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The interaction of parasites and resources cause crashes in a wild mouse population

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Summary

1. Populations of white-footed mice *Peromyscus leucopus* and deer mice *Peromyscus maniculatus* increase dramatically in response to food availability from oak acorn masts. These populations subsequently decline following this resource pulse, but these crashes cannot be explained solely by resource depletion, as food resources are still available as population crashes begin.

2. We hypothesized that intestinal parasites contribute to these post-mast crashes; *Peromyscus* are infected by many intestinal parasites that are often transmitted by density-dependent contact and can cause harm to their hosts. To test our hypothesis, we conducted a factorial experiment in natural populations by supplementing food to mimic a mast and by removal of intestinal nematodes with the drug, ivermectin.

3. Both food supplementation and the removal of intestinal nematodes lessened the rate and magnitude of the seasonal population declines as compared with control populations. However, the combination of food supplementation and removal of intestinal nematodes prevented seasonal population crashes entirely.

4. We also showed a direct effect on the condition of individuals. Faecal corticosterone levels, an indicator of the stress response, were significantly reduced in populations receiving both food supplementation and removal of intestinal nematodes. This effect was observed in autumn, before the overwinter crash observed in control populations, which may indicate that stress caused by the combination of food limitation and parasite infection is a physiological signal that predicts low winter survival and reproduction.

5. This study is one of the few to demonstrate that the interaction between resource availability and infectious disease is important for shaping host population dynamics and emphasizes that multiple factors may drive oscillations in wild animal populations.

Key-words: glucocorticoids, host–pathogen interactions, macroparasites, *Quercus*, resource pulses.

Introduction

Relatively few empirical studies have attempted to assess the role of infectious disease in natural populations. This is despite the fact that diseases often exhibit density-dependent transmission and have harmful effects on host fitness (Anderson & May 1992). Theoretical models suggest that macroparasites can cause oscillations in abundance (Anderson & May 1979; Anderson 1980), and a manipulative field experiment has strongly supported this hypothesis as an explanation for population cycles in red grouse *Lagopus lagopus scoticus* (L.) (Hudson, Dobson & Newborn 1998). It has also been suggested that parasites may play an important role in observed small mammal population oscillations (Boonstra, Krebs & Stenseth 1998). Positive correlations between small mammal density and a delayed increase in parasite prevalence or intensity have been found in several studies (Cavanagh *et al.* 2004; Burthe *et al.* 2006; Cerqueira *et al.* 2007). Additionally, individuals captured at the end of a population crash have been found to suffer heavy disease burdens (Soveri *et al.* 2000). However, while these observational studies suggest a role for parasites in oscillations of small mammal populations, they have not experimentally manipulated parasite infection levels and studied the consequences for population fluctuations.

Resource limitation is also known to affect small mammal population dynamics (Batzli 1983; Krebs *et al.* 1995; Elias,

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Witham & Hunter 2004). In the eastern USA, acorn masts have a direct positive effect on populations of small mammals, such as *Peromyscus*. White oaks *Quercus alba* and red oaks *Q. rubra* produce an acorn mast (abundant crop) on average of every 3–5 years, synchronized within a given species at a local scale (< 10 km, Sork, Bramble, & Sexton 1993; Liebhold *et al*. 2004). Population fluctuations in both the white-footed mouse *Peromyscus leucopus* (Rafinesque 1818) and the deer mouse *Peromyscus maniculatus* (Wagner 1845) are typically attributed to cyclical patterns of acorn masts (Wolff 1996a; Jones *et al*. 1998). During times of high acorn availability, *Peromyscus* breeds throughout the winter and has increased overwintering survival (Hansen & Batzli 1978), resulting in a population peak in the summer after an acorn mast (Hansen & Batzli 1979; Wolff 1996a). However, *Peromyscus* populations rapidly crash in high mast years while food is still in high abundance, and there has been no explanation for this pattern (Wolff 1996a). It is also unknown why some good mast years are not always followed by high summer *Peromyscus* densities (Elias *et al*. 2004).

At the Mountain Lake Biological Station, in western Virginia, *P. leucopus* and *P. maniculatus* populations have been monitored for the last 25 years (Fig. 1, Wolff 1996a; Clotfelter *et al*. 2008). Recent analyses of this long-term time series shows that while acorn availability plays a major role in the oscillations of *Peromyscus*, predation by raptors, as well as delayed density dependence are important in structuring the dynamics (Pedersen 2005). Possible factors causing the delayed density dependence include the many intestinal parasites that infect *Peromyscus* (Whitaker 1968; Forster 1984), where high host population densities can lead to increased prevalence of infection (Pedersen 2005). Among intestinal parasites, helminths (specifically nematodes) are highly transmissible, can persist outside their hosts (Anderson & May 1979), and are known to have negative impacts on host fitness (Anderson & May 1992; Hudson *et al*. 1998).

