

# Food resources and intestinal parasites as limiting factors for boreal vole populations during winter

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**Abstract.** Processes limiting the growth of cyclic vole populations have stimulated considerable research and debate over several decades. In Fennoscandia, the peak density of cyclic vole populations occurs in fall, and is followed by a severe winter decline. Food availability and intestinal parasites have been demonstrated to independently and synergistically limit wildlife populations. The purpose of this study was to directly compare competing food and parasite hypotheses on the limitation of overwintering high-density vole populations. Moreover, we evaluated the ability of food limitation and nematode infection to interact and thereby intensify population declines. A two-factor experiment with food supplementation and antihelminthic medication was conducted on replicated, enclosed field vole (*Microtus agrestis*) populations in central Finland over one full boreal winter. Population abundance, survival, and demographic attributes were monitored through live trapping. Vole feces were concurrently examined for the eggs of Heligmosomidae nematodes and oocysts of eimerian coccidians. We found that vole density declined in all treatment groups throughout winter. However, food supplementation mitigated this decline through positive effects on reproduction, and voles in food-supplemented populations were generally in better physiological condition than non-supplemented voles. Food supplementation and antihelminthic treatment reduced the prevalence of Heligmosomidae nematodes, while neither food nor medication affected the prevalence of eimerians, or infection intensity of either parasite group. Although food supplementation and antihelminthic medication aided in the clearance of Heligmosomidae nematodes, their prevalence did not influence vole population growth, and this parasite group is therefore unlikely to contribute to the cyclic winter decline of boreal vole populations. Instead food resources acting alone were the primary factor limiting vole population growth.

**Key words:** antihelminthic treatment; food; limitation; *Microtus agrestis*; nematodes; parasites; population cycles; resources; voles.

## INTRODUCTION

Malnutrition has been linked to reduced immunocompetence, and increased infection prevalence and intensity in several taxa, including humans (see Sheldon and Verhulst 1996, Katona and Katona-Apte 2008, Beldomenico and Begon 2009). This association is believed to primarily be due to a lack of nutrients to allocate to costly immune defenses in lieu of other processes, such as homeostasis, growth, and reproduction (Lochmiller and Deerenberg 2000, Zuk and Stoehr 2002). The relationship between poor condition and infection may also manifest as a vicious circle, analogous to a feedback loop, whereby poor condition predisposes an individual to infection, which further reduces condition and so on (Beldomenico et al. 2008a, Beldomenico and Begon 2009).

During the multi-annual density cycles of northern European vole species, populations usually display positive growth during two consecutive summers, and either stable or slightly negative population growth during the connecting winters (Myllymäki 1977, Hansson and Henttonen 1985a, Norrdahl and Korpimäki 2002). The peak density of a multi-annual cycle is attained in late summer to fall, following the second summer of positive growth, and is followed by a severe winter decline, which usually extends through the ensuing summer (Hansson and Henttonen 1988).

Grasses do not grow during the boreal winter (Myllymäki 1977). A depletion of food resources during winter has been demonstrated to limit the growth of high-density folivorous vole populations and induce a population decline (Huitu et al. 2003, Fey et al. 2008). During this cyclic decline, field vole (*Microtus agrestis*) populations may consume up to 90% of available vegetation, leading to low physiological condition and reduced survival (Huitu et al. 2007). During winter, vegetation is also often sequestered by ice and snow,

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which is detrimental to vole survival (Korslund and Steen 2006).

Parasites have been implicated in the limitation of animal population growth (Anderson and May 1979). For example, parasite-induced limitation of Red Grouse population growth in the UK was demonstrated by removal of a nematode that inhibits reproduction (Hudson et al. 1998). The potential influence of intestinal parasites on wild rodent population dynamics is also recognized (Boonstra et al. 1998, Laakkonen et al. 1998, Haukisalmi and Henttonen 2000), with infection associated with reduced condition, reproductive output, and survival (Scott 1987, Gregory 1991, Fuller and Blaustein 1996, Kristan 2002, Hakkarainen et al. 2007, Vandegrift et al. 2008, but see Haukisalmi and Henttonen 2000).

