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A POTENTIAL ROLE FOR GUT MACROPARASITES IN THE POPULATION DYNAMICS

OF CENTRAL PENNSYLVANIA WHITE-FOOTED MICE *PEROMYSCUS LEUCOPUS*

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By

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ABSTRACT:

In an era of bio-terrorism and emerging novel pathogens including AIDS, Ebola, SARS and avian influenza, the need to more closely address disease dynamics is clear. In the past 25 years, the world has seen an unprecedented increase in the number of emerging infectious diseases throughout the world, and this has implications for both human and animal health. Wild animal populations can be very useful in investigating zoonotic diseases and human epidemiology. Determination of the prevalence of macro- and microparasites in a wildlife model system could provide useful insight into how these pathogens flow through and persist in a natural population of animals. We can evaluate the demographics and try to address questions such as: Are there key hosts responsible for a disproportionate amount of the transmission? Are there hot spots of transmission? How does this pathogen flow through the population?

Peromyscus leucopus (the white-footed mouse) provides a unique opportunity to investigate these patterns in the wild because of its ease of handling, high trappability, and inter-annual population dynamics. This allows us to intensively monitor individual interactions between reservoir hosts, both spatially and temporally, and it is these interactions that are key in understanding transmission dynamics of directly transmitted diseases. Additionally, by trapping every two weeks we can closely monitor or track infection and determine any peak transmission periods. These large and replicated monitoring areas we use encompass the home range of many individuals; thus, we can investigate our questions at both the individual and population level. These characteristics make the system conducive to manipulations whereby we can perturb these populations away from equilibrium and disrupt transmission.

The goals of this thesis were to first identify the parasites and outline the seasonal demographics, distribution, and vital rates of the infected and uninfected hosts and we accomplished this with both intensive and extensive trapping efforts. Then, in 2004, we sought to manipulate the populations to see the response to additional food in the form of periodical cicadas or sunflower seeds. In the third year (2005) we investigated the influence of both habitat quality and helminth removal. The 2004 results indicated that *P. leucopus* does respond to a springtime pulse of protein rich cicadas by increasing in density, but they do not respond to the carbohydrate rich seeds as is typical in the autumn. In 2005, parasite removal led to increased size, breeding, and body condition of animals and caused a reversal of the mid-summer breeding hiatus, while increased habitat quality did not appear to influence any vital rates or demographic characteristics.

There is growing evidence that parasites play an important role in shaping population dynamics, and that chronic parasite infection can influence host fitness by reducing breeding output or perhaps by shaping seasonal breeding strategies. We have shown that there are significant impacts of nematode worms on the body condition, mass, growth, and breeding of *P. leucopus*, and that parasites can account for the mid-summer breeding hiatus commonly observed in female mice. These results imply that parasites may play a more important role in the vital rates and temporal dynamics of *P. leucopus* than resource abundance. We also show that the dominant parasite in the community (*Pterygodermatities peromysci*), exhibits a random distribution amongst the mouse population and this coupled with the impact upon summer breeding should lead to destabilization of the relationship between parasite and host. Further studies are needed determine if and what role parasites play a role in the unstable dynamics of *P. leucopus*.

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INTRODUCTION:

The Greek origin of the word ecology comes from *oikos* or household and *logos* meaning knowledge. Ecology is commonly defined as the scientific study of the distribution and abundance of life and the interactions between organisms and their environment. This thesis touches upon all these aspects and delves more deeply into how parasites might play a role in the dynamics of small mammals. Historically, ecologists have assumed parasites were benign specialized predators that played a trivial, if any role in host dynamics (Lack 1954). In fact, an oft quoted argument is that if the parasites were highly virulent then they would simply die within their host. However, there is now empirical and theoretical evidence that the lifetime reproductive success of a parasite is not solely based on survival, but is a result of interactions between reproduction, survival, and transmission (Tompkins et al. 2001).

In his classic book, The Natural Regulation of Animal Numbers, David Lack (1954) proposed that parasites would be unlikely to regulate animal populations unless they act in conjunction with food shortage. Lack considered only parasite-induced mortality and yet there is evidence that gastrointestinal helminths, particularly in moderate levels of infection, induce morbidity rather than mortality and tend to reduce host body condition (Tompkins et al. 2001; Stien et al. 2002) and fecundity (Hudson 1986). When parasite-induced reduction in fecundity is large relative to the impact on host mortality this can lead to instability and generate population cycles (May and Anderson 1978; Dobson and Hudson 1992).

Very few organisms exist at stable levels of abundance and some vertebrate populations exhibit cyclic patterns. The most classic example of these population cycles is that of the snow shoe hare (*Lepus americanus*) and Canadian lynx (*Lynx canadensis*) and the data were compiled from the pelt-trading records of the Hudson Bay Company over 90 years (Elton 1924). These

data do show cycles, but they are only correlational and do not allow us to decipher causal relationships. Very few studies have found the causal relationships that drive these oscillations. In snowshoe hares, an interaction between predation and food availability has now been proposed as the driver of the cyclic dynamics (Krebs et al. 1995) while the multi-annual oscillations of rodent populations in Fennoscandia are described as chaotic and are likely due to delayed density dependence imposed upon them by their mustelid predators **(**Gilg et al. 1993**).** However, the only rigorous empirical field evidence is with red grouse (*Lagopus lagopus scoticus*) in Northern England where removal of the gut macroparasite *Trichostrongylus tenuis* with an oral antihelmintic drastically reduced the extent of the cyclic population crashes which occur every four to eight years (Hudson et al. 1998).

A key advancement in epidemiology was the union of parasitology with the more quantitative science of population biology and this was largely due to the work of Anderson and May (1978). They synthesized these two fields by pointing out that the parasite-host relationship was not simply the impact a parasite had on an individual host, but an integral of these interactions at the population level (Hudson, 2001). The models of Anderson and May (1978) identify the conditions necessary for a parasite to regulate a host population and when the effects are stabilizing or destabilizing. In essence, the models predict that parasitic nematodes will regulate the host population if the growth rate of the parasite is faster than the growth rate of the host. Instability occurs when the relative impact of the parasite on the breeding production of the mice is greater than the impact on host survival, relative to its distribution in the host population (Dobson & Hudson 1992). One of the strengths of the grouse study was that they used these models to generate predictions and to determine the proportion of the population that needed to be de-wormed in order to shift the equilibrium.

Theoretically, it is now clear that if a parasite influences the birth and death rates of the host population in a density dependent manner, then the parasite has the potential to regulate the host population (Tompkins et al. 2001). However, the distribution of the parasites within the host population also plays a key role. Typically, macroparasitic helminths have a variance to mean ratio of greater than 1 and are considered aggregated or over- dispersed such that a minority of hosts harbor a majority of the parasites (Shaw and Dobson 1999). In this scenario, and under the assumption that the impact of the parasite upon the host is dose dependent, theory suggests regulation is unlikely and that the parasites will have a stabilizing influence on the host population because parasite induced mortality effectively cuts off the tail of the distribution and so decreases transmission. However, parasites can be distributed randomly, as is the case with *Trichostrongylus tenuis*, where the variance is approximately equal to the mean. This type of distribution is destabilizing because a greater proportion of the host population experiences sub-lethal effects caused by moderate levels of infection (e.g. decreased fecundity). With this kind of distribution, the parasites are more likely to be an important selection pressure and may indeed play a regulatory role in population dynamics.

