Stress and Demographic Decline: A Potential Effect Mediated by Impairment of Reproduction and Immune Function in Cyclic Vole Populations

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ABSTRACT

The stress response is initially adaptive, operating to maintain homeostasis. However, chronic long-term exposure to stressors may have detrimental effects. We proposed that chronic stress may be a major factor in demographic vole cycles, inducing decline in high-density populations. We monitored four populations of the fossorial water vole *Arvicola scherman*, which undergo pluriannual demographic cycles in the Jura Mountains (France). Sampling was conducted during the high densities and the decline. We measured fecal corticosterone metabolites (FCMs) to assess stress levels and injected phytohemagglutinin to estimate the cell-mediated immune response. We demonstrated that stress levels increase between the high densities and the decline in most of the vole populations. At the individual level, FCM concentrations varied with reproductive status and body condition. During the outbreak, we observed significantly higher levels of FCM concentrations in nulliparous females than in females that had previously reproduced. Moreover, a significant negative correlation was observed between concentrations of FCMs and both immunocompetence and body condition during population decline. These results might reflect an impairment of the female reproductive capability in high densities and accelerated senescence affecting immune function during decline, both arising from chronic stress.

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Introduction

For the past twenty years, population biology has increasingly integrated physiological aspects (Chown and Storey 2006). In particular, the influence of stress is of great concern in evolutionary ecology and conservation biology studies because of its potential effects on fitness and life-history strategies (for a recent review, see Reeder and Kramer 2005). Although the term "stress" is very controversial, we define this term as the physiological state of an animal in relation to the stressor experienced (Buchanan 2000). In vertebrates, the hypothalamic-pituitary-adrenocortical (HPA) axis is one of the main stress response systems for maintaining homeostasis. It permits a rapid and short-term response to acute stressors, leading to the reestablishment of baseline conditions through negative feedback to the brain (Sapolsky 1992). Alternatively, chronic long-term exposure to stressors may lead to some pathological consequences. In this case, feedback signals are weak, and the system remains activated for long periods. This results in the chronic overproduction of glucocorticoids, which damages regulatory centers in the hippocampus, compromising the animal's ability to maintain homeostasis (Sapolsky 1987). Consequently, chronic stress has detrimental effects on cognition functions and individual physiology.

Chronic stress has been proposed as a possible factor in population regulation (Christian 1950). Crowding often involves direct interactions between conspecifics and considerably increases intermale and interfemale aggressive behavior. This induces social stress, which profoundly modifies endocrine parameters in animals. It may slow down the rate of sexual maturation (Marchlewska-Koj 1997) and impair reproduction (for reviews, see Christian and Davis 1964; Marchlewska-Koj 1997). Chronic stress may also affect survival through the effects of immunosuppression caused by a dysfunctional HPA axis (Bradley et al. 1980; Dhabhar and McEwen 1997; Boonstra 2005). Chronic stress is thus regularly invoked to explain the decline

of populations experiencing high densities and multiannual demographic cycles. This hypothesis has rarely been tested in natural populations (but see Lee and McDonald 1985; Boonstra and Singleton 1993; Moshkin et al. 2003). Two nonexclusive processes described in the literature support the role of chronic stress. (1) The "senescence hypothesis" (Boonstra 1994) proposes that suppression of maturation during peak densities causes a shift in age structure toward older animals, leading to senescence and population decline. Several mechanisms, in particular chronic stress, could explain this inhibition. (2) The "maternal-effect hypothesis" proposes that maternal condition could worsen during population peak densities as a consequence of chronic stress and that this could have long-term consequences for the following generations through maternal inheritance (Boonstra et al. 1998*b*).

The fossorial water vole (*Arvicola scherman* Shaw) is an arvicoline species that typically inhabits grasslands. Its breeding season usually extends from early spring (March–April) to early autumn (September–October). This species exhibits 5–8-yr demographic cycles in the Jura Mountains of France and Switzerland (Saucy 1994; Giraudoux et al. 1997). Booms spread spatially as a "traveling wave" from epicenters across 2,500 km^2 (Giraudoux et al. 1997). In this article, we present the results of an analysis of fecal corticosterone metabolites (FCMs)—a noninvasive indicator of adrenocortical activity and thus stress—of four synchronous populations of water voles from the Jura (France). They were sampled at the end of the autumn reproductive period in 2003 and 2004. These years correspond, respectively, to high densities and the beginning of population decline. We examine whether FCM concentrations vary between these years. We analyze the relationships between FCM levels and individual factors, considering the possible changes in population structure. Age structure, for example, is known to change during demographic cycles (Chitty 1960; Krebs and Meyrs 1974). These changes could have confounding effects that prevent the detection of the relationship between demography and stress levels. A decrease in FCM levels associated with the decline would indicate a direct effect of abundance on stress levels. Alternatively, an increase in FCM levels during the decline would suggest a delayed effect of abundance on stress levels. This delay could result from chronic stress, which we would expect to suppress less essential physiological energy costs, such as immune function (for reviews, see Sheldon and Verhulst 1996; Råberg et al. 1998; Lochmiller and Deerenberg 2000).

