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Phase dependence in winter physiological condition of cyclic voles

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Lack of food resources has been suggested as a factor which limits the growth of cyclic vole populations. During peak phases of the cycle, vole population growth typically ceases during late autumn or early winter, and is followed by a decrease in density over the winter. To investigate whether this decrease is due to increased mortality induced by a depletion of food resources, we studied overwinter food consumption and physiological condition of field voles (*Microtus agrestis*) in western Finland in both an increase and a decrease phase of a three-year population cycle. The growth rate of vole populations was negatively related both to prevailing vole densities and to densities six months earlier. The condition index of voles, as well as their blood levels of haematocrit, proteins, free fatty acids and immunoglobulin G, were positively related to population growth rate when populations were declining. When populations were increasing, these parameters tended to be negatively related to population growth rate. The overall physiological condition of voles was lower in the winter of the decrease phase as compared to the increase phase. The return rate of voles, a proxy of survival, was also lower in the decrease than in the increase phase of the cycle and positively related to haematocrit levels. Almost 90% of all green vegetation shoots were consumed by voles during the winter of the decrease phase while only two thirds were eaten in the increase phase. Our results suggest that the winter decrease phase of cyclic vole populations is associated with both a deterioration in the physiological condition of voles and a significant depletion of winter food resources. This implies that malnutrition induces poor physiological condition in voles, which in turn may increase mortality either directly through starvation or indirectly through increased susceptibility to predators and pathogens.

Arvicoline rodents commonly undergo 3–5 year cyclic, high-amplitude, fluctuations in population density (Hansson and Henttonen 1985, Hanski et al. 1991, Stenseth 1999, Korpimäki et al. 2005). It is currently widely believed that in pronouncedly seasonal areas with long winters, such population cycles are caused by the combined effects of predation and winter food limitation (Hanski et al. 1993, 2001, Norrdahl and Korpimäki 1995, Korpimäki and Norrdahl 1998, Korpimäki et al. 2002, 2005, Huitu et al. 2003, Ekerholm et al. 2004).

The growth of vole populations may become limited by a shortage of food resources, primarily so during seasons when food is not being regenerated, i.e. during winter (Myllymäki 1977a, Hansen et al. 1999, Stenseth et al. 2002, 2003, Huitu et al. 2003, Lima et al. 2006). This is congruent with the fact that peak density phases in vole population cycles typically occur in late autumn – early winter (Myllymäki 1977a, Henttonen et al. 1987, Yoccoz et al. 2001, Huitu et al. 2003), and are followed by considerable overwinter decreases in density (Hansen et al. 1999, Korpimäki et al. 2002). Huitu et al. (2003) prevented this overwinter decrease under experimental predator-free conditions by food supplementation, which indicates that high density vole populations are indeed capable of depleting their natural food resources beyond levels that cause malnutrition, and thereby reduced survival (Myllymäki 1977a, Hansson 1979, Laine and Henttonen 1983, Huitu et al. 2003, Korslund and Steen 2006).

What remains hitherto largely unexplored is whether food limitation also affects peak density vole populations in unmanipulated natural environments. In addition to food limitation, the overwinter population declines of cyclic herbivores may plausibly be attributable also to predation. In this case, predators may terminate the increase and initiate the population decrease well before the herbivores deplete their food resources (Myllymäki 1977a, Hansson 1979, Sinclair and Arcese 1995, see also Korpimäki et al. 2002, 2005). The effects of food depletion and natural enemies are also likely to be interactive; malnourishment has repeatedly been implied to increase the susceptibility of individuals to predators or pathogens (Chan et al. 1996, Tompkins and Begon 1999, Soveri et al. 2000, Murray 2002, Wirsing et al. 2002, Demas et al. 2003).

Prolonged food limitation will eventually result in malnutrition, and following this, a reduction in the health state of an individual. A deterioration in health state can be assessed in vertebrates by a number of physiological measurements. Commonly, low body mass in relation to physical measurements, such as length or head width, is considered indicative of low energy reserves (primarily protein and fat), and hence poor body condition (Schulte-Hostedde et al. 2001). Detrimental processes leading to poor body condition can be identified at an earlier stage of malnutrition from haematological indices. For example, decreased blood haematocrit levels may indicate nutritional deficiencies and/or chronic infection (Svensson and Merilä 1996, Ots et al. 1998, Potti et al. 1999). Concentrations of proteins and free fatty acids (FFA) in blood plasma reflect the size and utilization of the protein and fat reserves available for energy metabolism (Boismenu et al. 1992, Ots et al. 1998, Voltura and Wunder 1998). Low plasma protein levels have recently also been implied as indicative of infection (Johnson 1999, Fuhrman et al. 2004).

A malnutrition-induced deterioration of body condition almost invariably also leads to a deterioration of the immune system, which predisposes individuals to infections and disease (Chan et al. 1996, Demas and Nelson 1998, Tompkins and Begon 1999, Demas et al. 2003). Immunoglobulins, circulatory antigen-binding proteins, are a key element of the vertebrate immune system (Tonegawa 1985, Janeway et al. 2001). Elevated levels of IgG, the most common type of immunoglobulin, may indicate, e.g. a chronic state of infection. Malnutrition, on the other hand, may result in decreased levels of IgG, which translates into reduced immunocompetence (Nelson et al. 2002, Demas et al. 2003).