Parasitism also interacts with other biological processes by increasing vulnerability to predators (Hudson et al. 1992; Lafferty and Morris 1996; Yunger 2002; Kissui and Packer 2004), reducing aggression (Fox and Hudson 2001), increasing transmission of secondary infections (Bentwich et al. 1995; Glass et al. 2002; Cattadori et al. 2005), reducing access to food or other resources (Kristan 2002), and by changing the outcomes of competitive interactions (Park 1948). In nature, many of these factors interact so that the observed dynamics are a tension between these stabilizing and destabilizing forces (Hudson et al. 2003). These studies identify the

importance of focusing on the sub-lethal effects of parasitism and, in particular, the parasite induced reduction in host breeding production.

If we are to make progress in understanding the underlying mechanisms that drive these dynamics then this complex network of potential interactions should be examined in components with a rigorously controlled scientific approach. The best way to do this is to find a suitable organism and use population level replicated experimental manipulations in the field to test between competing hypotheses. Unfortunatley, these experiments are labor intensive and so are very expensive. "Sadly, ecologists rarely have the resources to grasp the nettle and go for such large-scale experiments because it is only with these data that we can put the theories to the test and further refine the models to mimic the patterns observed in the field (Hudson & Bjørnstad 2003)." We were afforded one of these opportunities thanks to a grant by the NSF to study the disease transmission in a wild small mammal system here in the Appalachian highlands of Central Pennsylvania.

The white-footed mouse (*Peromyscus leucopus*) provides a unique opportunity to investigate these patterns in the wild because of their high abundance, ease of handling, and high trappability. These characteristics allow us to intensively monitor interactions within the same individual, over time and between hosts as well as both spatially and temporally. It is these interactions that are the key to understanding transmission dynamics. Another vital characteristic of this model system is that *P. leucopus* exhibit unstable dynamics and are infected with parasites that can be removed with anthelmintics (Ferrari et al. 2004; Vandegrift et al. 2008).

It is generally thought that the dynamics of *P. leucopus* are governed by periodic pulses of resource availability in the form of oak masting (acorns). These acorns provide an important

winter food resource for mice and studies have shown that this food resource is not only associated with increased survival (Bendell 1959; Hansen and Batzli 1979; Pucek et al. 1993; Jones et al. 1998; McCracken et al. 1999; Falls et al. 2007) but also increased breeding during the following spring and summer (Smyth 1966; Hansen and Batzli 1979; Gashwiler 1979; Pucek et al. 1993; Wolff 1996; Ostfeld et al. 1996; Jones et al. 1998; McCracken et al. 1999; Elias et al. 2004; Falls et al. 2007).   This relationship has been recorded in Virginia (Wolff 1996a), New York (Jones et al. 1998, Ostfeld 1996), Ohio (Vessey 1987), Maine (McKracken et al. 1999, Elias et al. 2004) and Ontario (Falls et al. 2007). Alas, only one of these studies provides more than correlational evidence (Jones et al. 1998) and the strength of the relationship varies among sites. The strongest correlation ($r^2 = 0.79$) was found with 14 years of data in the highlands of Virginia (Wolff 1996) where the habitat is remarkably similar to the Appalachian highlands of Pennsylvania.

Chapter 1 addresses an aspect of the relationship between pulsed resources and *P. leucopus*. The periodic cicadas (*Magicada* sp.) provided an exceptional opportunity to investigate a natural pulse of resources. The responses of a small mammal community to emerging *Magicada* sp. has been studied previously in a multi-year comparison of *Peromyscus* spp. abundance. Khrone et al. (1991) found no response by mice, but a four fold increase in the insectivorous shrew *Blarina brevicauda* in the year of the cicada emergence.   As mentioned above, very strong correlations exist between the oak mast crop and population levels of *Peromyscus leucopus*, *Peromyscus maniculatus*, and *Tamias Striatus* (Wolff, 1996). The vast majority of these studies have dealt with the oak mast and thus the pulse is in the autumn rather than the springtime. We know the nutritional value of cicadas and acorns differ, especially with respect to the carbohydrate to protein ratio, so we experimentally provided sunflower seeds to

mimic the nutritional value of a typical acorn mast and compared our results to the control and cicada emergence populations. We monitored these six grids (three cicada, three additional sunflower seeds) as well as three controls weekly during the month of cicada emergence and then biweekly into August. These data allowed us to address three questions. First, we wanted to know if *P. leucopus* would respond to a pulse of resources when it arrives in the springtime rather than in the autumn. Secondly, we wanted to know how the response of the host may differ between a carbohydrate and a protein rich pulse. Finally, we wanted to know if and how natural enemies respond to an increase in density and, for this we focused on the gut macroparasites.

Chapter 2 of the thesis focuses on my main experiment. We sought to investigate the influence of macroparasitic nematodes and habitat quality on the vital rates and density of *P. leucopus*. Food and parasites can independently play a role in destabilizing population fluctuations of animals and yet more than 50 years ago David Lack proposed that these two factors should act in concert (1954). We examined the role of these factors on the vital rates of free-living *P. leucopus* over the summer and autumn months. To accomplish this, we used a replicated factorial experiment in which deer exclosures doubled acorn availability and anthelmintic application reduced gastro-intestinal helminths. Our *a priori* predictions were that more acorns over the winter would produce females in better breeding condition that would have a greater reproductive output and that worm removal would interact to increase production. More specifically, we wanted to know if either factor or an interaction between the two accounted for the mid-summer breeding hiatus observed in this species.

In the 3rd chapter of this thesis, we examine the population biology of the dominant parasitic helminth *P. peromysci* as well as the other helminths of *P. leucopus* with the objective of providing details on the parasite distribution within the host population and how this varies in

time and space. *P. leucopus* is infected with a diverse community of parasites and so we also asked general questions about the population biology of these species and how they interact with *P. peromysci*. One critical bit of data we wanted to appreciate was how the parasites are aggregated within the host population. The frequency distribution is an important piece of information because an aggregated helminth distribution has a stabilizing effect while a random or Poisson distribution will tend to destabilize the relationship between parasite and host (Tompkins et al. 1999). We also wanted to use demographic data to explain the variation in parasite intensity as well as between infected and uninfected hosts. We did this by using binomial and negative binomial generalized linear models (GLMs), respectively, with parameters such as sex, body size, breeding condition, and age. We evaluated the age prevalence and age intensity curves of *P. peromysci* in order to understand how infection status and intensity vary with age. Finally, we looked at co-infections between macroparasites because there is accumulating evidence that there are significant competitive interactions between parasites and with pathogens that can be either direct or host mediated (e.g. cross immunity and immunosuppression) that may alter the dynamics of each individual parasite (Cattadori et al. 2005; Holmstad et al. 2004; Lello et al. 2004).

In order to disentangle these mechanisms, in it is necessary to use this blend of theory and field empiricism. Ideally, and as David Bradley puts it: "For real progress, the modeler as well as the epidemiologist must have mud on their boots."

Chapter 1

Responses to enrichment: Small mammals vary in their response to a springtime cicada emergence.

