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Spring declines in *Microtus pennsylvanicus* and the role of steroid hormones

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Summary

1. Spring declines are a common feature of small mammal demography. We tested the hypothesis that meadow voles from populations experiencing severe spring declines should exhibit severe stress responses as indicated by high free corticosterone levels.

2. Three populations in southern Ontario were live-trapped intensively in the spring of 1985, all animals were bled each time, and measurements of total corticosterone, corticosterone-binding globulin (CBG), and total androgen (testosterone and dihydrotestosterone) were obtained by radioimmunoassay.

3. Population density was correlated to stress responses: in populations with the highest densities, both males and females had the highest total corticosterone, lowest CBG, and highest free corticosterone levels; males had the highest wounding levels. However, these populations declined the least. In contrast, the population with the lowest density had males and females with the lowest total corticosterone, highest CBG, and lowest free corticosterone levels and males with the lowest wounding levels. This population declined the most.

4. Total corticosterone and CBG levels in males were about half those in females, and in females varied significantly with reproductive condition, with lactating and pregnant females having the highest levels. Only in pregnant females was body mass positively related to total corticosterone and CBG levels and this is probably related to impending parturition. In males, androgen levels were similar among populations and not correlated to total corticosterone or CBG levels nor to body mass. In females, androgen concentrations were only 4% that of males. In males, CBG, free corticosterone, androgen levels, and wounding rates showed significant repeatability.

5. We conclude that stress responses were not related to population demography in our study and reject the Christian stress hypothesis and the adaptive stress hypothesis of Lee and Cockburn as explanations for spring declines in this species. We propose an adaptive model to account for the differences in the hormonal response to breeding between species in which the males are semelparous and those in which males are iteroparous.

Key-words: corticosterone, microtine fluctuations, population regulation, social behaviour, stress.

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Introduction

Numerous hypotheses have been posed to explain population fluctuations in microtines (lemmings and voles), e.g. food, disease, predation, behaviour, rapid selection (Krebs 1985; Taitt & Krebs 1985; Tamarin 1985; Cockburn 1988; Hansson & Stenseth 1988). At present, there is no consensus on their cause. Our purpose was to test whether stress could explain spring declines in meadow voles (*Microtus pennsylvancius* (Ord)). Stress can be defined as the set of responses by mammals to potentially harmful environmental changes (Selye 1946; Lee & McDonald 1985). There are two major views with respect to the role of stress in small mammal demography. The first is that stress responses by small mammals are ultimately non-adaptive, causing increased mortality and decreased reproduction (Christian

1980); the other, that stress responses are adaptive, acting to promote reproductive fitness (Lee & Cockburn 1985a).

Christian (1980) proposed that social strife at peak microtine densities had direct effects on the pituitary–adrenocortical endocrine system resulting in an increased production of glucocorticoids, such as corticosterone. Glucocorticoids play predominantly two physiological roles: (i) they are critical in normal day-to-day activities associated with the diurnal cycle of waking such as increased locomotion, exploratory behaviour, appetite, and food-seeking behaviour (McEwan, Brinton & Sapolsky 1988); and (ii) they play a central role in allowing animals to adapt to acute stressors by stimulation of hepatic gluconeogenesis, inhibition of glucose uptake by peripheral tissues, suppression of inflammatory responses, suppression of immune reactions, and inhibition of secretion of several hormones and neuropeptides (Munck, Guyre & Holbrook 1984). Thus, the normal stress response is geared to short-term challenges. However, in response to the chronic stress of social conflict at high densities, Christian (1980) proposed that high corticosterone levels precipitated declines because they increased mortality by suppressing the immune and anti-inflamatory responses, thus making the animals more vulnerable to disease, and decreased reproduction by impairing reproductive capacity of both adults and offspring. This 'stress' hypothesis has been supported by numerous laboratory experiments (see reviews by Christian 1978, 1980), but field evidence has been weak and inconclusive (see reviews by Krebs & Myers 1974; Lee & McDonald 1985).

Recent studies involving direct measurements of endogenous hormone levels have revived Christian's hypothesis and provide a mechanism by which social stress could produce population declines in microtines. Bradley, McDonald & Lee (1980) and McDonald *et al.* (1981) found that mortality after breeding of male dasyurid marsupials of the genus *Antechinus* Macleay was associated with excessive amounts of free corticosterone. Normally circulating corticosteroids are firmly bound to a carrier protein called plasma corticosteroid-binding globulin (CBG) and only a small fraction is free or weakly bound to plasma albumin (Tait & Burstein 1964; Westphal 1971). Only free or weakly bound corticosteroid is believed to be biologically active (Rosner 1990) and thus simply measuring total corticosteroids (e.g. Bronson 1973; Vivas 1980; Caro, Fitzgibbon & Holt 1989) may not be a good indication of its impact on the body. In *Antechinus*, a persistent high concentration of free corticosteroid occurred for three reasons: (i) intense competition amongst males for access to females resulted in high corticosterone levels; (ii) the pituitary–adrenocortical feedback system failed and did not respond to rising corticosterone levels (McDonald *et al.* 1986); and (iii) the CBG levels declined in response to rising testosterone levels. The high free corticosterone levels resulted in immunosuppressive and anti-inflammatory effects accounting for the spectacular mortality observed in males. Bradley, McDonald & Lee (1980) proposed that similar mechanisms might contribute to breeding-related population declines in other species of mammals. Consistent with this idea, McDonald *et al.* (1988) found that endocrine imbalances were associated with poor male survival during the breeding season in the Australian bush rat (*Rattus fuscipes* Waterhouse). Similarly, McDonald & Taitt (1982) suggested that endocrine imbalances could also be a significant factor in the regulation of population density of *M. townsendii* (Bachman).

In contrast to the non-adaptive role of stress proposed by Christian (1980), Lee & Cockburn (1985a,b) proposed an adaptive stress hypothesis to first explain *Antechinus* demography, and then to account for spring declines in other small mammals. They viewed high corticosteroid levels as a mechanism for animals to mobilize energy reserves from body protein by gluconeogenesis during the onset of the breeding season. Thus, the animals would be freed from having to forage as much as normal, thus allowing more time to compete for access to mates. The net consequence would be enhanced reproductive success at the expense of post-mating survival.