Material and Methods

Study Area, Sampling Locations, and Vole Demography

The survey was carried out in late September of 2003 and 2004. The study area covered about 50 km^2 in east-central France (Franche-Comté, Jura, Nozeroy canton: 46°47′N, 6°03′E). The mountains in this area are of medium height (370–970 m). The landscape consists to a large extent of open grassland interspersed with forests and mixed habitat areas (countryside with many hedges, trees, and small fields).

We conducted population abundance surveys twice a year between October 2002 and October 2006 at four sites in the Nozeroy canton, following the index method developed by Giraudoux et al. (1995). Briefly, two perpendicular transects of 250 m were examined for the presence of water vole surface activity at 5-m intervals. The presence of mounds (tumuli) indicates vole fossorial activity. The index of abundance corresponds to the percentage of intervals containing water vole tumuli on the site. The four study sites (A–D) consist of 1–2 ha of grassland each, 3–10 km apart. They experienced synchronous demographic cycles, with a boom in autumn 2003 and a demographic decline between autumn 2004 and autumn 2005 (Fig. 1).

Measurement of FCMs

At least 20 individuals (10 males and 10 females, when possible) of more than 50 g (subadults and adults) were live-trapped at each study site. To avoid degradation of FCMs, we collected only fresh fecal samples. Trapping was carried out between 11 a.m. and 3 p.m. Traps were set in different colonies to avoid the capture of closely related individuals and were checked every 90 min. Since in small rodents, fecal steroids have a 4–12-h lag in response to changes in hormonal secretion (Harper and Austad 2000; Palme 2005; Palme et al. 2005; Touma and Palme 2005), FCM concentrations estimated from these samples should reflect the pretrapping endocrine status of the animals.

Feces were kept on ice in closed tubes in a thermos for a few hours after collection in the field. They were then stored at −20°C until analysis, 1–2 mo after the field survey. A validation experiment was performed to select an appropriate enzyme immunoassay (EIA) to measure FCMs and monitor the adrenocortical activity of the fossorial water voles (Palme 2005; Touma and Palme 2005). We collected fresh fecal samples from eight individuals (four males and four females) both in the field at the time of trapping and 8 h after they underwent an acute stress (handling and shaking for 1 min). We analyzed these samples with three different group-specific EIAs, all described previously (corticosterone EIA: Palme and Möstl 1997; tetrahydrocorticosterone EIA: Quillfeldt and Möstl 2003; and 5α-pregnane-3β,11β,21-triol-20-one EIA: Touma et al. 2003). The tetrahydrocorticosterone (5β-pregnane-3α,11β,21-triol-20-one) EIA was selected for further analyses, because a more pronounced increase in concentrations of FCMs was observed in the second samples, which reflect the stress experienced by the animals during handling (mean difference = −10.237; t ratio = −4.066, df = 7, $P = 0.002$). Steroids were extracted following the method of Palme and Möstl (1997), with slight modification. A total of 0.1 g of feces per sample was used; 1 mL 80% methanol was added to each sample and vortexed for 30 min. After centrifugation (15 min, 2,500 *g*), the supernatant was transferred into new tubes, diluted 1 : 10 with a buffer solution (Tris/HCl 20 mM, pH 7.5), frozen, and stored at