The objective of this study is to determine whether the winter decrease of cyclic vole populations from peak densities is associated with food limitation followed by a deterioration in the physiological condition of voles (Hansson 1979, Laine and Henttonen 1983, Huitu et al. 2003). Considering that field vole reproduction during winter is virtually non-existent and dynamically inconsequential in our study area (Norrdahl and Korpimäki 2002) and that voles largely refrain from dispersing during midwinter (Beacham 1980), the demographic mechanism affected by food depletion will predominantly be survival (Stenseth et al. 2003, Korslund and Steen 2006). Since recording actual sources of mortality in subnivean vole populations during winter is difficult, we opted to elucidate causal mechanisms behind the vole population decrease by quantifying predisposition to mortality through measurement of nutritive status and health state indices (body condition, haematocrit, blood proteins, FFA, IgG), and how these relate to population growth rate.

To do this, we trapped and sampled field voles (*Microtus agrestis*, L.) during both an increase phase winter and a decrease phase winter of a three-year population cycle (Korpimäki et al. 2005), as well as evaluated the degree of subnivean grazing. Since the possible adverse effects of winter food depletion on the physiological condition of voles can be expected to cumulate through the season, sampling sessions were carried out in midwinter and at the end of winter, some time following the attainment of peak densities. We predicted that if predation terminates the growth of vole populations prior to food depletion, no physiological indications of malnutrition or poor health would be detected in individual voles from decreasing populations. Conversely, if voles do exhaust their food resources, individuals should exhibit reduced physiological condition and health state.

Material and methods

Study sites and field protocol

The study was conducted on two unfenced 1-ha agricultural field sites situated 2.5 km apart in the Alajoki farmland plain of Lapua, western Finland (63°N, 23°E). The sites have been uncultivated for over ten years and are currently naturally vegetated primarily by graminoids (Norrdahl et al. 2002). The most common small mammal species in the area are the field vole, the sibling vole (*Microtus rossiaemeridionalis*), the bank vole (*Clethrionomys glareolus*) and the common shrew (*Sorex araneus*). The most important predators of small mammals are least weasels (*Mustela nivalis*), stoats (*M. erminea*), Eurasian kestrels (*Falco tinnunculus*), short-eared owls (*Asio flammeus*), long-eared owls (*A. otus*) and Tengmalm's owls (*Aegolius funereus*) (Korpimäki and Norrdahl 1991, Norrdahl and Korpimäki 1995). Voles and common shrews exhibit temporally synchronous three-year

population cycles in the study area (Korpimäki et al. 2005) (Fig. 1a).

Both study sites contained 100 trap stations arranged in a grid with 10 m between stations. Each station consisted of one Ugglan multiple-capture live trap (Grahnab, Sweden), covered by a $40 \times 30 \times 25$ cm plastic box which reduced exposure to rain, wind and temperature extremes. Vole abundances in the study sites were monitored at 5–13 week intervals between June 2001 and August 2003. The trapping protocol included baiting with Rat/Mouse Breeding Diet pellets (Altromin GmbH, Germany), and checking the traps three times a day (at 06:00 h, 14:00 h and 22:00 h) for three days. Prior to release at the point of capture, all trapped voles were weighed, sexed and individually marked by toe clipping. Site-specific population density estimates (no. field voles ha^{-1}) were obtained for each trapping occasion separately using the jackknife estimator for model M_h (including heterogeneity in individual capture rates) in program CAPTURE (Otis et al. 1978).

All field voles trapped in February (midwinter; mean temperature $-8.0^{\circ}C$, Drebs et al. 2002, mean day length ca 9 h) and April (end of winter, before growing season of vegetation; mean temperature $1.9^{\circ}C$, Drebs et al. 2002, mean day length ca 15 h) of 2002 (increase phase) and 2003 (decrease phase) were brought from the trap stations to a mobile laboratory (Toyota Motor Co., Japan) for measurement and sampling. The body mass of these voles was measured to the nearest 0.1 g using an electronic scale, and head width to the nearest 0.1 mm with a digital calliper; all head width measurements were made by the same person (TM). Three retro-orbital blood samples (1×18 µl, 2×75 µl) were collected from each individual into heparinized capillary tubes (I.D. 0.5–0.6 mm). After sampling, voles were returned to the point of capture, following a post-trap confinement of no more than 30 min.

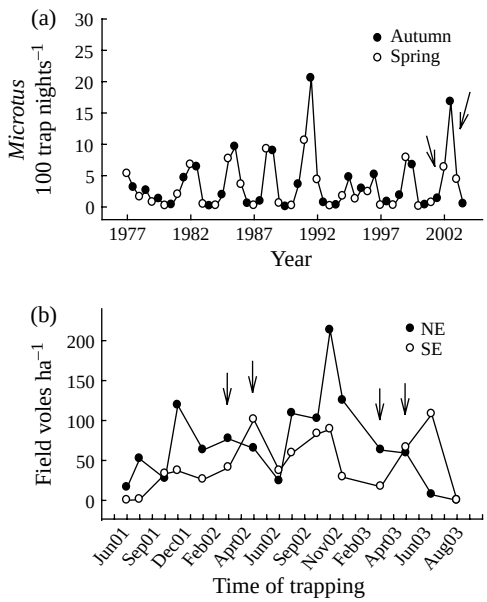


Fig. 1. (a) Long-term fluctuations in populations of *Microtus* voles in the study area, expressed as voles trapped per 100 trap nights. Open symbols are spring (May) values, closed symbols autumn (October) values. (b) Live-trapping-based estimates of field vole population size at two separate sites (NE = north-east, SE = south-east) during the experiment. In both panels, arrows denote trapping occasions when individual voles were sampled for determination of physiological condition.

The capillary tubes were centrifuged at 12 000 g for 5 min in a haematocrit centrifuge (Heraeus Biofuge, Thermo Electron Co., Finland) to separate the plasma from the blood cells. Haematocrit was thereafter measured and expressed as the percentage of packed red blood cells in total blood volume. The plasma was stored at $-20^{\circ}C$ for analyses of plasma proteins, FFA and IgG (below). Altogether 39, 73, 47 and 73 blood samples from field voles were available for analyses in February 2002, April 2002, February 2003 and April 2003, respectively.

