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Title:

Temporal variation of ***Ixodes ricinus*** intensity on the rodent host ***Apodemus flavicollis*** in relation to local climate and host dynamics

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Abstract

The risk to humans of contracting tick-borne zoonotic diseases depends on the risk of a bite from an infected tick, which can be broken down into its component parts as the number of host-seeking ticks in the environment, in particular nymphs, and the prevalence of tick-borne pathogens they are carrying. In turn, the prevalence of tick-borne pathogens is dependent upon tick biting intensity on hosts that support transmission between ticks; namely rodents. These ticks once fed moult into the next life stage and search for the next blood meal, thus posing a zoonotic risk. Here, we analyse tick biting intensity on rodents in a known tick-borne encephalitis (TBE) focus in Trentino (northern Italy). We examine patterns of tick demography and the influence of host densities and climate on ticks'generation time, development rates, tick density and intensity. During the period 2000-2004 a population of the yellow-necked mouse, ***Apodemus flavicollis,*** the most important TBE transmission host, was intensively monitored. Ticks feeding on individual rodents were counted, distinguishing between the larval and nymph life-stages. Local temperature and relative humidity was calculated using both dataloggers in the field site and regional weather stations. We investigated which factors had a predictive value both on feeding tick intensity and on the overall density of larvae or nymphs feeding on rodents in a year. We observed a negative effect of rodent density on tick intensity, while temperature influenced positively both larvae and nymph intensity. Overall larval density was higher in the years and trapping grids where rodent density was higher, while for nymphs no such effect was observed. The best explanatory variable for nymph density was the larval density in the previous year, confirming the discrete nature of tick demography. This provides important information in terms of monitoring the risk to humans of acquiring pathogen infected ticks.

Running title: host and climate effects on tick dynamics

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1. Introduction

The tick, ***Ixodes ricinus***, is one of the most important vectors of emerging zoonotic pathogens in Europe. Tick-borne pathogens include the agent of Lyme Borreliosis (***Borrelia burgdorferi*** s.l) (e.g. Gern and Humair, 2002), the virus causing Tick-Borne Encephalitis (TBE virus) (e.g. Randolph, 2000), ***Anaplasma phagocytophilum*** , causing Human Granulocytic Ehrlichiosis (HGE) (e.g. Strle, 2004), and other less common, but not less important, pathogens such as ***Babesia microti*** and ***Rickettsia*** spp (e.g. Gray ***et al***., 2002a; Parola, 2004).

Both biotic and abiotic factors influence tick population dynamics and the persistence of tick-borne pathogens (Randolph ***et al***., 2002a; Randolph, 2004; Perret ***et al***., 2004). Indeed, tick questing (host-seeking) in the environment is highly dependent on local climatic conditions, especially temperature and humidity, which affect the demographic processes of recruitment and loss (Randolph ***et al***., 2002b). The density of questing ticks is also influenced by the local abundance of hosts that support tick-feeding, while infection prevalence in the tick popoulation depends on the pattern of tick infestation on these hosts (Randolph ***et al***., 2002a). Among the various ***I. ricinus*** hosts recognised in Europe, small mammals, especially voles and mice, play a crucial role both as reservoirs of infections and as feeding host for larvae and nymphs (Gray ***et al***., 2002b). In particular, the yellow-necked mouse (***Apodemus flavicollis***) supports transmission of the most important pathogens carried by ***I. ricinus*** (Randolph ***et al***., 1999, 2002a). In northern and central Europe, this rodent species displays large temporal variation in abundance, with periodical population peaks mostly related to seed production (mast) (Jensen, 1985; Angelstam ***et al***., 1987).

Due to this interplay between ticks, the environment and hosts the temporal fluctuations in rodent density have been shown to result in changes in tick intensity and their infection prevalence with various pathogens. For example, in the case of Lyme borreliosis, it has been

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observed that variation in numbers of the rodent reservoir ***Peromyscus leucopus*** over time affected both the number of nymphs and their infection rate the year following population peaks, while climatic variables either had no significant effect on the intensity of nymphs or marginally increased the explanatory power of models that included seed production (acorns) or rodent density as independent variables (Ostfeld ***et al***., 2006). Density and ***Borrelia burgdorferi***-infection prevalence of questing nymphs was affected by rodent density in the prior year and by acorn production 2 years previously (Ostfeld ***et al***., 2001; 2006). Similarly, it has been seen in Russia that the years following rodent population peaks, both the number of questing nymphs and their rate of infection with ***Borrelia burgdorferi*** sl, increased (Kovalevskii ***et al***., 2004).