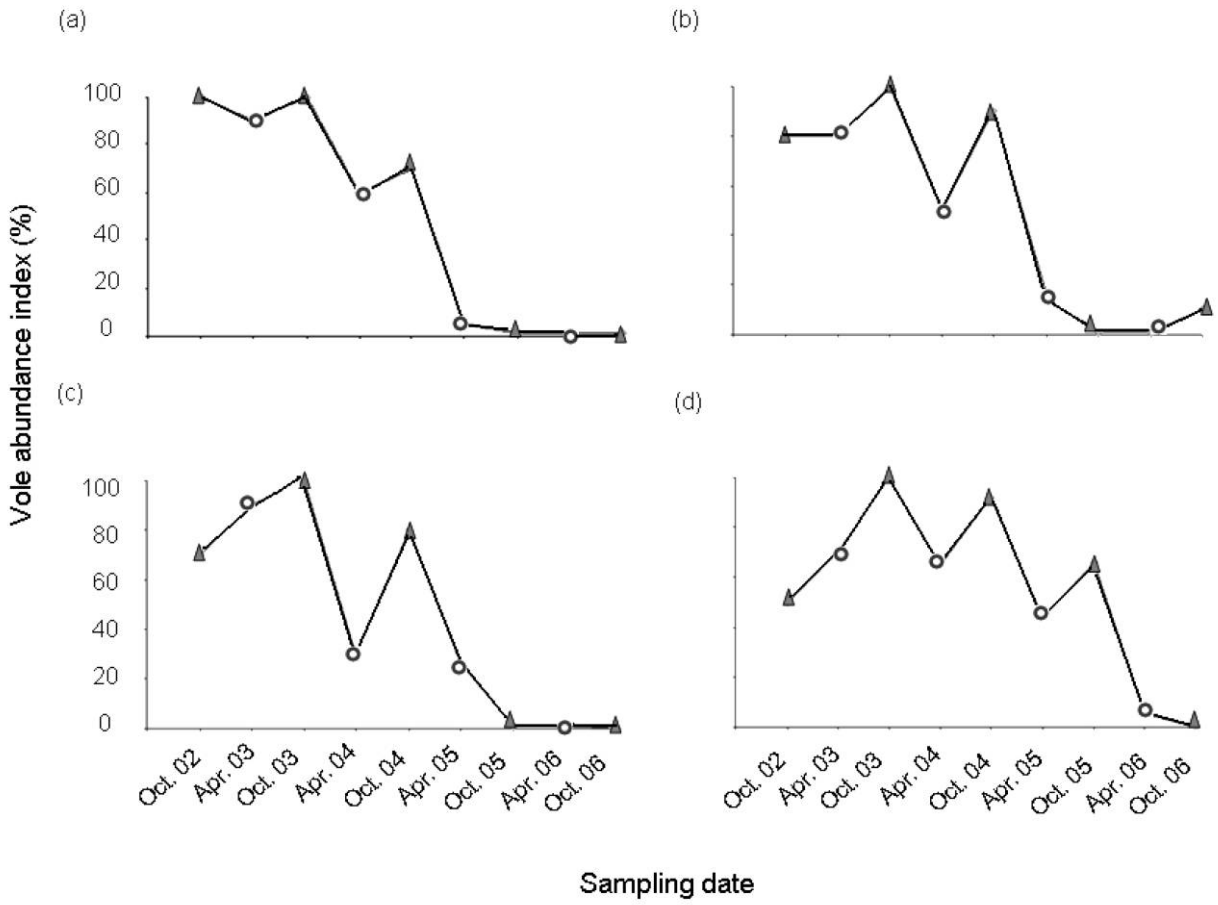

Figure 1. Estimated vole abundance index before, during, and after the monitoring period in localities A, B, C, and D, corresponding to plots *a–d*. The vole abundance index is estimated as the percentage of 5-m intervals containing vole surface index over two transects of 250 m (Giraudoux et al. 1995; Berthier et al. 2006). The triangles and the circles correspond to autumn and spring data, respectively.

−20°C until the analysis with the tetrahydrocorticosterone EIA (Quillfeldt and Möstl 2003).

Cell-Mediated Immune Response (CMI): The Phytohemagglutinin Test

The CMI function was assessed using the phytohemagglutinin delayed-hypersensitivity response 4 d after voles were housed in the laboratory. This provides a measure of the proliferative response of the circulating T lymphocytes to the injected mitogenic substance (Goto et al. 1978). This particular immune response is very useful for assessing the overall competence of cell-mediated components of immunity within a host. It provides a reliable indicator of in vivo cellular immunity in rodents (Mendenhall et al. 1989; Sinclair and Lochmiller 2000; Webb et al. 2003). Each animal was injected intradermally in the center of the right foot with 0.1 mg of phytohemagglutinin (PHA; Sigma L8754) dissolved in 30 μL of physiological phosphate-buffered saline (PBS). Foot thickness was measured with a pressure-sensitive micrometer (Mitutoyo 547-301) to the nearest 0.01 mm before and 24 h ($\pm$15 min) after the injection. The swelling was estimated as the change in thickness of the right foot between the day of injection with PHA and the following day (minus the change in thickness of the left foot during the same period for the control). One person carried out all the injections and thickness measurements. A previous control experiment had been conducted on 40 individuals, which were also injected in the left foot with 30 μL PBS alone. As there was no detectable change in the left-foot thickness (mean change = 0.61 $\pm$ 0.20 [SD] mm; paired *t*-test: $t = 0.412$, df = 33, $P = 0.683$), there was no need to adjust the measurement of the right foot for changes in the control left foot (see also Smits et al. 1999). In addition, the repeatability of the test, the variation attributable to measurement errors, was investigated by performing three measures before and 24 h after the injection (Lessels and Boag 1987). The repeatability observed ($r = 0.96$) was similar to that reported in other studies (0.94 and 0.99 in Zuk and Johnsen 1998; 0.94 and 0.99 in

Smits et al. 1999). Note that within-individual variation would give another estimation of the repeatability of such challenge (Siva-Jothy and Ryder 2001; Granbom et al. 2004). However, measuring two different swellings on the same individual is hard to interpret both spatially, since it induces a modification of the PHA dose injected, and temporally, since the immune system has a memory and responses to a second injection of PHA would not give an independent repetition of the first injection (Smits et al. 2001).

