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Spatial and temporal variation of hantavirus bank vole infection in managed forest landscapes

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Abstract. Zoonoses are major contributors to emerging infectious diseases globally. Hemorrhagic fever with renal syndrome (HFRS) is a zoonosis caused by rodent-borne hantaviruses. In Europe, *Puumala hantavirus* (PUUV) carried and shed by the bank vole (*Myodes glareolus*), is the most common cause of HFRS. We explore the relationship of PUUV infection in bank voles, as measured by PUUV antibody detection, with habitat and landscape scale properties during two successive vole cycles in boreal Sweden. Our analysis revealed that PUUV infection in the population was not uniform between cycles and across different landscapes. The mean density index of PUUV antibody positive and negative bank voles were highest in old forest, second highest in cut-over forest (approx. 0–30 years old) and lowest on mires. Most importantly, old forest was the core habitat, where PUUV antibody positive bank voles were found through the low density phase and the transition between successive vole cycles. In spring, occurrence of antibody positive voles was negatively related to the proportion of cut-over forest in the surrounding landscape, suggesting that large scale human induced land-use change altered the occurrence of PUUV infection in voles which has not been shown before. Dependence of PUUV infection on habitat and landscape structure, and the variation in infection load within and between cycles are of importance for human risk assessment.

Key words: bank vole; forest management; hantavirus; infection load; landscape change; land-use change; *Myodes glareolus*; Puumala virus; Sweden.

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INTRODUCTION

Hantaviruses (family Bunyaviridae) cause human diseases such as hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS) with up to 150,000 diagnosed cases globally (Jonsson et al. 2010). Hantaviruses are considered emerged pathogens. In spite of the large interest regarding hantavirus disease and biology, considerable gaps of knowledge still exist regarding e.g., the ecology of the viruses and how they persist in fluctuating reservoir populations. Nephropathia epidemica (NE) is a milder variant of HFRS caused by the hantavirus Puumala virus (PUUV) present in many parts of Europe (Olsson et al. 2010), and bank voles (*Myodes glareolus*) are the natural reservoir (Brummer-Korvenkontio et al. 1980,

Yanagihara et al. 1984). The epidemiology of NE is closely linked to the temporal and spatial dynamics of the bank vole (Niklasson et al. 1995, Olsson et al. 2005, Voutilainen et al. 2012). In Sweden, approximately 90% of all diagnosed cases are found in the northern part of the country (Olsson et al. 2003).

For zoonotic diseases in general, there is a need to understand how landscape features affect the occurrence of infected individuals at large spatial scales. With such ecological knowledge, landscape patterns that facilitate or impede transmission of virus between different sub-populations of host animals can be revealed (Langlois et al. 2001) and also linked to the risk for human exposure (Ostfeld et al. 2005). In Belgium, Linard et al. (2007*b*) found that infected bank voles were common in the parts of the country that had a large proportion of broad-leaved forests. In Germany, habitat structures associated with old forests such as dead wood was linked to increased probability of PUUV in bank voles within homogenous forest landscapes (Heyman et al. 2012, Thoma et al. 2014). Also, bank voles in wooded recreation areas and forested city parks were found to have high PUUV prevalence (Essbauer et al. 2007, Ulrich et al. 2008). In northern Sweden, bank voles prefer moist spruce forests (Hansson 1978, Olsson et al. 2005) but to our knowledge, no boreal landscape studies focusing on PUUV infected voles and forest landscape structure have been done.

Forestry is changing forest landscapes around the world (Lindenmayer and Franklin 2002). Old boreal forest is transformed into young forest which is generally deficient in natural ground structures (Caruso et al. 2008, Stenbacka et al. 2010). The forest landscape in northern Sweden has changed fundamentally since clear-cutting became the main harvesting method in the 1950s (Ebeling 1959). Prior to the 1950s, the landscape was dominated by old forests that had never been clear-cut, but by 2005 the landscape consisted of approximately 40 % cut-over forests (Ecke et al. 2013). Bank voles are affected by forestry, as high densities of bank voles are associated with dead wood providing shelter (Ecke et al. 2002, Olsson et al. 2005). Furthermore, the winter survival of voles is higher in old forests (Ecke et al. 2002, Savola et al. 2013) and in spring, voles are found in these core habitats where winter survival is best. In summer, reproduction in disturbed habitats, such as clear-cuts, could be as high as in old forest or even higher (Ecke et al. 2002). Voutilainen et al. (2012) found that PUUV infection rate of bank voles was higher in young than in old forest habitats. However, since the population density of bank voles was highest in old forests, these habitats had higher densities of PUUV infected voles (Voutilainen et al. 2012).

During the reproductive season, female bank voles defend territories greater than 4000 m^2 in northern Sweden (Löfgren 1995*a*). Because of this density-dependent factor, bank voles need large forest stands to be able to build up large local populations. As large populations usually are accompanied by a high density of infected bank voles (hereafter infection load, IL; Olsson et al. 2005, Voutilainen et al. 2012), we hypothesize that the occurrence of virus infected individuals may be positively related to the size of the forest patch surrounding the sampling plot (i.e., the focal patch size of old forest). Since bank voles' overwinter survival is poor in young forests (Ecke et al. 2002, Savola et al. 2013), and overwinter survival is lower for infected than non-infected bank voles (Kallio et al. 2007), we also hypothesize that winter survival and subsequent numbers of infected voles in spring would be decreased by poor-quality landscapes with high proportions of young forest.

In northern Sweden, environmental monitoring of vole populations dates from 1971 (Hörnfeldt 1994, 2004), creating good opportunities for linking bank vole densities to NE-incidence (Niklasson et al. 1995, Olsson et al. 2009). As trapped animals are stored deep frozen in the Swedish Environmental Specimen Bank (ESB; Odsjö et al. 1997), presence of hantavirus antibodies in bank voles can be studied retrospectively. Niklasson et al. (1995) found that the proportion of PUUV antibody positive voles in spring was dependent on the bank vole abundance in the previous fall. Following up on other findings, especially by Kallio et al. (2007) and Voutilainen et al. (2012), we now reevaluated the PUUV data published by Niklasson et al. (1995) in relation to local habitat and landscape structure during two successive vole cycles.

Our main aim was to identify core habitats and suitable landscapes where winter survival is

good and where the virus survives low bottleneck phases of the vole cycles. Furthermore, we had three specific aims. We explored whether PUUV antibody positive individuals especially in spring were related to (1) local habitat, (2) focal patch size of old forest, and (3) the proportion of cut-over forest in the surrounding landscape. Finally, we discuss whether there may be long-term changes in PUUV IL in addition to the 3–4 year cyclic changes based on (1) our current study, (2) recent changes in occurrence of NE, and (3) long-term changes in the dynamics of the bank vole and its main predator, Tengmalm's owl (*Aegolius funereus*).