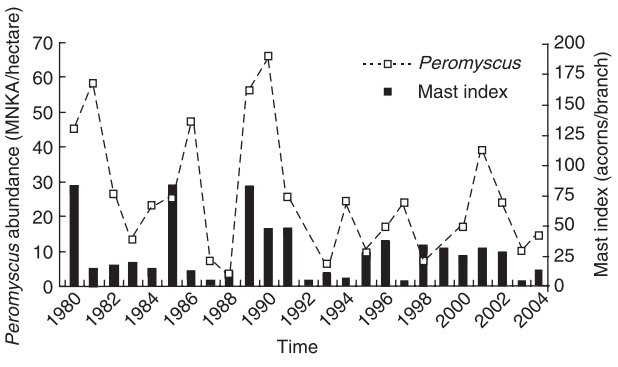

Fig. 1. Long-term patterns of acorn mast intensity (black bars) and annual summer *Peromyscus* abundance (measured as the minimum number known alive (MNKA)/ha – dotted line) from 1980 until 2004 at the Mountain Lake Biological Station (MLBS) (Pedersen 2005). Data on acorn production were obtained from the yearly census carried out by the Virginia Department of Game and Inland Fisheries (D. Martin, pers. comm.). The mast index is the average number of acorns per branch (200 branches), summed for 10 white oak and 10 red oak trees. The Mountain Lake Biological Station is within 5 km of the Stony Creek census site (high elevation – south-west region). Wolff (1996a) has shown that the Stony Creek mast index is highly positively correlated with an independent measure of mast production at MLBS ($r = 0{\cdot}997$).

Understanding the factors that cause oscillations in small mammal abundance has been a long-standing interest to ecologists (Elton, Davis & Finlay 1935; Elton 1942). However, pinpointing the causes of the oscillations has been difficult, and they have been variously ascribed to interspecific or intraspecific competition (Connell 1983; Hansen *et al*. 1999), resource limitation (Batzli 1983; Krebs *et al*. 1995; Elias *et al*. 2004) or predation (Hanski *et al*. 1993; Krebs *et al*. 1995; Hanski & Hentonnen 1996; Turchin & Hanksi 2001). It is becoming clear that multiple factors may be important in driving long-term population fluctuations (Lidicker 1988); however, few studies have experimentally manipulated several factors at one time in natural populations to understand how they interact to affect the population dynamics. Here we hypothesize that the interaction of intestinal parasites and food resource pulses significantly affect population fluctuations in wild *Peromyscus* populations. To test this, we used a replicated factorial design manipulating both food levels and parasite loads in a natural population of *Peromyscus* over 2 years. We measured effects on demographic parameters and stress hormone levels in response to the treatment and our results provide evidence that the intestinal parasites and resource availability interact to play an important part in small mammal population dynamics.

Methods

This study was conducted at the Mountain Lake Biological Station (MLBS) in south-western Virginia (37 10′N, 80 20′W, 1200 m), which is set in an oak–maple forest, with a 60% white and red oak cover capable of supporting high densities of *Peromyscus maniculatus* and *P. leucopus*.

TRAPPING PROTOCOL

In August 2002, 12 trapping grids (0·5 ha) were established in three spatial replicates, each consisting of four grids (Fig. 2). These grids were located in close proximity (within 0·3–0·6 km) to the grids used in the long-term studies of population cycles (illustrated in Fig. 1). Within each grid, 64 live traps (H.B. Sherman $2 \times 2{\cdot}5 \times 6{\cdot}5$ inch folding trap, Tallahasee, FL), were arranged in an 8×8 array, with 10 m spacing between each trap. Throughout the 2-year period of the experiment, beginning in August 2002, the grids were live-trapped for 2–3 consecutive nights every 4–8 weeks (less frequently during the winter). Traps were set between 16.00 and 18.00 h, baited with crimped oats, and checked at sunrise the following morning. To estimate the treatment effects, all captured individuals were permanently ear-tagged (National Band & Tag, Newport, KY, USA), and the following data were recorded for each individual: species, sex, age, weight, length, body condition and reproductive condition. *Peromyscus maniculatus* was distinguished from *P. leucopus* based on the following characteristics: longer tail length than body length, sharply bi-coloured tail, and presence of a hair tuft at the end of the tail (Choate 1973). For both species, developmental age (juvenile, subadult, adult) was denoted by the pelage colour, based on juvenile moulting patterns.