The degree of overwinter food consumption was assessed immediately after snowmelt in both 2002 and 2003, by counting remaining numbers of intact (i.e. not clipped by voles) wintered green base stems and shoots of grasses and dicotyledons (hereafter called green shoots). Counting was conducted in both sites on 16 randomly placed 0.25 m^2 quadrats and on one 0.25 m^2 quadrat inside each of five permanent plant sampling plots (2×2 m), which were protected from vole grazing by a separate fence (Huitu et al. 2003). Quadrats were not counted in permanent plots if signs of vole grazing were detected.

Determination of plasma protein, FFA and IgG

The concentration of protein in plasma (mg ml^{-1}) was determined with the Bradford method, using a microplate modification of BioRad Protein Assay (Bio-Rad Laboratories Inc., California, USA). Bovine serum albumin (BSA, Sigma Chemical Co., Missouri, USA) served as the calibrating protein. Free fatty acids in plasma (mmol l^{-1}) were analyzed using an enzymatic, photometric method (Wako NEFA C ACS-ACOD Method, code 994-75409E, WAKO Chemicals GmbH, Germany) and a Konelab 60i Clinical Chemistry Analyzer (Thermo Electron Co., Finland).

Plasma IgG was determined using a microplate enzyme-linked immunosorbent assay (ELISA). We established a strong cross-reactivity of anti-mouse IgG antibodies (M-8642, Sigma Chemical Co., Missouri, USA) with field vole immunoglobulin, and a double antibody method based on these antibodies was set up.

First, flat-bottomed 96-well microtiter plates (Immuno Plate Maxisorp, Nunc Co., Denmark) were coated with anti-mouse IgG antibody (10 μg ml^{-1}) in 0.5 M carbonate buffer pH 9.4. After saturation with 1% bovine serum albumin (BSA, Roche Diagnostics, Germany) in phosphate buffered saline (PBS, pH 7.4), the samples were diluted in 1% BSA/PBS and incubated in wells. The bound vole immunoglobulin was then detected with anti-mouse IgG immunoglobulin alkaline phosphatase conjugate (A-2179, Sigma Chemical Co., Missouri, USA). Washing was performed between each step with PBS-Tween 20 (0.05%). P-nitrophenyl phosphate (1 mg ml^{-1}, Sigma Chemical Co., Missouri, USA) was used as the substrate, and after the enzyme reaction the optical density was read with a Titertek Multiskan Plus plate reader (Flow Laboratories, California, USA) at 405 nm. A pool of plasma collected from 20 voles in the study was used to calibrate the assay, thus providing an arbitrary measure against which the concentration of samples [expressed as artificial units per ml plasma (U ml^{-1})] was compared.

Statistical analyses

The growth rate of field vole populations (r_t; unit per year) between successive trapping occasions was determined as $r_t = 1/T \times \ln(n_{t+1}/n_t) \times 52$, where n_t is field vole density at time t, n_{t+1} is field vole density in the following trapping occasion and T is the number of weeks between successive trapping occasions (Turchin and Ostfeld 1997). We analysed how the growth rate was related to the current and past densities of field voles by linear regression (PROC MIXED, SAS®; maximum likelihood method), with site as a random factor. Due to uneven time intervals between successive trapping occasions, we interpolated past population density estimates at four-week intervals for 4–24 weeks (n_{t-4}, $n_{t-8} \ldots n_{t-24}$) prior to n_t by first dividing vole density changes into one-week intervals (change in vole density between two successive trapping occasions divided by the number of weeks between the occasions) and then subtracting the sum of the respective number of consecutive retrospective weekly changes from n_t.

We used a model averaging procedure (Burnham and Anderson 2002) to estimate the effects of the explanatory factors n_t, $n_{t-4} \ldots n_{t-24}$ on population growth rate r_t. Briefly, we first determined Akaike's model weight values for that subset of models (out of models containing all possible combinations of the explanatory variables) which contain the variable of interest. Akaike's model weight values indicate the relative probability of a given model being the best model out of those compared for the observed data. The parameter estimates from these models are then multiplied with their respective model weight. A model averaged parameter estimate is obtained by summing these weighed estimates across the subset of models (Burnham and Anderson 2002, Johnson and Omland 2004). Confidence intervals for the averaged parameter estimates are calculated in a similar manner (Burnham and Anderson 2002, Johnson and Omland 2004). Akaike's model weight values (calculated across the entire model set) were used to provide additional information on the relative importance of individual explanatory variables by summing the weight value of all models containing the variable in question (Burnham and Anderson 2002, Johnson and Omland 2004).

The condition index of field voles was determined as the individual residual values from a linear regression of body mass on head width (Schulte-Hostedde et al. 2001). Values of the condition index were $\ln(x+10)$, and blood proteins, FFA and IgG ln(x) −transformed for normality prior to analyses. Outliers were removed from all response variables when their values exceeded four standard deviations from the mean (three individuals for proteins, one each for FFA and IgG).

We constructed linear mixed models (PROC MIXED, SAS®) to analyze the effects of cycle phase (increase and decrease), month (February and April) and sex on condition index, haematocrit, blood proteins, FFA and IgG. Site was included in the models as a random factor, as was analysis batch or kit number or date for proteins, FFA and IgG. Individuals trapped both in February and April of the same year (no voles were trapped during both winters) were included in these analyses only for either of the two months by randomization to avoid pseudoreplication (Hurlbert 1984). To determine whether population-level changes in mean physiological parameter values during the two winters reflect actual changes in the physiological condition of individuals, rather than mirror population turnover, we compared parameter values of those individuals that were sampled both in February and April of either of the winters to the population mean values of the same time periods. This was done by standardizing (PROC STANDARD, SAS®) the mean parameter values from the four sampling occasions to mean 0, standard deviation 1, separately for the whole populations and the twice-sampled individuals, and for each physiological parameter. The resulting values were then collectively analyzed with a Pearson correlation coefficient (PROC CORR, SAS®).

