole cycles typically involve two successive years of variable but positive population growth in summer and negative or zero population growth in winter [2,15–16]. The peak of a multiannual cycle is usually attained in late summer to autumn of the latter increase year, after which winter food depletion initiates a population decline [17–18]. The year following peak density is characterized by a summer decline, when populations typically decrease in size from spring to autumn [1–2,15].

The growth rate of vole populations varies profoundly between years, including years representing the same cycle phase. The overall amplitude of multi-annual cycles (i.e. the difference between maximum and minimum densities) also varies markedly within and between sites [1–2,8,19]. Cycle amplitude is generally greater in cooler and more continental areas than in temperate, mild coastal areas, where density variations are predominantly seasonal [1,20]. These differences have traditionally been attributed to winter severity and amount of snowfall, which are negatively associated with the stabilising effect of generalist predators on voles and their specialist predators [1,8].

Recent reports have documented a widespread collapse of small rodent population cycles [21], often attributed to changing winter climate [22–24]. Korpela et al. [19] presented evidence to challenge this association, and instead, using extensive time-series vole monitoring and climatic data from Finland, highlighted a connection between weather conditions during spring and summer, and vole population growth. The latter relationship is potentially mediated by variation in forage quality (e.g. [25–26]).

For herbivores, forage quality is to a considerable degree determined by nitrogen content, which is often a primary limiting factor for the growth of populations (nitrogen limitation hypothesis [27–30]). Nitrogen levels in plants vary in response to a range of

biotic and abiotic factors, such as weather, leading to both spatial and temporal variation in its availability to herbivores [31–32]. For example, Cole and Batzli [33] identified that different vegetation types altered the density, reproductive performance and survival of wild prairie vole populations, and concluded that highly nutritional forage can elevate peak population densities. Additionally, a midsummer cessation of breeding, often occurring in cyclic folivorous voles during the height of the summer growing season (termed a 'midsummer crisis' for voles), is hypothesized to result from nutritional changes in plants during their reproductive phenology [15].

The physiological health state of individuals may vary before translating into changes in population demography. For example, populations of small mammals are characterised in decline years by small individual body size, as well as low reproductive output and adult survival (the Chitty effect [34–36]). Haematological indices, e.g., levels of albumin, haematocrit and immunoglobulins, can also reflect the quality of individual dietary intake [37–40].

The objective of our study is to evaluate the extent to which protein availability limits the density and population growth of small mammals during northern European summer, a time of seemingly superabundant food resources. We hypothesise that protein supplementation will have positive effects, proximately on the physiological condition of voles and ultimately on population growth, as compared to non-supplemented populations. Specifically, we predict that the positive response will be more pronounced in populations that receive high protein food than in those that receive supplemental food with equivalent levels of energy but low protein. As model species we use the folivorous field vole (*Microtus agrestis*), the most widely distributed of fluctuating small rodents throughout Fennoscandia, and often considered the driver of population cycles in northern Europe [41].

Materials and Methods

Ethics statement

The experiment was conducted on private land near the town of Suonenjoki in Central Finland [lat 62° 45.672′, lon 27° 6.015′; ETRS89 geographic coordinates (~WGS84)]. Permissions for carrying out experiments at this location were obtained from the land owner, whose contact information is available from the authors upon request. The study did not involve endangered or protected species. The experiment was approved by the Finnish Animal Ethics Council (permit ESAVI/1437/04.10.03/2011). Field technicians were trained prior to the experiment and took all possibly precautions to minimise animal stress during trapping and sampling.

Enclosures and experimental design

The experiment was conducted in 18 adjoining field enclosures (20×25 m each) with natural meadow vegetation, dominated by the grasses *Phleum pratense* and *Deschampsia caespitosa*. Enclosures were constructed of sheet metal rising approximately one meter above ground and extending 50 cm underground. Thereby, vole movement between enclosures was prevented and access by mammalian predators of voles (mustelids) restricted. Avian predators had access to the enclosures but were very rarely observed in the area during the experiment. Each enclosure contained eight sheet-metal shelter boxes (40×40×50 cm, with two entrance hole at the base) approximately 10 m apart, in a 3×2×3 configuration. An Ugglan Special live trap (Grahnab, Sweden) was placed in each shelter box.