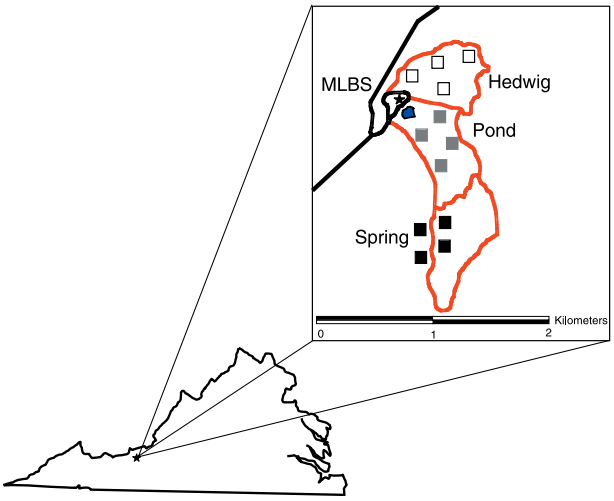

Fig. 2. Map of the study area located at the Mountain Lake Biological Station (MLBS, represented by the star), Virginia, USA; black lines represent the MLBS station road, and grey lines represent established trails. The four experimental treatments were grouped into three spatial blocks (Pond, Hedwig and Spring).

Males were considered to be reproductive if testes were greater than 6 mm × 4 mm, and females if they had a perforate vagina, lactating nipples, or were pregnant.

EXPERIMENTAL DESIGN

To test the effect of periodic food pulses and intestinal nematode parasitism on the population dynamics of *Peromyscus*, we used a 2 × 2 factorial design of food supplementation and ivermectin treatment, with three spatial replicates. The experimental treatments were applied each year from 2002 to 2004.

FOOD SUPPLEMENTATION

In this design, two grids in each of three spatial replicates were supplemented with food, while the other two grids remained unmanipulated. The goal of the food supplementation was to mimic an oak acorn mast year. Owing to the difficulty in obtaining sufficient acorns, we used sunflower seeds to supplement food. Before this experiment, we measured the average density and crown radius of both red *Quercus rubra* and white *Quercus alba* oaks in similar habitats at the MLBS. From this we estimated that one 0·5-ha grid would have approximately 50 oaks, with an average seed shadow radius of 4 m. To simulate acorn availability in a mast year, we randomly selected 50 random points on each food supplementation grid, and each became the centre of a simulated 'masting oak', from which we distributed seeds in a 4-m radius around this point. Based on previous estimates of the weight of acorns in mast years (Jones *et al.* 1998), we delivered over 800 kg of sunflower seeds in fortnightly intervals, each of 12 kg per 0·5-ha grid. Food supplementation was carried out in 2002 and 2003 from early September through late March (the seasonal pattern of acorn availability in mast years).

INTESTINAL NEMATODE REMOVAL

The intestinal parasite community has been identified and monitored in the population of *Peromyscus* species at MLBS for the past several years (Forster 1984; Pedersen 2005). Four major parasite groups have been identified: intestinal nematodes, coccidial protozoans, cestodes and ectoparasites. Over a 4-year period at MLBS, 33·8% ($n = 422$) of *Peromyscus* were infected with at least one species of intestinal nematode: including *Aspicularis americana*, *Capillaria americana* and *Syphacia peromysci*, and two unidentified morphospecies (Pedersen 2005). The former three species require larval development in the soil and are transmitted through both faecal–oral and direct contact.

We previously demonstrated that a single oral dose of ivermectin removes these intestinal nematodes for at least 4 weeks in wild populations of *Peromyscus* (Pedersen 2005). Treatment also reduced ectoparasite infections (fleas, ticks and botflies *Cuterebra* spp.) late in the summer season. In the ivermectin-treated grids (two in each of three spatial replicates), treated individuals received a single oral dose of ivermectin (200 g kg^{-1}, Generic Ivomec, Lambriar Animal Health Care, Fairbury, NE, USA) using a gavage. Treatment with ivermectin began in August 2002 and continued through the remainder of the experiment. All mice caught in the ivermectin treatment grids received a single oral dose at each capture, while animals from control grids received water. Ivermectin is a nontoxic antihelminthic drug that has been proven to remove parasites such as nematodes, trematodes, some cestodes, as well as fleas, ticks and other ectoparasites (Smith & Burgman 1997).