In order to understand the risk to humans of exposure to tick-borne zoonoses and to better understand the demography of ticks we carried out longitudinal monitoring of the sheep tick, ***I. ricinus*** and the rodent host ***A. flavicollis*** in northern Italy, where some of the most common tick-borne diseases, such as Lyme disease and tick-borne encephalitis have been recorded (Hudson ***et al***., 2001; Rizzoli ***et al***., 2002). Here, we report observations of the dynamics of ticks (larvae and nymphs) feeding on rodents over a five year period coupled with analyses of the effects of local climatic parameters and rodent density on tick dynamics.

2. Material and Methods

Study area

This study was carried out in a mixed broadleaf woodland (see Perkins ***et al.*** 2003 for a description of the site), located in Valle dei Laghi within the Province of Trento in the north-eastern Italian Alps (grid reference: 1652050E 5093750N, altitude 750-800m a.s.l.). Within this site eight randomly selected areas (8x8 trapping grids, with a 15 m inter-trap interval, i.e. 64

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traps per grid) were used to monitor the rodent population during the period 2000-2002, with only four trapping grids monitored in 2003 and 2004.

Rodent and tick monitoring

Individual rodents were intensively monitored using capture-mark-recapture techniques during the period 2000-2004. Trapping effort consisted of 75 trap sessions, occurring fortnightly, including the period April-October in all years. Rodent densities were estimated at each trapping session and at each grid over 5 years using the standard open population Jolly-Seber model (Krebs, 1989). Grid size and location remained constant from 2000 to 2004 (14.400 m^2); densities were expressed in number of rodents per hectare.

For each rodent captured a careful assessment of the intensity of ticks feeding on that rodent was carried out, this included counting the number of different tick life stages on that particular rodent. For the purpose of this study the ticks were not removed from the rodents. The biting ticks are easily observed as they typically attach around the head. For this reason we are confident that our intensity counts reflect true tick intensity.

Three different measurements of tick feeding were calculated; the tick intensity, tick density and the integrated tick density, as explained below. Tick intensity, distinguishing between larvae and nymphs, was estimated by calculating the average number of ticks per host, trapped in a fortnightly session. Tick density was calculated by multiplying the tick intensity by the rodent density, within a 15-day period; i.e. a trapping session. The integrated tick density was calculated as the total density of larvae [nymphs] that have fed over a 1-year period. Since larvae complete their feeding in 2-5 days whereas the nymphs take 2-7 days (Balashov, 1972) , we considered that ticks found feeding in a fortnightly trap session could not be the same as those found in a subsequent trap session a fortnight later. Hence, summing the tick densities estimated for all trapping sessions over a year, we obtain the integrated tick density, which is an

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estimate of the total number of ticks (of all the life stages) that have fed on the rodents in that year. Because of the discrete nature (discussed below) of tick demography, we deem that this is the most relevant demographic quantity, and most of the following analyses are focused on it. Questing ticks, distinguishing between larve and nymphs, were collected fortnightly from vegetation only during the period 27th May - 20th Augut 2003. Collection of questing ticks was carried out using a dragging blanket (of one square meter) through six different linear transects of 100 meters near rodent trapping site.

Temperature and humidity data

Temperature and relative humidity (RH) values were collected within the study area using a datalogger (Hobo® Pro Temp/RH). Hourly data were recorded during the period 1 January 1999 - 16 September 2003. In order to obtain a dataset of manageable size the daily averages for the minimum, maximum and mean temperature and RH were computed. However, because of technical problems, the time series, especially for RH data, recorded by the datalogger was not continuous. As such we supplemented, only temperature data, with measurements from nearby weather stations of the provincial governmental offices, according to the procedure described below. For relative humidity no further data were available.

Estimate of development rate for larvae, nymphs and adults

Since individual ticks cannot be followed through development stages, other information is needed to estimate ticks' generation time. Therefore, we employed the procedure used by Randolph ***et al.*** (2002b) to estimate minimal inter-stadial development times using temperature-dependent development rates found in the laboratory, and extrapolated to the field using the field recorded temperatures. We used the equations for development rates estimated by Campbell (1948) and reported by Randolph ***et al***. (2002b). To find the length of the development periods of engorged larvae at any given time, we sum the 10-day development

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rates, until we reach the value 1 (=development completed). It is assumed that if development is not completed in the same year, then ticks will enter diapause and start development in the following spring starting from the development stage reached in the previous year. To obtain an estimate for the expected period between the time when a larva feeds, and the time when it will feed as a nymph 50 days were added. This was based on the assumption that it takes on average 20 days for a tick to harden off before starting questing, 20 days to find a host, and 10 days to feed (Randolph ***et al***. 2002a).

Statistical analysis

We used a regression analysis to predict the temperatures in the field site when the datalogger data were missing. We regressed hourly temperatures from the data logger within the field site against hourly temperatures from the local weather stations.