Spring declines are a characteristic feature of the demography of small mammals and are associated with the social reorganization that accompanies the onset of the breeding season (Chitty & Phipps 1966; Fairbairn 1977; Krebs, Halpin & Smith 1977; Gurnell 1978; Krebs & Boonstra 1978; Cockburn 1981; Taitt & Krebs 1983). Meadow vole populations show similar spring declines; in years after a peak, the decline may be severe and the populations remain low for a year or more; in other years and in populations undergoing only annual fluctuations, it may also be substantial but the populations recover rapidly (Boonstra 1985; Getz *et al.* 1979; Mihok, Turner & Iverson 1985). In this paper, we test the adaptive and non-adaptive stress hypotheses but will not attempt to distinguish between them, as both predict that the severity of the meadow vole spring decline should be directly related to the levels of free corticosterone. In addition, we also test the prediction by Christian that the magnitude of the corticosterone response should vary directly with density, as high density populations are expected to experience a greater amount of social strife (Christian 1975).

Methods and materials

Three populations of meadow voles were trapped once in late November 1984 and four times in spring

1985 from 28 March to 10 May. The sites were abandoned pastures located near Kingston, Ontario on Amherst Island (67 km^2), on Wolfe Island (135 km^2), and on the mainland 2 km west of Amherstview. All sites had a similar complement of mammalian predators (short-tailed weasel *Mustela erminea* L., raccoon *Procyon lotor* L., red fox *Vulpes vulpes* L., coyote *Canis latrans* Say), garter snake *Thamnophis sirtalis* L., and a variety of hawk and owl species. Each trapping grid had 49 trapping stations spaced at intervals of 7·6 m, arranged in a 7×7 pattern (approximately 0·4 ha). One Longworth live trap was placed at each trap point, baited with oats, provided with cotton for warmth, and locked open when not in use. Because of high vole densities, an additional 28 traps were placed on the Wolfe Island grid on 10 April. During the spring, traps were set for 2 days every second week. All new voles were ear-tagged and on each capture we recorded: tag number, location, mass, sex, breeding condition (males, testes abdominal or scrotal; females, vagina perforate or non-perforate, lactating or not, and obviously pregnant or not), and number of wounds on the lower back. We used the complete enumeration method of Krebs (1966) to compare grids and the minimum trappability estimate of Krebs & Boonstra (1984) to assess how readily animals were caught.

During the spring we collected an 80 µl blood sample using a heparinized pipette by puncturing the suborbital sinus. On first capture during each trapping session, a blood sample was taken from each overwintered animal within 2 min of handling; young of the year were not bled. The blood was stored at 4 °C in a portable refrigerator for a maximum of 8 h and centrifuged at 8800 rpm for 4 min in an Eppendorf Micro Centrifuge. The separated plasma was then stored at −20 °C until analysis.

We measured total plasma corticosterone by the radioimmunoassay method of Etches (1976), using his antibody to corticosterone. All reagents were redistilled. The following modifications were made to the protocol: 40 µl double distilled water and 20 µl NH_4OH (to saponify triglycerides) were added to 10 µl plasma. This mixture was extracted twice with 1 ml iso-octane (BDH) to remove progesterone, vortexed for 30 s, and each time the aqueous layer was frozen in an ethanol-dry ice bath and the iso-octane discarded. Two ml dichloromethane (Fisher) were added to the washed plasma, vortexed, centrifuged at 1000 rpm, and the aqueous layer aspirated. Fifty and 200 µl subsamples of the dichloromethane extraction mixture were removed, transferred to new tubes, dried in a 37 °C water bath under nitrogen, and 300 µl of phosphate buffer (pH 7) were added and allowed to equilibrate at room temperature for 1 h. Blank values of charcoal-stripped plasma and of solvent did not differ significantly from zero. The assay was sensitive to 20 pg per 10 µl. The mean recovery of [1,2,6,7-^{3}H] corticosterone (Amersham) added to plasma was 98% (SE 1·7) (range 91·9–99·2). Recoveries of authentic corticosterone above 20 pg per 10 µl added to charcoal-stripped plasma were within 16% of that expected and the intra- and inter-assay coefficients of variation were 10% and 16%, respectively. This variation is comparable to that reported by Etches (1976).

Plasma corticosteroid-binding globulin (CBG) was measured as the maximum corticosterone-binding capacity (MCBC) using [1,2,6,7-^{3}H] corticosterone, diluted in non-radioactive corticosterone (0·72 µM) to known specific activity 5 to 20-fold in excess of the expected capacity (see McDonald *et al.* 1981). In calculating the MCBC, we included the endogenous corticosterone in the plasma to the total amount of non-radioactive corticosteroid added to the sample. The high-affinity fraction in 10 µl sample of plasma, diluted in 0·5 ml phosphate buffer, was measured by liquid scintillation after separation from the free- and albumin-bound fractions with dextran-coated charcoal (Tan & Mulrow 1975). The high-affinity-bound corticosterone was then calculated knowing the specific activity and the radioactivity in the bound fraction. We calculated the amount of free corticosterone present using the CBG-binding constant of 1×10^7 M^{-1} determined for the laboratory rat (Westphal 1967) and the binding equation developed by Tait & Burstein (1964) (program provided by I.R. McDonald, pers. comm.).

Plasma testosterone plus dihydrotestosterone was measured by radioimmunoassay using diethyl ether extracts of plasma. The antibody (P43/11) was produced by Croze & Etches (1980) and showed a major cross-reaction with dihydrotestosterone (62%) relative to testosterone. The protocol for the radioimmunoassay of testosterone was based on that of Abraham *et al.* (1971) with double diethyl ether extraction of plasma samples. Each plasma sample (50 µl) was treated with 20 µl NH_4OH prior to extraction. Blank values of charcoal-stripped plasma and of solvent did not differ significantly from zero. The assay was sensitive to 20 pg per 50 µl plasma. The mean recovery of [1,2,6,7-^{3}H] testosterone (Amersham) added to plasma was 99% (SE 0·4) (range 98–100%). Recoveries of authentic testosterone above 10 pg per 50 µl added to charcoal-stripped plasma were within 6% of that expected and the intra- and inter-assay coefficients of variation were 5% and 8%, respectively.

The data were not distributed normally, so we calculated the Box-Cox transformation for each factor. For male androgen levels, corticosterone levels, and MCBC, and for female MCBC, the lambdas were all near 0 and the 95% confidence limits included 0; hence, we used the log (x) transformation. For male free corticosterone, female total and free corticosterone levels, we used lambda transformations of −0·14484, 0·18137 and −0·12311,

respectively. All ANOVAS were performed with transformed values using Super ANOVA (Gagnon *et al.* 1990). We calculated the intraclass correlation coefficient (Sokal & Rohlf 1981; Lessells & Boag 1987) to estimate the repeatability of hormone levels and wounding within males among weeks for all animals sampled more than once.