Vole Examination

After this immunological experiment, animals were euthanized by cervical dislocation. They were sexed and weighed to the nearest 0.1 g. We estimated the age of individuals using eye lens mass and the formula obtained by Boujard (1982) in a fossorial water vole population geographically close to our study area. For each site and sampling date, the body condition was estimated as the residuals of the body mass (log transformed) over the age (log transformed). Finally, the reproductive status of all the animals was checked during the dissection. Males with testes longer than 6 mm and developed seminal vesicles were considered sexually mature and in breeding condition (Morel 1969). Maturity and sexual activity of the females were determined as a function of the size of the uterus, presence of embryos and placental scars, and development of the mammary glands. Since we found only one female in gestation and none in lactation, we removed these individuals from the data set. Females were categorized as immature (nulliparous) or mature (having previously reproduced). All animals used in these experiments were housed and maintained in accordance with the Institut National de la Recherche Agronomique (INRA) animal care guidelines (Veissier 1999), and all procedures were approved by the Departmental Veterinary Service (B34-169-1), an institution accredited by the National Ministry of Agriculture and Fisheries.

Statistical Analyses

We used linear models to investigate the influence of individual measures (body condition, log-transformed age, reproductive status, and sex), locality, and year on FCM levels. Since FCM concentrations have greater variance with increasing expected values, we log transformed the data to normalize their distribution. We verified the absence of significant correlations (Pearson r) between the individual predictor variables to assess their independence and prevent further problems due to colinearity.

We then investigated the influence of FCM levels on the CMI, using linear models with CMI as the dependent variable and individual measures (body condition, log-transformed age, FCM level, reproductive status, and sex), study site, and year as the independent variable. We square root–transformed CMI values to normalize their distribution.

For both analyses, we included the two-way interactions that were biologically relevant. Those including year were kept because the relationship between FCM levels and individual fitness is expected to vary with changes in vole population demography. We also kept those including age because such interactions would reflect the effects of senescence. We used the Akaike Information Criterion (AIC) to select the most parsimonious model, the one explaining most of the variance with the fewest parameters (Burnham and Anderson 1998; Johnson and Omland 2004). Models with $\delta\text{AIC} < 2$ compared to the model with the lowest AIC were selected.

Significance of explanatory variables and their interactions was determined by using deletion testing, with the significance of a term determined by the log-likelihood ratio test (McCullagh and Nelder 1989). When an interaction term was found to be significant, the lower-order terms involved in that interaction were also retained (Crawley 1993). By checking the probability plots, we ensured that the residuals were normally distributed. Finally, we used the sum of squares to test model fit (F statistic). In a posteriori pairwise comparisons for least square means, a multiple comparison adjustment for the P values was done with the Tukey-Kramer method. All analyses were performed using GENSTAT 7.1 (Lawes Agricultural Trust, Rothamstead).

Results

Demography

Figure 1 shows the variation of vole abundance index observed between autumn 2002 and autumn 2006 in the four sites monitored. The decrease of vole abundance observed during the winter indicates the dominance of mortality over reproduction during this period. Voles experienced booms in 2003, characterized by a saturation of the abundance index. A decrease in abundance was observed between 2004 and 2005. The vole populations nearly became extinct in three localities in autumn 2005 (Fig. 1). In locality D, the population was extinct in spring 2006. All four populations exhibited very low abundance in autumn 2006.

Analysis of FCM Level Variability in Autumn, 2003 and 2004

After the selection procedure, the single model with the most parsimonious explanation of the spatiotemporal variations of FCM levels includes body condition, sexual maturity, and the interaction between year and locality (Table 1*A*). Higher FCM levels were associated with poorer body condition ($P = 0.002$). Nonreproductive individuals (immature males and nulliparous females) had significantly higher FCM concentrations than reproductive ones ($P = 0.001$). This was mainly due to females, as the data set included only six nonreproductive males. This result was not explained by differences in age structure or sex ratio in the samples (Table 2). With the spatiotemporal variations taken into account, FCM levels increased significantly between 2003 and 2004 at sites C and D (Fig. 2). A

Table 1: Parameter estimates for the models explaining fecal corticosterone metabolites variation

Effect	Standard Estimate	Error	df	t	Pr > $\lvert t \rvert$
A. Autumn 2003 and autumn 2004:[a]					
Intercept	2.034	.045	…	45.07	$<10^{-3}$
Year (2003)	−.089	.059	1	−1.51	.132
Locality			3	2.57	.057
Year × locality			3	7.35	$<10^{-3}$
Body condition	−1.122	.358	1	−3.14	.002
Sexual maturity	−.1314	.039	1	−3.36	.001
B. Autumn 2003 only:[b]					
Intercept	2.038	.033	…	62.33	$<10^{-3}$
Sexual maturity	−.117	.041	1	−2.81	.006
Sex	−.071	.038	1	−1.86	.066
C. Autumn 2004 only:[c]					
Intercept	1.829	.056	…	32.54	$<10^{-3}$
Locality			3	6.88	$<10^{-3}$
Body condition	−1.566	.572	1	−2.74	.008

Note. The reference for the factor "sexual maturity" is the nonreproductive category, and that for "sex"' is the female category. Pr > $\lvert t \rvert$ is the two-tailed probability associated with the t-test.

[a] 2003 and 2004: $n = 143$, AIC = 152.29, % variance = 29.0, global model $F_{9,133} = 7.43$, $P < 10^{-3}$.