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Summary

1. Autumnal masting events provide a pulse of carbohydrate rich resources that influences the dynamics of small mammals and their natural enemies. Similar patterns are observed when cicadas emerge in spring and provide a protein rich pulse but comparisons are confounded by timing and food type.

2. We compared the influence of a naturally occurring spring pulse of cicadas with an experimental spring pulse of synchronous carbohydrate rich seeds. We used a replicated population level field experiment and capture mark recapture techniques to record the vital rates, demographics, and abundance of *Peromyscus leucopus* and other small mammals and their parasites.

3. The presence of cicadas increased *P. leucopus* density and appeared to be acting through early recruitment but was followed by reduced breeding and a fall in recruitment. Other small mammals including the Eastern Chipmunk, *Tamias striatus* and The Short-tailed Shrew *Blarina brevicauda*, increased in density but there was no affect on The Masked shrew *Sorex cinereus*. Coupled with the increase in *P. leucopus* was an increase in the prevalence of the nematode *Pterygodermatities peromysci* in males.

4. In contrast to the presence of cicadas there was no influence of sunflower seed supplementation on small mammal density, vital rates, or reproduction with the exception of an increase in *B. brevicauda* density.

5. The periodic spring pulse of protein rich cicadas had a short term effect on abundance which resulted in increased parasite prevalence, reduced breeding, and lower recruitment while the unpredictable carbohydrate pulse had no significant effects compared to the fall

pulses in seed masting. Timing of pulses and food type appear important but further experimentation is needed.

Keywords- *Peromyscus*, *Tamias*, *Blarina*, Cicadas, nematode, *Pterygodermatities peromysci*, pulsed resources, food addition, seasonal nutrition

Introduction

Seasonal breeding is a dominant pattern in nature and invariably coincides with temporal or spatial variation in resource abundance (Asdell 1964). The predictable, seasonal perturbation in resources has consequences for population structure and demographics, but then unpredictable pulses (those that do not occur annually) can perturb the system dramatically and have long term consequences for dynamics and the community. For example, unpredictable pulses in the fall, such as mast seed production, may influence dispersal and survival whereas an early spring pulse is more likely to influence the timing of breeding and productivity. Theory applied to the process of resource enrichment has identified a paradox whereby resource enrichment not only destabilizes the relation between the consumer and its natural enemy, but also can lead to extinction (Rosenzweig 1971). However, empirical work has rarely been able to support these predictions and much of the research has focused on the tensions between the stabilizing and destabilizing forces that could lead to extinction Roy & Chattopadhyay (2007). Identifying and understanding the consequences of unpredictable enrichment and the nature of these pulses (protein or carbohydrate) is an interesting and sometimes non-intuitive challenge for ecologists and has important ramifications for pest management, biocontrol, and conservation.

Peromyscus leucopus responds to acorn masting enrichment by increasing density, breeding, and survival (Bendell 1959; Ostfeld et al. 1996; Wolff 1996; Ostfeld et al. 1998; Jones

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et al. 1998; Pucek et al. 2006). Longitudinal studies have found strong associations between the summer density of *P. leucopus* and a mast index (available carbohydrate) in the preceding fall (Wolff 1996, Elkington 1999, McKracken et al. 1999, McShea 2000). This association has been tested experimentally by adding carbohydrate rich foodstuffs such as sunflower seeds and recording changes in abundance and vital rates. In one study, an over-winter supplement decreased mass loss and home range size while tripling immigration rates, increasing fecundity and allowing wintertime breeding (Taitt 1981). Other studies found that female mice responded to extra carbohydrates whereas males did not (Fordham 1971; Galindo-Leal and Krebs 1998) in contrast to other studies that found adding carbohydrate rich food had no influence (Bendell 1959, Hanson and Batzli 1979; Wolff 1986). Most of these studies cite immigration as the likely source of increased density. There is no clear explanation for the variation in the responses which may imply that the response depends on how the mouse population is embedded in the ecological community and the response of natural enemies (Hudson and Bjornstad 2003).

In a recent paper, Marcello et al. (2008) examined if the unpredictable spring pulse of perishable cicadas had the same affect on *P. leucopus* as an autumnal pulse of cacheable acorns. They found good evidence that the spring emergence resulted in increased density similar to that observed in the fall (Ostfeld et al. 1996; Wolff 1996; Pucek et al. 2006) but unlike the fall, this increase was a consequence of increased breeding. Observational studies, focused on such complex effects are naturally confounded in time, space and community structure and finding the right comparative controls are difficult. Cicada emergence is patchy and may be associated with secondary factors and then natural enemies maybe responding to both cicadas and mice. Spring time pulses influence breeding condition and production (as recorded by Marcello et al. 2008) whereas fall pulses tend to influence winter survival and dispersal. Carbohydrate pulses provide

an energy rich food source for over winter survival, whereas protein rich cicadas provide the limiting amino acids necessary for breeding. The relative response is expected to vary between insectivorous and omnivorous species. A longitudinal study by Khrone et al. (1991) has examined the relationship between cicada emergence and small mammal abundance and they recorded a four fold increase in the shrew *Blarina brevicauda* associated with cicada emergence, but no response by *P. leucopus*. To examine the interaction of timing, food and community structure we would ideally want a fully randomized experimental protocol but this is technically difficult (cicadas emerge in spring not fall) and logistically difficult over large scales.

We studied the 2004 emergence of Brood X cicadas (*Magicada septendecim, cassini, septendecula*), one of 20 cicada broods in eastern North America as studied by Marcello et al. (2008). This emergence occurred in the northeastern USA in an area stretching from Princeton (New Jersey) south to Baltimore (Maryland) west into Ohio and across southern Pennsylvania (Figure 1). To evaluate the effects of pulsed food type on the small mammal dynamics we compared control populations to those with introduced sunflower seeds and no cicadas at the same time as the cicadas were present on the other sites. We recorded the density response of each small mammal species on a weekly basis in each site during the emergence. We used capture mark recapture techniques to estimate abundance, recruitment rates and survival. Furthermore, we evaluated a response by parasites as a proxy for natural enemies, not only because of the close association with their host but also because their transmission, in broad terms, is often density-dependent (Arneberg et al. 1998). We predicted an increase in density, breeding, and immigration in areas with supplemental food or cicadas but, with greater protein we expected a stronger response in the cicada grids.

Materials and Methods

Experimental Design and Trapping

Small mammal live trapping was set up to monitor relative abundance before, during, and after the cicada emergence. We started on June 4th and ended on August 19th 2005. During this time period, nine 8 x 8 grids of multi-capture live traps (Ugglan, Graham, Sweden) were established in 15-meter intervals and then checked for two consecutive days each week until July 7th and then bi-weekly until mid-August. We returned to the control and cicada grids two years later in late May and reset the control and cicada grids with snap traps for three days to obtain abundance estimates. All experimental grids were established in open hardwood forest typical of the northeastern Appalachians where the forest is dominated by *Quercus* spp. and *Acer* spp. and all grids were separated by at least 250 m. of woodland. Three of the grids were located in Huntingdon County, PA where the periodical cicadas emerged. The other six grids (3 controls and 3 sunflower seed addition) were in Centre County, PA just north of the emergence where no cicadas were present (Fig 1). The three food addition grids were randomly assigned to receive supplemental sunflower seeds (18 kg/week/grid) for the 1st three weeks of trapping, for the same period that cicadas were present on the three grids 80 km south in Huntingdon County. During the 4th week when the cicadas die and fall to the ground, an additional 45 kg of sunflower seeds were spread on each of these three supplemental food grids.