Methods

Ethics statement

Small mammals have been monitored by snap-trapping in northern Sweden since 1971, and since 1979 as a part of the National Environmental Monitoring Programme (NEMP; see below). Permission to trap small mammals have been obtained from the Swedish Environmental Protection Agency (SEPA; latest permission: Dnr 412-4009-10) and from the Animal Ethics Committee in Umeå (latest permission: Dnr A-61-11). Since 1971, trapped animals have been stored deep frozen at −20°C in the ESB (Odsjö et al. 1997), and all tests for PUUV antibodies were performed on specimens from the ESB; see Niklasson et al. (1995) for further details.

Study site and design

The bank voles were trapped in the County of Västerbotten, northern Sweden (Hörnfeldt 1994; 64° N, 20° E; Appendix: Fig. A1), in 1979–86 as part of the ongoing monitoring of small rodents within the NEMP run by the SEPA (Hörnfeldt 1994, 2004). The data on PUUV antibody-positive bank voles were obtained from Niklasson et al. (1995). This existing dataset was carefully reanalyzed in relation to local habitat (cut-over forest, old forests and mires) and surrounding landscape structure as obtained by infra-red aerial photo interpretation (Ecke et al. 2013; see also Appendix: Fig. A2). The study area of 100 × 100 km contained 16 regularly distributed 5 × 5 km areas separated by 20 km. Each 5 × 5 km area was divided into four non-overlapping 2.5 × 2.5 km sub-areas with one 1-ha sampling plots in the center of each (for coordinates, see Fig. 1 and Appendix: Fig. A1). Each 1-ha plot was represented by a 90 m trapping line with 10 trap stations centered along one of the diagonals. Each station contained five snap traps placed within a circle with a 1 m radius (Hörnfeldt 1994; Appendix: Fig. A1). In total, 58 plots were trapped each spring and fall for three consecutive nights except in spring 1981 and 1984 (57 plots). The habitat composition of the 16 sub-areas within the study area in the early 1980s (i.e., when the voles whose antibody status was quantified were collected) was 49% old forest, 23% cut-over forest, 14% mires and 6% meadows. Also, in the early 1980s, mean patch size of old forest was ~15 ha in the eastern (K in Appendix: Fig. A1) and ~50 ha in the western (J in Appendix: Fig. A1) part of the study area (Ecke et al. 2013). This difference was due to the earlier adoption of large scale clear-cuttings practices in the eastern part (Ecke et al. 2013) thus making it interesting to explore if differences also existed in presence/absence of PUUV infected bank voles.

Bank vole data

Bank vole data from 1979–86 were analyzed, representing different phases/years of two successive and complete vole cycles, I: 1979–82 and II: 1983–85 and the first year of a third cycle (III: 1986). The transition between cycles was characterized by a major shift in rate of change in numbers during reproduction season in summer, from low values in the last year of the previous cycle to high values in the first year of ensuing cycles (here: 1979, 1983 and 1986; Appendix: Fig. A3, redrawn from Hörnfeldt 1994: Fig. 3 and Hörnfeldt 2004: Fig. 8). Subsequent cycles were denoted with Roman numerals and subsequent years within each cycle were numbered sequentially by Arabic numerals. For example II:3 refer to the third year in cycle II. The bank vole density index was expressed as number of animals trapped per 100 trap nights (Hörnfeldt 1994).

Hantavirus antibody data

Data on PUUV antibody-positive bank voles were obtained from Niklasson et al. (1995). Antibodies to PUUV had been screened in 2412 bank voles trapped in spring and fall 1979–86 (starting in fall 1979), with 422 voles (17%) being seropositive (Niklasson et al. 1995). In contrast to

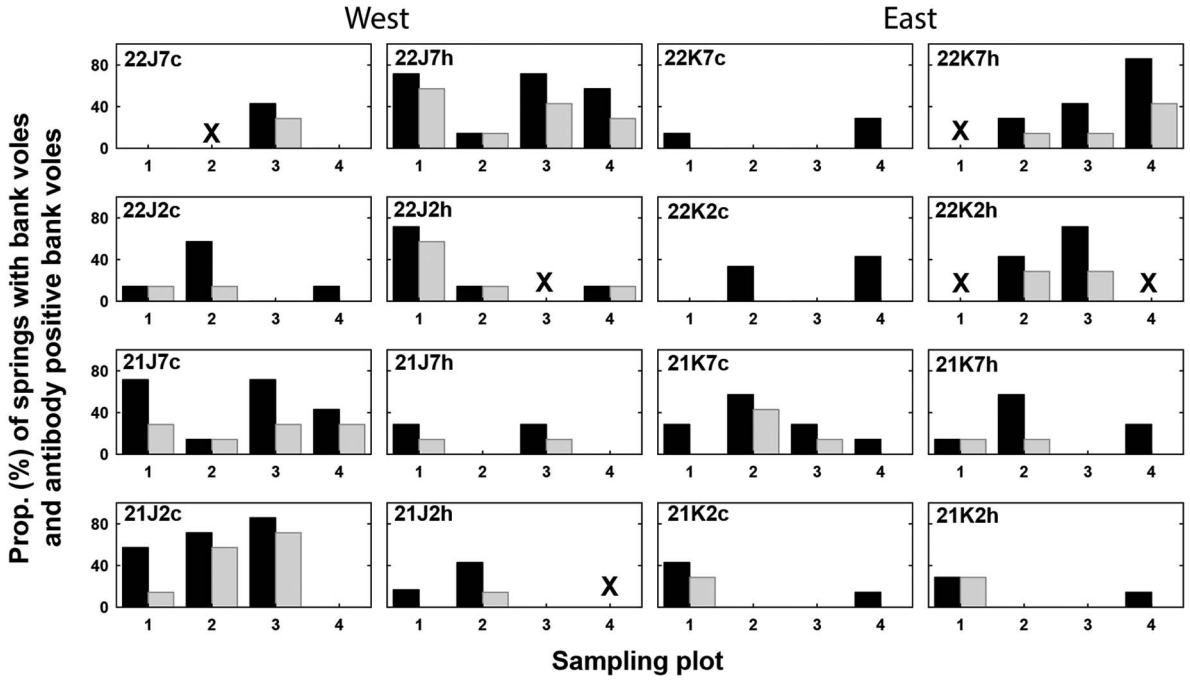

Fig. 1. Proportion (%) of springs with any trapped bank voles (black) and trapped PUUV antibody positive bank voles (grey) for each of the four sampling plots in each of the 16 5 × 5 km areas during 1980–1986. The study area is divided into a western ($n = 8$) and eastern ($n = 8$) region. The 5 × 5 km areas contains four 2.5 × 2.5 km subareas usually with one sampling plot in the center of each. In total, 58 sampling plots were trapped in denoted with coordinates of the Swedish National Grid as explained in Appendix: Fig. A1b, c and Hörnfeldt (1994). X denotes plots that were not sampled.