To avoid overfitting the aforementioned models (Ginzburg and Jensen 2004), we analyzed the relationship between the physiological parameters and population growth rate of field voles separately by linear regression (PROC MIXED, SAS®). As explanatory variables, we used linear and quadratic terms of preceding population growth rate, calculated as

$\ln(n_{t-4}/n_t)$, where n_t is a population density estimate at the time of blood sampling and n_{t-4} an interpolated density estimate four weeks earlier (above). Site entered the models as a random factor. The decision to use a second-order polynomial explanatory variables was made on the basis of the corrected Akaike's information criterion AICc, which indicated a second-order model as the most parsimonious in all cases (difference between most and second most parsimonius model, $\Delta AICc > 3$) except FFA, where first- and second-order models were deemed equally good ($\Delta AICc = 0.9$). $\Delta AICc$ values exceeding 2 are considered indicative of substantial differences in support for the compared models (Burnham and Anderson 2002).

To estimate a proxy of survival, we examined the effects of cycle phase and field vole physiological parameters in February on the likelihood of a subsequent recapture in April (i.e. return rate; Lebreton et al. 1993) with generalized linear mixed models (GLIMMIX −macro, SAS®). The recapture data were used as a binary response variable (logit link function), while cycle phase, vole sex and each physiological parameter in turn were used as explanatory variables. Site was again used as a random factor.

Winter food consumption was analyzed from green shoot count data using linear mixed models (PROC MIXED, SAS®), with cycle phase and quadrat protection (protected or not from grazing) as fixed factors and site as a random factor. All statistical analyses were carried out with SAS® statistical software (v. 8.2).

Results

Field vole populations at both studied sites remained either relatively stable or increased in size through the first winter (2001−2002), decreased briefly in the beginning of the summer 2002 and increased towards late autumn 2002. Thereafter both populations decreased considerably until February 2003, after which the NE population continued to decrease while the SE population increased in size until June 2003. No field voles were caught at either site at the end of the study in August 2003 (Fig. 1a−b). Population growth was most parsimoniuously modelled by the negative effects of current (n_t) densities and densities 24 weeks (n_{t-24}) earlier (Table 1). While the model-averaged parameter estimate for n_{t-24} differed from zero, the 95% confidence limits for n_t marginally included zero. Also, of these two variables, n_{t-24} had a stronger effect on the response variable, as judged by its higher variable weight (Table 1). Due to their insignificant explanatory contribution, the values of the other candidate parameters were not estimated. The variance component parameter estimate for the random effect of site on population growth rates was zero in all models. No newborn individuals (<15 g) were trapped in February or April in either year. One female (out of 21 sampled) was pregnant in February of the increase year and eight (out of 41) in the same April. No pregnant females were encountered in the decrease winter. Collectively, these findings indicate that voles were in the increase phase of their population cycle in 2002, and in the decrease phase in 2003.

The body condition of field voles was generally lower in females than in males, lower in February than in April and lower in the decrease phase than in the increase. Lowest body condition indices were recorded for females in April of the decrease phase (Fig. 2a, see Appendix 1, Table 1 for full ANOVA table). Blood haematocrit levels were also generally lower in females, lower in February and lower in the decrease phase than in the increase phase (Fig. 2b, Appendix 1, Table 1). Levels of blood proteins were lower in the decrease phase than in the increase phase. We found an interaction between phase and month in protein levels, such that the highest overall levels were measured in February of the increase phase and lowest levels in February of the decrease phase (Fig. 2c, Appendix 1, Table 1). FFA levels were overall lower in April than in February, with females exhibiting higher levels in the decrease than in the increase phase (Fig. 2d, Appendix 1, Table 1). IgG levels were lower in the decrease phase than in the increase; levels tended to be lower in males than in females (Fig. 2e, Appendix 1, Table 1). Changes in the physiological parameters of those individuals that were sampled both in February and April of either of the two years (2002: 8 ind., 2003: 5 ind.) were closely related to changes on a population level ($r_p = 0.49$, $p = 0.03$, $n = 20$).

In the populations as a whole, body condition and FFA levels correlated negatively ($r_p = -0.18$, $p = 0.01$, $n = 198$), haematocrit and plasma proteins positively ($r_p = 0.30$, $p < 0.0001$, $n = 201$) and IgG and plasma proteins positively ($r_p = 0.21$, $p = 0.003$, $n = 202$).

All measured indices of physiological condition in field voles were similarly related to the direction and magnitude of population change during the four weeks preceding blood sampling occasions (Fig. 3, see Appendix 1, Table 2 for estimates; note that FFA values are expected to exhibit inverse numerical values compared to the other indices with changing physiological condition). The condition indices were positively related to population growth rates when populations had been declining. The relationship disappeared or even reversed when populations were increasing (Fig. 3, Appendix 1, Table 2). Generally, field voles exhibited highest indices of physiological condition when populations were stable or increasing slightly.

The return rate of field voles between February and April was higher in the increase than in the decrease phase in all five tested models with the different

Table 1. Results from model selection procedure to identify the effects of current (n_t) and earlier population densities (n_{t-4}, $n_{t-8} \ldots n_{t-24}$ = interpolated population density estimates 4–24 weeks prior to n_t, respectively) on the growth rate (r_t; see Methods for formula) of field vole populations. The symbol × indicates that the variable is included in the model. K = number of estimated parameters in the model, AICc = Akaike's information criterion corrected for small sample size, ΔAICc = difference in AICc units between the model and the most parsimonious model of the candidate set (smallest AICc value; the two most parsimonious models are displayed in bold face), model weight = the relative likelihood of the model given the data. Model-averaged parameter estimates across all candidate models containing the variable in question, with respective 95% confidence limits, are given only for n_t and n_{t-24} on the basis of their variable weights. Variable weights indicate the relative importance of the variables and are calculated as the sum of all model weight values in which the variable appears (Burnham and Anderson 2002).