Vital Rate Measures Density and Macroparasite Dissections

On capture of each *P. leucopus* and *T. striatus*, individual standard body measures were taken and recorded. These measures included body length, tail length, body mass, sex, body condition, and breeding status. Animals were considered in breeding condition if they had descended testes (males), or if they were lactating, pregnant, or had a perforate vagina (females).

Individuals were identified using a Trovan™ (Electronic ID Devices, Ltd., Santa Barbara, California) passive induced transponder (PIT) tag inserted into the scruff of each animal. Individuals caught in more than one trapping session were considered residents. Population size estimates for *P. leucopus* and *T. striatus* were estimated using Jolly-Seber (see Pollock et al. 1990). The abundance estimates for the *Sorex cinereus* and *B. brevicauda* are transformed with natural logarithms ($\ln(x + 1)$); to avoid stress and conform to IACUC requirements these animals were not marked but released immediately upon capture. All *P. leucopus* caught during the last trapping session were euthanized with an overdose of isofluorane and the intestinal tracts of these animals (n=150) were dissected and all gastrointestinal worms were collected, identified, and enumerated.

Statistical Analyses

Statistical analyses were carried out with use of the statistical package R. Nested analyses of variance (ANOVA) (Error term = grid) were used to analyze response variables such as body mass, body condition, days known alive, recruitment, and time in breeding condition, in order to ensure that grid was the unit of replication. We obtained estimates of recruitment for each grid using a Pradel model (Pradel 1996) in the program MARK (White and Burnham 1999). Abundance data and the Jolly Seber population size estimates were analyzed with the nested ANOVA and in these models week was included as a covariate. When the response variable was binomial (i.e. proportion in breeding condition and proportion pregnant), the proportions were calculated for individual grids and arcsine square root transformed before analysis with Gaussian linear models and week was included as a covariate in these models. The best predictors of each response variable were selected using backwards stepwise selection, retaining variables with P <

0.05 based on F-statistics. When significant differences were detected in the full model we divided the dataset to see which treatments effects were significant.

Results

Experimental Treatment Trapping and Density

We undertook 6912 trap nights with 1527 captures of small mammals: 827 *P. leucopus,* 241 *T. striatus*, 247 *B. brevicauda*, and 182 *S. cinereus*, 24 *Clethrionomys gapperi*, 3 *Zapus hudsonius* and 3 *Glaucomys volans*. A lack of abundance of the last three species prevented detailed analysis.

Peromyscus leucopus abundance

Abundance of *P. leucopus* was not altered by the addition of sunflower seeds (coef. = -0.097, F = 0.00, d.f. = [1, 4], P=0.98) but, as predicted, was significantly greater on the grids with cicadas as compared to the controls (coef. = -5.21, F = 6.85, d.f. = [1,4], one-tailed P=0.03; Fig. 2A). The difference in abundance between the sunflower addition grids and the cicada grids was not significant (coef. = -5.31, F = 1.81, d.f. = [1, 4], P=0.25; Fig. 2A). Jolly-Seber methods do not permit an estimate of population size for the first trapping period, prior to cicada emergence, so we used the log transformed number of animals caught as the response variable and there was no significant difference between control and cicada grids during this week of trapping (coef. = 7.66, F = 4.44, d.f. = [1, 4], P=0.10). We repeated this analysis for the period while cicadas were present (sessions 2, 3, and 4) and compared with the subsequent period when cicadas were absent (sessions 5 and 6) and found significantly more *P. leucopus* when cicadas were present (coef. = 10.78, F = 61.10, d.f. = [1, 4], P<0.01) but not after they had gone (coef. = -2.17, F = 1.57, d.f. = [1, 4], P=0.28). We also returned to the control and cicada sites two years

after the study and found no significant difference in the density index of *P. leucopus* between sites (coef. = 1.06, F = 0.039, d.f. = [1,4], P=0.86).

Peromyscus leucopus vital rates

There was no significant effect of treatment (either cicada presence or the additional sunflower seeds) on body mass, body length, or body condition when we examined all animals and there was no effect of sex on these findings (all P>0.05) (Table 1).

A Gaussian linear model identified a significantly lower proportion of animals in breeding condition on the cicada grids than either the fed (coef. = -0.26, t = -6.74, P<0.01) or the not fed control grids (coef. = -0.23, t = -14.72, P<0.001) but no significant difference between fed and control grids (coef. = -0.01, t = -.34, P=0.75; Fig 3A). This pattern holds for both males (coef. = -0.32, t = -3.54, P=0.02) and females (coef. = -2.04, t = -3.01, P=0.04) when we compare the control and cicada supplemented populations. There was no significant difference between sunflower seed fed grids and control grids for males (coef. = -0.10, t = -0.90, P=0.42) or females (coef. = -0.05, t = 0.25, P=0.81) nor were there significant differences in the proportion breeding between fed and cicada grids for either males (coef. = -2.04, t = -2.35, P=0.0) or females (coef. = -0.25, t = -1.35, P=0.24; Fig 3B-D).

To examine breeding effort in more detail we examined the proportion of time individual residents were in breeding condition. In the full model the effect of treatment were significant (coef. fed = 0.52, coef. unfed = 0.47, F = 35.71, d.f. = [2,6], P<0.001) and this pattern holds for both males (coef. fed = 0.43, coef. unfed = 0.453, F = 5.88, d.f. = [2,6], P=0.04) and females (coef. fed = 0.72, coef. unfed = 0.45, F = 13.99, d.f. = [2,6], P<0.01). The animals on both the sunflower fed (coef. = 0.44, F = 115.15, d.f. = [2,6], P<0.001) and control (coef. = 0.44, F = 89.17, d.f. = [2,6], P<0.001) grids were in breeding condition for a significantly greater

proportion of time than animals on grids where the cicadas emerged. The difference between sunflower fed and not fed control grids was not significant (coef. = 0.004, F = 0.04, d.f. = [2,6], P=0.95; Fig 3E).

The analysis of the Pradel model estimates of recruitment indicated the effect of treatment was close to being statistically significant but counter to what was expected. The control populations had higher recruitment than the sites supplemented with cicadas (coef. = 0.44, d.f. = [1,4], F = 6.75, P=0.06) or sunflower seeds (coef. = 0.47, d.f. = [1,4], F = 4.92, P=0.09). The recruitment onto cicada grids did not differ from the fed grids (coef. = 0.66, d.f. = [2,6], F = 1.27, P=0.32; Fig 4A). MARK does not permit examination of the recruitment of individual cohorts of animals because it will not allow stratified input. Instead, we used the log transformed number of captures through time of the three mass classes of mice and estimated recruitment rate as the slope of the relationship for each grid. The models with recruitment rate as the response variable indicate the effect of treatment was highly significant for juveniles (coef. = 0.44, 0.54, F = 34.59, d.f. = [2,6], P<0.001), approached significance for sub-adults (coef. = 0.075, 0.18, F = 3.77, d.f. = [2,6], P=0.09) and was not significant for adults (coef. = 0.09, 0.10, F = 3.77, d.f. = [2,6], P=0.57; Fig 4B). This enables us to parse out which cohorts were responsible from the increase in abundance seen on the cicada grids. The recruitment of juveniles onto cicada grids was high initially and then tapered to below control levels whereas control grids had a steady increase in juvenile recruitment throughout the course of the study (Fig 4C). Table 2 provides the recruitment rate model results for the between treatment analyses.