Enclosures were randomly allocated to one of three treatment groups: 1) *ad libitum* high-protein (30 per cent dry weight crude protein) food supplementation, 2) *ad libitum* low-protein (1 per cent) food supplementation, or 3) control (no food supplementation). The energy content of the two protein treatments (30% and 1%) was unchanged at 3500 kcal/kg. Food supplementation was supplied through specifically formulated pellets (Altromin, Lage, Germany) available from a wire mesh feeder placed in each shelter box.

At the beginning of June 2011, six field voles (three males, three females) were introduced to each enclosure. The first trapping occasion was conducted two weeks later to obtain baseline abundance estimates representative of established individuals. A total of two male and three female voles were introduced to four enclosures (2 high protein and 2 low protein) to replace voles that had apparently died between introduction and baseline trapping. Food supplementation began immediately following baseline trapping on June 16, 2011, and continued until immediately prior to the final trapping in mid-September (13th) 2011. The experiment thus encompassed the primary reproductive period of field vole populations in central Finland [15].

Vole monitoring and sampling

Abundance monitoring and vole blood sampling was conducted every fourth week for a total of four trapping occasions. On each occasion, traps were baited with oats and checked consecutively at 7 am, 2 pm and 9 pm, for a total of 8–9 times over three days. An electronic PIT-tag (EID Aalten BV, Aalten, Netherlands) was subcutaneously injected into every vole upon first capture and the unique identification number recorded at each encounter. Voles were placed into ventilated buckets and taken to an on-site field laboratory where their sex and reproductive status (males: subadult, mature; females: subadult, mature, pregnant and/or lactating) was determined through external examination. Body mass and head width were measured (to nearest 0.1 g and 0.1 mm, respectively), and approximately 150 µl of blood was collected from the retro-orbital sinus with heparinized capillary tubes. Blood was not collected from juvenile individuals weighing under 15 g. Voles were then released into the same enclosure as captured, except on the final trapping occasion when voles were removed from enclosures. Upon encountering an individual that had already been sampled for blood during the trapping occasion, the vole was immediately released at the point of capture after recording its identification number, sex, reproductive status and weight.

Vole abundance (hereafter density) was estimated separately for each enclosure and trapping occasion (18 enclosures × 4 occasions = 72 population density estimates) using the program CAPTURE [42]. M_h models (which incorporate heterogeneity in capture rates) with the jackknife estimator were employed for trapping occasions one to three. Throughout this period, four enclosures experienced one trapping occasion in which no individual was recaptured after their initial capture. In these cases, density was estimated with removal (M_{bh}) models (Pollock and Otto's estimator [43]). During the final trapping occasion, voles were removed from enclosures upon first capture and density was estimated with removal models. Rarely, voles were found dead in traps or died during sampling (approximately 3% of captures). These individuals were excluded from the density estimation models, but added to the final estimate [42]. A population growth rate was calculated for each trapping interval based on the formula, $R_t = \ln(N_{t-1}/N_t)$, where N_t is the population density at time t [18,44].

Vole survival rate was calculated separately for each enclosure and trapping interval using program MARK 7.0 [45]. Since survival estimates partially depend on recapture rate, Akaike's information criterion (AIC) -based model selection was employed [46] to compare recapture rate models including enclosure, trapping occasion, their permutations or only the intercept. Due to a small difference in AIC values between the two most parsimonious models (ΔAIC <2), final survival estimates were obtained using a weighted model averaging procedure, taking model selection uncertainty into account [46].

Condition indices

Body condition index was expressed as the studentized residuals of a random coefficients regression model of individual body mass on head width [47]. Identity of the head width measurer was entered as a random factor in the model to adjust for potential individual variation in head width measurements. Only mature males were included in the analysis of condition index to avoid confoundment by juveniles and reproducing females.