Two groups of intestinal parasites common to rodent species in northern Europe, including voles, are coccidians of the genus *Eimeria* and nematodes of the family Heligmosomidae (Haukisalmi and Henttonen 1990, Haukisalmi et al. 1994, Laakkonen et al. 1998, Hakkarainen et al. 2007). A study of 20 mainland and 27 island populations in central Finland identified an association between infection with *Eimeria* spp. and reduced condition in bank vole (*Myodes glareolus*) mothers and pups (Hakkarainen et al. 2007). While this finding alone is not obviously relevant to population dynamics, size at birth has been identified as one of the most important determinants of reproductive output in bank voles, decreasing maturation age and increasing breeding probability and size of the first litter (Mappes and Koskela 2004).

Anthelmintic treatments, such as ivermectin medication, can be used to remove nematodes and thereby evaluate their effects on host populations (Hudson et al. 1998, Haukisalmi and Henttonen 2000, Ferrari et al. 2004, Pedersen and Greives 2008). A factorial experiment on 12 white-footed mouse (*Peromyscus leucopus*) populations in North America found that anthelmintic treatment prevented a midsummer breeding hiatus, as well as increased individual growth, body condition, and survival (Vandegrift et al. 2008). Anthelmintic treatment is not effective against coccidians, but removal of one parasite group may indirectly increase the prevalence and intensity of untreated parasites through competitive release, or alter their potential interactive effects (Yan 1996, Pedersen and Antonovics 2013). In other words, the prevalence or intensity of eimerians may increase after removal of nematodes (Knowles et al. 2013, Pedersen and Antonovics 2013).

Food limitation and intestinal nematode infection additively exacerbated seasonal crashes in *Peromyscus* mouse populations in North America (Pedersen and Greives 2008). Congruently, there exists clear potential for a synergistic effect of winter food resource limitation and intestinal nematode infection on high-density vole populations. Factors driving cyclic vole population fluctuations represent one of the greatest mysteries of

modern population ecology (Krebs 2013). Although predation is undoubtedly critical (Hanski et al. 1991, 1993), the multitude of potential complementary factors, including parasitism (Haukisalmi and Henttonen 2000, Hakkarainen et al. 2007, Burthe et al. 2008) and food resources (Huitu et al. 2003), has stimulated much research and debate over several decades (Krebs 2013).

We report on a replicated, two-factor enclosure experiment with food supplementation and anthelmintic treatment to directly compare competing food and disease hypotheses on the limitation of overwintering vole populations. Moreover, we evaluated the ability of food limitation and nematode infection to interact and thereby intensify population declines. The relationship between food resources, individual health, and parasite infection has only rarely been evaluated experimentally at the population level (see Pedersen and Greives 2008, Vandegrift et al. 2008, Eberhardt et al. 2013). This is the first study to examine such an interaction in boreal vole populations. We hypothesize that vole populations subjected to resource limitation (no food supplementation) will encounter greater prevalence and intensity of intestinal parasite infection than populations with ample food (food supplementation), and that populations exposed to both food limitation and nematodes will display lower population growth and survival than those exposed to either factor alone.

## METHODS

### *Enclosures and experiment design*

The experiment was conducted in 32 (20 × 25 m) adjoining field enclosures in central Finland (62°37'30" N, 27°7'30" E; Forbes et al. 2014). The enclosures were constructed on one continuous 2-ha tract of set-aside agricultural grassland, which had been uncultivated for over 10 years, receiving no agricultural practices except a single mowing once per summer. At the time of the experiment, the enclosures were primarily vegetated with the grasses *Phleum pratense* and *Deschampsia caespitosa*. The site was surrounded on three sides by managed coniferous forest, with a narrow gravel road and farmhouse nearby.

Enclosures were made of sheet metal, which rose ~1 m aboveground and extended 50 cm underground. As such, vole movement between enclosures was prevented, and access by mammalian predators was largely restricted. Each enclosure contained eight aluminum shelter boxes (40 × 40 × 50 cm), with two entrance holes at the base. An Ugglan Special live trap (Grahnb, Gnosjö, Sweden) was placed in each shelter box.

Field voles (*Microtus agrestis*; see Plate 1) were selected as model species for this experiment because they are one of the most abundant and widely distributed of fluctuating small mammals in Fennoscandia (for example Hansson and Henttonen 1985b). Voles were live-trapped from fields in the surrounding area prior to the experiment and housed for 0–3 weeks at the

animal facility of the Finnish Forest Research Institute in Suonenjoki while trapping was being conducted. These voles were born during the preceding summer/fall (aged <8 weeks), except for a small number (1–2 per enclosure) of younger voles (aged ~4 weeks), which were spread evenly amongst the enclosures to increase numbers. Existing parasite fauna were not evaluated or manipulated prior to introduction into the experimental enclosures. At the beginning of November 2011, 7 male and 11 female field voles were introduced to each enclosure. The number of voles introduced to each enclosure was selected to approximate the density of cyclic peaks, and the proportion of each sex captured prior to the experiment dictated the sex ratio of the introducees.