A linear model was used in order to see whether each years' temperatures and relative humidity were consistently different from one another. Minimum, maximum and average daily temperature together with average daily relative humidity, grouped fortnightly, were used as response variable and year and fortnight (trapping) period as explanatory variables. The variance explained by each explanatory factor and its significance were calculated using stepwise back-wards deletion test (Crawley, 2002). Multiple comparisons were carried out to identify the years in which the temperature differed significantly.

A generalized linear model (GLM) with negative binomial errors (S-PLUS 6.2 Version, Insightful®) was used to study the influence of several explanatory variables on the intensity of ticks counted on rodents (the response variable) from 2000 to 2004, restricting to the period May to October, the main period of tick activity; the variables used are rodent density, temperature (min, max and mean), relative humidity, the year, the fortnight period and the specific grid of

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trapping. For the period May to August 2003, when data on questing ticks were available, we added to the analysis the density of questing larvae and nymphs as explanatory variables.

Finally, to investigate if integrated tick density was significantly affected by rodent density and/or tick density in the same or previous years, we used a GLM with negative binomial errors. Both integrated density of feeding larvae and nymphs were used as the response variable while average rodent density and integrated tick density in previous years were selected as fixed explanatory variables to identify the model that best explained the variance observed.

3. Results

Rodents and tick monitoring

The trapping effort (from 5 April 2000 till 22 September 2004) resulted in 1960 captures of 754 rodents of different species. Almost all captured rodents were yellow-necked mice, ***Apodemus flavicollis,*** with 1906 captures of 726 individuals. Other species, ***Clethrionomys glareolus*** (31 individuals), ***Apodemus spp*** (6 individuals) and ***Apodemus sylvaticus*** (3 individuals), were very rare and were excluded from the analysis.

The mean number of ***A. flavicollis*** varied among years (Fig. 1), so that the estimated rodent density shows a peak in the year 2000 (mean=11.72, SE=1.61) and a minimum in year 2003 (mean=1.79, SE=0.42).

The ticks observed on ***A. flavicollis*** in this study site are mostly ***Ixodes ricinus***, as identified from previous work (Perkins ***et al***., 2003). Ticks feeding on rodents were observed in every sampling session, with peaks in the tick intensity for both larvae and nymphs recorded during the period May-June followed by a decrease through the summer; a pattern that continued during the 5 years of the study (Fig. 1). However, it is notable that in the year 2002 larvae showed a second peak in their intensity during the period August-September (Fig. 1).

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Significant variation in the intensity of larvae and nymphs feeding on rodents was observed between years (see Tab. 1 and Fig. 1). The average intensity of larvae per rodent, in the main activity period May-October, ranged between 13.05 and 23.57 and the number of nymphs between 0.13 and 0.48. The number of adult ticks feeding on rodents is not reported as this was so rarely observed as to be deemed insignificant. Larvae and nymph intensity vary in relation to rodent density, with the intensity of feeding larvae and nymphs decreasing with increasing rodent density (see Tab. 1 and Fig. 1).

Intensity of feeding ticks, especially for larvae, seems to increase with increasing density of questing ticks, but this positive correlation is not significant (P=0.09 for larave), possibly due to the short time series of questing ticks,

Analysis of tick density

As discussed in the Methods Section, we estimated densities of feeding ticks by multiplying tick intensity by rodent density. The temporal variation in tick density is shown in Fig. 2, and appears rather different from Fig. 1. Summing the values of tick densities (Fig. 2) over each year, we obtain the integrated tick densities, an index of the overall number of ticks that have fed on rodents that year.

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site. The resulting regression is shown in Tab. 2 since its R^2 is very close to 1, we are confident that these data can be used to supplement the field site temperature, when datalogger data were missing (Fig. 6). This was not possible for humidity since these weather stations do not report humidity.

We found that the maximum, minimum and mean daily temperatures together with mean daily relative humidity over the period May-October were significantly different between years and fortnight periods (see Tab. 3). Multiple comparisons show the year 2003 to be significantly hotter (for the maximum, minimum and mean temperature) than the others, while 2002 had a significantly colder maximum temperature than other years. In addition, 2003 resulted also drier than 2001 and 2002 (see Tab. 3).

Finally, we found that temperature influence positively the intensity of feeding ticks, both for larva and nymphs (see Tab. 1), while no significant effect of relative humidity has been observed.

Estimate of development rate for larvae, nymphs and adults

Combining the temperature data with estimated tick development rates and observed tick densities, we can predict when ticks seen as feeding larvae should be expected to be found as feeding nymphs, according to relation estimated by Campbell (1948) and reported by Randolph ***et al***. (2002b). The results, not shown, indicate that larvae feeding between May and July in each year could in principle feed as nymphs in the autumn of the same year. However, we do not see any peaks in the density of feeding nymphs in the autumn of the same year (Fig. 2). Instead we see a peak in feeding nymphs in the following year during the spring (April to June). Thus, it appears that the development period between larvae and nymph usually lasts at least one year, probably because of diapause occurring in periods where the temperature would not allow development to occur.