FAECAL CORTICOSTERONE ASSAY

Resource availability and parasite infection are environmental stressors that can affect steroid hormone levels, such as glucocorticoids (Sapolsky, Romero & Munck 2000). Exposure to elevated levels of glucocorticoids over prolonged periods leads to decreased immune function, leaving individuals more susceptible to infection (Ader & Cohen 1993; Black 1994; Webster, Tonelli & Sternberg 2002) with a concomitant increase in mortality (Romero & Wilkeski 2001). In addition, elevated levels of glucocorticoids depress activity of the hypothalamic–pituitary–gonadal axis that is important in regulating reproductive physiology and behaviour (Sapolsky *et al.* 2000). Thus, circulating levels of glucocorticoids respond to environmental conditions (e.g. food availability, infection status) and serve as important physiological mechanisms mediating population dynamics through changes in mortality and or reproductive activity.

To measure the stress response of mice in different treatment groups, a random subset of faecal samples were collected during the fall of 2002 (October) and the spring of 2003 (March and May) and stored at –20 C in ethanol until samples could be processed ($n = 80$). Faecal corticosterone, the predominant circulating glucocorticoid in *Peromyscus* species, and related metabolites were measured from faecal pellets using a corticosterone enzyme immunoassay (EIA) kit (Assay Designs, Ann Arbor, MI, USA). This kit has high cross-reactivity with corticosterone metabolites deoxycorticosterone (21·3%) and desoxycorticosterone (21%), low cross-reactivity (< 0·5%) with progesterone, testosterone, tetrahydrocorticosterone, aldosterone, and negligible (< 0·1%) cross-reactivity with cortisol. Glucocorticoid extraction was performed using methods outlined by Harper & Austad (2000) and the samples were then re-suspended in 95% ethanol and stored at –20 C until assayed. The EIA assay was performed according to the manufacturer's instructions, using 0·01 g dry faecal matter homogenized in 4·0 mL of assay buffer. Samples were run in duplicate and concentrations of faecal corticosterone and metabolites were obtained using 96-well optical density plate-reader (Bio-Rad, Hercules, CA, USA), calibrated against a five-point logistic standard curve with concentrations from 32 to 20 000 pg mL^{-1}. Intra-assay variability was less than 9% on all plates and interassay variability

was 22·6%; the interassay variability was due largely to the fact that EIA kits were from two different lots. Assay sensitivity was 26·99 pg mL^{-1}.

DATA ANALYSIS

Peromyscus leucopus and *P. maniculatus* have been studied at MLBS for over 25 years (J. Wolff, J. Cranford and A.B. Pedersen), and previous studies have shown that these species are similar in many ways, including patterns of reproductive success and demographic features (Wolff 1985, 1996b), intestinal parasite infection prevalence and intensity (Pedersen 2005), microhabitats (Wolff 1986), nest sites (Wolff & Hurlbutt 1982), territoriality (Wolff 1984) and food habits (Wolff, Dueser & Berry 1985). Thus, due to small sample sizes, data for *Peromyscus leucopus* and *P. maniculatus* were combined in the analyses.

Abundance of *Peromyscus* was measured as the minimum number known alive (MNKA) per trapping grid (0·5 ha) (following Wolff 1996a). In addition to MNKA counts, we estimated mouse abundance using Lincoln–Peterson mark–recapture methods (Petersen 1896; Lincoln 1930), but due to low population abundance and lack of convergence, many estimates throughout the time series could not be obtained. However, *Peromyscus* abundance as measured by MNKA is highly positively correlated with abundance based on the Lincoln–Peterson estimates at MLBS (Pearson's $r = 0{\cdot}96$, $P < 0{\cdot}001$, $n = 11$ years, Clotfelter *et al.* 2008). Therefore, the MNKA abundance was used for the analyses. Previous studies have shown that count data are likely to perform as well as mark–recapture estimates when the trapping protocols and study plots remain unchanged (Slade & Blair 2000), as was the case here.