Results

POPULATION DEMOGRAPHY

Population density was inversely related to the rate of the spring decline (Fig. 1 and Table 1): Wolfe Island had the highest density but declined the least, whereas Amherstview had the lowest density and declined the most; Amherst Island was intermediate. The trappabilities were 69%, 83%, and 85% for the animals on the Amherstview, Amherst Island, and Wolfe Island grids, respectively. Therefore, the estimates of density and survival should be comparable among areas.

The more rapid loss of animals from Amherstview population was also indicated by their lower survivorship. We calculated the life expectancy for all animals alive from March until May (Leslie *et al.* 1955). Males and females from Amherstview could expect to live only about half as long as those on Wolfe Island (Table 2). Amherst Island males had intermediate life expectancies; Amherst Island females had similar life expectancies to those on Wolfe Island.

We used the frequency of back wounding as an index of the severity of aggressive interactions. Wounding was largely restricted to males, which had average rates about 11 times higher than those of females (Table 3). To compare among areas, we calculated the mean wounding rate only of males (Table 1). Wounding rates were highest on Amherst and Wolfe Islands and lowest at Amherstview. Thus, the higher density areas also had the highest wounding rates.

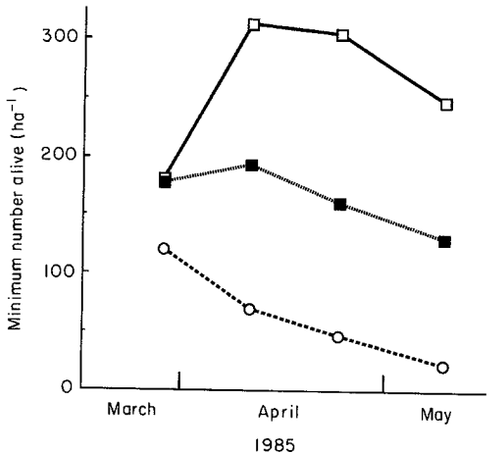

Fig. 1. Population changes in *M. pennsylvanicus* on Wolfe Island (□), Amherst Island (■) and Amherstview (○), Kingston, Ontario.

All overwintering males were in breeding condition in the first trapping session in March and remained so throughout the study. No females on any area were either lactating or obviously pregnant

Table 2. Expectation of further life (weeks ± SE) for cohorts of *M. pennsylvanicus* alive in March 1985* (sample size in parentheses)

	Males	Females
Amherstview	$2{\cdot}6^{a} \pm 0{\cdot}4$ (23)	$2{\cdot}8^{a} \pm 0{\cdot}4$ (18)
Amherst Island	$4{\cdot}2^{b} \pm 0{\cdot}4$ (33)	$5{\cdot}2^{b} \pm 0{\cdot}4$ (33)
Wolfe Island	$5{\cdot}9^{c} \pm 0{\cdot}3$ (28)	$5{\cdot}6^{b} \pm 0{\cdot}4$ (37)

* Significant differences among sites and within a sex were tested by ANOVA ($P < 0{\cdot}05$) using $\log(x+1)$ transformation. Identical superscript letters indicate means that are not different.

Table 1. Population characteristics and associated hormone levels in male *M. pennsylvanicus*. n = number of males

	Maximum spring density (number ha^{-1})	Instantaneous rate of population growth (week^{-1})	Mean ± SE* (range) in males				
			Number of wounds	Total corticosterone (μmol l^{-1})	MCBC (μmol l^{-1})	Free corticosterone (μmol l^{-1})	Androgen (nmol l^{-1})
Amherstview ($n = 27$)	129	−0·28	$1{\cdot}02^{a} \pm 0{\cdot}28$ (0–5)	$0{\cdot}74^{a} \pm 0{\cdot}07$ (0·06–2·00)	$4{\cdot}02^{a} \pm 0{\cdot}18$ (1·60–5·71)	$0{\cdot}003^{a} \pm 0{\cdot}0004$ (0·0001–0·008)	$24{\cdot}54^{a} \pm 4{\cdot}58$ (2·26–90·52)
Amherst Island ($n = 40$)	207	−0·06	$2{\cdot}30^{b} \pm 0{\cdot}40$ (0–8)	$0{\cdot}88^{a} \pm 0{\cdot}18$ (0·09–7·33)	$2{\cdot}91^{b} \pm 0{\cdot}16$) (1·00–5·34)	$0{\cdot}062^{b} \pm 0{\cdot}056$ (0·0002–2·253)	$18{\cdot}58^{a} \pm 1{\cdot}88$ (4·62–47·12)
Wolfe Island ($n = 85$)	336†	−0·04	$1{\cdot}71^{b} \pm 0{\cdot}15$ (0–5·5)	$1{\cdot}04^{b} \pm 0{\cdot}06$ (0·23–4·32)	$2{\cdot}09^{c} \pm 0{\cdot}07$ (1·23–5·35)	$0{\cdot}054^{c} \pm 0{\cdot}021$ (0·002–1·679)	$18{\cdot}75^{a} \pm 1{\cdot}40$ (2·60–52·40)

* Significant differences among grids tested with Kruskal-Wallis ANOVA for wounding ($H = 6{\cdot}84$, $P = 0{\cdot}03$) and with one-way ANOVA on transformed values for total corticosterone ($F_{2,149} = 9{\cdot}30$, $P < 0{\cdot}001$), MCBC ($F = 29{\cdot}44$, $P < 0{\cdot}001$), free corticosterone ($F = 35{\cdot}56$, $P < 0{\cdot}0001$), and androgen ($F = 0{\cdot}11$). Identical superscript letters indicate means that are not different.

† The minimum number of animals caught on Wolfe Island in the March trapping session was an underestimate, as too few traps were available. Thus, we use the maximum number present in early April as the minimum number that must have been present at the start of trapping in the spring.