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4. Discussion

As shown in Fig. 1, tick intensity varied considerably during the study period. The analysis of the data shows that, after accounting for year, fortnight and grid factors, rodent density and air temperature influence significantly tick intensity (similarly for larvae and nymphs). Tick intensity increases with temperature and it could be a consequence of the documented positive influence of temperature on tick activity (Randolph, 2004; Daniel ***et al.***, 2006). On the other hand, tick intensity decline with increasing rodent density; this effect is predicted through simple models of the encounters between hosts and questing ticks (Rosà and Pugliese, in press), when recruitment of questing ticks is decoupled from feeding rates, as it is within a season. We did not detect a significant effect of the density of questing ticks on the intensity of feeding ticks (as expected through the same models), but the time series of questing ticks was very short.

In order to understand long term dynamics, tick density is probably more relevant than tick intensity and an estimate of the generation time is necessary. From the analysis of Fig.2, it quickly appears that, in the area of study, larvae that feed in one year generally quest and feed as nymphs in the following year (Fig. 2). It is true that the temperatures recorded in the area would, according to the documented relation between temperature and development rate (Randolph ***et al.***,2002b), often allow for two meals in the same year, but, as discussed before, this seems not to occur. Presumably, larvae feeding in a given year enter diapause at some developmental stage in order to emerge as nymphs in the following spring. It thus appears that tick dynamics can be described through discrete year-to-year transitions, at least as far as the transition from larvae to nymph is concerned; such a pattern had been established in more Northern habitats, such as England and Wales (where, indeed, sometimes the transition requires more than one season)

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(Randolph ***et al***., 2002b), but one could expect a quicker cycle in a warmer climate, such as is found in our field site in Italy, at least compared to Scotland.

From this observation we conclude that one year of observations can be synthesized, as far as the overall population dynamics is concerned, into a few indices, such as the integrated densities of feeding ticks.

We examined which variables had a predictive value on the integrated densities of larvae [or nymphs]. Although there are not many years to look at, it is apparent from Fig. 3 that larval density is higher when and where host density is higher, while (Fig. 4) there is no such effect for nymph density. We stated above that tick intensity declines with rodent density, while we report here that integrated tick density increases with average rodent density. The two results are not in contradiction, because they refer to different time scales (fortnight vs. year) but especially because tick density is computed multiplying tick intensity by host density. Hence, it is perhaps not too surprising finding a positive correlation between integrated larva density and average rodent density. It is then a very strong negative result not finding a positive effect of rodent density on integrated nymph density; the fact that the highest densities of nymphs were estimated in 2001, when host density was lower than in 2000, yields a strong support for 2001 truly being a year of high nymph density.

We next examined what are the main determinants of nymph density. The only significant explanatory variable was larval density the year before, as shown in Fig. 5, suggesting a very simple demography. We did not find instead a positive effect of rodent density the year before, although it positively influences larval density the year before; the lack of significance is probably due to the indirect influence. Ostfeld ***et al.*** (2006), on the contrary, did not find any relation between the density of nymphs one year and larval density the year before but found an effect of host density. This may be due to the difference in geographic area and species studied,

For Peer Review

but more likely is due to the tick sampling method. Ostfeld and colleagues measured densities of ***questing*** larvae, i.e. those that are host-seeking, whilst we used measures of actual feeding ticks, which we posit will have a greater predictive power for the number of emerging nymphs.

It is difficult to detect an effect of temperature on integrated tick densities, since our time series was very short, as far as years are concerned. It is interesting to note, however, that 2003 was markedly hotter and drier than all other recorded years. Correspondingly, the nymph density recorded in 2003 (Fig. 6) is much lower than predicted by the linear model on the basis of the number of larvae the previous year. In fact, the 2003 points are all markedly under the straight line of the linear regression model, while for all other years the points are equally distributed on both sides of the regression line; a regression of integrated nymph density on larval density of the year before and on a factor contrasting year 2003 with all others (in which temperature was similar) gives both factors significant. Although the evidence is very weak, there seems to be a negative effect of extremely hot and dry seasons on tick populations, supported by laboratory experiments that show low humidity to negatively affect larval development and survival (Perret ***et al***., 2000).

From the point of view of predicting risk from tick-borne pathogens to humans, it appears that high density of nymphs (the tick stage that transmits infection to humans) is likely to occur the year after high rodent density, although certainly a longer time series would be needed to reach more definite conclusions. It is true that the risk to humans comes from questing nymphs and not from those already feeding on rodents, but it seems likely that a strong correlation exists between the two densities. Additionally, the tick intensity is crucial for the study of tick-borne encephalitis (TBE) since transmission of this flavivirus occurs only between ticks feeding on a host. The rodent ***Apodemus flavicollis*** has been experimentally determined to be the most important hosts in terms of supporting transmission of TBE between feeding ticks (Labuda ***et al***.