Niklasson et al. (1995), we refrained from using animals from fall 1987, as that sample did not cover the whole vole monitoring area. The density of bank voles carrying PUUV antibodies were referred to as PUUV infection load (IL) as PUUV causes a life-long persistent infection in bank voles (Meyer and Schmaljohn 2000). Maternal antibodies are important for postponing PUUV infection in young rodents (Kallio et al. 2006). Consequently, following Voutilainen et al. (2012), we treated all hantavirus positive animals <14.4 g in the data set used by Niklasson et al. (1995) as carrying maternal antibodies ($n = 46$) and thus not being infected. Overall PUUV prevalence per season was calculated as: (number of hantavirus positive individuals weighing ≥14.4 g)/(no. of all bank voles).

Data on habitat and landscape scales

Large scale clear-cutting in Sweden started in the 1950s (Ebeling 1959). In the beginning of the study period in 1979, cut-over forest was composed of single-layered monocultures 0–30 year old and the old, multi-layered forest was approximately 81–120 years (Swedish National Forest Inventory 2014). On the habitat scale, sampling plots were characterized by the dominant habitat along the trapping line using a combination of forest vegetation data from Ecke et al. (2013) and field protocols from the NEMP, where it was noted when sampling plots had been clear-cut. Four habitats were used; (1) cut-over forest ($n = 15$–19 plots), (2) old forest ($n = 28$–32 plots), (3) mires ($n = 9$ plots) and (4) meadows ($n = 2$ plots); note that four old forest stands were clear-cut during the study period (in 1979, 1983, 1985 and 1986, respectively) so the sample sizes for cut-over and old forest changed over time (see Appendix: Table A1 for numbers of trapped voles in different habitats). Meadows were not included as the low sample size ($n = 2$) did not allow for comparison with the other

habitat types. The mire types (peatlands) included treeless mires and sparsely forested mires.

On the landscape scale, we used available landscape structure data from Ecke et al. (2013) in 1985. Two landscape metrics were used: (1) focal patch size of old forest (ha) and (2) proportion (%) of cut-over forest in the 2.5 × 2.5 km sub-area. The former was defined as the size of a forest patch intersecting or situated close to the trapping line (≤50 m from the trapping line). If the trapping line was >50 m from old forest, the focal patch size was set to zero. In the analyses, focal forest patches were not allowed to extend outside of the borders of the individual 2.5 × 2.5 km sub-areas and were consequently truncated if that was the case.

Statistical analyses

Chi-squared tests were used to test for differences in presence/absence of bank voles and PUUV infected bank voles in spring between the western and eastern part of the study area. The Mann-Whitney *U* test was used to test for significant differences between proportion of springs during our study period with PUUV infected and non-infected voles in the eastern and western part of the study area. We used Student's *t* test to test for significant differences in vole density index (log-transformed) between different habitat types in each year and season.

On the local habitat scale and in order to test if habitat (cut-over forest, old forest and mires) contributed to the variation in the number of infected bank voles among different sampling plots, we fitted a generalized linear mixed effects model. Separate models were fitted for spring and fall as we expected the pattern to differ between them. Raw counts of infected individuals were used in each plot after removing plots where trapping effort was lower than 100 trap nights, where traps had not been sampled in all years and seasons during our study period or a change of habitat type from old forest to cut-over forest happened during the study period. To correct for repeated measures on the same sampling plots we included "plot" as a random effect. Year was also included as a random effect as we had interest in the variation of infected bank voles that could be attributed to habitat differences. The model for both spring and fall was fitted using a Poisson probability distribution for the count response variable. However, for the spring model and due to the presence of many sampling plots with zero infected bank voles, we also incorporated a term to adjust for zero inflation. Mires were excluded from the spring model, as there were no infected bank voles trapped then. The analyses were carried out in R using the "lme4" package (Bates et al. 2014) for the fall model and the "glmmADMB" package (Fournier et al. 2012) for the spring model.

On a landscape scale and for testing the effect of focal patch size of old forest and proportion of cut-over forest in the surrounding landscape on occurrence of PUUV infected bank voles in individual sampling plots, we also controlled for the effect of (1) local habitat and (2) the proportion of springs with any bank voles in the same model. This was necessary since the occurrence of PUUV infected bank voles is dependent on occurrence of bank voles. This is also in line with Linard et al. (2007*b*), emphasizing the importance of controlling for non-infected individuals in prediction models of hantavirus infected bank voles based on environmental variables. A generalized linear model with binomial errors and a logit link function in the "lme4"-package (Bates et al. 2014) in R was used, allowing for the combination of categorical and continuous predictor variables in the same model. Prior to running the model, all variables included in the model were checked for co-linearity using a cut-off correlation value of ±0.7 (Dormann et al. 2013; Pearson product moment correlation test). All analyses were made using either Statistica 12 (StatSoft 2013) or R.

Results

Spatial and temporal distribution of infected voles

The spatial distribution of PUUV antibody positive bank voles trapped in spring (based on presence/absence data for all years combined) was significantly skewed toward the western part of the study area (Fig. 1; $\chi^2 = 5.61$; $P < 0.05$), where mean patch size of old forest was considerably larger than in the eastern part in the early 1980s (see above and Ecke et al. 2013). Similarly, the median proportion of springs with PUUV antibody positive voles was higher in west (Fig. 1; Mann-Whitney *U* test; $P < 0.05$). In

contrast, the distribution of non-infected bank voles was not skewed ($\chi^2 = 0.35$; $P = 0.56$), neither was the proportion of springs with non-infected voles higher in the west (Mann-Whitney *U* test; $P = 0.29$). Some areas seemed to be PUUV "hot spots" (for example sampling plot #3 in 21J2c, #1 in 22J7h and #1 in 22J2h; which were located in old forest), while no PUUV IL in spring was found in some sampling plots (for example within 22K2c and 22K7c; Fig. 1). All sampling plots experienced local extinction events of both infected and non-infected voles; not a single sampling plot was occupied during all seven springs (Fig. 1).