Vole blood was centrifuged at 12 000 g for five minutes, and haematocrit expressed as the percentage of packed red blood cells in total volume. Blood plasma was then separated and frozen (<−20°C) before enzyme-linked immunosorbent assays (hereafter, ELISA).

Total IgG antibody titres were measured according to the following protocol. Solid anti-mouse conjugate was pushed through a 0.22 µm syringe filter and dissolved in 0.135 M NaCl. Plate wells were then coated with 50 µl of anti-mouse IgG (M-8642, Sigma, lot 060M6082) solution (1 mg/ml) and incubated for a minimum of 12 hours at +4°C. Wells were emptied, and masked with 100 µl 1% bovine serum albumin in phosphate-buffered saline (BSA-PBS) and incubated for 60 minutes at room temperature. Wells were then emptied, washed and pat dried. 50 µl of plasma sample (diluted at 1:40000 with BSA) was added to duplicate wells. A standard was prepared by combining 2 µl from each sample over all trapping occasions. Duplicate standard concentrations of 200, 150, 100, 50, 25, 10, 5 and 0 were run on each plate simultaneously with samples. Plates containing samples and standards were then incubated for 3 hours at room temperature. Following incubation, solutions were removed and the wells washed. 50 µl of alkaline phosphatase conjugated anti-mouse IgG (A-2179, Sigma, lot 31K4852), diluted at 1:4000 with BSA-PBS, was added to each well and plates incubated for a minimum of 12 hours at +4°C. Following incubation, wells were washed and pat dried, and 50 µl of substrate (1 mg pNPP [P4744, Sigma, lot 109K6076] to 1 ml DEA buffer) was added to each. Plates were then incubated in the dark and read at 405 nm with a Thermo Labsystems Multiskan Ascent 354 platereader after 15, 30, 45, 60 and 75 minutes. An absorbance approximately mid-way between the standard dilutions is most desirable. After comparing absorbance levels, 45 minutes of incubation was deemed the most appropriate. The mean absorbance of the sample duplicates was used as the final measure. On rare occasions when an anomalous result occurred, the plausible duplicate was used alone.

A commercially available mouse-albumin ELISA kit (Alpha Diagnostics International, Texas) was used to measure the albumin concentration of vole plasma as per manufacturer's instructions. An anti-mouse albumin-HPR conjugate was used and plates were read at 450 nm using the Thermo Labsystems Multiskan Ascent 354 platereader.

Statistical analyses

Random coefficients regression models (PROC MIXED) were used to evaluate the individual and interactive effects of time (week of year as a continuous variable) and treatment on vole density, with the intercept and week as random effects. The effect of population mean condition index on density was evaluated in a separate model. For this, data were restricted to the final three trapping occasions, and condition at the previous trapping occasion (t-1) set as an initial explanatory variable, along with time, treatment and their interactions. The intercept and time were again used as random effects.

Due to the positive correlation between density and week ($P < 0.001$), analyses of growth rate (R_t), survival, condition index, total IgG, haematocrit and albumin content were carried out with repeated-measures mixed ANOVA models (PROC MIXED) with trapping occasion as a repeated categorical variable. Other initial fixed explanatory variables were treatment, density and all possible interactions. Enclosure and enclosure × trapping occasion were included as random factors (for IgG and albumin models the ELISA plate number was also included as a random factor). Repeated covariate type (autoregressive, unstructured, compound symmetry or toeplitz) selection was based on AIC of the full model. Model selection was thereafter based on a stepwise reduction approach, guided by AIC values and biological importance, using Kenward and Roger estimation [48]. Model comparisons were made using the maximum likelihood (ML) method, and final values obtained from the most parsimonious model with restricted maximum likelihood (REML). Sexes were analysed separately when possible, and model validity was verified via the residual distribution. To assess for delayed effects of density, the data were restricted to the final two trapping occasions and each response model incorporating current density compared to models including densities for the two preceding trapping occasions (t-1, t-2). Unstructured repeated covariate type was employed in these reduced models.