For Peer Review

1997). Therefore the intensity of ticks gives us a proxy measure of the transmission potential of the pathogen.

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Table 1. Output of Generalized Linear Model (GLM) with Negative Binomial Error on the intensity of larvae and nymphs counted on rodents from 2000-2004 restricting to the period May-October.

Response variable	Explanatory variable	Coefficient	Chi-square	*df*	P-value
Larval intensity	Year		12.6	3	<0.01
	Fortnight		31.49	11	<0.01
	Grid		8.01	3	<0.05
	Rodent density	-0.04	16.77	1	<0.01
	Mean daily temperature	0.08	4.66	1	<0.05
Nymph intensity	Year		14.22	3	<0.01
	Fortnight		9.32	11	0.61
	Grid		5.19	3	0.15
	Rodent density	-0.13	4.51	1	<0.05
	Mean daily temperature	0.36	4.82	1	<0.05

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Table 2. Regression of hourly temperature in the Cavedine field site with the temperatures at the meteorological stations (managed by the Istituto Agrario di San Michele all'Adige: see http://217.222.71.209/meteo/) of Aldeno, Arco , Cavedine (village) and Viote Bondone, and on the time of day (***Day*** = hh 10-16; ***Night*** = hh 22-6; ***Intermediate*** others). The values of group ***D*** (day), ***I*** (intermediate) and ***N*** (night) represent the intercept for that group.

Coefficients	Estimate	Std. Error	T-value	P-value
Temperature_aldeno	0.224	0.008	28.93	< 0.001
Temperature_cavedine	0.191	0.009	20.25	< 0.001
Temperature_montebondone	0.287	0.005	55.91	< 0.001
Temperature_arco	0.241	0.010	24.31	< 0.001
group***D***	-2.007	0.042	-48.34	< 0.001
group***I***	-1.177	0.039	-30.25	< 0.001
group***N***	-0.729	0.039	-18.93	< 0.001

R^2 = 0.9841. ***F***-statistic = 9696 on 7 and 10986 ***df***, ***p***-value < 2.2e-16

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Table 3. Analysis of variance for the maximum, minimum and mean temperatures and relative humidity at the Cavedine study site over the period May-October for the years 1999-2003.

Response variable	Factor	F-value	*df*	P-value
Maximum temperature	Year	20.23	4	< 0.001
	Fortnight	87.25	11	< 0.001
Minimum temperature	Year	15.62	4	< 0.001
	Fortnight	88.49	11	< 0.001
Mean temperature	Year	21.47	4	< 0.001
	Fortnight	117.22	11	< 0.001
Relative humidity	Year	20.29	2	< 0.001
	Fortnight	5.53	11	< 0.001

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Figure legends

Figure 1: Variation of rodent density, feeding tick intensity and questing density of larvae and nymphs over a period of five years.

Figure 2: Larval and nymph density over a five-year period.

Figure 3: Integrated larvae density (see text for explanation) against average rodent density for five years of live-trapping, where each data point refers to a trapping grid.

Figure 4: Integrated nymph density (see text for explanation) against average rodent density for five years of live-trapping, where each data point refers to a trapping grid.

Figure 5: Integrated nymph density of year ***t*** against integrated larval density of year ***t***-1 for five years of live-trapping, where each data point refers to a trapping grid.

Figure 6: Variation of fortnightly average minimum, mean and maximum temperature over the period 1999-2003 and fortnightly average relative humidity over the period 2001-2003.