[b] 2003: $n = 83$, AIC = 83.06, % variance = 16.6, global model $F_{2,80} = 9.16$, $P < 10^{-3}$.

[c] 2004: $n = 60$, AIC = 65.12, % variance = 26.7, global model $F_{4,55} = 6.38$, $P < 10^{-3}$.

similar trend was observed at site B but not at site A (Fig. 2). Because of the complex influence of the factor "year" on the relationships between FCM levels and the other variables, we analyzed the data for autumn 2003 and autumn 2004 separately.

Year Differences in FCM Level Variability

After the selection procedure, two factors, "sexual maturity" and "sex," composed the model with the most parsimonious explanation of FCM variations in 2003 (Table 1*B*). Higher FCM levels were observed in nonreproductive voles ($P = 0.006$; Fig. 3) and in females, although this effect was only marginally significant ($P = 0.066$). Note that there was no difference in FCM levels among sites ($P = 0.911$; Fig. 2).

In 2004, the most parsimonious model selected included body condition and site (Table 1*C*). Higher FCM levels were observed in voles with poor body condition ($P = 0.008$; Fig. 4). Individuals from sites C and D exhibited higher FCM concentrations ($P < 10^{-3}$; Fig. 2). Also note that the nonsignificant differences between males and females in FCM levels in 2004 could be explained by an increase in FCM levels in males in 2004 compared to 2003 (ANOVA, effect of the year on male FCM level: $F_{1,66} = 13.036$, $P = 6 \times 10^{-4}$) and a similar level in both years in females (ANOVA, effect of the year on female FCM level: $F_{1,74} = 1.718$, $P = 0.194$; Fig. 3).

Table 2: Population structure considering age, sex, and sexual maturity within the different localities

Year of Sampling, Locality	Latitude, Longitude	Mean Age (mo)	Sex Ratio (% males)	Sexually Mature Voles (%)	Number of Individuals
2003:					
A	46°49′N, 6°03′E	5.98	.48	68	29
B	46°48′N, 6°00′E	4.18	.41	72	22
C	46°46′N, 6°01′E	5.77	.48	67	21
D	46°44′N, 6°04′E	4.11	.35	73	23
2004:					
A	46°49′N, 6°03′E	8.08	.55	85	20
B	46°48′N, 6°00′E	6.26	.55	90	20
C	46°46′N, 6°01′E	8.51	.57	67	21
D	46°44′N, 6°04′E	5.36	.43	86	21

Note. Age was estimated using the formula established by Boujard (1982) for water voles sampled near the study area, age = exp [(EL − 3.39)/2.14], where EL is the weight of the eye lenses in milligrams.

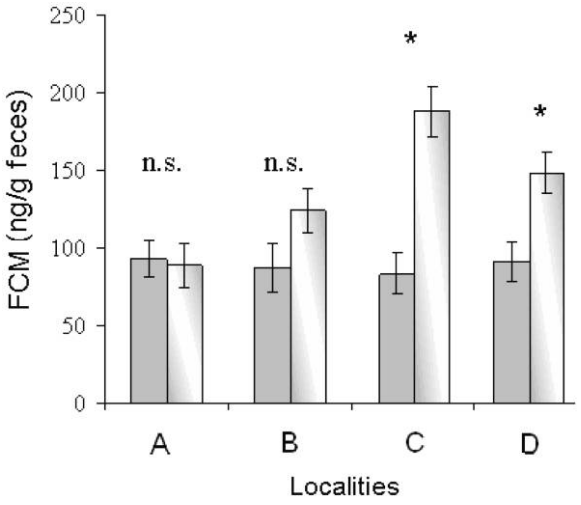

Figure 2. Spatiotemporal variation of fecal corticosterone metabolite levels (mean ± SE) between autumn 2003 (*dark gray*) and autumn 2004 (*light gray*) in the four localities monitored. "n.s." indicates nonsignificant differences, whereas an asterisk indicates significant differences ($P < 0.05$).

Analysis of CMI Variability in Autumn, 2003 and 2004

After the selection procedure, the factor "locality" ($P = 0.047$) and the interaction between FCM levels and year ($P = 0.043$) composed the model with the most parsimonious explanation of the CMI variations (Table 3*A*). In light of this interaction, the data were reanalyzed for each year separately. In 2003, none of the independent variables included in the model significantly explained differences in CMI responses. In 2004, FCM level was the only variable selected that significantly explained CMI variations (Table 3*B*). Higher FCM levels were associated with a lower immune response ($P = 0.006$; Fig. 5).

Discussion

Demographic Changes and Variations of FCM Levels

This spatiotemporal survey focused on the particular and enigmatic phase of the demographic cycles, the beginning of the decline. Different nonexclusive scenarios have been proposed to link demography and stress. First, stress and consequently FCM levels may be directly related to density, as the latter may quantitatively reflect interactions between conspecifics (e.g., in *Microtus pennsylvanicus* [Boonstra and Boag 1992] and in the great gerbil [Rogovin et al. 2003]). Under this hypothesis, we expected a decrease of FCM levels associated with the demographic decline. We did not observe this direct relationship in our data. The decline in water vole populations between 2003 and 2004 was also associated with an increase in FCM levels at two sites. This result suggests that density does not translate directly into social stress in water voles. A similar absence of a direct relationship between stress and density has been observed in *Arvicola scherman*. Moshkin et al. (2003) observed in a long-term study that the highest stress levels occurred 2 yr after peak densities of water voles, suggesting that the maximum manifestation of stress in the decline phase was related to factors other than social pressures alone. Observations suggest that the predominant factors were feeding conditions and food quality (Moshkin et al. 2003).