To test the effects of food supplementation and ivermectin treatment on the abundance of *Peromyscus* and the number of reproductive mice, we used a repeated measures analysis of variance over the trapping periods, testing specifically for the role of an ivermectin treatment by food supplementation interaction. This model included a spatial block (three replicates) and a year effect (2 years). The August 2002 sample served as the pre-food and pre-treatment control and was not included in the repeated measures analysis. The time series was measured for the October, March, May and June samples for 2002–3 and 2003–4. Faecal glucocorticoid samples were collected in October 2002, and in March and May 2003. The effect of food, ivermectin treatment, and their interaction on faecal glucocorticoid levels were analysed using separate general linear models (GLMs) for each sampling period. Statistics were run using SAS (SAS Institute 2000), and MINITAB 14 (Minitab Inc. 2006).

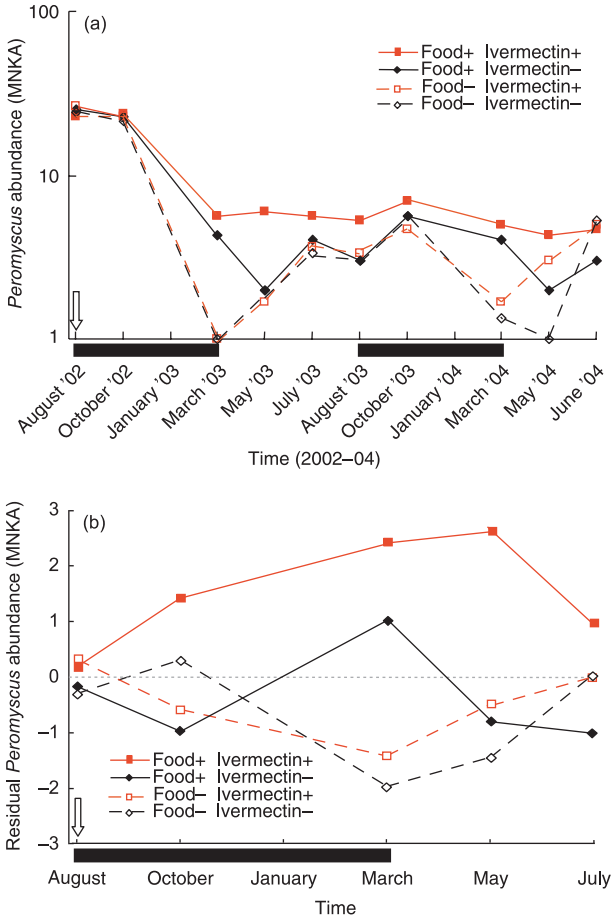

Fig. 3. (a) *Peromyscus* abundance on the experimental grids measured as the mean number known alive/grid from August 2002 until June 2004. Ivermectin treatment is indicated by grey lines, and food supplementation is indicated by solid line. To highlight the main effects of ivermectin treatment and food supplementation (b) shows average residual *Peromyscus* abundance (MNKA)/grid based on a repeated measures analysis of variance model, where variation due to spatial (block) and time (year) is controlled. Ivermectin treatment was initiated in August 2002 (arrow denotes start); however, the effect of the treatment is not evident until the following trapping interval due to the time lag needed to clear nematode infections. Food was supplemented during the period of available acorns in oak mast years (August–March) and is denoted by the black bar below the *x*-axis.

Results

Between August 2002 and July 2004, 639 *Peromyscus leucopus* and 168 *P. maniculatus* were captured in the experiment grids. Prior to food supplementation and ivermectin treatment, *Peromyscus* abundance did not differ between the four experiment grids [Fig. 3; August 2002, mean = 24·7 mice per grid ± 0·6 (SE based on spatial replicates); block, $P = 0{\cdot}19$; food supplementation, $P = 0{\cdot}64$; ivermectin treatment, $P = 0{\cdot}874$]. After the treatments were initiated there was a significant positive effect of food supplementation ($P = 0{\cdot}0084$), ivermectin ($P = 0{\cdot}0245$), and an interaction between food supplementation and ivermectin treatment ($P = 0{\cdot}0483$) on the abundance of *Peromyscus* (Table 1a, Fig. 3a,b). The treatment effects on *P. leucopus* and *P. maniculatus* were not significant when each species was considered individually, but the directions of the effects were the same: *P. leucopus*, food supplementation ($P = 0{\cdot}012$), ivermectin treatment ($P > 0{\cdot}15$), and *P. maniculatus*, food supplementation ($P > 0{\cdot}15$), ivermectin treatment ($P = 0{\cdot}124$).