The survival of resident animals was not significantly altered by either the addition of sunflower seeds or the periodic cicada emergence (coef. = 0.26, F = 0.85, d.f. = [1,4], P=0.41)

and this pattern was also recorded for both males (coef. = 0.19, F = 0.33, d.f. = [1,4], P=0.60) and females (coef. = 0.38, F = 0.85, d.f. = [1,4], P=0.40; Fig 3F).

Peromyscus leucopus **Natural enemies**

We evaluated the intestinal parasite intensity of the 150 *P. leucopus* captured and euthanized on the last trapping session. Four nematode species were recorded: *Pterygodermatities peromysci*, *Capillaria americana*, *Syphacia peromysci*, and *Heligmosomoides vandegrifti;* one cestode species (*Hymenolepsis s. str.*) and one trematode species (*Brachylaima peromysci*). The most common helminth (*P. peromysci*) was twice as prevalent (52%) on the cicada grids when compared to the fed (24%) and not fed (19%) grids. A generalized linear model identified a significant difference in prevalence of this helminth between treatment grids (χ^2 = 13.7, d.f. = [2,147], P = 0.001). We examined the sexes separately and the influence of treatment on *P. peromysci* prevalence is significant for male mice (χ^2 = 18.8, d.f. = [2,77], P < 0.001) and not females (χ^2 =1.9, d.f. = [2,56], P = 0.39). The difference in prevalence between the fed and not fed grids was not significant for males (χ^2 =0.02, d.f. = [1,53], P = 0.90) or females (χ^2 =1.1, d.f. = [1,35], P = 0.28). The pattern of higher prevalence of *P. peromysci* on the cicada grids holds for the comparisons between both fed (χ^2 =9.4, d.f. = [1,94], P<0.01) and not fed (χ^2 =10.8, d.f. = [1,101], P<0.01) grids and in both of these comparisons we find the prevalence is only significant in males (fed: χ^2 =13.2, d.f. = [1,46], P = 0.0003; not fed: χ^2 =14.6, d.f. = [1,55], P = 0.001) and not females (fed: χ^2 =0.01, d.f. = [1,37], P = 0.87; not fed: χ^2 =1.7, d.f. = [1,40], P = 0.20; Fig 4D).

Tamias Striatus

The abundance of *T. striatus* and the effect of treatment approached significance when we considered all treatments (coef. = -1.39, -4.37, F = 4.74, d.f. = [2,6], P=0.058); with no

statistical difference between sunflower fed and not fed grids (coef. = -2.98, F = 4.44, d.f. = [1,4], P=0.10) nor between cicada grids and the controls (coef. = -1.39, F = 1.67, d.f. = [1,4], P=0.27). There was a tendency (0.1> P >0.05) towards greater abundance in the cicada grids when compared with fed grids (coef. = -0.43, F = 6.07, d.f. = [1,4], P=0.07) (Fig 2B). We again used the log transformed capture data for each grid to circumvent the lack of a population size estimate for the first trapping session and found the abundance of *T. striatus* on cicada grids was not different to the control during the first trapping session (coef. = 0.33, F = 0.1, d.f. = [1,4], P=0.76) or the final 2 trapping sessions (coef. = 3.00, F = 4.70, d.f. = [1,4], P=0.01). However, the abundance of *T. striatus* was higher on cicada grids while the cicadas were present (coef. = 3.56, F = 14.62, d.f. = [1,4], P=0.02) and the effect of cicadas approaches significance in the full model with week as a covariate (coef. = -4.37, 1.39, F = 4.74, d.f. = [2,6], P=0.06).

Blarina brevicauda and *Sorex cinereus*

The insectivores (*B. brevicauda and S. cinereus*) were not PIT tagged and so capture-mark-recapture techniques could not be used to estimate abundance. We examined log abundance of animals caught per grid per trapping session. The effect of treatment is highly significant on the abundance of both *B. brevicauda (*coef. = -1.71, -1.13, F = 87.0, d.f. = [2,6], P<0.0001) and *S. cinereus* (coef. = 0.97, 1.04, F = 16.3, d.f. = [2,6], P<0.003). The abundance of *B. brevicauda* was highest on cicada grids and this was significantly greater than both control (coef. = -1.70, F = 139.6, d.f. = [1,4], P<0.001) and fed grids (coef. = -1.13, F = 141.26, d.f. = [1,4], P<0.001). Interestingly, this insectivore was also significantly more abundant on the sunflower seed fed grids than the not fed controls (coef. = 0.574, F = 14.92, d.f. = [1,4], P=0.02) (Fig 2D). *S. cinereus* showed no such effect between fed and unfed grids (coef. = 0.08, F = 0.15, d.f. = [1,4], P=0.72) but had significantly higher abundance on both fed (coef. = 1.05, F = 47.72,

d.f. = [1,4], P<0.001) and not fed grids (coef. = 0.97, F = 15.46, d.f. = [1,4], P=0.017) than on the cicada grids (Fig 2C). Thus the cicada treatment has a positive influence on the abundance of one insectivore (*B. brevicauda*) and a negative influence upon the other (*S. cinereus*) and the former was the only species that responded to the sunflower seed enrichment.

Discussion

We examined the hypothesis that the abundance and vital rates of small mammals would be increased by a springtime pulse in resource availability as per Marcello *et al*. (2008). Specifically, we tested between the effects of protein rich cicadas and carbohydrate rich sunflower seeds and predicted that both should increase the vital rates and abundance of the small mammal community, but we postulated this influence would be greater on the grids with cicadas. We predicted a larger influence of cicadas not only because the timing of the pulse coincides with the beginning of the breeding season, but also because the higher protein content would provide amino acids which have been shown to be limiting nutrients in the springtime (McAdam & Millar 1999a; McAdam & Millar 1999b). We found that the springtime emergence of cicadas led to a brief increase in abundance of *P. leucopus*, *T. striatus*, and *B. brevicauda* but not *S. cinereus*. This appeared to be caused by reproduction before the cicada emerged and was followed by reduced breeding and reduced immigration. In contrast, on the sunflower seed grids, the abundance of small mammals did not increase with the exception of the shrew *B. brevicauda*. Overall, the spring response of *P. leucopus* to cicadas and seed was different to the fall seed pulses; the response to cicadas was short lived and led to a decrease in breeding and an increase in parasitism. We conclude from this that the effects of a protein rich pulse do indeed have different effects on small mammal dynamics than a carbohydrate rich pulse in spring.

In the study by Marcello et al. (2008), the demographic process giving rise to the increased density appeared early and increased breeding production. We provide further evidence to suggest that increased breeding occurred earlier on grids where cicadas emerged and we also found a high rate of juvenile recruitment during the first 2 weeks of the emergence. It is interesting to note that overall there was a tendency ($P=0.06$) after the emergence had started for more animals to be recruited onto the control grids than either the cicada or fed grids and this was counter to our *a priori* predictions based on observations from the fall pulses of food (Fig 4A). The analyses of the temporal patterns of recruitment indicated the recruitment rate onto cicada grids declined throughout the study and was indeed negative overall (Fig 4B). Given these findings, we suppose the increased density observed was a consequence of improved breeding before the emergence (Fig 4B). Analyses of juvenile recruitment onto cicada grids shows a high initial recruitment which was then followed by a decline in contrast to what was observed on the control and carbohydrate supplemented grids (Fig 4C).