A two-factor study design was employed whereby enclosures were randomized to one of four treatment groups: ad libitum food supplementation and antihelminthic treatment (F+A+), ad libitum food supplementation alone (F+A-), antihelminthic treatment alone (F-A+), and control (F-A-). Food supplementation consisted of rodent chow pellets (22.5% crude protein, 5% crude fat, 4.5% crude fiber, and 6.5% crude ash) distributed from a wire mesh feeder and an open aluminum tray in each shelter box. Voles could remove and hoard pellets from the trays, but had to consume them through the wire mesh of the feeder. Food supplementation was initiated immediately following baseline trapping in mid-November 2011, and continued until the experiment conclusion in April 2012. Baseline trapping was conducted two weeks after voles were introduced to the enclosures. This delay was to ensure that abundance estimates represented established voles (our experiences have demonstrated that some field voles die due to stress associated with introduction to a new environment), and to account for any existing voles in the enclosures.

Ambient weather conditions during the experiment were not recorded at the enclosure site. According to the Finnish Meteorological Institute (the nearest weather station is ~30 km from the enclosures; more information *available online*).<sup>6</sup> the winter of 2011–2012 was warmer than the long-term mean (1981–2010), largely due to an abnormally warm December, which delayed the onset of thermic winter by almost two months, to the end of December. The mean December–February temperature in the general area was  $-6.0^{\circ}\text{C}$  to  $-7.5^{\circ}\text{C}$ , which is  $1.0$ – $1.5^{\circ}\text{C}$  warmer than the long-term mean. Precipitation during the experiment winter was 20–40% greater than the long-term mean. Snow arrived relatively normally at the beginning of December. Maximum snow depth was 50–60 cm, which is greater than the average.

#### *Vole monitoring and sampling*

Vole sampling and abundance monitoring was conducted every sixth week (except for a four-week interval

between trapping occasions three and four), for a total of six trapping occasions throughout the experiment. Three days prior to trapping, supplementary food was removed and traps were pre-baited with oats. Traps were then set and successively checked at 07:00, 14:00, and 21:00 hours, 8–9 times, for each trapping occasion. Upon first capture, voles were injected with a passive integrated transponder (PIT; EID Aalten BV, Aalten, The Netherlands), and the unique identification number was recorded at each encounter. Voles were placed into individual ventilated containers, with a small piece of turnip, and taken to an on-site laboratory where their sex and reproductive status (subadult, mature, post-mature for males; and subadult, mature, pregnant and/or lactating, post-mature for females) was determined through external examination. Mass and head width were measured (nearest 0.1 g and 0.1 mm, respectively), and ~150  $\mu\text{L}$  of blood was collected from the retro-orbital sinus with heparinized capillary tubes. Individuals weighing under 20 g were not sampled for blood. Feces were collected from containers, which were wiped clean and sterilized with 70% alcohol between voles, and voles were released into the same shelter box from which they were captured. If an encountered individual had been previously sampled during the trapping occasion, the identification number was recorded in the field and the vole was immediately released.

Ivermectin antihelminthic treatment (Ivomec Vet 10 mg/mL [Merial Animal Health, Lyon, France]; dose: 200  $\mu\text{g}/\text{kg}$  per vole diluted in linseed oil at 1:60; 3 drops per dose) was administered orally with disposable pipettes concurrent to vole sampling. Individuals from non-antihelminthic treatment groups were given the same dose of linseed oil alone. Voles were treated once per trapping occasion, and individuals weighing under 15 g were excluded.

Robust trapping methods were employed, with few previously uncaught voles encountered by the end of each trapping occasion. However, since individuals may display behavioral variation in their likelihood of being recaptured, population density estimates (32 enclosures  $\times$  6 trapping occasions = 192 estimates) were calculated with program CAPTURE using the  $M_h$  estimator, which incorporates heterogeneity in capture rate (Otis et al. 1978). Rarely, voles were found dead in traps or died during sampling (<1% of captures). These individuals were omitted from abundance models, but added to the final estimate (Otis et al. 1978). Growth rate was calculated using the formula  $R_t = \ln(N_{t-1}/N_t)$ , where  $N_t$  is the population density at time  $t$  (Sibly and Hone 2002, Huitu et al. 2003). Enclosure-based survival estimates were calculated for each trapping interval using program MARK (White and Burnham 1999). Akaike's information criterion (AIC) was used to compare recapture rate models, including enclosure, trapping occasion, their permutations or only the intercept (Burnham and Anderson 2002). Population survival estimates were

<sup>6</sup> <http://ilmatieteenlaitos.fi/talvi-2011-2012>

then obtained from the most parsimonious recapture rate model.