Before food supplementation and ivermectin treatment, the experimental grids did not differ in the number of reproductive adults based on pooled results for both species of *Peromyscus* (August 2002; block, $P = 0{\cdot}001$; food supplementation, $P = 0{\cdot}48$; ivermectin treatment, $P = 0{\cdot}29$). However, after the treatments were initialized, there was a significant positive effect of food supplementation ($P = 0{\cdot}0183$), ivermectin treatment ($P = 0{\cdot}0017$), as well as their interaction (food × ivermectin, $P = 0{\cdot}0269$, Table 1b) on the abundance

Table 1. Results from the repeated measures analysis of variance test of the effect of food supplementation and ivermectin treatment on (a) *Peromyscus* abundance, and (b) the abundance of reproductive adults over the four post-treatment trapping samples (October, March, May and June)

Source	d.f.	Type III SS	*F*	*P*
(a) *Peromyscus* abundance				
Block	2	89·1	7·8	0·0041
Year	1	337·5	58·7	0·0001
Food supplementation	1	51·0	8·9	0·0084
Ivermectin treatment	1	35·0	6·1	0·0245
Food × ivermectin	1	26·0	4·5	0·0483
Error	17	97·8		
(b) Reproductive adults				
Block	2	33·8	32·6	0·0001
Year	1	4·7	8·9	0·0084
Food supplementation	1	3·6	6·8	0·0183
Ivermectin treatment	1	7·4	13·9	0·0017
Food × ivermectin	1	3·1	5·9	0·0269
Error	17	9·1		

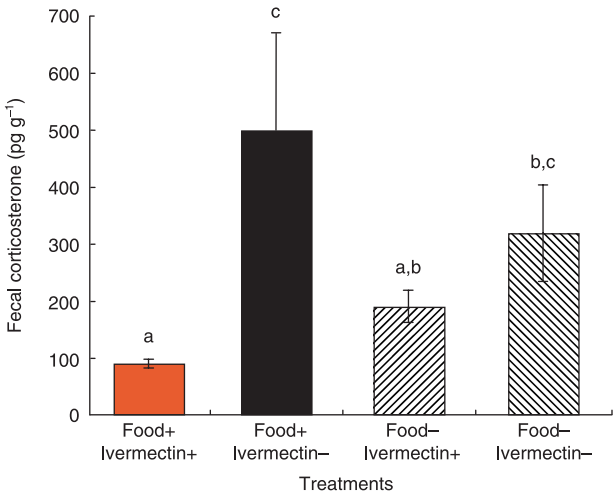

Fig. 4. Faecal corticosterone levels in *Peromyscus* sp. from each of the four experimental treatments in October 2002. Letters denote treatment averages that are not significantly different from each other based on a *post-hoc* analysis, while error bars represent standard error. Samples size for the treatments were food+, ivermectin+ = 5, food+, ivermectin– = 9, food–, ivermectin + = 8, and food–, ivermectin+ = 7.

of reproductive adults. In addition, while a similar qualitative pattern was found for the number of juveniles/subadults captured on the four grid types, with higher numbers of young mice found in the treated, food supplemented grids; the differences were not significant ($P > 0{\cdot}05$ for all treatments).

Using individual mouse recapture rates as a proxy for survival, we found a higher recapture rate on food supplemented, ivermectin-treated grids, however this was also not significant ($P > 0{\cdot}05$ for all treatments and the food supplementation × ivermectin interaction).

Faecal glucocorticoid levels differed among treatments after the initialization of food supplementation and ivermectin treatment, but prior to the onset of winter (October 2002; Table 2, Fig. 4). Faecal glucocorticoid levels in *Peromyscus* were significantly decreased by ivermectin treatment ($F_{1,21} = 16{\cdot}78$, $P < 0{\cdot}001$), and the interaction between ivermectin treatment and food supplementation ($F_{1,21} = 4{\cdot}66$, $P = 0{\cdot}043$). However, there was no main effect of food supplementation ($F_{1,21} = 0{\cdot}83$, $P = 0{\cdot}372$).

Post-hoc analysis for October 2002 faecal glucocorticoid levels revealed that animals from food supplemented and ivermectin-treated grids significantly differed from controls ($P = 0{\cdot}02$), and only food supplemented grids ($P = 0{\cdot}002$). Additionally there was a trend for mice that had ivermectin treatment only (food control) to have lower glucocorticoid levels compared with food supplementation treatment only ($P = 0{\cdot}08$).