For Peer Review

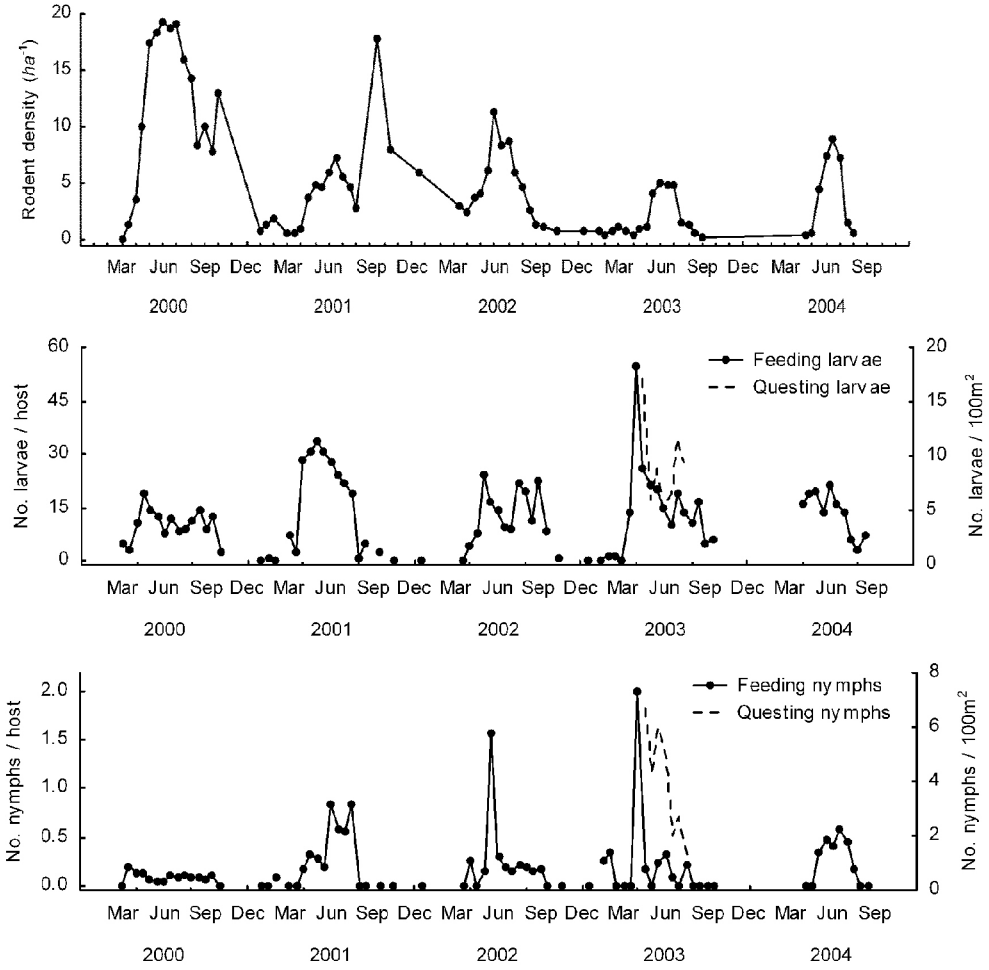
Rodent density (ha-1)
20
15
10
5
0
Mar Jun Sep Dec Mar Jun Sep Dec Mar Jun Sep Dec Mar Jun Sep Dec Mar Jun Sep
2000
2001
2002
2003
2004
No. larvae / host
60
45
30
15
0
Feeding larvae
Questing larvae
No. larvae / 100m2
20
15
10
5
0
No. nymphs / host
2.0
1.5
1.0
0.5
0.0
Feeding nymphs
Questing nymphs
No. nymphs / 100m2
8
6
4
2
0