#### *Parasite identification*

Two intestinal parasite groups, oocysts of *Eimeria* coccidians and eggs of Heligmosomidae nematodes (probably *Heligmosomoides laevis* and possibly some *Heligmosomum costellatum*) were found sufficiently prevalent to permit statistical enquiry. Both groups are known to occur widely in *Microtus agrestis* in Finland (Haukisalmi et al. 1994, Laakkonen et al. 1998). The prevalence of *Eimeria* spp. in field voles in Finland has been found to vary from 5% to 56%, with peaks in early fall, when young susceptible voles shed large numbers of oocysts (Laakkonen et al. 1998). In several of our samples, there appeared to be two distinguishable *Eimeria* species based on size. However, as comparison based on morphology alone is not adequate in determining *Eimeria* (or Heligmosomidae) species and molecular methods for the eggs of these specific taxa were not at our disposal, species were not separated in the analyses. To our knowledge, the *Eimeria* species infecting field voles in Finland have never been identified.

The prevalence of Heligmosomidae nematodes in field voles in Finland has been observed at 10% (Haukisalmi et al. 1994). Seasonal dynamics of Heligmosomidae nematodes have been thoroughly studied in *Myodes glareolus* in Finland, with peaks of infection occurring in winter (Haukisalmi et al. 1988). Other intestinal parasites that are known to infect field voles in northern Europe include coccidian *Cryptosporidium*, nematodes *Carolinensis minutus*, *Syphacia* sp., *Aoncotheca* (*Capillaria*) sp., *Trichuris arvicoliae*, *Mastophorus muris* (Tenora et al. 1983, Laakkonen et al. 1994, Haukisalmi et al. 2004, Callejón et al. 2012), and cestodes including *Paranoplocephala gracilis*, *P. blanchardi*, *P. omphalodes*, *Anoplocephaloides dentata* complex, *Microcephaloides* (*Anoplocephaloides*) *variabilis* complex, and *Hymenolepis* (*Rodentolepis*) *asymmetrica* (Haukisalmi et al. 1994, 2008, 2009, 2010).

In the current study, salt flotations were used to isolate the eggs and oocysts of intestinal parasites. Parasite identification, to the genus level in eimerians and family level in Heligmosomidae, was achieved through visual inspection with a light microscope. The intensity of infection was determined by summing the number of eggs or oocysts observed in a slide transect, and standardized per gram of feces. A small number of eggs of other parasites were sporadically seen in vole feces, primarily cestodes belonging to the family Anoplocephalidae and, very rarely, the nematode *Trichuris arvicoliae*.

#### *Data analyses*

Tracks of mustelid predators were occasionally, but rarely, observed inside enclosures. Density estimates from enclosures where mustelid tracks were recorded,

and where a marked decrease in vole density had occurred, were excluded from analyses from that point forward (10 enclosure occasions; see Appendix for a description of the excluded data). Throughout all trapping occasions, eimerians were absent from two enclosures (F+A+ and F-A-), Heligmosomidae nematodes were absent from one enclosure (F+A-), and both parasite groups were absent from one enclosure (F-A-). These enclosures were also excluded from statistical analyses.

Generalized linear mixed models were used to evaluate the individual and interactive effects of food, ivermectin antihelminthic treatment, and week on the proportion of voles infected with each parasite group and the proportion of males. Enclosure, week, and the intercept were included as random factors. Due to the low prevalence of reproducing females (pregnant and/or lactating) and new voles (<20 g) and their strongly biased occurrence in food-supplemented, rather than non-supplemented populations, statistical analysis of these data was not possible. Instead, raw values are reported.

Random coefficient regression models were used to evaluate the individual and interactive effects of food, ivermectin antihelminthic treatment, and week on the burden of each parasite group (including only infected individuals), population density, and the population survival rate. Enclosure, week, and the intercept were again included as random factors.