During March or May 2003, neither food supplementation nor ivermectin treatment had a significant effect on glucocorticoid levels (Table 2): (1) March: food supplementation ($F_{1,22} = 1{\cdot}04$, $P = 0{\cdot}318$), ivermectin treatment ($F_{1,22} = 0{\cdot}34$, $P = 0{\cdot}564$), food × ivermectin interaction ($F_{1,22} = 0{\cdot}22$, $P = 0{\cdot}642$), and (2) May: food supplementation ($F_{1,19} = 1{\cdot}12$, $P = 0{\cdot}304$), ivermectin treatment ($F_{1,19} = 0{\cdot}35$, $P = 0{\cdot}560$), food × ivermectin interaction ($F_{1,19} = 0{\cdot}64$, $P = 0{\cdot}433$).

Discussion

By experimentally manipulating food availability and treating individuals with an antihelminthic drug, we have shown that seasonal crashes in wild *Peromyscus* populations are affected by the interaction of resource pulses and intestinal parasites. While previous work has demonstrated the potential for food availability or parasites to cause population cycles (Krebs *et al.* 1995; Hudson *et al.* 1998), our study is the first to manipulate both parasites and resource availability to explore how multiple factors, including their interaction, can

Table 2. Effect of food supplementation and nematode removal moval on mean faecal glucocorticoid concentrations (ng g^{-1}) (± SE) at each of the three trapping periods

Resource supplementation	Parasite treatment	October 2002	March 2003	May 2003
+Food	+Ivermectin	88·3 (± 8·1)	221·8 (± 69·2)	161·3 (± 64·1)
+Food	–Ivermectin	497·5 (± 68·7)	236·7 (± 99·2)	394·2 (± 273·7)
–Food	+Ivermectin	188·6 (± 27·8)	114·9 (± 38·5)	258·9 (± 206·8)
–Food	–Ivermectin	316·8 (± 84·3)	68·8 (± 21·7)	100·9 (± 46·9)

affect small mammal oscillations in a natural population. Specifically, we found that control grid populations (no food supplementation or ivermectin treatment) suffered more dramatic seasonal population declines than treated grids, crashing to very few individuals each spring. When populations received only food supplementation or ivermectin treatment, the seasonal crashes were delayed and less dramatic. However, seasonal crashes were significantly reduced from populations that received both food supplementation and were treated for intestinal nematode infections.

Parasitism has been an often-neglected factor when trying to understand the forces driving population dynamics, even though Elton (1942) gave much attention to the 'epidemic hypothesis' as a cause of long-term population oscillations. In the current study, we demonstrate that although food resource pulses can drive population booms (Wolff 1996a; Jones *et al*. 1998), population crashes are intensified by intestinal parasite infection, suggesting that multiple factors, and not food alone, affect population oscillations in *Peromyscus*. Thus far, only one previous experimental study has demonstrated the role of parasites in stabilizing host population dynamics (Hudson *et al*. 1998). In the latter study, elimination of parasites removed population cycles in the red grouse. In contrast, here we show that the elimination of parasites plus the addition of food greatly reduced winter population crashes, which may have implications for the longer-term cycles seen in wild *Peromyscus* populations. Previous studies in *Peromyscus* have clearly demonstrated that reproductive activity is very dependent on food availability and acorn mast patterns, such that food supplementation can both initiate reproductive conditions and lengthen the breeding period (Merson & Kirkpatrick 1981; Blank & Desjardins 1984). Importantly though, in the current study there was a significant interaction between the food pulse and intestinal nematodes, where only the combined effect of food supplementation and ivermectin treatment reduced the seasonal crash. This combined treatment also showed a higher abundance of reproductive adults, especially during the spring when the other treatments had very few, nonreproductive individuals.

The intestinal nematodes removed in this study by a single oral dose of ivermectin are members of a taxonomically diverse parasite community (Pedersen 2005). Laboratory studies have suggested that many of these nematodes (i.e. *Aspicularis americana*, *Syphacia peromysci* and *Capillaria americana*) cause few overt symptoms, and may only cause digestive tract problems and death in individuals with heavy parasite burdens (Raush & Tiner 1949). However, the effects of these parasites have not been well studied in wild populations where energetically stressful functions, such as thermoregulation, reproduction or foraging, may deplete energy stores needed for adequate immune defences. Investment in immunity directly relates with energy availability (Demas 2004), and thus parasites may have a greater impact on their hosts under field conditions, particularly over the winter when individuals are likely to be energetically stressed. In addition, the average recapture rates throughout the experiment were approximately 33%; thus it is possible that the affect of ivermectin treatment could be even stronger with higher treatment coverage. Nematodes comprise just one of three taxonomic groups of intestinal parasites in *Peromyscus* at the MLBS, and a previous experiment demonstrated that while ivermectin removed nematodes from the treated individuals, this resulted in a reciprocal increase in the prevalence of coccidial protozoans and cestode parasites (Pedersen 2005). It is therefore possible that if all three intestinal parasite groups were removed, the pattern between food supplementation pulses and parasitism may be even more dramatic. Other diseases that could not be monitored, for example bacterial and viral infections, whose transmission also increases at high mouse density, could be having a substantial effect.