The proportion of sampling plots with PUUV antibody positive voles increased from spring to fall (i.e., over summer) in the first years, but decreased in the last year of cycle I and II. Similarly, in winter (from fall to spring), the proportion of sampling plots with infected voles increased in year 1 of the cycles and often decreased in the latter part of the cycles (Fig. 2c and Appendix: Fig. A4). There was a high proportion of sampling plots (overall >50%) with antibody positive voles in fall in I:2 and I:3 but only in fall in II:2 (Fig. 2c and Appendix: Fig. A4). The IL was twice as high in cycle I as in cycle II, after pooling data for the corresponding phases of the two cycles (fall in year 1 to fall in year 3; Fig. 2b). In both these cycles, bank voles reached a wide distribution in the landscape, with the highest proportion of sampling plots occupied in fall in I:2, I:3 and II:3 (Fig. 2c and Appendix: Fig. A4). Niklasson et al. (1995) noted but did not emphasize that prevalence gradually rose and fell in cycle I, while the pattern was strictly seasonal in cycle II, with consistent decreases from spring to fall, i.e., during the reproduction season in summer (Appendix: Fig. A4).

Habitat scale effects on PUUV IL in voles

Mean bank vole density index was most often highest in old compared to cut-over forest and mires for all seasons and years. Densities were significantly higher in old than cut-over forest in spring and fall in I:3, I:4, II:2 and spring II:3 (Student's *t* test: $P < 0.05$; Fig. 2a). Mires were rarely occupied in spring (Fig. 2a), but were occupied at high densities in fall in I:2, I:3 and II:2. There were strong winter declines in 1980/81 and 1981/82 in cycle I and in 1984/85 in cycle II (Fig. 2a). The mean IL showed a similar pattern as density index of bank voles and was normally highest in old compared to cut-over forest and mires (Fig. 2b). The overall habitat analysis, considering all years and seasons (Table 1a, b), showed that IL were significantly higher in old forests than in cut-over forest during spring but not fall. IL on mires in spring, when PUUV antibody positive voles were absent, and fall were significantly lower than in both old forest and cut-over forest (Table 1a, b). The cumulated IL across all years in old forest (excluding four plots that were clear-cut in 1979–86) was approximately 2-fold that in cut-over forest and 16-fold that in mires. Early and late in both cycles at low densities (in fall I:1, I:4, spring II:1 and fall II:3) old forest was the main habitat for PUUV antibody positive voles, the only exception being spring in III:1 when they also occurred in cut-over forest (Fig. 2c; see also Fig. 2d).

In contrast, the highest PUUV prevalence was found in cut-over forest especially in spring with 50% in four cases (I:3, I:4, II:3 and III:1) but based on few animals (see Appendix: Table A1), next highest in old forest and lowest on mires (Fig. 2d). In cycle I, prevalence rose successively in old forest until it dropped in the end of the cycle (I:4), while there was a strictly seasonal pattern with summer declines throughout cycle II (Fig. 2d).

Landscape scale effects on PUUV antibody positive voles

In spring, PUUV antibody positive bank voles were expected to be found in suitable sampling plots and landscapes. After statistically controlling for the expected positive effect of the proportion of springs with any bank vole ($P < 0.01$), we found that the proportion of cut-over forest in the surrounding 2.5×2.5 km landscape negatively influenced occurrence of PUUV antibody positive voles in spring (i.e., presence of infected voles in one or more springs; Table 2; $P < 0.01$). These two variables significantly explained the occurrence of PUUV antibody positive bank voles in certain sampling plots during spring (Table 2). Local habitat (old forest, cut-over forest and mires) and focal patch size were both non-significant.