Figure 1

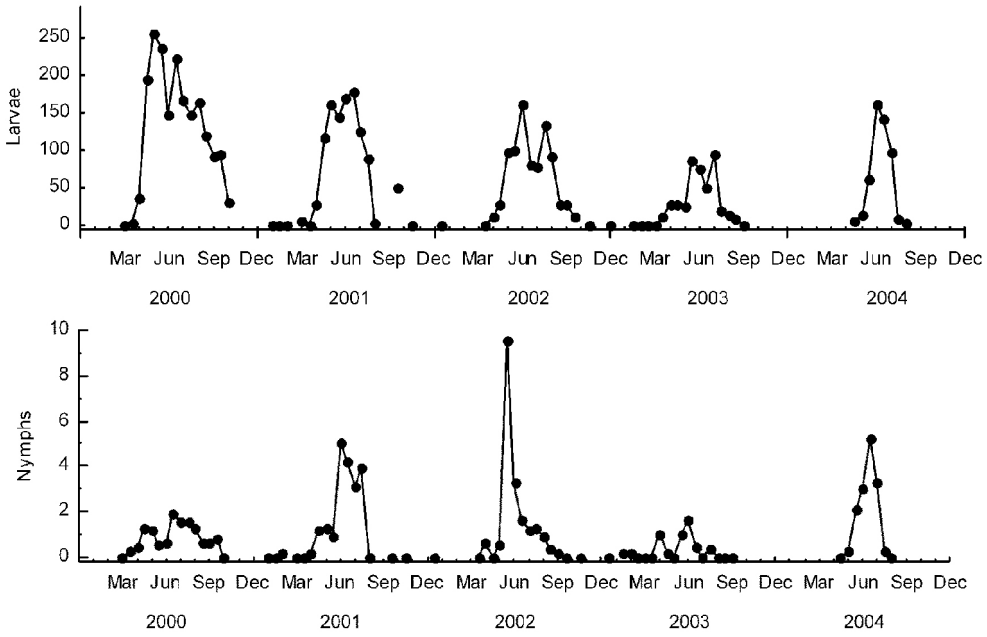

Figure 2

Review

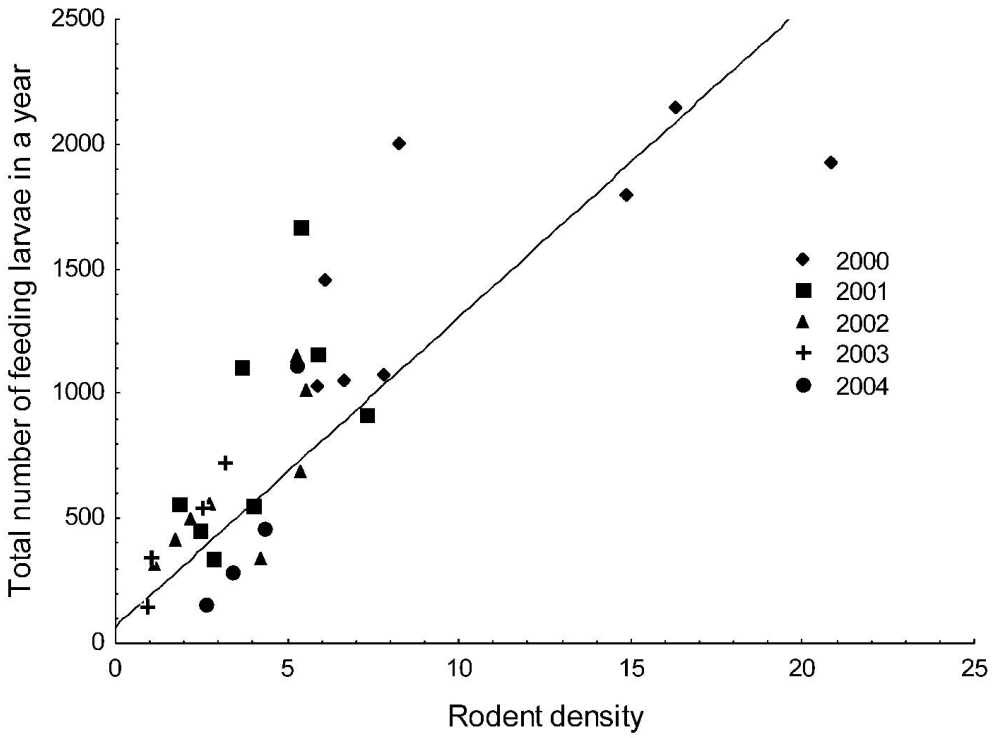

Figure 3

eview

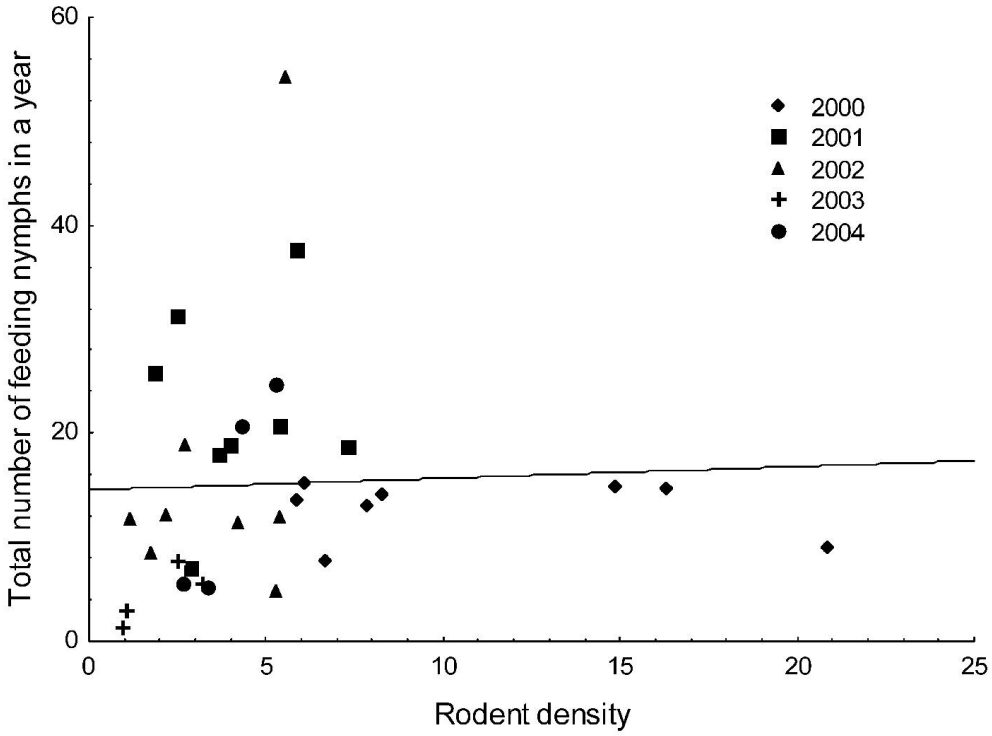