		Independent variable							−2 log likelihood	K	AICc	ΔAICc	Model weight
		n_t	n_{t-4}	n_{t-8}	n_{t-12}	n_{t-16}	n_{t-20}	n_{t-24}					
Dependent variable	r_t	×	×	×	×	×	×	×	113.8	9	154.3	26.9	0.00
		×	×						166.2	4	176.2	48.8	0.00
		×		×					159.6	4	169.7	42.3	0.00
		×			×				144.2	4	154.5	27.1	0.00
		×				×			144.5	4	154.8	27.4	0.00
		×					×		132.2	4	142.8	15.4	0.00
		×						×	**116.4**	**4**	**127.5**	**0.1**	**0.49**
		×							173.5	3	180.6	53.2	0.00
			×						169.2	3	176.3	48.9	0.00
				×					163.1	3	170.3	42.9	0.00
					×				146.2	3	153.6	26.2	0.00
						×			147.4	3	154.7	27.3	0.00
							×		135.0	3	142.5	15.1	0.00
								×	**119.7**	**3**	**127.4**	**0.0**	**0.51**
									178.9	2	183.4	56.0	0.00
Model-averaged values	Estimate	−0.061	.	.	.	.	.	−0.094					
	Lower 95% CLl	−0.125	.	.	.	.	.	−0.176					
	Upper 95% CLl	0.002	.	.	.	.	.	−0.013					
	Variable weight	0.487	0.00	0.00	0.00	0.00	0.00	0.999					

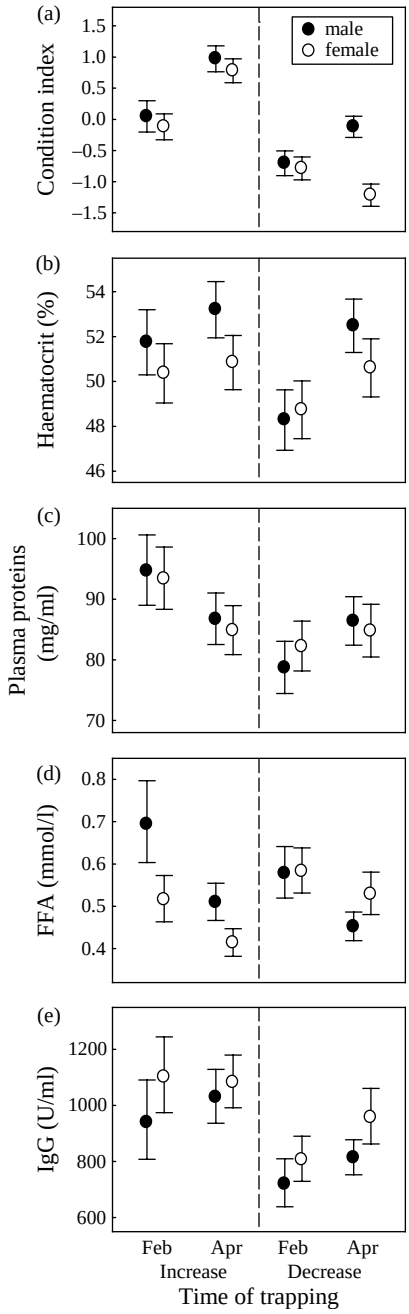


Fig. 2. Mean (±SE) values of body condition index (a), haematocrit (b), plasma proteins (c), free fatty acids (FFA; d), and immunoglobulin G (IgG; e) of field voles sampled in February and April during both an increase phase and a decrease phase of their population cycle. Statistics in Appendix 1, Table 1.

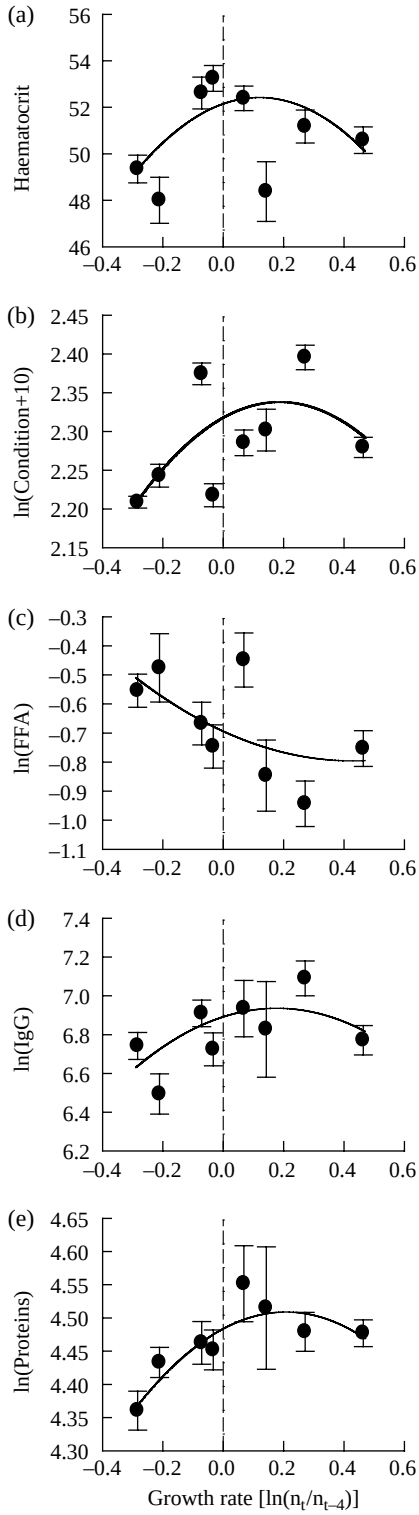
physiological measurements as covariate (mean back-transformed predicted return rate estimates for increase and decrease: 0.34 and 0.06, respectively; mean 95% confidence limits for difference in increase and decrease least squares means: 0.03–0.39; all p-values < 0.03). The predicted return rate from February to April was positively related to the haematocrit levels of field voles in February (backtransformed slope estimate for main effect ±SE: 0.55 ± 0.02) ($F^{haematocrit}_{1,75.8} = 4.47$, p = 0.038). The return rate of females was positively related to FFA levels in the increase phase, and negatively in the decrease phase, while no such relationships were observed for males ($F^{FFA \times sex \times phase}_{1,63.9} = 4.60$, p = 0.036). Return rate was not related to body condition, plasma protein levels or IgG.