During the period of cicada emergence there was reduced breeding amongst the adults with a smaller proportion of the animals in breeding condition and moreover, those that were breeding, did not remain in breeding condition for as long as those animals on the control grids. *P. leucopus* exhibits a midsummer breeding hiatus when they do not breed for a period of several weeks in midsummer (Burt 1940; Wolff 1985; Terman 1998). The evidence from this study coupled with that provided by Marcello et al. (2008) suggests that the mice commenced breeding on the cicada grids before emergence and much earlier than usual and as such may have entered the summer breeding hiatus earlier than normal hence we observed reduced breeding during the emergence. Another recent paper found that the extent of this summer hiatus can be reduced significantly through the experimental removal of gastrointestinal worms and in particular the

nematode *P. peromysci* (Vandegrift et al. 2008). Interestingly the *P. leucopus* on the study sites with cicadas exhibited increased levels of parasitism with *P. peromysci* suggesting that these animals may have entered their summer breeding hiatus early because of the increased levels of parasitism. The interesting feature of the life cycle of this nematode is that it involves an intermediate host, the camel cricket (*Orthoptera: Gryllidae*) that becomes infected when it feeds on mouse feces. It is most unlikely that the worms use cicadas as an intermediate host so these mice got infected eating camel crickets. The data we have do not allow us to disentangle whether this was a consequence of increased exposure to camel crickets associated with cicada emergence or a reduction in host susceptibility.

The design of these pulsed resource experiments is confounded by a number of variables that need to be addressed. We expected the cicadas to emerge further north than they actually did and indeed as they have in the past (Cooley et al. 2004), so grids we established to measure initial density and breeding effort had to be abandoned. This meant that the grids further south had only one trapping session before emergence and this not only prevented us from using the Jolly-Seber techniques to estimate relative abundance before the pulse but also prohibited us seeing the ramped up reproduction prior to the emergence observed by Marcello et al. (2008). It also means that strictly speaking, the data are spatially confounded, since the sites where the cicadas emerged could not be selected at random, so the differences observed could be a reflection of site rather than treatment. We attempted to ameliorate this problem by first analyzing the data with respect to when the cicadas were present and also second returned 2 years after the effects of the cicadas had passed so we could estimate density again. Together these data support the explanation that the significant differences in density were associated with the cicada emergence rather than the site or other effects.

A key finding in this study is that the addition of carbohydrate rich sunflower seeds in the spring had no effects on density of *P. leucopus* or *T. striatus* compared with the controls. We suspect that this is related to differences in the protein and carbohydrate levels but we must also note that the response of animals to experimental fall pulses (as described in the introduction) is highly variable and it would have been informative to have a comparative fall experiment. Also, if we would have provided a foodstuff nutritionally equivalent to the cicadas rather than sunflower seeds we could have provided more information on whether small mammals can predict these pulses and their relative responses. Interestingly, *B. brevicauda* did increase in response to the addition of sunflower seeds; there is no clear explanation for this result unless the seeds were attracting an insect species the shrew fed on.

We predicted that these 2 spring time pulsed resources would increase the breeding, density, and survival of *P. leucopus* but, with greater protein we expected a stronger response in the cicada grids. We also predicted other members of the small mammal community would increase as a result of the increased availability of these foodstuffs. Further, we predicted the increases in density would be coupled with an increase in the prevalence of the major helminth P. peromysci.

In summary, we found the pulse of protein rich cicadas led to earlier breeding and high levels of juvenile recruitment and thus increased abundance. This was quickly followed by an increase in *P. peromysci* prevalence, a cessation of breeding, and a return to control levels of abundance. In contrast, the sites with supplemental sunflower seeds had no such influence and were indistinguishable from the controls in nearly all analyses. Given that animals of the Genus *Peromyscus* have been shown to be limited by protein in the field, out results are not surprising

especially when the arthropods which compose the major portion of their diet are not available until about the time when the breeding season typically starts.

Table 1. The results our ANOVA for the body morphometric data of *Peromyscus leucopus*. The enrichments of additional sunflower seeds and emerging cicadas did not result in any increases in body size or condition (all P>0.05). The degrees of freedom reflect our use of grid as the error structure in the models.

Response	Coef. fed	Coef. not fed	F	d.f.	P
Male Mass	-0.51	0.55	1.32	2,6	0.34
Female Mass	1.46	0.63	0.52	2,6	0.62
Male Condition	-0.002	-0.001	0.18	2,6	0.84
Female Condition	0.004	0	0.18	2,6	0.84
Male Body Length	1.73	2.28	1.54	2,6	0.29
Female Body Length	1.49	-0.07	0.45	2,6	0.66

Fig. 1. The emergence of *Magicada* spp cicadas in 2004 (a) broad distribution in North East America with location of study areas in blow up; (b) emergence in Pennsylvania where filled circles represent positive cicada presence and unfilled circles are negative reports.
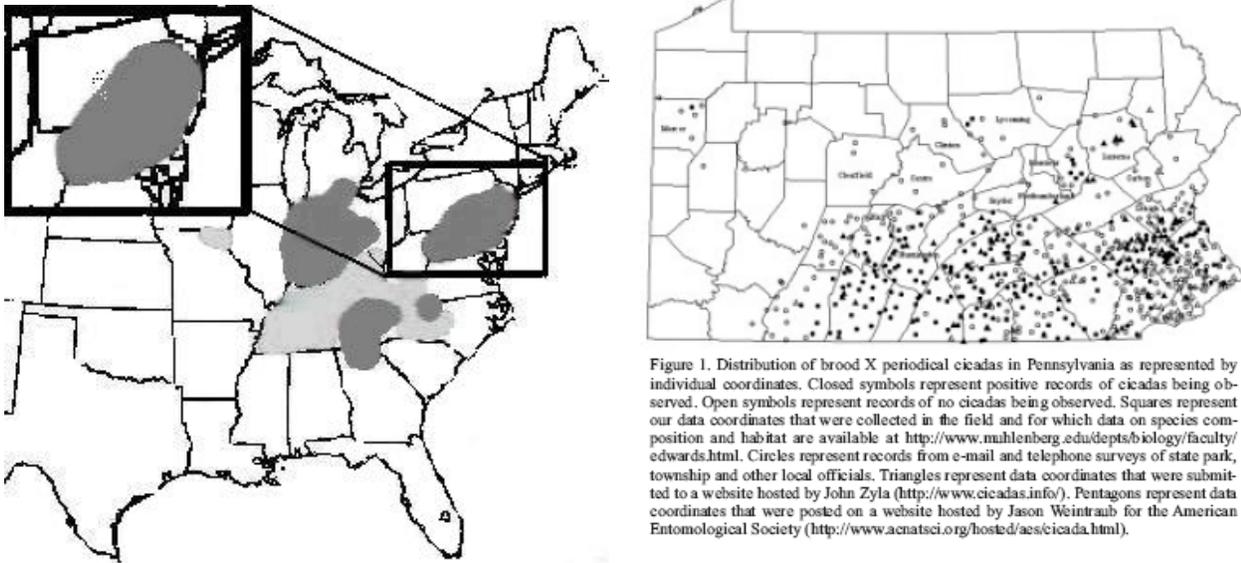
Figure 1. Distribution of brood X periodical cicadas in Pennsylvania as represented by individual coordinates. Closed symbols represent positive records of cicadas being observed. Open symbols represent records of no cicadas being observed. Squares represent our data coordinates that were collected in the field and for which data on species composition and habitat are available at http://www.muhlenberg.edu/depts/biology/faculty/edwards.html. Circles represent records from e-mail and telephone surveys of state park, township and other local officials. Triangles represent data coordinates that were submitted to a website hosted by John Zyla (http://www.cicadas.info/). Pentagons represent data coordinates that were posted on a website hosted by Jason Weintraub for the American Entomological Society (http://www.acnatsci.org/hosted/aes/cicada.html).