Table 3. Average hormone levels and wounding rates in *M. pennsylvanicus** (range in parentheses). Repeatability calculated only for males. Androgens include testosterone and dihydrotestosterone. n = number of animals. Degrees of freedom for repeatabilities were 98,168

	Males ($n = 152$)	Females ($n = 141$)†	Repeatability	F	P
Total corticosterone ($\mu mol\,l^{-1}$)	0·95 ± 0·06 (0·06–7·33)	3·36 ± 0·19 (0·28–14·71)	−0·02	0·94	NS
MCBC ($\mu mol\,l^{-1}$)	2·65 ± 0·09 (0·19–8·40)	6·03 ± 0·25 (1·14–17·97)	0·22	1·77	<0·001
Free corticosterone ($\mu mol\,l^{-1}$)	0·047 ± 0·019 (0·0004–2·253)	0·101 ± 0·030 (0·001–4·494)	0·15	1·48	<0·02
Androgens ($nmol\,l^{-1}$)	19·74 ± 1·24 (2·26–90·52)	0·83 ± 0·12 (0·14–2·60)	0·21	1·63	<0·003
Wounds	1·7 ± 0·2 (0–8)	0·2 ± 0·04 (0–4)	0·20	1·68	<0·002

* Individual animals were caught and bled up to four times, so mean values for each animal were calculated and then these were averaged.

† Androgen levels were determined only in 27 females.

in March. However, by 10 April 30% ($n = 27$) of the females on Amherst Island were pregnant or lactating. No females were lactating or pregnant on the other areas until 23 April and by May, at least 75% of all overwintered females on all areas were producing litters. Young of the year were caught only on Amherst Island and were first caught on 22 April.

PLASMA CORTICOSTERONE AND CBG CONCENTRATIONS

Males generally had much lower concentrations of both corticosterone and MCBC in plasma than females and in both sexes average levels of MCBC exceeded those of corticosterone by 2–3 times (Table 3). Thus, animals on all areas were generally well buffered from the effects of high levels of corticosterone by high levels of high-affinity binding globulin. We calculated the amount of free corticosterone present and these were low in both sexes, with females averaging levels about two times those of males.

We examined whether there were major differences in the ratio of total corticosterone to MCBC in each week. In some small mammal species, periods of rapid population decline occur when total corticosterone levels exceed the corticosteroid binding capacity, resulting in large amounts of free corticosterone (Bradley, McDonald & Lee 1980; McDonald *et al.* 1981; McDonald *et al.* 1988). Figure 2 shows that in both males and females in all populations and all weeks except one, average MCBC always exceeded average corticosterone levels. The exception was in the Amherstview females on 9 May, when six females were caught (one non-perforate; five lactating and/or pregnant) and four had total corticosterone levels exceeding MCBC. However, the Amherstview population, which was the only one to decline drastically (Fig. 1), showed no excess corticosterone levels in other weeks. Thus, a severe imbalance in the ratio of total corticosterone to MCBC was not associated generally with differences in demography among the three populations.

A few animals from each trapping session had corticosterone levels exceeding MCBC and thus may have been at risk from the effects of immunosuppression. To examine whether these animals survived more poorly than those with excess MCBC, we pooled all areas, examined only those animals caught during the first trapping session in April (in others the sample size in various categories were too small), and determined how many survived to the end of the study. In neither males ($G = 1·24$) nor females ($G = 2·37$) did animals with corticosterone concentrations exceeding MCBC survive less well than those with MCBC exceeding corticosterone concentrations. Thus, the excess corticosterone levels observed in some animals may have been a temporary phenomenon not indicative of their normal condition.

To determine whether there were significant differences among areas, we first examined males because all were in breeding condition throughout the spring whereas females went from non-breeding in March to pregnant or lactating by May and this significantly affected hormone levels (see below). Ideally a balanced, repeated measures ANOVA would be suitable for this analysis, because each male could be caught and bled up to four times. However, because this analysis requires no empty cells (Gagnon *et al.* 1990), yet most males had empty cells as they were not caught every trapping session or did not survive throughout the study, we performed a simplified analysis. We obtained means of hormone levels and wounding for each male

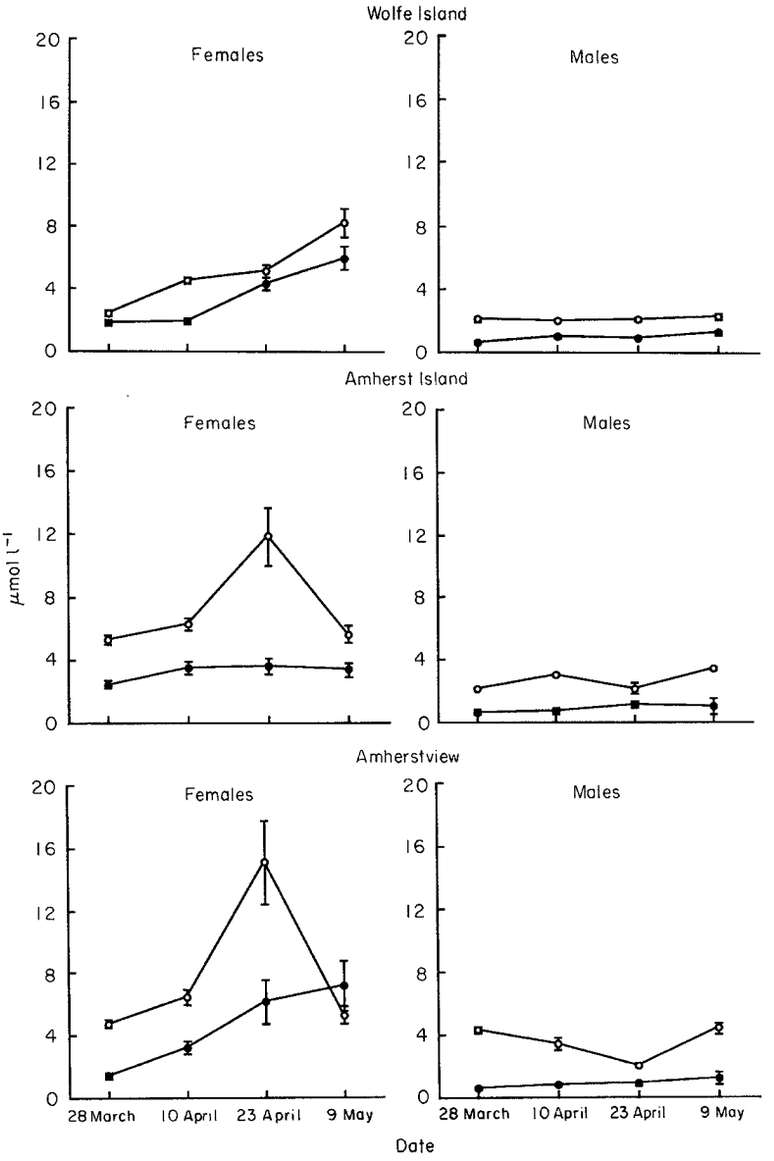

Fig. 2. Concentration of total corticosterone (•) and maximum corticosterone binding capacity (○) in plasma of male and female *M. pennsylvanicus* during spring 1985 on three areas. Vertical bars indicate ±SE.