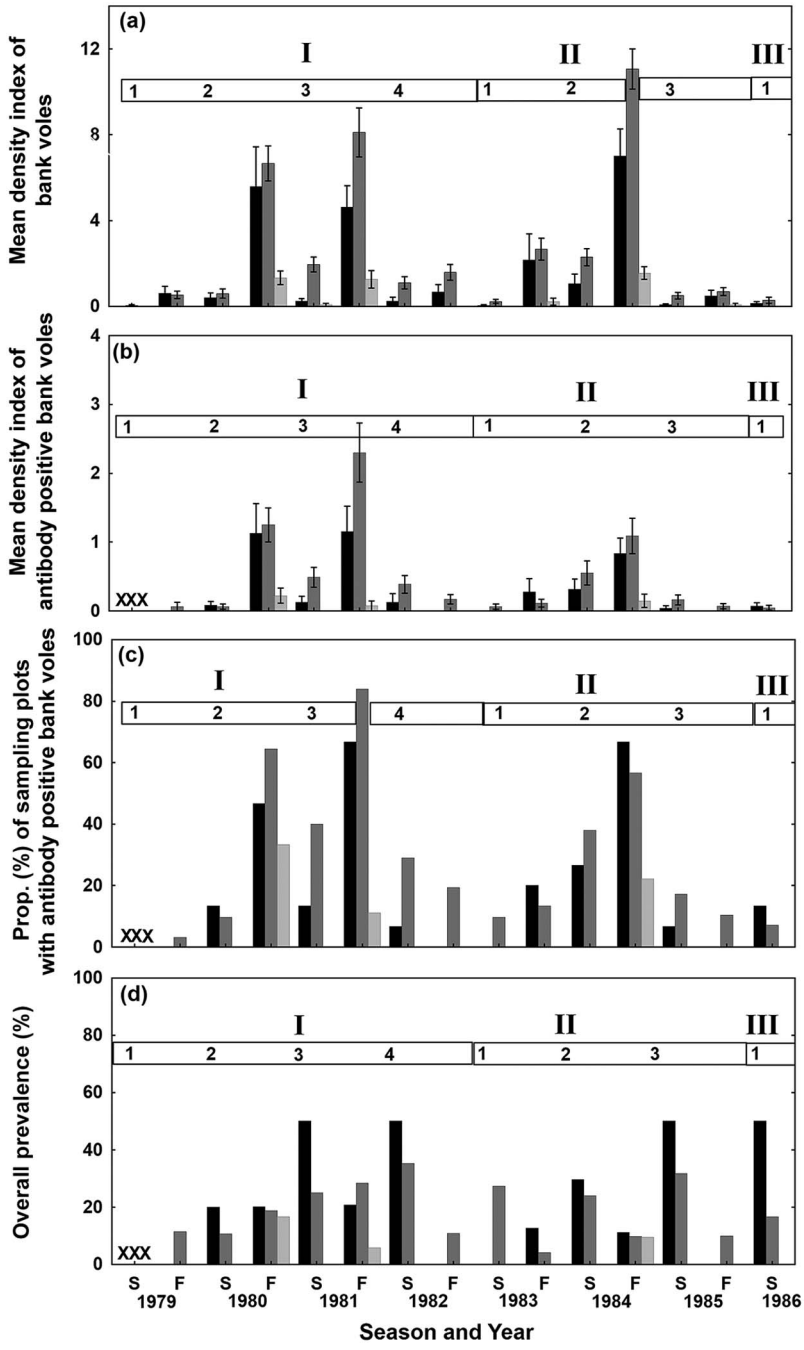

Fig. 2. (a) Mean density index of (a) any and, (b) PUUV antibody positive bank voles, indexed as number of trapped individuals per 100 trap-nights (mean ± SE), (c) proportion (%) of sampling plots with antibody positive bank voles and (d) overall PUUV prevalence (proportion of PUUV antibody positive individuals) in bank voles in each of three main habitats, i.e., cut-over forest (black; $n = 15$–19 plots), old forest (dark grey; $n = 28$–32 plots) and mires (light grey; $n = 9$ plots), in 1979–1986, representing different phases/years (1–4) of three successive vole cycles (I–III). F = fall; S = spring. X denotes that prevalence was not analyzed in spring 1979. Note that four plots with old forest were clear-cut during the study period.

Table 1. Summary of general linear mixed effect models for (a) spring and (b) fall using numbers of PUUV antibody positive bank voles as response variable to test whether habitat (cut-over forest, old forest and mires) contributed to the variation of infected bank voles among different sampling plots. Mires were excluded from the spring model since there were no infected voles trapped on mires in spring. For both models the "intercept" represents cut-over forest habitat. The spring model had a McFadden's pseudo R^2 of 0.19, and ΔAIC compared with the null model was 31.2. The fall model had a McFadden's pseudo R^2 of 0.36, and ΔAIC compared with the null model was 16.1.

Variables	Direction	SE	*P*
a) Spring			
(Intercept)	−1.755	0.670	**0.009**
Old forest	1.017	0.471	**0.031**
b) Fall			
(Intercept)	−1.648	0.611	**0.007**
Old forest	0.457	1.342	0.180
Mires	−2.009	0.622	**0.001**

Table 2. Summary of a generalized linear model showing only significant parameters using a binomial distribution and a logit link function to predict occurrence of PUUV antibody positive bank voles (n = 58 sampling plots) in *one or more* of the spring trappings in 1980–1986. Three continuous predictors were used: proportion of springs with bank vole occurrence (Prop. of Mg), cut-over forest in the surrounding landscape (Prop. of cut-over), focal forest patch size of old forest (focal patch size). In addition, we used one categorical predictor with four levels: habitat type (cut-over forest, old forest, mires and meadows). The model had a McFadden's pseudo R^2 of 0.63, and ΔAIC compared with the null model was 42.7.

Variables	Direction	SE	*P*
(Intercept)	−0.302	1.724	0.861
Prop. of Mg	12.061	4.057	**0.003**
Prop. of cut-over	−10.879	4.083	**0.008**

Discussion

Spatial variation of PUUV antibody positive bank voles

In the present report we have for the first time linked the variation PUUV antibody positive bank voles to differences in forest landscape structure suggesting that large scale human induced land-use change alters occurrence of PUUV infection in voles. Voutilainen et al. (2012), studied one vole cycle and concluded that the majority of Finnish forests provided suitable habitats and high connectivity between bank vole populations and viral strains. However, their results were based on habitat scale analyses not accounting for landscape metrics. The contrast between the eastern and western part of our study area, with a skewed distribution of PUUV antibody positive voles to the western part in the early 1980s, is interesting as it corresponds to a time lag in start of modern forestry and the adoption of large-scale clear-cutting, which started earlier in the eastern part and with higher logging pressure (Ecke et al. 2013). We show that the proportion of cut-over forest had a significant negative influence on the occurrence of PUUV antibody positive voles during spring suggesting that increased logging pressure can reduce PUUV distribution (Table 2). The observed local extinction dynamics of bank voles in general and of PUUV antibody positive bank voles in relation to habitat and landscape structure offer a unique study system to be further explored and compared with similar studies in other disease systems with regards to forestry effects (e.g., Allan et al. 2003, Suzan et al. 2008). Human risk assessments may be improved by combining such information on survival of PUUV antibody positive bank voles in different landscapes and where they emerge from following low-phases of the bank vole cycles. However, it must be noted that the infrequent sampling (twice per year in spring and fall) used in our study system can influence the interpretation of vole and disease dynamics (Krebs and Myers 1974, Carver et al. 2010).

Niklasson et al. (1995) only studied the temporal dynamics of hantavirus-prevalence in bank voles, not the spatial dynamics. However, in the 2000s satellite-image based forest landscape data for this region became available (Reese et al. 2003) and even more recently more detailed aerial photo-based landscape data, directly matched to the current sampling plots (Ecke et al. 2013) has changed the situation. Together, these have increased the potential to perform landscape-scale studies on vole populations (Hörnfeldt et al. 2006, Christensen et al.

2008) and, as in the present study, also on their hosted pathogens. So far, there are only a few studies worldwide on the dependence of occurrence and dynamics of hantaviruses on landscape features (e.g., Langlois et al. 2001, Glass et al. 2002, Linard et al. 2007*a*).

Bank voles in general and PUUV antibody positive bank voles occurred frequently on the habitat scale in old forest, followed by cut-over forest and mires as the least important. Old forest was evidently the core habitat for virus transmission and survival during low densities at the transition between cycles (Fig. 2b), and here most of the hot spots in the western part of the study area were found (Fig. 1). These results regarding the importance of old forest are in line with similar findings by Olsson et al. (2005) and Voutilainen et al. (2012). In contrast to both of these studies, we also studied the distribution of infected voles at the landscape scale and also the occurrence on mires on the habitat scale, which represent an important habitat in our study area and boreal areas in general (Ahti et al. 1968) and to our knowledge has never been studied previously with respect to PUUV distribution. PUUV antibody positive bank voles were never trapped on mires in spring, and although IL and proportion of sampling plots with infected voles in cut-over forest were often fairly high in fall, they were low in the ensuing spring (Fig. 2b, c) implying that the loss rates of antibody positive voles were particularly high in these habitats. However, as we do not know the fate of individual voles over winter, we could not determine whether these habitats were mainly "dead-ends" or contributed to virus transmission in the bank vole population.