Fig. 2. Jolly-Seber population estimates for (A) *Peromyscus leucopus*; (B) *Tamias striatus* on treated and untreated grids in Pennsylvania. Log abundance of 2 shrew species: (C) *Sorex cinereus;* (D) *Blarina brevicauda*. Points based on 3 grids +1 SE.
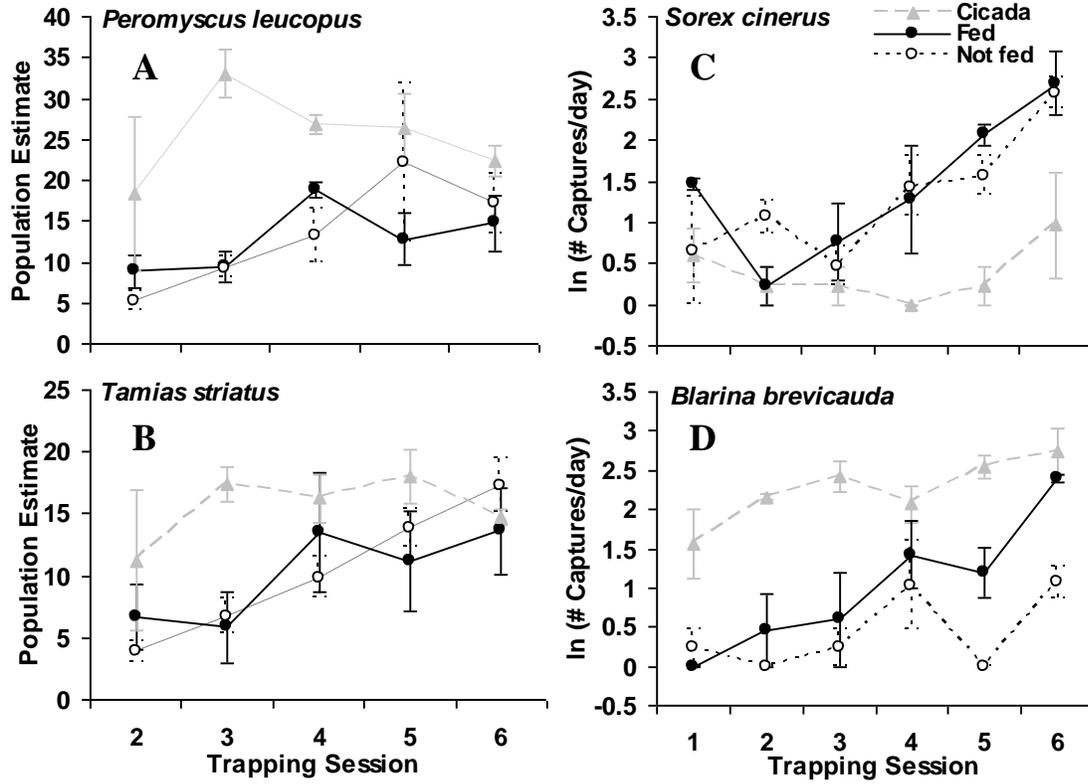
Fig. 3. The proportion of (A) all individuals; (B) males and (C) females in breeding condition with respect to treatment. (D), the average proportion of all male and female animals in breeding condition; (E) the average proportion of time an individual resident (caught in 2 trapping sessions or more) male or female is in breeding condition (F). The average number of days known alive for resident males and females in the three treatment groups. Error bars are standard error (n=3)
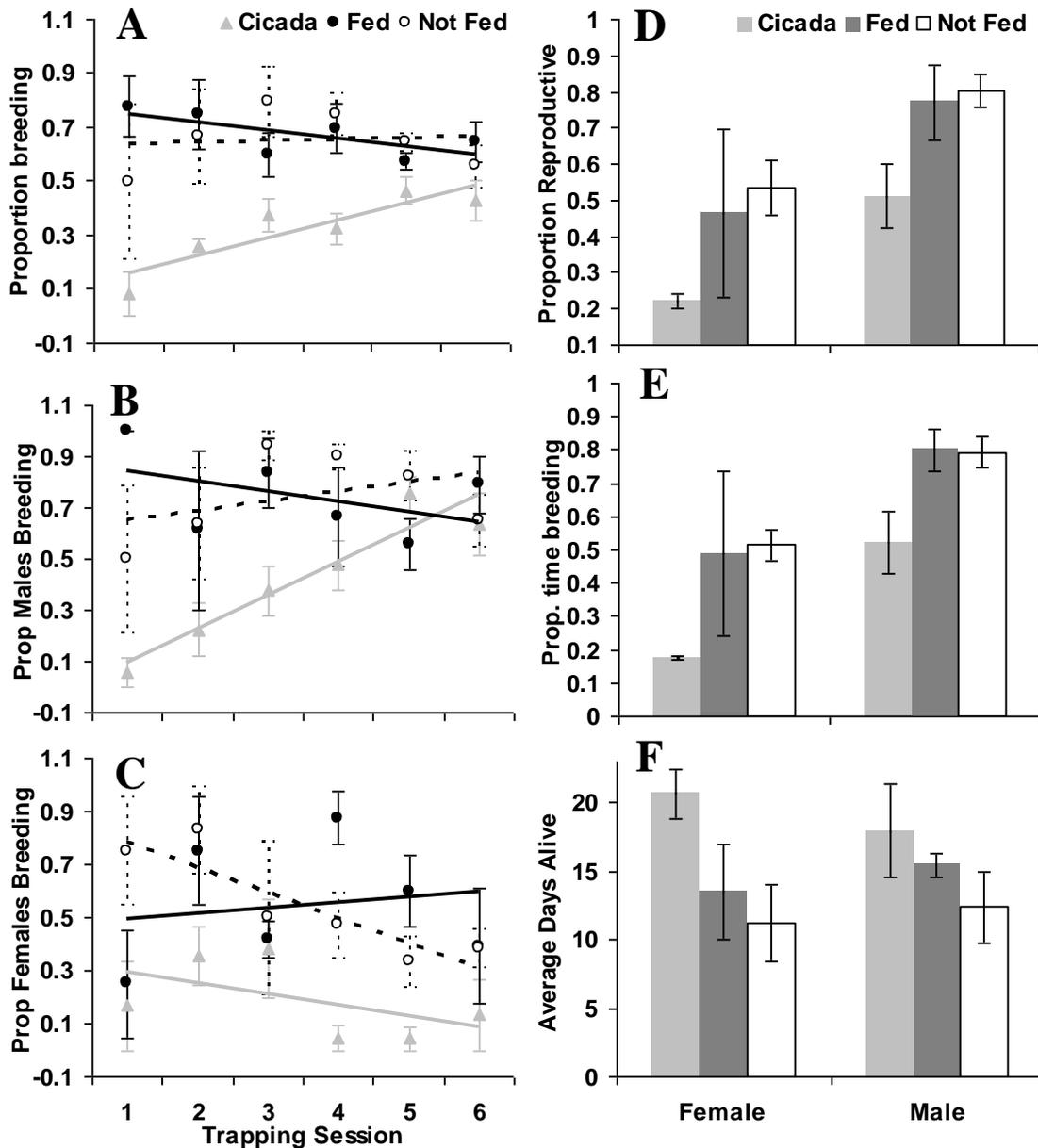
Fig. 4. (A) The Pradel estimates of recruitment for the 3 treatments indicated more animals were recruited onto the control grids than either the supplemental sunflower seed grids or the grids where the cicadas emerged and that these results approached significance (P=0.09 and P=0.06), respectively. (B) The average recruitment rates for 3 age classes of *P. leucopus* based on mass (juveniles <16g, sub-adults 16-20g, and adults >21g). Treatment is highly significant in the full model for juveniles (P = 0.0005), approaches significance for sub-adults (P = 0.087), and is not significant for adults (P = 0.57). (C) The rate of juvenile recruitment for the 3 treatments illustrates the much higher recruitment of juvenile animals onto cicada grids early in the study. This high rate then declines while the juvenile recruitment rate of the control and fed grids gradually increases throughout the study. (D) The prevalence of *Pterygodermatities peromysci* in *P. leucopus*. Males on grids where cicadas emerged had significantly higher prevalence than both the sunflower fed (P = 0.002) and control grids (P = 0.001). The error bars are standard error (n=3).
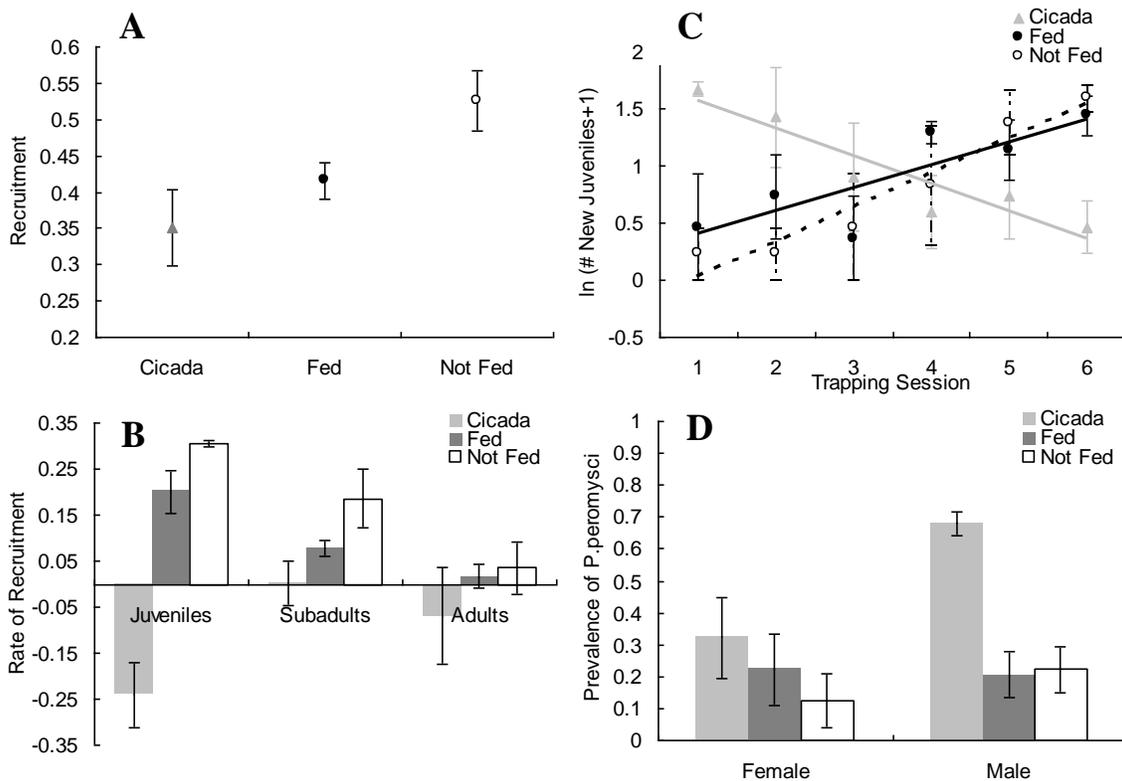
References