and performed a one-way ANOVA. We tested the robustness of this analysis by also carrying out a one-way ANOVA on information for the first week bled for each male, and by carrying out a balanced, repeated measures ANOVA for all males bled two times ($n = 99$) and three times ($n = 50$) (this analysis was not carried out for those bled four times ($n = 19$) as two of the areas only had one male each). The results from these latter analyses were similar to overall analysis and hence we present only the former. In males, there were significant differences among grids in both total corticosterone, MCBC, and free corticosterone (Table 1). Population density was directly related to corticosterone levels and inversely related to MCBC levels: the area with the highest population density (Wolfe) had the highest total and free corticosterone levels and the lowest MCBC; the area with the lowest population density (Amherstview) had the lowest total and free corticosterone levels and the highest MCBC.

Reproduction has a major impact on corticosteroid levels in female mammals (Challis *et al.* 1977; Martin *et al.* 1977; Liggins 1982). Females were grouped into four classes and each female was allowed to contribute to each class only once by randomly deleting other values. We assume that within a female, each hormone value in a different class was independent of other values. This was tested by examining the repeatability of hormone values within females bled more than once and, in all cases, there was no significant repeatability (total corticosterone, $F_{(83,124)} = 0{\cdot}77$; MCBC, $F = 1{\cdot}15$; free corticosterone, $F = 1{\cdot}31$). We recognize that our four reproductive classes were not entirely mutually exclusive as field determination of reproductive status is not as accurate as autopsy analysis

and, thus, the differences we found were minimum differences. Our classes were: obviously pregnant (some of these were also in the last week of lactation), lactating (since postpartum oestrus is common, some of these females may also have been in the early stages of pregnancy; McShea & Madison 1989), perforate, and non-perforate.

A two-way ANOVA was performed on total corticosterone, on MCBC, and on free corticosterone to examine for the differences between areas and among reproductive classes (Table 4) and there are two major conclusions. First, though average total corticosterone levels were similar on all areas, Wolfe Island had significantly lower MCBC and higher free corticosterone levels than the other two areas (Fig. 3). The significant interaction effect between total corticosterone levels and area was a direct result of lactating females on Amherstview having significantly higher levels than those on the other areas (Amherstview $8{\cdot}15 \pm 1{\cdot}76\,\mu\text{mol}\,l^{-1}$ ($n = 7$); Amherst Island $3{\cdot}15 \pm 0{\cdot}32$ ($n = 23$); Wolfe Island $4{\cdot}20 \pm 0{\cdot}44$ ($n = 26$) $P < 0{\cdot}001$); all other comparisons of total corticosterone levels within a class were not significant. Second, there were significant effects of reproduction on total and free corticosterone levels and on MCBC. Perforate and non-perforate females had the lowest levels and lactating and pregnant females the highest (Fig. 4). The analysis does not highlight the marked diference in free levels in pregnant females among areas. In the high density Wolfe Island population, pregnant females had average free corticosterone levels 14 times those on the other two areas, and thus these females may have been more vulnerable to immunosuppression and anti-inflammatory effects (Amherstview $0{\cdot}015 \pm 0{\cdot}006\,\mu\text{mol}\,l^{-1}$ ($n = 5$); Amherst Island $0{\cdot}017 \pm 0{\cdot}005$ ($n = 18$); Wolfe Island $0{\cdot}244 \pm 0{\cdot}069$ ($n = 35$) $P < 0{\cdot}0005$).

Individual males could have been bled up to four times in consecutive weeks, so we determined if

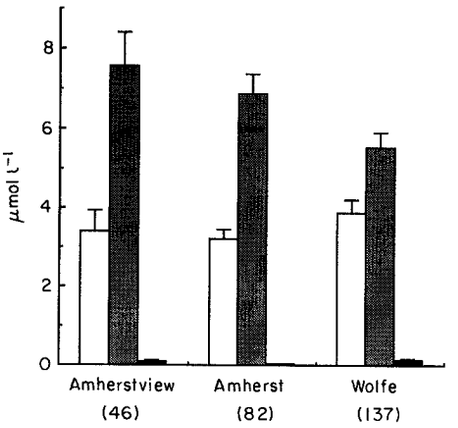

Fig. 3. Mean levels (±1 SE) of total corticosterone (□), MCBC (▩), and free corticosterone (■) of female *M. pennsylvanicus* on three areas. Sample size in parentheses.

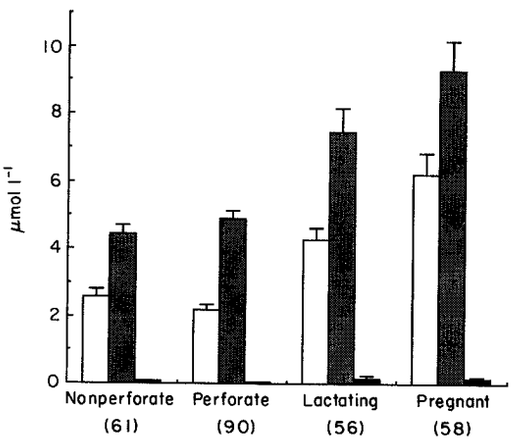

Fig. 4. Mean levels (±1 SE) of total corticosterone (□), MCBC (▩), and free corticosterone (■) for four reproductive classes of female *M. pennsylvanicus*. Sample size in parentheses.

Table 4. Two-way ANOVA testing for differences between areas and reproductive condition in female *M. pennsylvanicus*. Student-Newman-Keuls post-hoc tests were used if significant effects were found

	Source	df	SS	MS	*F*	*P**	Multiple range tests[a]
Total	Area	2	1·74	0·87	1·07		
corticosterone	Condition	3	51·14	17·05	20·96	<0·0001	Non-perforate[a] Perforate[a] Lactating[b] Pregnant[c]
	Area × cond.	6	17·73	2·96	3·60	<0·002	
	Error	253	205·81	0·81			
MCBC	Area	2	1·60	0·80	16·68	<0·0001	Amherstview[a] Amherst[a] Wolfe[b]
	Condition	3	2·01	0·67	14·02	<0·0001	Non-perforate[a] Perforate[a] Lactating[b] Pregnant[b]
	Area × cond.	6	0·45	0·75	1·56		
	Error	253	12·10	.05			
Free	Area	2	128·70	64·35	9·79	<0·0001	Amherstview[a] Amherst[a] Wolfe[b]
corticosterone	Condition	3	134·77	44·92	6·83	<0·001	Non-perforate[a] Perforate[b] Lactating[a] Pregnant[a]
	Area × cond.	6	13·41	2·04	2·04	<0·10	
	Error	253	1663·03	6·57			

* All absent probability values are non-significant.