To facilitate interpretation of a three-way interaction between density, treatment and trapping occasion in the final survival model (Table 1), a mixed model was constructed with density to explain survival. Enclosure, with intercept, was set as a random factor. Residuals of this model were then used as the response variable in a repeated ANOVA model in which survival was explained by treatment, trapping occasion and their interaction, as per the methods described above.

Generalized linear mixed models (PROC GLIMMIX), employing the same methodology and fixed and random factors, were used to evaluate changes in the proportion of males (sex ratio), voles weighing less than 20 g (as representative of juvenile recruitment), and reproducing females from the total female population. As external signs of reproduction or juveniles were not yet present at the onset of the experiment, baseline data were removed from these models. Generalized models were assessed for over-dispersion. Data were analysed in SAS version 9.3 (SAS Institute Inc., Cary, NC).

Results

Population size

The effect of treatment on density changed over time (Table 1). Densities were similar among treatment groups for the initial three trapping occasions (Figure 1a). However, by September densities were greater in high protein than low protein treatment populations, while control groups displayed an intermediate level of density, which did not differ from either supplementation group. Population density was not influenced by mean condition index ($F^{\text{condition index (t-1)}}_{1,\,38} = 2.47$, $P = 0.124$).

Mean population growth rates were predominantly positive throughout the experiment and varied between treatment groups

Table 1. Most parsimonious model to explain each response variable.

Response	Source of variation	Num. df	Denom. df	*F*	*P*
Density	week	1	56	45.35	<0.0001
	treatment	2	51	6.35	0.0034
	week × treatment	2	56	8.12	0.0008
Growth rate	occasion	2	22	3.08	0.07
	treatment	2	11	4.22	0.0445
	density	1	32	11.12	0.0022
Survival	occasion	2	32	6.55	0.0042
	treatment	2	35	4.98	0.0126
	density	1	36	4.71	0.0368
	density × treatment	2	35	4.60	0.0168
	density × occasion	2	34	7.20	0.0025
	treatment × occasion	4	31	1.67	0.18
	density × treatment × occasion	4	33	2.76	0.0434
Prop. males	occasion	3	44	2.53	0.07
	treatment	2	119	0.41	0.67
	density	1	119	1.41	0.24
	treatment × occasion	6	119	0.89	0.50
Prop. reproducing females	occasion	2	26	2.94	0.07
	treatment	2	51	0.78	0.47
	density	1	51	7.12	0.0102
	treatment × occasion	4	51	1.04	0.40
Prop. <20 g	occasion	2	227	3.06	0.06
	treatment	2	79	0.13	0.89
	density	1	79	7.62	0.0072
	density × occasion	2	79	3.36	0.0397
	treatment × occasion	4	79	5.27	0.0008

Final values were obtained with REML. Full models contained time (week or trapping occasion), treatment group, density, and all their interactions as initial explanatory variables. Trapping occasion is a categorical variable. Week denotes the week of year and is continuous. Enclosure and enclosure × time were set as random variables.
doi:10.1371/journal.pone.0091113.t001

(Table 1, Figure 1b). Population growth rate was negatively associated with density in all treatment groups (Table 1, Figure 1c).

Demographics and survival

Survival rates differed with density, treatment and time (Table 1). Survival was higher in low protein populations than in other groups from June to July (Figure 2). From July to September, all rates stabilized with approximately 70% of voles surviving between trapping occasions (Table 1). Neither treatment ($F^{treatment}_{2,18}$ = 0.80, P = 0.47) nor trapping occasion ($F^{occasion}_{2,33}$ = 0.96, P = 0.39) affected survival in the density-corrected model.