An individual body condition index was expressed as the studentized residuals of head width regressed by body mass. New and reproducing voles were removed from the analysis. Random coefficient regression models were then used to assess the individual and interactive effects of food, ivermectin antihelminthic treatment, and density on condition index (males and females were analyzed separately) and the population growth rates. Enclosure and the intercept were set as random factors. Two additional growth rate models were conducted with ivermectin replaced first by the prevalence of eimerians, and then Heligmosomidae nematodes.

Models were selected by sequentially removing terms from full models, beginning with highest order interactions, until attaining the most parsimonious model with the minimum AIC value. Models were selected using the maximum likelihood (ML) method, and final values obtained from the most parsimonious model with restricted maximum likelihood (REML), using Kenward and Roger estimation (Littell et al. 2006). Data were analyzed in SAS version 9.3 (SAS Institute 2011).

## RESULTS

Vole population density declined throughout the experiment in all treatment groups (Fig. 1). However, the decline was less severe in food-supplemented populations, which displayed higher densities than non-supplemented populations from January to April (Fig. 1, Table 1). Antihelminthic treatment did not affect



population density (Table 1). Population growth rates were inversely related to population density (Fig. 2a, Table 1) and higher with food supplementation (Fig. 2b), but not influenced by antihelminthic treatment. In growth rate models including eimerian and Heligmosomidae nematode prevalence as explanatory variables, density and food retained significance, but neither parasite group affected population growth (eimerians  $P = 0.4$ , Heligmosomidae  $P = 0.8$ ).

Neither population survival, nor the proportion of males, varied with time, food supplementation, or antihelminthic treatment (Table 1). Evidence of winter breeding was found, with more pregnant and/or lactating female voles clearly identified in food-supplemented than non-supplemented populations (total February to March, 17 F+A+, 11 F+A-, 4 F-A+, 0 F-A-). The same trend appeared for the number of new voles (16 F+A+, 15 F+A-, 10 F-A+, 2 F-A-). The mean body condition index of male voles was greater in food-supplemented than non-supplemented populations at low (10th percentile) and median densities, but no difference was seen at high densities (90th percentile; Table 1, Fig. 3a). At high densities, male condition index was also greater in antihelminthic-treated populations than non-antihelminthic-treated populations (Table 1). Female condition index was similarly greater at low and median densities in food-supplemented than non-supplemented populations (Table 1, Fig. 3b). There was no difference at high densities, and no influence of antihelminthic treatment on female condition index.

Eimerian prevalence ranged from 21% to 26% and did not change with time, food supplementation, or antihelminthic treatment (Table 1). The baseline prevalence of Heligmosomidae nematodes ranged from 15% to 38%, and was lowest in non-antihelminthic-treated populations (Fig. 4). Antihelminthic treatment then reduced the prevalence of Heligmosomidae nematodes over time (Table 1), as compared to non-antihelminthic-treated populations. The prevalence of Heligmosomidae nematodes was also lower with food supplementation (Table 1), and by the end of the experiment, most voles sampled from F+A+ treatment populations were uninfected with nematodes (Fig. 4). Eimerian and Heligmosomidae infection intensity was not influenced by time, food supplementation, or antihelminthic treatment (Table 1).

#### DISCUSSION

This was the first study to experimentally evaluate the interactive effects of food resources and nematode parasites on the winter decline of cyclic vole populations. Intestinal parasites belonging to two groups were consistently found in voles, both of which have been demonstrated to limit the growth of rodent populations (for example Fuller and Blaustein 1996, Vandegrift et al. 2008). However, their effects on vole populations in this

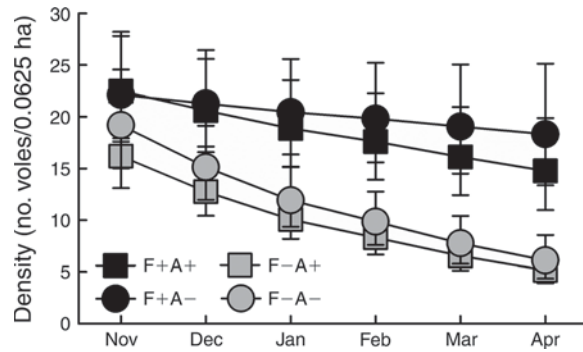


FIG. 1. Changes in field vole (*Microtus agrestis*) population density over time in central Finland. The four treatment groups were: ad libitum food supplementation and antihelminthic treatment (F+A+), ad libitum food supplementation alone (F+A-), antihelminthic treatment alone (F-A+), and control (F-A-). Black symbols denote F+ (food supplementation) populations, and A+ (antihelminthic treatment) populations are represented by squares. Error bars indicate standard error.

study were negligible and overshadowed by those of food alone.