[a] Identical superscript letters indicate means that are not different.

levels were similar within males from one week to the next. Free corticosterone levels and MCBC were repeatable, but total corticosterone levels were not (Table 3). Total corticosterone levels may not have been repeatable because we included samples obtained from the first time of capture when the animals had not yet habituated to the traps, and thus the animals may have been more stressed. However, when we eliminated the first sample for each male and all males only bled twice, total corticosterone levels were still not repeatable within males ($F_{(49,69)} = 1{\cdot}06$, $P = 0{\cdot}41$), whereas MCBC continued to be so ($F = 2{\cdot}16$, $P < 0{\cdot}002$). Thus, free corticosterone levels and MCBC showed predictability within individuals; total corticosterone levels did not.

Differences in social position within the population may be associated with differences in body mass or wounding and this may be reflected by differences in hormone levels (e.g. Buhl *et al.* 1978; Rose, Bernstein & Gordon 1975). In males, wounding rates were repeatable (Table 3) and thus, some were consistently more heavily wounded than others. We determined the relationship in males between number of wounds or body mass and total corticosterone levels or MCBC by examining individual weeks within grids, by examining all the data for each grid, and by examining all data for all grids combined. Though we found a small number of significant values ($P < 0{\cdot}05$) these disappeared when the Bonferroni adjustment (Rice 1989) was applied. In females, there was no relationship between number of wounds and either corticosterone level or MCBC for any reproductive category. However, body mass was correlated to both total corticosterone levels ($r = 0{\cdot}58$, $P < 0{\cdot}001$, $n = 58$) and MCBC ($r = 0{\cdot}39$, $P < 0{\cdot}005$) in pregnant females, but not in the other three classes of females. In summary, only pregnant females showed a positive relationship between total corticosterone levels and MCBC and body mass; all other correlations between body mass or wounding and total corticosterone or MCBC were not significant.

PLASMA ANDROGEN CONCENTRATIONS

Females had average androgen concentrations (testosterone and dihydrotestosterone) that were only 4% of those found in males (Table 3). Though all males were in breeding condition throughout the spring, the female sample (caught on 23 April) analysed for androgens was comprised of all reproductive states (pregnant, $n = 11$; lactating, $n = 11$; perforate only, $n = 3$; non-perforate only, $n = 2$) and there was no difference among them (Kruskal-Wallis ANOVA, $H = 0{\cdot}66$). Within males, androgen levels were repeatable over time (Table 3). Among the three areas, there were no overall differences in male androgen levels (Table 1).

COVARIATION IN HORMONE LEVELS

In other species of mammals, high androgen levels in males may depress the levels of MCBC which may then put the animals at risk from immunosuppression (Bradley, McDonald & Lee 1980; McDonald *et al.* 1981). For the entire sample we correlated androgen levels to corticosterone levels and MCBC. Total corticosterone showed a slight negative relationship to androgen levels ($r = -0{\cdot}14$, $F_{(1,318)} = 6{\cdot}5$, $P < 0{\cdot}05$), but there was no relationship to MCBC. We also examined each area separately and only Wolfe Island showed a slight negative relationship between androgen levels and corticosterone levels ($r = -0{\cdot}17$, $n = 209$, $P < 0{\cdot}02$). We conclude that androgen levels were only weakly related to corticosterone levels and were unrelated to MCBC.

Discussion

Our study provides no support for the hypothesis that spring declines in meadow vole populations are the direct result of mortality related to stress responses. This conclusion is based on two lines of evidence, which we will discuss in turn: (i) that individuals in high density populations do indeed suffer more from social interactions than low density populations as indicated by differences in hormonal levels and wounding rates; but, (ii) that this does not affect demography.

EVIDENCE ON THE ROLE OF STRESS

Our evidence is consistent with the prediction that high population densities lead to more 'social strife' (Christian 1975). In males and females of all reproductive classes, the MCBC, which represents the buffering capacity of the blood for corticosterone, is always inversely related to density, consistently being lowest in the high density Wolfe population (Tables 1 & 4, Fig. 2). Though corticosteroid-binding globulin is known to fall in response to a variety of stressors (Rosner 1990), this is the first time that population density has been shown to produce this effect. As discussed by Krebs & Boonstra (1978) density is a substitute variable which is probably tied to the frequency of social interactions. Free corticosterone levels also tended to be positively related to density, being highest in the Amherst and Wolfe Island males (Table 1), and in Wolfe Island females (Table 4, especially pregnant females, see Results). Moreover, wounding rates in males were higher in the high density populations (Amherst and Wolfe, Table 1). However, density in males was not linearly correlated to wounding rates and free corticosterone levels, as both were similar at Amherst and Wolfe even though density on Amherst was two-thirds that on Wolfe.

Though two of the study sites were on islands

(Amherst and Wolfe), we do not believe that any 'island effect' (Tamarin 1977) explains the differences we observed among the populations for the following reasons. Firstly, these islands were much larger (67 and 135 km^2, respectively) than Muskeget island (2·6 km^2) on which Tamarin worked. Second, these two islands are connected to the mainland in winter by ice and Lomolino (1984) has documented that meadow voles readily cross these ice bridges in this same region (the Thousand Islands area of the St. Lawrence River) to colonize islands. Third, unlike the Muskeget Island population of the beach vole (*M. breweri* (Baird)) which showed only minor changes in density over time, the meadow voles on our two islands have shown major fluctuations over the last 10 years (Plante, Boag & White 1989; L. Pavone & R. Boonstra, unpublished; R. Boonstra & P.T. Boag, unpublished). Thus, these two island populations were behaving more like mainland populations than the island population studied by Tamarin (1977).