The sex ratio of the populations was unaffected by the treatments (Table 1, Figure 3a). Meanwhile, the proportion of reproducing females, out of all females, decreased with increasing

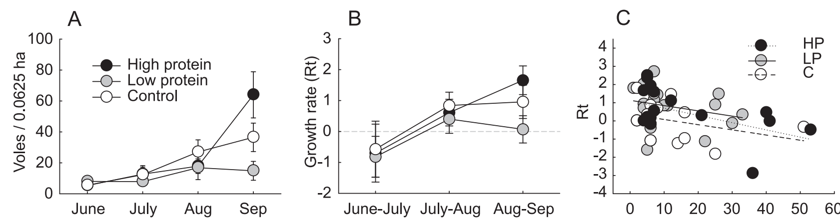

Figure 1. Size and growth of experimental field vole populations. (A) density (mean ± se), (B) growth rate (Rt) (least squared mean ± se), (C) population growth rate by density.
doi:10.1371/journal.pone.0091113.g001

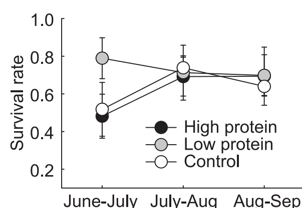

Figure 2. Treatment-wise population survival rate (mean ± se).
doi:10.1371/journal.pone.0091113.g002

population density, regardless of treatment (Estimate = −0.0161, s.e = 0.006, Table 1, Figure 3b). The proportion of juvenile voles (<20 g) varied between treatment groups and trapping occasions (Table 1, Figure 3c), being highest in control populations in July, and lowest by August. High protein populations displayed the greatest proportion of juveniles in August, but lowest in September.

Indicators of condition

No associations between treatment and body condition index were identified (Figure 4a). Nor were there significant differences between treatment groups in male haematocrit (Figure 4b). Male haematocrit was negatively density dependent at the beginning of the experiment, but the relationship dissipated with time ($F^{density\times occasion}_{3,\ 62} = 4.35$, $P = 0.008$). Density in the previous trapping occasion explained male haematocrit from August to September better than current density ($\Delta AIC = 2.0$). Male haematocrit thus exhibited delayed density dependence in high protein populations ($F^{density(t\text{-}1)\times treatment}_{2,17} = 4.22$, $P = 0.032$). A negative effect of density on male albumin, that was present at the beginning of the experiment, relaxed with time ($F^{density\times occasion}_{3,\ 86} = 2.62$, $P = 0.056$, Figure 4c). Current density explained male albumin levels better than past density ($\Delta AIC = 4.7$), but none of the explanatory variables reached significance. Meanwhile, male IgG was higher in June ($t = 2.69$, d.f $= 5$, $P = 0.046$) and September ($t = 3.55$, d.f $= 12$, $P = 0.004$) than July, but did not vary between treatment groups ($F^{occasion}_{3,\ 8} = 5.05$, $P = 0.032$, Figure 4d). Density in the previous trapping occasion was again a better predictor of male IgG than current density ($\Delta AIC = 3.2$), but none of the explanatory variables reached significance.

Female haematocrit consistently increased in high protein populations during the experiment (Figure 5a). However, interpretation is confounded by three-way interactions with both current and past density ($F^{density\times occasion\times treatment}_{6,\ 34} = 33.8$, $P = 0.039$; $F^{density(t\text{-}2)\times occasion\times treatment}_{2,\ 77} = 3.18$, $P = 0.047$). Meanwhile, no significant effects on female albumin were identified (Figure 5b), including density two occasions prior, which explained the data better than current density ($\Delta AIC = 6.6$). No effects of treatment group were identified in female total IgG in the full model (Figure 5c). However, a delayed density-dependent decrease in female IgG present in August, had disappeared by September ($F^{density(t\text{-}1)\times occasion}_{1,\ 93} = 7.56$, $P = 0.007$).

Discussion

Consistent with our hypothesis, the summer growth of vole populations was limited by the availability of dietary proteins. Food resources are of great importance to the population dynamics of herbivores [49–51], including cyclic small mammals [17,52–53]. In general, the quantitative effects of resources on vertebrate herbivore populations have been extensively studied [54], while the effects of food resource quality remain little investigated. Similarly, in voles the limiting effects of food on population demography has manifested through quantity, and predominately only during winter [18]. As such, our experimental results offer important insights into processes contributing to variation in herbivore density – namely the availability of high-quality food during the growing season.