Our results demonstrate that food resources limited vole population growth in winter through effects on reproduction. The amount of natural food in the enclosures was sufficient to maintain adequate health for vole survival in the absence of food supplementation, even though initial density levels were comparable to peak abundances of wild vole populations. This contrasts earlier studies, which have found winter food limitation to be mediated primarily through differential survival (Huitu et al. 2003, 2007, Korslund and Steen 2006; but see Aars and Ims 2002). The amount of available vegetation is highly dependent on the length of the growing season, and substantial interannual variation can occur in winter conditions, which may also affect population demographic rates (Aars and Ims 2002, Korslund and Steen 2006). Indeed, Aars and Ims (2002) found that, while varying winter conditions had a strong effect on individual survival, vole recruitment was strongly density dependent, possibly through food limitation. The degree to which interannual variations in weather conditions impact the carrying capacity of the environment and/or the demographic processes determining population growth rates remains to be investigated.

Vole reproduction is rare during the winter period in northern Europe (Myllymäki 1977, Kaikusalo and Tast 1984, Norrdahl and Korpimäki 2002). The high protein content of supplemented food, which is beneficial for small-rodent breeding (Cole and Batzli 1979, Taitt and Krebs 1981), is likely to explain winter reproduction during the current experiment. Voles that received food supplementation were also in better physiological condition than non-supplemented voles. Although this effect was not present at high densities, which seems counterintuitive, it is important to

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

Response and source of variation	Numerator df	Denominator df	F	P
Density				
Food	1	133	4.31	0.0398
Ivermectin	1	32	0.24	0.63
Week	1	154	27.07	<0.0001
<b>Week × food</b>	<b>1</b>	<b>154</b>	<b>8.76</b>	<b>0.0036</b>
Growth rate				
<b>Food</b>	<b>1</b>	<b>129</b>	<b>6.63</b>	<b>0.0112</b>
Ivermectin	1	129	0.22	0.64
<b>Density</b>	<b>1</b>	<b>129</b>	<b>22.04</b>	<b>&lt;0.0001</b>
Survival				
Food	1	130	0.43	0.51
Ivermectin	1	130	0.01	0.94
Week	1	130	0.24	0.63
Proportion of males in population				
Food	1	1310	0.02	0.88
Ivermectin	1	1257	0.89	0.35
Week	1	2173	2.10	0.15
Male condition index				
Food	1	82	10.01	0.0022
Ivermectin	1	86	2.55	0.11
Density	1	416	17.81	<0.0001
<b>Density × ivermectin</b>	<b>1</b>	<b>361</b>	<b>5.16</b>	<b>0.0237</b>
<b>Density × food</b>	<b>1</b>	<b>417</b>	<b>5.40</b>	<b>0.0206</b>
Female condition index				
Food	1	59	13.34	0.0005
Ivermectin	1	26	0.01	0.94
Density	1	493	6.08	0.0140
<b>Density × food</b>	<b>1</b>	<b>494</b>	<b>4.46</b>	<b>0.0352</b>
Eimerian prevalence				
Food	1	337	0.09	0.77
Ivermectin	1	363	0.19	0.67
Week	1	624	0.03	0.87
Heligmosomidae prevalence				
<b>Food</b>	<b>1</b>	<b>449</b>	<b>12.13</b>	<b>0.0005</b>
Ivermectin	1	615	6.83	0.0092
Week	1	615	6.71	0.0098
<b>Week × ivermectin</b>	<b>1</b>	<b>615</b>	<b>7.40</b>	<b>0.0067</b>
Eimerian intensity				
Food	1	131	0.72	0.40
Ivermectin	1	131	1.54	0.22
Week	1	131	0.43	0.51
Heligmosomidae intensity				
Food	1	110	1.87	0.17
Ivermectin	1	110	0.36	0.55
Week	1	110	0.31	0.58

Note: Highest order significant interactions or effects are marked in boldface type.

remember the densities were highest at the beginning of the experiment when natural food resources were still abundant. Voles in best condition have been previously found to initiate spring reproduction (Beldomenico et al. 2008b).