We found that food supplementation and the removal of intestinal parasites lowered glucocorticoid stress hormone levels and increased the abundance of reproductive adults compared with other treatment groups. This analysis of faecal glucocorticoids demonstrates that infection status combined with food availability alters stress physiology. Glucocorticoids are known to affect fitness-related traits. Specifically, elevated glucocorticoids down-regulate the reproductive neuroendocrine axis (Sapolsky *et al*. 2000), reduce immunocompetence (Webster *et al*. 2002) and reduce survivorship (Romero & Wikelski 2001). For example, in lizards stress-induced elevations of glucocorticoids negatively affect immune defences against parasitic infections (Oppliger *et al*. 1998), and in corvid songbirds, food supplementation decreases circulating glucocorticoids, which is correlated with earlier initiation of reproduction (Schoech, Bowman & Reynolds 2004). Similarly, the decreased levels of glucocorticoids in the treated mice prior to the onset of winter, an energetically costly and stressful time period, may act as a physiological mechanism affecting population dynamics through greater overwinter survival and/or earlier initiation of reproduction in the late winter/early spring. No effect of treatment was found on glucocorticoid levels during the March and May trapping periods. Comparable glucocorticoid levels across treatment groups at these times may be due to the early increase in energetic costs associated with reproductive activities in these populations, as significantly more adults were reproductive earlier on ivermectin-treated, food-supplemented grids.

Although the experimental food treatment was designed to mimic acorn masting, it did not produce the dramatic population increases seen in actual mast years (Wolff 1996a; Elias *et al*. 2004; Clotfelter *et al*. 2008). It is possible that sunflower seeds were not an entirely suitable substitute for acorns on an equivalent mass basis. Additionally, there may have been local immigration from surrounding areas of lower food abundance, or there might have been higher local predation due to the pulse of resources and higher mouse density (Jones *et al*. 1998). Jones *et al*. (1998) mimicked an oak acorn mast and found significantly higher population densities on supplemented grids, but similar to this study, the supplementation did not increase the densities as seen in actual mast years, and both food supplemented and control populations were at equal densities the following summer. While it is clear that acorn mast events cause substantial

increases in density, supplementing food in *Peromyscus* populations has had variable results, with many studies having little effect on density (Flowerdew 1972; Hansen & Batzli 1978, 1979; Gilbert & Krebs 1981; Young & Stout 1986; Terman 1999).

While several theoretical investigations have demonstrated that infectious disease (Anderson & May 1979; Anderson 1980) as well as specialized predators (Hanski *et al*. 1993; Turchin & Hanksi 2001) can drive population cycles, few have explored cases with multiple interacting factors to determine affects on population dynamics. Parasitism has been an often neglected factor when trying to understand the forces driving population dynamics; however, it is becoming clear that both 'bottom up' and 'top down' factors can drive trophic interactions and structure community dynamics (Ostfeld & Keeling 2000). In a large experimental manipulation, Krebs *et al*. (1995) found a more than additive effect of predator exclusion and food addition for increasing hare density, suggesting that the population cycles of snowshoe hares were driven by both the interaction of lynx predation and resource limitation. More generally these results suggest that multiple factors or tri-trophic interactions, can be important determinants of population fluctuations and cycles (Lidicker 1988).

In conclusion, we demonstrate that removal of a group of intestinal parasites and food supplementation alleviates population crashes in a wild population of *Peromyscus*, indicating a key role for disease and stress in initiating population crashes even in the presence of abundant resources. Thus multiple factors affect population oscillations in *Peromyscus*, and while we have identified two such factors by experimental manipulation, observational studies suggest that other pathogens and parasites, or predation by raptors might also play a substantial role (Pedersen 2005). Our findings based on concentrations of faecal glucocorticoids also demonstrate that lack of food and parasite infection induce substantial stress in wild mice, and that this may serve as an important physiological mechanism mediating population crashes, perhaps through their effects on reproduction and immunocompetence. Our conclusions may challenge the idea that intestinal parasites have little effect on host health or population level consequences, and demonstrate that multiple factors may often be driving long-term small mammal oscillations.

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