Survival rates of voles did not differ between treatment groups during the experiment. Therefore, the observed differences in density are largely attributable to increased recruitment through reproduction. This is supported by the tendency of high protein populations to consist of few males and many reproducing females as compared to the other treatment groups. However, increased rates of reproduction were not reflected in the proportion of juvenile voles, which were lowest in September when the population growth rate was highest. Desy and Batzli [12] identified the same peculiarity which they attributed to faster growth of juveniles with food supplementation. In other words, high quality food enables voles to grow faster than lower quality food. Indeed, protein supplementation has been found to accelerate the growth of small rodent individuals [55]. For this reason, it was not appropriate to evaluate functional group differences in survival and condition between juvenile and adult voles in the current experiment. It should be noted that the quality of available food resources may affect the demographic rates of vole populations differently in winter than in summer – this remains a topic for further experimentation.

Interestingly, control treatment groups in our experiment attained approximately half the densities of high protein

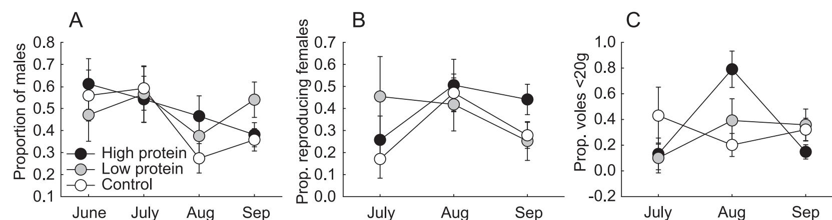

Figure 3. Demographic attributes of experimental populations (least squared mean ± se). (A) proportion of males in total population, (B) proportion of reproducing (pregnant and/or lactating) females in total female population, (C) proportion of voles <20 g from total population.
doi:10.1371/journal.pone.0091113.g003

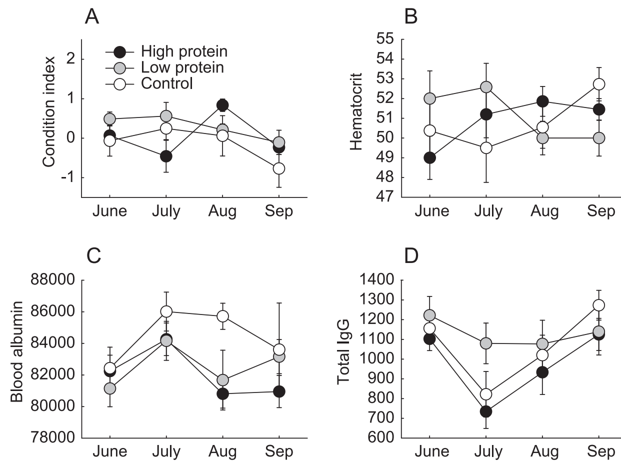

Figure 4. Condition indices of male voles from experimental populations (mean ± se). (A) body condition index, (B) haematocrit, (C) plasma albumin, (D) total IgG antibody titer.
doi:10.1371/journal.pone.0091113.g004

supplemented groups (Fig. 1). The protein content of our high-quality supplementation was 30%, while crude protein levels in grasses (including *Phleum pratense*) at the end of the growing season are about 10–15% of dry weight [56]. It is therefore tempting to entertain the idea that summer vole densities closely reflect the levels of dietary protein available to voles in their forage.

Contrary to our predictions, neither body condition nor haematological indices were clearly associated with experimental treatments or population density. Nevertheless, the identification of changes over time, and interactions with current and past density, highlight the complex interactions and potential utility of these measures in population ecology research. It should be noted that interpretation of haematological indices is difficult and several parameters are usually required to provide an adequate representation of health status [57]. For example, elevated total IgG could represent high baseline immunity levels resulting from good health