A second scenario linking stress and demography assumes that chronic stress during peak densities has long-term, negative consequences on demography, through the impairment of life-history traits such as survival (Boonstra 2005), reproduction

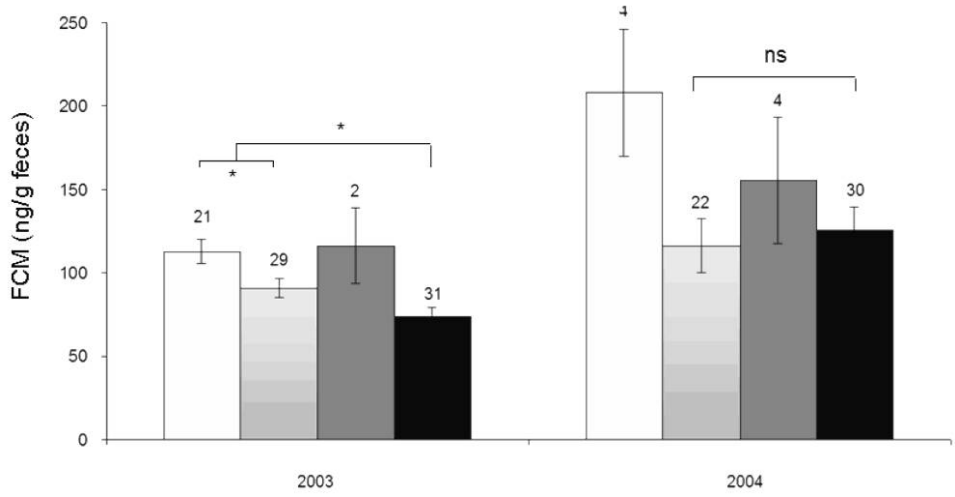

Figure 3. Influence of sex and reproductive status on fecal corticosterone metabolite concentration in four vole populations in autumn 2003 and autumn 2004. Females are represented by white (no reproduction yet) and light gray (reproduction) bars and males by dark gray (immature) and black (mature) bars. Numbers indicate the number of voles sampled per category. "n.s." indicates nonsignificant differences, whereas an asterisk indicates significant differences (Tukey-Kramer tests, $P < 0.05$).

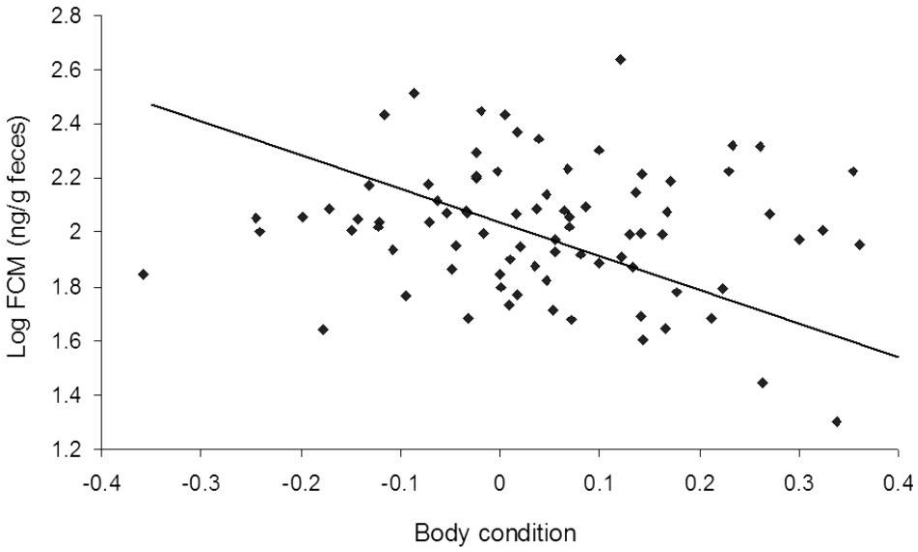

Figure 4. Negative relationship between fecal corticosterone metabolite concentrations and body condition, estimated as the residuals of weight over age, in the four localities surveyed in autumn 2004.

(for reviews, see Christian and Davis 1964; Marchlewska-Koj 1997), and immune function (Bradley et al. 1980; Dhabhar and McEwen 1997). The high FCM levels maintained during the decline in the localities surveyed could support this hypothesis. The relationships observed between FCM concentrations and individual characteristics, such as reproduction, body condition, and immune function, corroborate the negative effects of chronic stress on vole fitness.