As old, often spruce-dominated forest is the main habitat of bank voles (Hansson 1978, Olsson et al. 2005), the negative effect of forestry on the occurrence of PUUV antibody positive voles in spring has likely resulted from clear cutting and converting such forest into cut-over forest. As reported by Stenbacka et al. (2010), young, thinned forests lack many forest structures commonly found in old forest, such as coarse downed logs, implying a decrease in shelter against predators and increasing predation risk. Also, arboreal lichens are lost in the young, cut-over forest (Esseen et al. 1996), and thereby important winter food for the voles (Viro and Sulkava 1985) which may decrease their over-winter survival. In addition, the cover of the important food plant bilberry, *Vaccinium myrtillus*, is estimated to increase with increasing age of the forest stand (Miina et al. 2009). In North America, abundance of berry crops in late summer and fall have been proposed to increase over-winter survival for *Myodes rutilus* (Boonstra et al. 2001), a closely related species to the bank vole. Boonstra and Krebs (2011) also suggested that year-round feeding experiments should improve over-wintering survival of *Myodes*-populations since food limitation is an important factor driving population change.

The high PUUV prevalence in cut-over forest in spring should probably not be over-emphasized as it occurs at very low bank vole densities (Fig. 2d). However, an important aspect of the varying PUUV prevalence in different habitats is its bearing on the design and interpretation of studies on prevalence. Comparisons of studies based on pooled overall prevalence without considering habitat type may be hampered by different habitat representation and therefore needs to be considered. This is line with Douglass et al. (2001) that suggest a more local than general approach in Sin Nombre hantavirus models due to large variation in host species abundance and Sin Nombre hatanvirus antibody prevalence among habitat types.

Temporal variation of PUUV antibody positive bank voles

Niklasson et al. (1995) pointed out that the overall prevalence of PUUV in bank voles was consistently higher in spring than the previous fall, suggesting horizontal virus transmission. This type of delayed density dependent antibody prevalence pattern has been found in studies of Sin Nombre hantavirus also (see Madhav et al. 2007, Carver et al. 2011). As found here, this pattern holds true for old and cut-over forest, but not for mires as infected bank voles were only trapped there in fall. PUUV prevalence dynamics clearly differed between cycle I and II. PUUV prevalence increased smoothly in cycle I in old forest (Fig. 2d), while it was strictly seasonal with regular summer declines in cycle II in both cut-over forest and old forest (Fig. 2d). This also translated into a difference between cycles of the IL in the bank vole population, as the density

index of PUUV antibody positive infected voles (Fig. 2b) and the proportion of sampling plots with infected voles in the landscape (Fig. 2c) were higher in cycle I than cycle II, especially in old forest. Niklasson et al. (1995) focused on analyzing variation of infection rates within cycles and the dependence on bank vole densities. One of the key findings in our study was the temporal variation of IL also between cycles which, as far as we know, have not been shown explicitly before. This variation between cycles speaks in favor of more comparative and long-term studies of the temporal dynamics of PUUV infection in bank vole populations, and of the underlying mechanisms (preferably with frequent trapping sessions if possible). At present we have no strong hypothesis for the variation between cycles of the IL in the bank vole population. However, it is worth noting that the shift to seasonal PUUV dynamics, in cycle II compared to cycle I, occurred during a period with a more general disturbance and shift from cyclic to seasonal vole dynamics in this and other areas of Europe (e.g., Hörnfeldt et al. 2005, Cornulier et al. 2013).

Long-term changes of infection load in the bank vole population?

As mentioned above, our results suggest that increased logging pressure reduces the distribution of PUUV antibody positive bank voles. However, to assess the relative importance of forest management on long-term changes in prevalence and distribution of PUUV other external factors also have to be considered. In 2007/08 we had the largest NE outbreak recorded in Sweden so far, with 1483 human cases (Olsson et al. 2009, Khalil et al. 2014*b*). The outbreak was associated with adverse weather and harsh snow conditions in winter, thought to trigger bank vole behavior to enter human dwellings more than normal and thereby increasing human contacts with infectious voles. However, it was also suggested that this extreme outbreak could have been related to higher PUUV prevalence, and implicitly higher IL in the bank vole population than in the early 1980s (Olsson et al. 2009).

Other sympatric small mammal species and predators may also affect prevalence and distribution of hantavirus in host species populations (e.g., Suzan et al. 2009, Voutilainen et al. 2012). In that context it is interesting to recall three major, and highly relevant, changes that have occurred in our study area. Since the early 1980s, the long-term decline of vole populations has led to (1) more or less an extinction of the grey-sided vole (*Myodes rufocanus*) population, (2) a severe and persistent decline of the Tengmalm's owl population, and (3) an elevation of the cyclic low phase of bank voles in spring, applying both to density index and especially to the distribution of the population in the landscape (Hörnfeldt et al. 1990, Hörnfeldt 1994, 2004, Hipkiss et al. 2013). The grey-sided vole is a sympatric species to the bank vole in this boreal forest landscape (Hörnfeldt 2004, Hörnfeldt et al. 2006, Christensen et al. 2008), as is the Tengmalm's owl, which is also an important predator on the bank vole (Hipkiss et al. 2013).

Ostfeld and Holt (2004) made a strong argument that predators may be good for human health by reducing host density. According to their review, loss of predators should increase the number of infected prey. The role of predators in this sense has obviously been overlooked so far (Khalil et al. 2014*a*), and we only know of the study by Orrock et al. (2011) who reported a negative relationship of prevalence of Sin Nombre virus (SNV) in deer mouse (*Peromyscus maniculatus*) populations with species richness of predators on different islands. However, other recent studies point in the same direction. Dizney and Dearing (2013) made a critical observation, finding that SNV infected deer mice were more likely to be involved in aggressive interactions and travelled longer distances than other mice (but see Lonner et al. [2008]; Waltee et al. [2009] found no effect from SNV infection on movement of deer mice). Such risky behaviors make infected animals more vulnerable to predation. In addition, Kallio et al. (2007) recently found that overwinter loss rate was higher for infected than non-infected bank voles. Although the cause of losses/mortality was not studied, predation seems to be a strong candidate and we suggest that captured animals in future studies are examined for wounds or scars. With the above recent findings in mind, we hypothesize that PUUV prevalence, and IL, in the bank vole population has increased in later years compared to the early 1980s, partly as a result from relaxed predation due to the decline of the Tengmalm's owl.