The mean number of green shoots counted in the protected quadrats after snowmelt did not differ between the increase and decrease years (Fig. 4, $F^{phase}_{1,72} = 0.57$, p = 0.45). The mean number of green shoots was lower on unprotected quadrats than on protected quadrats after both winters, but more pronouncedly so after the decrease winter (Fig. 4, $F^{phase \times protection}_{1,72} = 5.79$, p = 0.02). The variance component estimate for site was again small relative to the estimate for residual variance (158.6 and 900.2, respectively). Effectively, almost 90% of all green vegetation shoots had been consumed by voles during the winter of the decrease phase, and only two thirds in the increase phase.

Discussion

The physiological condition of field voles was lower in the winter of the decrease phase than in that of the increase phase of the three-year population cycle. Physiological condition was positively related to previously experienced population growth rates when populations were declining, whereas the relationship tended to be negative when populations were increasing in size. Voles exhibited a lower return rate in the decrease phase than in the increase, suggesting poorer survival. The fact that voles had consumed roughly 90% of all vegetation during the decrease winter, compared to only two-thirds in the increase winter, suggests that the overwinter decrease in vole population cycles is strongly influenced by a depletion of food resources, which compromises the physiological condition of voles. This implies that malnutrition induces poor physiological condition in voles, which in turn may increase mortality either directly through starvation or indirectly through increased susceptibility to predators and pathogens.

Both studied populations exhibited a general pattern typical of a multiannual population cycle; they increased gradually in size from autumn 2001 to autumn 2002, whereafter they decreased to zero by autumn 2003 (Fig. 1a–b). Contrary to expectations, the SE field vole population appeared to increase briefly in size between February and April of the decrease phase



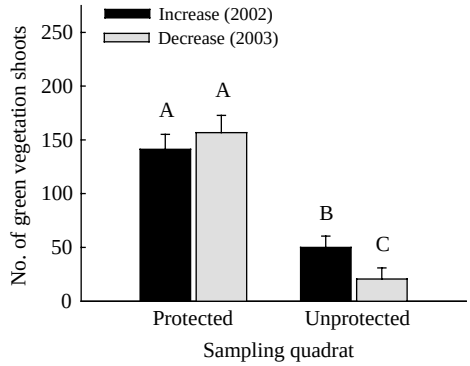


Fig. 4. Overwinter food consumption by voles in the increase phase (2002; black bars) and decrease phase (2003; grey bars) of their population cycle, as measured by least squares mean (±SE) numbers of green vegetation shoots remaining after snowmelt in 0.25 m^2 quadrats. Protected quadrats excluded vole grazing by means of a fence while the randomly selected unprotected quadrats were unfenced. Different letters above the bars indicate significant ($\alpha = 0.05$) differences between shoot numbers.

(Fig. 1b). As reproduction had not yet begun in the population in the decrease April, a possible explanation might be an increase in the mobility of resident voles, which lead to higher numbers of individuals being trapped; low and decreasing population sizes are often associated with increased movement rates in small mammals (Ims and Andreassen 2005). However, we cannot exclude immigration as a contributing mechanism. Having been uncultivated for several years, the SE site may have offered more cover from predation at the end of winter than the surrounding cultivated farmland, thus potentially making it an attractive patch for voles facing exposure by a diminishing snow cover.

Despite these inter-population differences in dynamics, vole population growth was negatively related to both current densities as well as densities ca six months earlier (Table 1). In other words, vole populations exhibited both direct and delayed density dependence, respectively, which fulfills the theoretical prerequisites for multiannually cyclic dynamics (Turchin 2003). Direct density dependence, which is considered the mechanism through which population growth becomes limited, has been found to be strong during winter seasons (Hansen et al. 1999, Stenseth

Fig. 3. Mean (± SE) population-wise values of body condition index (a), haematocrit (b), plasma proteins (c), free fatty acids (FFA; d), and immunoglobulin G (IgG; e) measured in February and April of 2002 (increase phase) and 2003 (decrease phase) plotted against the population growth rate (r_t) during 4 weeks preceding blood sampling. Regression lines drawn on the basis of individual observations (see Appendix 1, Table 2 for parameter estimates). Vertical dashed lines indicate the point of zero population growth.

et al. 2002, 2003, Lima et al. 2006, but see Yoccoz et al. 2001) and suggested to result either from generalist predation (Hanski et al. 1991, Turchin and Hanski 1997, Korpimäki et al. 2002), or winter food limitation (Klemola et al. 2000, Stenseth et al. 2002, Huitu et al. 2003). Although predators are a major source of mortality in vole populations during most times of the year (Norrdahl and Korpimäki 1995, 2002), generalist predators at northern latitudes (mostly birds of prey) are not considered to exert strong mortality on vole populations in mid-winter due to the protection of snow cover and migratory behaviour (Hansson and Henttonen 1985, Aars and Ims 2002, but see Korpimäki et al. 2002, 2005). This leaves winter food limitation as a potential limiting factor of vole population growth.