Reproduction Impairment during Outbreak and Decline

The negative relationship observed between FCM concentrations and individual reproductive status provided evidence of reproductive impairment associated with high FCM levels. First, we demonstrated that nulliparous females in 2003 exhibited higher FCM levels than those that had previously reproduced. It is well known that reproductive status strongly influences the HPA axis through gonadal steroid hormones. However, the opposite pattern was expected, as estrogens are known to enhance the HPA axis (for a review, see Handa et al. 1994; Viau 2002; Touma and Palme 2005). These results might reflect that the female reproductive capacities are compromised in high-density populations as a result of chronic stress. A similar trend was observed during the decline in 2004, although few immature individuals had been sampled. More data are now required to confirm the potential negative effects of stress on vole reproduction during demographic declines.

Second, the differences observed between FCM concentrations in males and females were significant in 2003 only, with females exhibiting higher FCM levels than males regardless of their reproductive status. An animal's sex may influence FCM levels because of differing excretion or metabolism of gluco-

Table 3: Parameter estimates for the models explaining cell-mediated immune response variation

Effect	Standard Estimate	Error	df	t	Pr > $\lvert t \rvert$
A. Autumn 2003 and autumn 2004:[a]					
Intercept	.613	.156	…	3.94	$<10^{-3}$
Locality			3	2.72	.047
Year (2003)	.583	.204	1	2.85	.005
Log FCM	.010	.079	1	.13	.899
Year × log FCM	−.201	.103	1	−1.99	.043
B. Autumn 2004 only:[b]					
Intercept	1.186	.127	…	9.33	$<10^{-3}$
Log FCM	−.177	.061	1	−2.86	.006

Note. FCM = fecal corticosterone metabolite level. Pr > $\lvert t \rvert$ is the two-tailed probability associated with the t-test.

[a] 2003 and 2004: $n = 144$, AIC = 151.00, % variance = 36.1, global model $F_{6,137} = 14.45$, $P < 10^{-3}$.

[b] 2004: $n = 61$, AIC = 66.55, % variance = 10.7, global model $F_{1,59} = 8.19$, $P < 10^{-3}$.

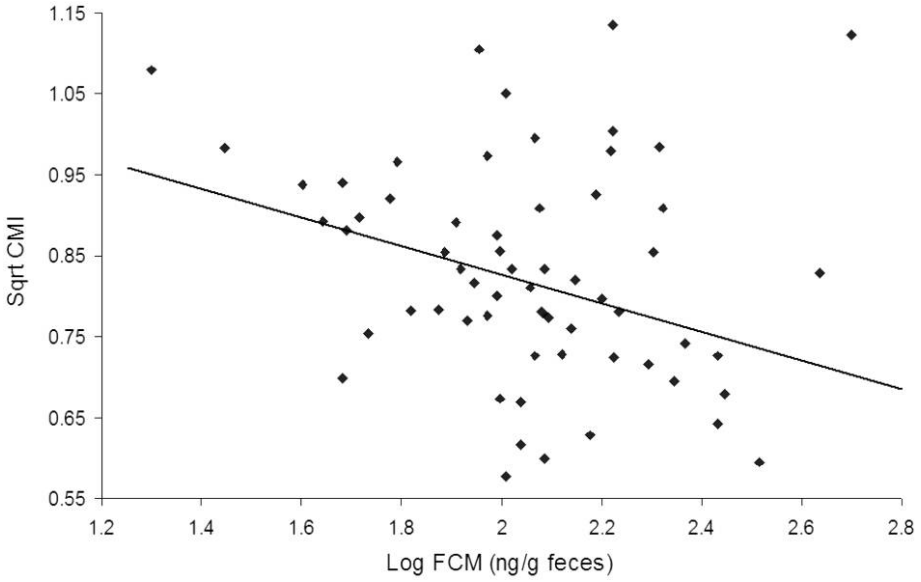

Figure 5. Negative relationship between the cell-mediated immune response (CMI) and fecal corticosterone metabolite (FCM) concentrations in the four localities surveyed in autumn 2004.

corticoids in the sexes (e.g., in mice; Touma et al. 2003) or differing plasma levels in the sexes. All of these influences seem highly species specific (for a review, see Touma and Palme 2005), and, to our knowledge, no data concerning the water vole are available. Higher basal glucocorticoid levels in females is still a general trend in mammals (Reeder and Kramer 2005). It is thus surprising that differences between males and females were no longer significant in 2004. The HPA axis is subject to gonadal influence. Simultaneous manipulation and assessment of these endocrine systems in male rats have revealed that testosterone can act and interact on different aspects of basal and stress HPA function (Viau 2002). In particular, high levels of testosterone, which occur when animals are in breeding condition, suppress corticosterone levels (Viau 2002). The low levels of FCM concentrations observed in males in 2003 could be explained by high testosterone levels. Consequently, a decrease in testosterone levels in males sampled in 2004 could explain the change in FCM pattern observed between 2003 and 2004. This could reflect the impairment of reproductive capacity of adult males during the decline, as observed in hares (Boonstra and Singleton 1993).