Voutilainen et al. (2012) found a dilution effect on PUUV prevalence in bank voles from the abundance of sympatric, non-reservoir small mammals in spring. The main dilution effect hypothesis in non-vector-borne disease systems such as ours is that a more diverse animal community will create fewer opportunities for individuals of the host species to meet with each other and transfer the pathogen, i.e., reduction in contact rate (Keesing et al. 2006). As the small mammal community in our study area and that in Voutilainen et al. (2012) is similar, we would expect a dilution effect here. However, we would also expect a relaxed dilution of it since the early 1980s, because of the severe decline of the grey-sided vole (see above), which is closely related, larger and dominant in space-use over the bank vole (Löfgren 1995*b*, Johannesen et al. 2002). We hypothesize that such a relaxation in turn may have contributed to increased PUUV prevalence and IL of the bank vole population. However, the concept of the dilution effect has been criticized (Randolph and Dobson 2012, Salkeld et al. 2013) which has led to a discussion of about the generality and applicability of the concept (Ostfeld 2013). In a recent review we found a consistent and strong dilution effect in all hantavirus systems (incl. PUUV) studied to date (Khalil et al. 2014*a*) but more studies of the dilution effect on PUUV prevalence are needed to understand the mechanism.

By analyzing more recent samples from the ESB (see above), studies are now under way to explore whether PUUV prevalence, distribution and IL in the bank vole population have increased in our study area since the 1980s (this study; Niklasson et al. 1995) and, if so, may be related to relaxed predation on bank voles by the Tengmalm's owl, and to a relaxed dilution effect from the grey-sided vole population and/or changes in logging pressure.

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Supplemental Material

Appendix

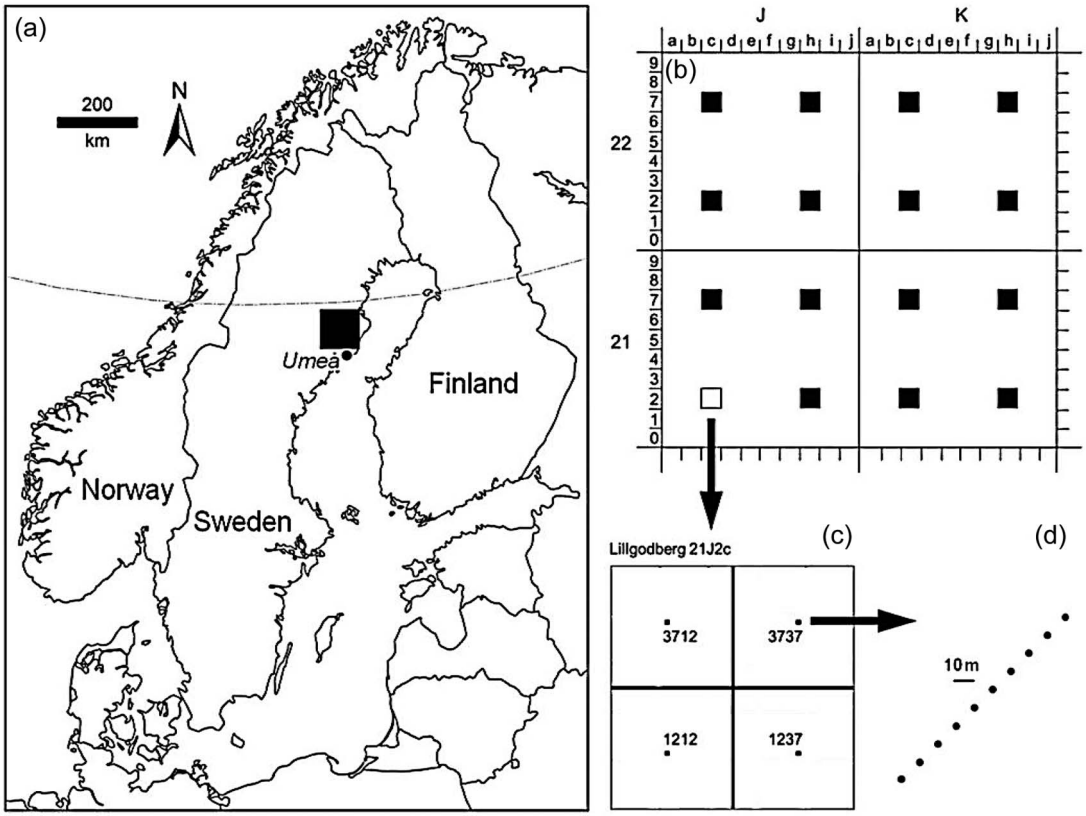

Fig. A1. (a) Study area (shaded) located in Västerbotten county, northern Sweden consisting of (b) 16 regularly-distributed 5×5 km areas. (c) Each 5×5 km area contained four 1-ha sampling plots, each in the center of one of four 2.5×2.5 km sub-areas, with plots denoted according to the coordinates of the Swedish National Grid. Each 1-ha plot was represented by (d) a 90 m trapping line with 10 trap stations centered along one of the plot diagonals. Each station contained five snap traps placed within a circle with a 1 m radius. In total, 58 out of 64 plots were sampled; six sampling plots fell on unsuitable land cover types such as water and were not trapped.