Anthelmintic treatment reduced the prevalence of Heligmosomidae nematodes, but did not affect eimerians. An increase in eimerian prevalence or intensity, due to competitive release, could have occurred following a reduction in nematodes, as has been found in mouse populations (Knowles et al. 2013, Pedersen and Antonovics 2013). Our lack of detection of an

interaction between parasite groups may be due to the six-week intervals between trapping occasions. Knowles et al. (2013) found that ivermectin treatment increased the intensity of coccidian infection in wood mice from one to three weeks posttreatment, but the effect had disappeared by four weeks. Eimerian prevalence in our vole populations was consistently ~25%, much lower than 60–80% summer coccidian prevalence reported in mouse populations (Pedersen and Antonovics 2013). Different seasonal dynamics are likely to have been operating between these study systems. Eimerian infection is greater in younger than

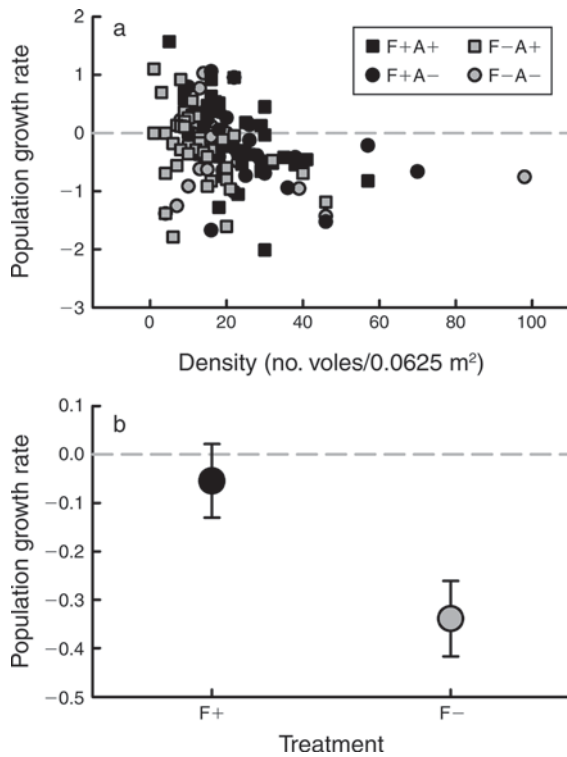


FIG. 2. Vole population growth rate in relation to (a) population density, and (b) food supplementation (estimated marginal means  $\pm$  SE). Black symbols denote F+ populations, and A+ populations are represented by squares.

older animals (Ball and Lewis 1984, Laakkonen et al. 1998), probably due to an absence of acquired immunity, and the recruitment of young during spring and summer probably increases population-wide infection pressure.

Food supplementation lowered the prevalence of Heligmosomidae nematodes, implying some level of resource quantity or quality limitation in immune response. However, the prevalence of neither parasite group impacted on the growth rate of vole populations. Negative effects of nematodes on the growth of mouse populations have been experimentally demonstrated in North America (Pedersen and Greives 2008, Vandegrift et al. 2008); however, prior to the current study, equivalent research had not been conducted on vole populations. Nematode species assemblages vary between these host systems and geographical areas (see Haukisalmi and Henttonen 1990, Pedersen and Antonovics 2013), which are likely to exert different effects on their hosts and explain the contrasting results. Interestingly, male antihelminthic-treated voles were in better physiological condition than non-treated voles at high densities, suggesting that nematodes may exert negative effects on the condition of voles in a highly stressful environment.

Since eimerian prevalence was not experimentally manipulated, either directly or indirectly (through

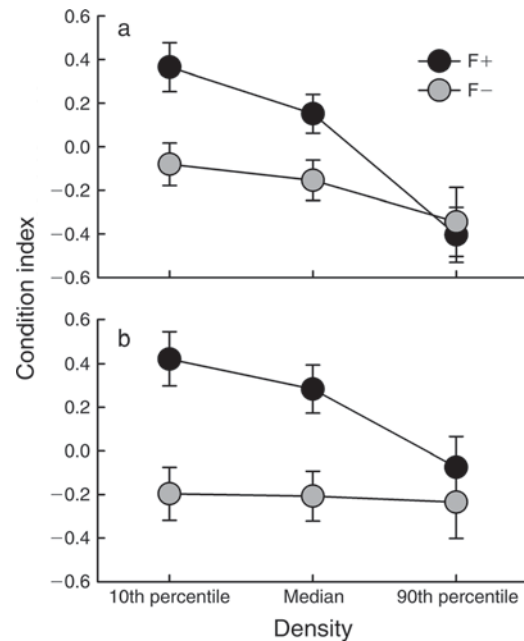


FIG. 3. Body condition index (estimated marginal means  $\pm$  SE; expressed as the residuals of a regression of body mass on head width, where positive values indicate individuals in relatively good physiological condition and negative values those in relatively poor condition) of (a) males and (b) females, at low (10th percentile), median, and high (90th percentile) densities in food-supplemented (black symbols) and non-supplemented (gray symbols) vole populations.