Alternatively, sex-biased dispersal may lead to sex differences in FCM levels because the act of dispersal induces stress (Boonstra and McColl 2000). It is well known from previous ecological (Saucy 1988) and population genetic studies (N. Charbonnel, Y. Chaval, K. Berthier, J. Deter, S. Morand, R. Palme, and J.-F. Cosson, unpublished data) that the dispersal rate is significantly biased in favor of young males in water vole populations. We could therefore expect young males to exhibit higher FCM levels. Under this hypothesis, the highest concentrations of FCMs observed in males sampled in 2004 would be explained by higher dispersal rates during the decline. This seems unlikely, as a recent spatiotemporal genetic study including localities A–D showed that demographic declines lead to small, isolated, and genetically differentiated vole populations (Berthier et al. 2006). This reflects the low numbers of migrants among *A. scherman* patches. The increasing phase was associated with the spatial expansion of vole populations and the increase in effective migration among them (Berthier et al. 2006). More migrants are thus expected during the demographic increasing phase of 2003 than in the decline of 2004.

These results revealed the negative effect of chronic stress on vole reproduction in outbreak and declining populations. Determining whether these deleterious changes affect a large number of vole populations and explain the demographic decline will require further analyses.

Negative Effects of High FCM Levels on Body Condition and Immune Function

Considering both outbreak and decline or decline only, we observed a negative relationship between FCM levels and body condition of voles. Body condition influences susceptibility to nutritional stress and therefore may affect the adrenal response (e.g., Heath and Dufty 1998). Alternatively, chronic stress induced by long-term perturbation of homeostasis leads to an excessive energetic mobilization, which helps to restore homeostatic equilibrium (Munck et al. 1984; Sapolsky 1992). This metabolic mechanism could be responsible for the poor body condition of voles experiencing high FCM concentrations. Similar results have been observed in other species experiencing pluriannual fluctuations with demographic decline, such as

snowshoe hares (Boonstra and Singleton 1993; Boonstra et al. 1998*a*) and voles (Norrdahl and Korpimäki 2002).

In 2004, a significant negative relationship was detected between FCM concentrations and the cellular immune response. Chronic stress could also compromise the CMI during decline. Immunocompetence is considered one of the most important determinants of fitness in many species (Lochmiller and Deerenberg 2000). Because it influences the prevalence of disease and survival, it has been suggested to play a mechanistic role in population regulation, and its relevance in explaining both annual and multiannual demographic fluctuations has been widely noted (Mihok et al. 1985; Dobrowolska and Adamczewska-Andrzejewska 1991; Lochmiller 1996). Although the link between immunocompetence and density has been studied extensively (for reviews, see Lochmiller 1996; Moshkin et al. 1998; Sinclair and Lochmiller 2000), the role of stress-induced alteration of the immune system remains scarcely understood. Whether the effect of chronic stress on immunity caused the water vole populations to decline remains an open question. In this study, the negative relationship observed between stress level and immune response might not affect as many voles as would be necessary to result in the decline of the whole population.

Senescence versus Maternal Effects

The senescence hypothesis (Boonstra 1994) proposes that age structure shifts accompany arvicoline cycles, with older individuals being present during population declines, and that these older individuals experience the effects of senescence mainly because of the degeneration of the HPA axis. This study does not provide strong evidence for this hypothesis, since there was no positive relationship between FCM levels and the age of voles. However, the impairment of reproduction due to chronic stress could contribute to a shift in age structure in vole populations, which is expected under this hypothesis. Further experiments are required to specifically test the senescence hypothesis.

The maternal-effect hypothesis (Inchausti and Ginzburg 1998) suggests that inherited environmental effects, including those operating during gestation and lactation, could act as a proximal source of time-lagged effects on population dynamics. This memory of environmental conditions may reside in social interactions or in interactions with food resources, predators, or pathogens. Our study revealed some marked differences among sites in 2004 despite the synchronous demographic changes. A direct estimation of predation pressure could help us determine the influence of this process on stress levels in the fossorial water vole. Changes in food quality or availability could cause delayed variation in stress and reproductive traits in arvicolines. However, very little evidence supporting this hypothesis has been found (but see Moshkin et al. 2003), and the sites studied consist only of sowed grasslands. Therefore, the role of food depletion may not be important here. Finally, pathogens, either by direct exposure or by modifying social or foraging behavior and thus increasing exposure to predation risk or social contacts, could exacerbate the effects described above and should be explored in more detail, too. Several recent studies have revealed marked differences in infection levels among water vole populations (Cerqueira et al. 2007; Deter et al. 2007).

Concluding Remarks

This study illustrates the relevance of noninvasive monitoring of glucocorticoid metabolites in feces to assess "stress" in wild small mammals and how it relates to population performance. An elevation in FCM concentrations is indicative of a physiological stress response. It also provides an accurate assessment of chronic stress (Harper and Austad 2000; Touma and Palme 2005). However, a large amount of variation remains unexplained by our models. Additional factors influencing FCM levels have to be identified, including pathogen and predation pressures; past experience, such as dispersal; and recent exposure to stressors.

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