Our results could also have been affected by capture and handling of the voles. Handling is unlikely to have influenced corticosterone levels as all animals were processed within 2 min of release from the trap and most (>90%) within 30 s. In laboratory rats, marked increases in corticosterone levels occurred 3–5 min after a variety of laboratory procedures, e.g. handling, electric shock, ether (Brown & Martin 1974; Levine & Treiman 1964). Confinement in traps could have influenced corticosterone levels. However, as all areas were trapped in the same way, any effect of trapping should have been similar among areas. It is likely that the animals were habituated to the traps, which remained in fixed locations throughout the study and were locked open when not in use. In addition, as the majority of animals had MCBC which exceeded corticosterone levels and thus the animals were well buffered from high corticosterone levels (Fig. 1, Table 1), we suggest that our trapping methods did not act as a major stressor and thus, that the differences we observed among populations were real.

Notwithstanding that our data are consistent with the prediction of more social strife and higher stress levels at higher densities, the populations responded demographically in the opposite way to that predicted by either Christian (1978, 1980) or Lee & Cockburn (1985b): the high density populations showed small declines, while the low density population showed a major decline (Fig. 1, Table 1). Thus, this evidence refutes the stress hypothesis and consequently other factors must explain the differences in demography among the populations.

The only other study on *Microtus* to measure both corticosterone and MCBC is that of McDonald & Taitt (1982). They measured these levels in a small number of dispersing *M. townsendii* after the animals had been removed from the field to the laboratory and found results similar to ours for males. However, a much higher proportion of females had total corticosterone levels which exceeded MCBC. The latter result could be related to the stress of movement to and confinement in the laboratory. We found this pattern in females only once, i.e. on Amherstview in mid-May (Fig. 2). Vivas (1980) measured only corticosterone levels in *M. ochrogaster* (Wagner) and, unlike our results, found no direct relationship between total corticosterone levels and changes in population density. However, his results are not directly comparable to ours as the proportion of the population breeding varied greatly over his study. In addition, it is clear that the most dramatic differences among our populations occurred in the MCBC and free corticosterone levels (Fig. 2, Tables 1 & 4) and thus these must be measured to understand the responses of animals to changing density and reproductive condition.

Our results contrast with those on the marsupial dasyurid *Antechinus* spp. (Bradley, McDonald & Lee 1980; McDonald *et al.* 1981), the Australian bush rat, *R. fuscipes* (McDonald *et al.* 1988), and the common rock rat, *Zyzomys argurus* (Thomas) (Bradley *et al.* 1988). In all of these, periods of poor survival were associated with high levels of total corticosterone relative to MCBC. In male *Antechinus* spp. (Bradley, McDonald & Lee 1980; McDonald *et al.* 1981), increasing concentrations of testosterone caused CBG levels to fall, and a failure of the pituitary–adrenocortical feedback system (McDonald *et al.* 1986) caused free corticosteroid concentrations to rise when aggressive, breeding interactions occurred. The net result was a collapse of the immune and inflammatory responses and a total male mortality after a brief mating period. Male *R. fuscipes* also showed an inverse relationship between testosterone levels and CBG levels and the associated breeding-related mortality (McDonald *et al.* 1988). In contrast, no relationship was found between testosterone concentrations and CBG levels in *M. pennsylvanicus* (this study), in *M. townsendii* (McDonald & Taitt 1982), or in another dasyurid marsupial, *Sminthopsis crassicaudata* (Gould) (McDonald *et al.* 1981). Thus, the relationship between testosterone and CBG levels appears to be a species-dependent characteristic: those species that show an inverse relationship between the testosterone and CBG levels show breeding-related mortality owing to stress symptoms; those species showing no relationship between the two, disappear for other reasons (e.g. death from predation or loss from dispersal).

Is there an adaptive explanation in small mammals for these two different hormonal responses in males? We believe there is and suggest the following modification of the ideas of Lee & Cockburn (1985a,b). We call the first strategy the 'adaptive stress response', and it is that employed by *Antechinus* and similar

small mammal species. It is ultimately caused by a predictable, but highly seasonal environment in which the length of time that the environment is suitable is approximately equivalent to that needed to rear only one litter successfully (Braithwaite & Lee 1979). Thus, there is an interplay between the environment and a life-history constraint, the length of time required for successful gestation and lactation. As a consequence, each adult female is normally only available for breeding once per year and males thus have an extremely narrow window for mating opportunities. As most small mammals are short-lived (generally less than 1 year; French, Stoddart & Bobek 1975) and the annual population turnover of 100% is common (Christian 1971), the probability of a male mating in two successive years is zero or extremely low. The response by males is to maximize their fitness by investing most or all of their reproductive effort in early bouts of reproduction. Natural selection operates in this system to maximize the amount of energy available to these males. The hormonal consequences of this are high amounts of free circulating corticosteroid resulting from a failure of the negative feedback of corticosteroid secretion by the adrenals on the pituitary during the breeding season (McDonald *et al.* 1986) and from the high androgen levels which depress the CBG levels. The benefit of these high corticosteroid levels is the mobilization of energy through gluconeogenesis of protein, which frees the males from having to forage as much as normal and allows them additional time for intense competition for mates. The cost is drastically reduced survival and hence future reproduction because of inhibition of the immune and inflammatory responses.

We call the second strategy, the 'homeostasis stress response', and it is that employed by *Microtus* and most other small mammals. The ultimate cause of this strategy is an environment which, though it may be predictable and seasonal, is suitable for long enough to rear two or more litters of young. It requires a female life history in which the length of maternal investment (gestation and lactation) is short relative to the length of time that the environment is suitable. Thus, the number of mating opportunities for males is spread out over a breeding season as adult females (and possibly their daughters) breed a number of times. Hence, the highest reproductive fitness by males can be attained by spreading out breeding effort. The hormonal consequences of this strategy for males is that the negative feedback of corticosterone on the pituitary should continue to function well during the breeding season, that rising testosterone levels during the breeding season should not depress CBG levels, and that these males should continue to derive energy from the normal sources of food rather than from the gluconeogenesis of protein. Therefore, unlike Lee & Cockburn (1985b) who predicted that adaptive stress responses should occur in *Microtus* spp. at the onset of the breeding season, we predict that such small mammal species should never exhibit these responses under normal circumstances. If high levels of free corticosterone and hence gluconeogenesis do occur, we predict that it would be pathological in nature and caused by an external agent such as disease, severe climate, etc.