The body condition indices of field voles were lower in the decrease phase winter than in the increase phase winter and lower in midwinter (February) than in the end of winter (April) in both phases of the cycle (Fig. 2a). Inter-annual variations in body condition have been suggested to be related to variations in food abundance or quality (Norrdahl et al. 2002, Ergon et al. 2001, 2004), with low values indicating that individuals are encountering prolonged periods of reduced energetic intake (Schulte-Hostedde et al. 2001). Seasonally low values of body condition, on the other hand, may indicate that individuals are optimizing their body mass as an adaptation to metabolically challenging environmental conditions, such as cold winters, in order to maximize survival (Iverson and Turner 1974, Hansson 1990, Aars and Ims 2002, Ergon et al. 2004). Taken together, it appears that voles are both optimizing body mass between seasons as well as experiencing food limitation between years.

Although the condition indices of both males and females increased towards the end of the winter in the increase phase, possibly due to the onset of the breeding season, only males exhibited the same positive response in the decrease phase (Fig. 2a). A possible explanation may be that males may be more able to discover and monopolize unexploited food patches when they increase their mobility in spring (Myllymäki 1977a, Erlinge et al. 1990, Norrdahl and Korpimäki 1998), while the more sedentary females continue to reside in overgrazed areas. Nonetheless, our results indicate that in particular female condition is negatively affected by low winter resource levels. This, in turn, may have important implications for reproduction and hence the further dynamics of the populations (Myllymäki 1977b, Andreassen and Ims 1990, Ergon et al. 2001, Koskela et al. 2004).

Changes in the levels of haematocrit and plasma proteins were positively intercorrelated and analogous to changes in the body condition index of voles (Fig. 2b–c). Both parameters are commonly believed to reflect primarily the nutritive and health status of an individual, with low values corresponding to malnutrition, reduced health state or inflammatory processes (Svensson and Merilä 1996, Ots et al. 1998, Johnson 1999, Potti et al. 1999, Nelson et al. 2002, Fuhrman et al. 2004). FFA levels, on the other hand, were negatively correlated with the body condition index of the voles and overall higher in February than in April and in females in the decrease phase than in the increase phase winter (Fig. 2d). An increase in the levels of plasma FFA indicates that voles are mobilizing fat reserves during times of reduced food intake (Torbit et al. 1985, Batzli and Esseks 1992, Voltura and Wunder 1998); this appears to be occurring during the population decrease, particularly so in field vole females.

Field voles had lower IgG levels in the decrease than in the increase phase winter (Fig. 2e). Interpreting the ecological significance of immunological parameters is not straight forward, particularly in unmanipulative studies (Adamo 2004). For example, high values of IgG may indicate that an individual is currently battling an infection, or that a non-infected individual has a high baseline level of immune defence (Ots et al. 1998, Nelson et al. 2002). Unfortunately, histological samples from live voles were unavailable to enable determination of whether voles were in fact infected or not. Nonetheless, maintenance of an effective immune system is energetically costly, and therefore a reduction in food intake may lead to a suppression of immune defences (reviewed by Sheldon and Verhulst 1996, Lochmiller and Deerenberg 2000, Nelson et al. 2002). Based collectively on our other measures of physiological condition, it appears that our measures of IgG indeed reflect a suppression of the immune system. The trend for males to have a lower level of immune defence than females is consistent with earlier observations on hormonally determined sex-bias in mammalian immunity (Schalk and Forbes 1997).

All measured physiological parameters were non-linearly related to the direction and degree of population change that the voles had experienced during the four weeks preceding blood sampling (Fig. 3). In declining populations, physiological parameters were positively related to population growth rate; the steeper the decrease had been the poorer physiological condition the voles exhibited. The dependence of physiological parameters on population growth either disappeared or became reversed when population growth rates increased in positive values (Fig. 3). In other words, voles appeared to be in best physiological condition when populations were either stable or increasing slightly, while both pronounced decreases or increases in population size resulted in poorer physiological condition.

A positive relationship between physiological condition and population growth rate during a population decrease is consistent with the idea that voles are exhibiting symptoms of malnutrition. The symptoms may also be partly explained by an elevated risk of predation (Boonstra et al. 1998, Carlsen et al. 1999), which may contribute to the nutritional stress of voles during non-breeding seasons by, e.g. decreasing foraging movements (Norrdahl and Korpimäki 2000). However, the effects of predation risk on vole physiology, especially during winter seasons, remain controversial and in need of further study (Mappes et al. 1998, Carlsen et al. 1999, Ergon et al. 2004). Although breeding had apparently ceased in the populations during the winter, a possible explanation for the lack of or a negative relationship between condition and population growth rate during a population increase may be that lowered condition reflects costs of increasing inter- or intraspecific competition.

The return rate of field voles from February to April was lower in the decrease than in the increase phase and positively related to levels of haematocrit. Rather than a proximate cause of mortality per se, levels of haematocrit can in this context be regarded more a general indicator of low health or infection (Svensson and Merilä 1996, Potti et al. 1999). Return rates were also positively related to increasing levels of FFA, but only for females in the increase phase. This may indicate that females were able to successfully acquire energy from adipose tissue in the winter of the increase phase, while their body condition in the decrease phase may not have permitted this due to exhaustion of available fat reserves (Batzli and Esseks 1992, Voltura and Wunder 1998) (Fig. 2a). Return rates were not obviously affected by sex (contrary to Aars and Ims 2002), body condition, plasma proteins or IgG. We acknowledge the fact that return rates may be a biased estimator of survival when recapture rates are low (Lebreton et al. 1993, Martin et al. 1995), and emphasize that our aim is not to provide absolute estimates of survival but rather a proxy for survival with which to compare possible inter-annually coincident changes in winter return rates relative to the cycle phase.