competitive release), we cannot make firm inferences on the effects of this parasite group on vole demography. Eimerians have been demonstrated to inhibit the overwinter survival of male deer mice (Fuller and Blaustein 1996), thereby demonstrating their potential to influence the population dynamics of wild rodents. Nonetheless, the temporal and spatial patterns of eimerian prevalence and intensity in cyclic vole populations do not support this concept in Finland (Laakkonen et al. 1998).

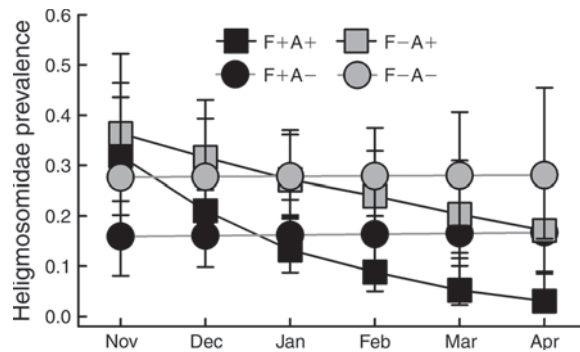


FIG. 4. Changes in Heligmosomidae nematode prevalence over time. Black symbols denote F+ populations, and A+ populations are represented by squares. Error bars indicate standard error.



PLATE 1. Field vole *Microtus agrestis*. Photo credit: copyright ©Metla/Erkki Oksanen.

Intensity of parasite infection was not influenced by food or antihelminthic treatments, indicating that antihelminthic treatment was able to completely clear voles of nematodes. Indeed, it was unusual for an antihelminthic-treated vole to be nematode infected at a later time point: Only three such cases were recorded. Pedersen and Antonovics (2013) similarly found no difference in infection intensity in *Peromyscus* mice, despite strong treatment effects on prevalence.

The low number of new voles that we recorded does not clearly indicate greater reproduction in food-supplemented populations. However, high-quality food resources have been shown to speed the growth of individual rodents (Cole and Batzli 1979, Desy and Batzli 1989, Cameron and Eshelman 1996). In other words, food-supplemented voles probably exceeded the mass-based criteria used to recognize new juvenile voles more rapidly than non-supplemented voles. During the experiment, only a small proportion of the total female voles were classified as visibly reproducing. This may mask negative effects of parasite infection on reproduction. For example, nematodes have been demonstrated to inhibit summer reproduction in white-footed mice (Vandegrift et al. 2008). It is therefore possible that the influence of parasites on the growth of vole populations will more clearly manifest in summer when a far greater

proportion of female voles are reproducing, although this potential effect may also be offset by a lower prevalence of nematodes in summer than winter (Haukialmi et al. 1988).

Although lack of food is elementally associated with poor individual health (Sheldon and Verhulst 1996, Katona and Katona-Apte 2008, Beldomenico and Begon 2009), the results of our experiment indicate that there exists no pronounced reciprocal interaction between a seasonal lack of food resources, physiological condition, and intestinal nematodes that might reflect onto cyclic vole population dynamics. We demonstrate that winter food resources are a key factor limiting the growth of cyclic vole populations, and that this effect appears to be greater than the negative impacts of intestinal nematodes alone. Although our study had less power to evaluate the effects of eimerians, their influence on vole winter dynamics also appears minimal. We acknowledge the existence of several other important factors, beyond those under investigation here (stress, co-infections, more severe food limitation, and so on), which could strengthen the negative impacts of intestinal parasites on their hosts and their dynamics in the wild. However, these fall outside of the scope of this study.

Our results are directly applicable to cyclic vole populations of northern Europe, and by extension, into populations with similar dynamics elsewhere in the



world. Cornulier et al. (2013) demonstrated that, during recent decades, vole populations of some species across Europe have exhibited dampening cyclicity, most noticeable in reduced spring densities. This may plausibly be explained by temporal changes in the quantity or quality of winter food resources, and as such warrants further investigation.

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## SUPPLEMENTAL MATERIAL

## Ecological Archives

The Appendix is available online: <http://dx.doi.org/10.1890/13-2381.1.sm>