Few investigators have carried out the necessary hormonal measurements to test these ideas. The most extreme example of the adaptive stress response comes from males of the dasyurid *Antechinus* spp. (Braithwaite & Lee 1979; Lee & McDonald 1985). Males of *R. fuscipes* also appear to use primarily this hormonal strategy (McDonald *et al.* 1988). This species lives in the same area as the *Antechinus* spp. studied by Bradley, McDonald & Lee (1980) and thus may experience some of the same environmental pressures. During the short breeding season, confined mainly to December and January (Lee & McDonald 1985), most *R. fuscipes* males have high levels of free corticosterone. The best examples of the homeostasis stress response comes from males of *M. pennsylvanicus* (this study; life history reviewed in Hasler 1975; Keller 1985) and from males of *S. crassicaudata*. The latter is a small dasyurid marsupial in which females breed twice over a 6–8 month breeding season in 1 year (McDonald *et al.* 1981; Lee & McDonald 1985).

High levels of free corticosterone have been reported at times not principally associated with reproduction, but their cause then appears to be pathological. Bradley *et al.* (1988) did not distinguish between the sexes of *Z. argurus* but simply indicated that high levels of free corticosterone were present in January, occurring when reproductive activity was low and infection rates with *Salmonella* were high. They conclude that the high levels of free corticosterone were environmentally induced and occurred independent of population density. McDonald *et al.* (1988) report that in female *R. fuscipes* high levels occurred in the non-breeding season in winter and speculated that it was associated with ageing.

STEROIDS IN BREEDING FEMALES

Breeding (lactation and pregnancy) causes a major increase in corticosteroid and MCBC levels in a variety of rodent species (Rosenthal, Slaunwhite & Sandberg 1969a,b; Westphal 1971; McDonald *et al.* 1988) and the absolute amount of free corticosteroid increases as parturition becomes imminent (Rosenthal, Slaunwhite & Sandberg 1969a; Oakey 1975; Martin *et al.* 1977; Savu, Nunez & Jayle 1977; Gewolb & Warshaw 1986). In microtines, this general relationship was initially observed as an increase in adrenal gland size in breeding females (for a review see Lee & McDonald 1985; Seabloom 1985). Our evidence also indicates that hormone levels show

the same response as found in other species. In *M. pennsylvanicus*, lactating and pregnant females had total corticosterone and MCBC (though not free corticosterone) significantly above those of non-breeding (non-perforate) females or those in the early stages of reproduction (Table 4). In the preliminary study by McDonald & Taitt (1982) on *M. townsendii* breeding females (two of 14 known to be pregnant) were also found to have high levels, with free corticosterone values being two to four times above those we found. These high levels may have been a response to the stress of bringing the females into the laboratory before collection of the blood sample. The period near parturition may be a particularly vulnerable time for the females and their embryos, as the naturally high levels of corticosteroids (especially free corticosteroids) may be exacerbated by external stresses. Prenatal stress is known to cause irrevocable changes in the embryos which impair subsequent fertility and behaviour (e.g. Ward & Weisz 1984; Crump & Chevins 1989; Takahashi, Baker & Kalin 1990). If similar natural stresses occur in breeding vole populations owing to intraspecific conflict or food limitation, it could affect offspring behaviour, fertility, and viability. Nonetheless, for our populations, we conclude that the majority of our females were well buffered from the effects of stress at all phases of the reproductive cycle by having high MCBC.

We found a positive correlation between body mass and total corticosterone and MCBC only in pregnant females. We suggest that this relationship was caused by the growing embryos as the adult females neared parturition. In species of mammals such as rats and guinea-pigs, corticosteroids produced by the foetal adrenals increase near parturition, cross the placenta (e.g. Dupouy, Coffigny & Magre 1975; Cohen *et al.* 1990; Rosenthal, Slaunwhite & Sandberg 1969b), and in sheep, play a crucial role in the initiation of parturition (Challis *et al.* 1977; Liggins 1982). Thus, the high levels of corticosteroids we observed in pregnant females may in part be foetal corticosteroids.

McDonald & Taitt (1982) made the surprising observation that dispersing female *M. townsendii* voles had androgen levels that were intermediate between the lowest and highest male values. They speculated that this androgen was produced by the adrenals, especially in pregnant females (Chitty & Clarke 1963), and that it might play a role in the maintenance of aggression and territoriality. Our evidence does not support these findings. Female *M. pennsylvanicus* are known to be strongly territorial (Madison 1980; Webster & Brooks 1981; Ostfeld *et al.* 1988) yet androgen levels in females were only about 4% of that found in mature males and there was no relationship between reproductive state and androgen level.

STEROID VARIATION AMONG MALES

The repeatability in MCBC, free corticosterone levels, and androgen levels (Table 3) indicate that individual males tend to have a consistent hormonal pattern over the critical spring breeding period. It is tempting to suggest that these hormonal differences among males should then be reflected in differences in behaviour and ultimately in breeding success. In laboratory studies on rodents, the typical pattern is that dominant males have high testosterone levels and low corticosterone levels (e.g. Bronson 1973, Buhl *et al.* 1978; Machida, Yonezawa & Noumura 1981; Raab *et al.* 1986; Sachser & Lick 1989). Turner & Iverson (1973), from a field study on *M. pennsylvanicus*, found that large breeding males maintained consistent levels of aggression throughout the breeding season.

We also found that wounding rates were also repeatable (Table 3) but these were not closely correlated to hormone levels. This repeatability in wounding rates is not the result of counting the same wounds in successive trapping sessions, as they heal within a week (Rose & Hueston 1978) and we trapped every 2 weeks. Thus, wounds on the lower back may be too crude an index of an animal's position in the social system. Wounds on the nose and head may be a better index of dominance and these should be looked for in future studies. Clarke (1956) found that dominants received such wounds from subordinates turning to defend themselves. A more detailed longitudinal study throughout the breeding season incorporating behaviour tests, radiotelemetry, hormone analysis, and paternity analysis would clarify how hormone levels are related to dominance and ultimately to reproductive success. To provide maximum discrimination between animals in terms of their dominance, we suggest a standardized stress be given to animals to observe their hormonal response. Both Caro, Fitzgibbon & Holt (with cheetahs, 1989) and Sapolsky & Ray (with baboons, 1989) have used this technique and found dramatic differences between dominants and subordinates.

In summary, our evidence refutes the stress hypotheses – both the non-adaptive (Christian 1980) and the adaptive (Lee & Cockburn 1985a,b) models – as explanations for spring declines in the meadow vole. Population density clearly aggravates stress responses, but these responses do not translate into declines.

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