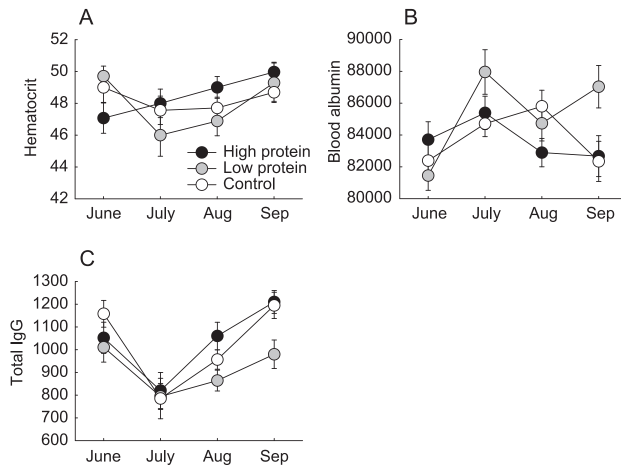

Figure 5. Condition indices of female voles from experimental populations (mean ± se). (A) haematocrit, (B) plasma albumin, (C) total IgG antibody titer.
doi:10.1371/journal.pone.0091113.g005

or an immune response to infection [39]. Similarly, low albumin may be a sign of a protein deficient diet or infection [58]. Since we employed several health indicators without observing treatment effects, it appears that voles were able to maintain good physiological condition during the breeding season on natural food resources alone (for contrasting results during the non-breeding season, see [39]). In the context of our treatments, it seems plausible that voles which received supplemental protein were allocating it foremost to reproduction, as opposed to elevating their own physiological condition (i.e., income breeding [59]).

Korpela et al. recently highlighted the association between summer growing conditions and the dynamics of vole populations [19]. However, their study did not identify proximate mechanisms acting during summer. Climate, amongst other things, has been shown to alter nitrogen levels in plants [31–32], and we have demonstrated here that summer protein levels are a plausible mechanistic link between climate and vole population demography. In further support, a 'midsummer crisis', hypothesized to result from a shortage of high-quality food due to graminoid senescence [15], did not present in high protein treatment groups. Meanwhile, the growth rate of low protein populations was clearly reduced between the final two trapping intervals without obvious changes in survival rates. Further research is nonetheless needed to elucidate causalities between climate and herbivore diet quality.

Considerable debate has focused on factors which limit and regulate cyclic populations of small mammals. A common line of differentiation is between intrinsic (for example age structure and maternal or juvenile environment: see [60–63]) and extrinsic factors (the environment, including predation: see [6,9]. However, recent transplant experiments have provided compelling evidence to support an important effect of the immediate environment on the life history traits of voles [64–65] (see however [63]). Our identification of diet quality limitation on increasing vole populations is consistent with the latter findings. Specifically, we have demonstrated that protein availability limits the growth of summer vole populations. We therefore conclude that diet quality, ultimately determined by stochastic variation in climate, is likely to have a hitherto underestimated influence on the population dynamics of small mammals.

Acknowledgments

We thank Jean-Emmanuel Fournier, Stephen Ryan, Ilkka Taponen and Anaïs Zimmer for their contributions to field work, Nuria Blanco and Sami Kyröläinen for assistance with ELISA tests, and Tero Klemola for advice regarding abundance modelling and statistical analyses.

Author Contributions

Conceived and designed the experiments: KF PS TM HH OH. Performed the experiments: KF PS TM OH. Analyzed the data: KF KH OH. Contributed reagents/materials/analysis tools: TM OH. Wrote the paper: KF PS TM KH HH OH.

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III

FOOD RESOURCES OVERRIDE INTESTINAL PARASITISM IN WINTER LIMITATION OF BOREAL VOLE POPULATIONS

by

Kristian M. Forbes, Peter Stuart, Tapio Mappes, Heikki Henttonen & Otso Huitu 2014

Submitted manuscript

IV

COMPLEX INTERACTIONS DRIVE PATHOGEN LIMITATION OF VOLE POPULATIONS

by

Kristian M. Forbes, Heikki Henttonen, Varpu Hirvelä-Koski, Anja Kipar, Tapio Mappes, Peter Stuart & Otso Huitu 2014

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