Vegetation censuses showed that vole grazing had been considerably more intense during the decrease phase winter than in the increase, and unrelated to interannual climatic differences in primary production (Fig. 4). Although field voles appear less selective in their foraging habits during winter than in summer (Myllymäki 1977a), the ca 10% of all green vegetation remaining after the decrease phase winter probably contains plant species avoided by voles (Moen et al. 1993, Norrdahl et al. 2002), or physically unavailable to them due to ground ice cover (Aars and Ims 2002, Korslund and Steen 2006). Our findings thereby indicate that food resources had become severely depleted during the winter of the decrease phase. Such intense grazing by voles has seldom been documented in natural habitats at northern latitudes (reviewed by Turchin and Batzli 2001). We provide here the first clear indication that such a phenomenon may occur in grassland habitats following high vole densities.

Our study has demonstrated holistic interannual differences in the wintertime physiological health state of field voles during the increase and decrease phases of a multiannual population cycle, which can arguably be largely attributed to variations in density-dependent ecological processes, namely winter food resource utilization. However, we wish to explicitly acknowledge the fact that the study encompassed the aforementioned phases of only one population cycle, and thus only two winters. As interannual variations in weather conditions, also in winter (Drebs et al. 2002), undoubtedly contribute to variation in vole population growth (Aars and Ims 2002, Korslund and Steen 2006), a degree of caution should be exercised when interpolating our results into a more generalized framework of theory on small rodent population dynamics. In any case, our results suggest that winter food depletion limits the growth of vole populations by predisposing voles to malnutrition, which, in turn, increases mortality either directly through starvation or indirectly through increased susceptibility to predators (Korpimäki et al. 2002, 2005) and infections (Soveri et al. 2000). What remains to be investigated through more detailed, temporally extensive work is to what degree the observed deterioration in physiological condition is a general phenomenon of decrease phase voles. Also, experimental work is needed to verify the link between physiological condition and malnutrition and/or infection, and what kind of influence these factors might have on the population dynamics of cyclic voles.

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Appendix 1.

Table 1. Linear mixed model ANOVA table for the effects of population cycle phase (P; increase, decrease), trapping month (M; February, April), sex (S), and their interactions on physiological measurements of health state in field voles. Study site was included in all models as a random factor, as was analysis kit number or date for plasma proteins, free fatty acids (FFA) and immunoglobulin G (IgG). Results are plotted in Fig. 2.

		Phase (P)	Month (M)	Sex (S)	P × M	P × S	M × S	P × M × S	Site	Kit[c]	Residual
Body condition	DDF[a]	209	209	208	208	209	208	208			
	F	115.25	19.13	14.4	14.75	4.68	6.78	6.51			
	P	<0.001	<0.001	<0.001	<0.001	0.03	0.01	0.01			
	VarComp[b]								<0.01	n.a.	0.01
Haematocrit	DDF[a]	206	207	206	206	206	206	206			
	F	8.59	14.76	6.28	4.08	1.25	2.59	0.45			
	P	0.004	<0.001	0.01	0.04	0.26	0.11	0.5			
	VarComp[b]								2.29	n.a.	12.18
Plasma proteins	DDF[a]	187	187	188	186	186	187	186			
	F	12.29	0.43	0.01	11.56	0.45	0.58	0.37			
	P	<0.001	0.51	0.91	<0.001	0.5	0.45	0.55			
	VarComp[b]								0	0.01	0.03
FFA	DDF[a]	190	188	190	189	190	189	189			
	F	0.07	11.97	1.84	0.56	7.16	0.91	0.06			
	P	0.79	<0.001	0.18	0.45	<0.01	0.34	0.81			
	VarComp[b]								<0.01	0	0.15
IgG	DDF[a]	189	189	193	194	190	192	188			
	F	10.68	1.64	2.8	0.57	0.05	0.04	0.31			
	P	0.001	0.2	0.1	0.45	0.82	0.84	0.58			
	VarComp[b]								0	0.02	0.21

[a]Denominator degrees of freedom; NDF (numerator degrees of freedom) = 1 in all models
[b]Variance component estimates for random effect variables
[c]Identifier for the analysis kit number or date for plasma proteins, FFA and IgG

Table 2. Estimates with 95% confidence limits for the first- and second-order effects of preceding population growth rate (r_{t-4}) on the values of physiological health state indices in field voles in winter. All dependent variables excluding haematocrit were ln-transformed for normality. Preceding population growth rate is calculated as $\ln(n_t/n_{t-4})$, where n_{t-4} is an interpolated population density estimate four weeks prior to the blood sampling occasion n_t.

Dependent variable	Intercept			r_{t-4}			r^2_{t-4}		
		95% CL			95% CL			95% CL	
	Estimate	Lower	Upper	Estimate	Lower	Upper	Estimate	Lower	Upper
Haematocrit	52.15	51.46	52.84	4.54	1.78	7.3	−18.95	−27.78	−10.12
ln(condition + 10)	2.32	2.3	2.34	0.22	0.15	0.28	−0.58	−0.8	−0.35
ln(FFA)	−0.69	−0.77	−0.61	−0.47	−0.78	−0.17	0.55	−0.44	1.55
ln(IgG)	6.89	6.8	6.99	0.5	0.14	0.86	−1.4	−2.56	−0.23
ln(proteins)	4.48	4.45	4.52	0.24	0.11	0.37	−0.58	−0.99	−0.17