Figure 4

Review

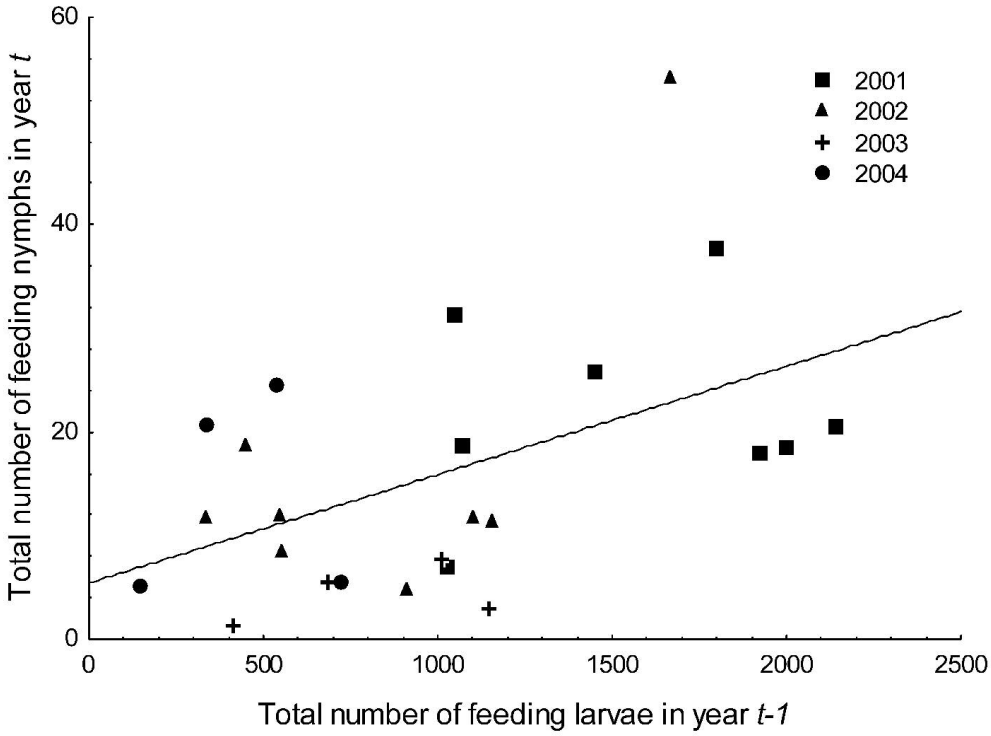

Figure 5

Review

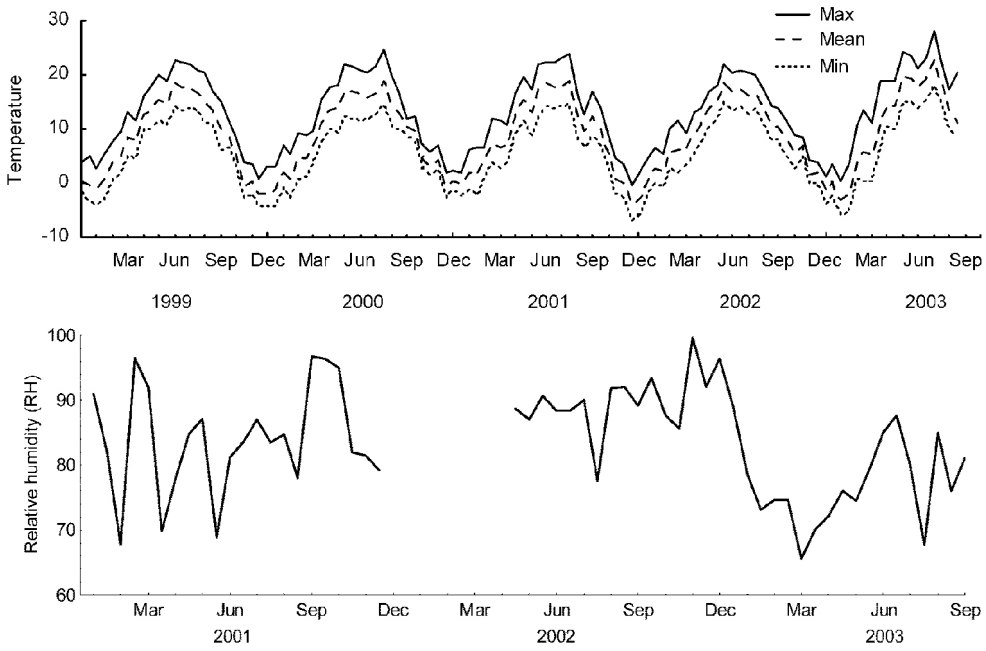

Figure 6

Review