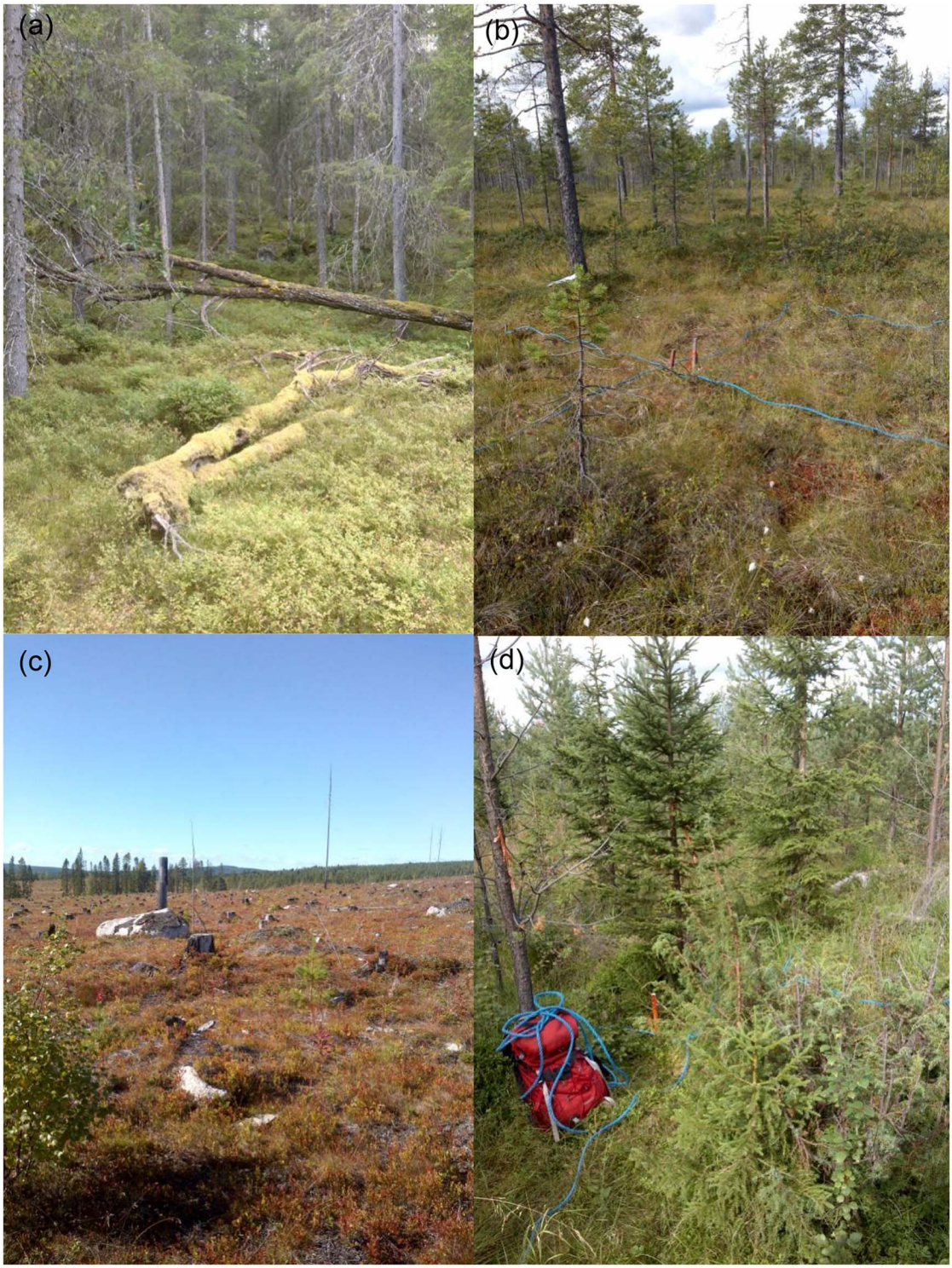

Fig. A2. Representative photographs of the main studied habitat types. (a) old forest, (b) mire, (c) cut-over forest (new clear-cut), (d) cut-over forest (young plantation).

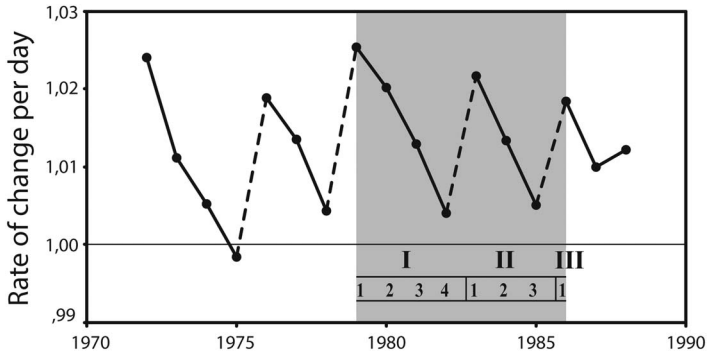

Fig. A3. Rate of change in summer 1972–1988 (according to trapping indices in spring and autumn) for *Myodes glareolus*. The horizontal lines denote stability in numbers. Broken lines indicate the major shifts in rate of change, from low to high(er) values, at the transition between successive cycles. Redrawn from Hörnfeldt (1994, 2004).

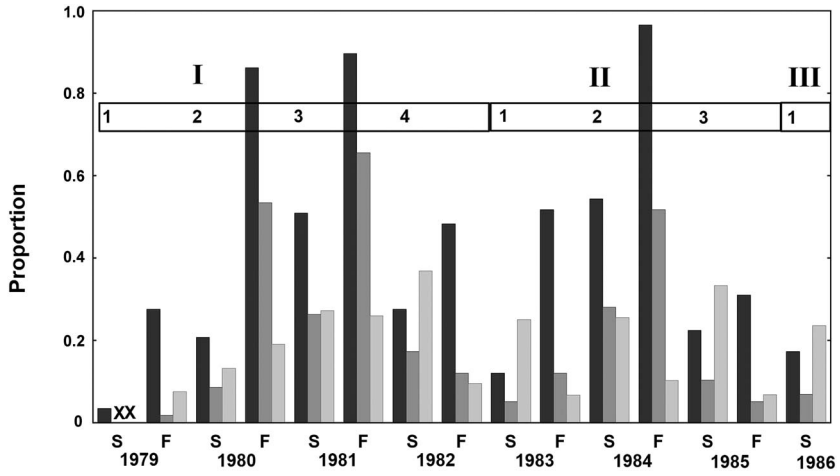

Fig. A4. Proportion (%) of sampling plots in falls and springs with all bank voles (black), PUUV antibody positive bank voles (dark grey) and overall proportion (all sampling plots combined) of PUUV antibody positive bank voles (light grey) in 1979–1986, representing different phases/years (1–4) of three successive vole cycles (I–III). F = fall; S = spring. X denotes that antibodies were not analyzed in spring 1979.

Table A1. Number of bank voles carrying antibodies to PUUV (body weight >14.4 g) and the total number of trapped bank voles in each habitat and season 1979–86 (starting in fall 1979) used for the habitat analyses. F = fall; S = spring. Note that four plots with old forest were clear-cut during the study period and that meadow sampling plots were excluded due to low sample size ($n = 2$).

	No. antibody positive voles >14.4 g			Total no. trapped bank voles		
Season and year	Cut-over forest (n = 15–19)	Old forest (n = 28–32)	Mires (n = 9)	Cut-over forest (n = 15–19)	Old forest (n = 28–32)	Mires (n = 9)
F79	0	3	0	14	26	0
S80	2	3	0	10	28	0
F80	27	58	3	134	310	18
S81	3	22	0	6	88	1
F81	23	107	1	111	377	17
S82	3	18	0	6	51	0
F82	0	8	0	16	74	0
S83	0	3	0	1	11	0
F83	7	5	0	55	120	3
S84	8	24	0	27	100	0
F84	20	49	2	179	498	21
S85	1	7	0	2	22	0
F85	0	3	0	13	30	1
S86	2	2	0	4	12	0