Arnebreg, P., Skorping, A., Grenfell, B.T.,& Read, A.F. (1998) Host densities as determinants of abundance in parasite communities. *Proceedings of the Royal Society of London Series B Biological Sciences*, 265, 1283-1289.

Asdell, S. A. (1964) *Patterns of mammalian reproduction*. Cornell Univ. Press, Ithaca, New York, 2nd ed., viii 670 pp.

Burt, W.H. (1940) Territorial behavior and populations of some small mammals in southern Michigan. *Misc. Publication of the Museum of Zoology*, University of Michigan, 45, 1-58.

Bendell, J.F. (1959) Food as a control of a population of white-footed mice, *Peromyscus leucopus noveboracensis* (Fisher). *Canadian Journal of Zoology*, 37, 173-209.

Cooley, J.R., Marshall, D.C. & Simon, C. (2004) The Historical contraction of periodical cicada brood VII (Hemiptera: Cicadidae: *Magicada*). *Journal of the New York Entomological Society*, 112, 198–204.

Edwards, M.J., Faivre, A.E., Crist, R.C., Sitvarin, M.I. & Zyla, J. (2005) Distribution of the 2004 emergence of seventeen year periodical cicadas (Hemiptera: Cicadidae: *Magicada* spp., Brood X) in Pennsylvania. *Entomological News*, 116, 273-282.

Elias, S. P., Witham, J. W. &. Hunter, M.L. (2004) *Peromyscus leucopus* abundance and acorn mast: population fluctuations over 20 years. *Journal of Mammalogy*, 85, 743-747.

EIDAP Inc. of Sherwood Park, Alberta, Canada. http://www.eidap.com/

Elkington, J. S., Healy, W. M., Buonaccorsi, J.P., Boettner, G.H., Hazzard, A.M., Smith, H.R. & Liebhold, A.M. (1996) Interactions among gypsy moths, white-footed mice, and acorns. *Ecology*, 77, 2332–2342.

Falls, J.B., Falls, A.E. & Fryxell, J.M. (2007) Fluctuations of deer mice in Ontario in relation to seed crops. *Ecological Monographs*, 77, 19–32.

Fordham, R.A. (1971) Field populations of Deer Mice with supplemental food. *Ecology*, 51, 138-146.

Galindo-Leal, C., & Krebs, C.J. (1998) Effects of food abundance on individuals and populations of the Rock Mouse (*Peromyscus difficilis*). *Journal of Mammalogy*, 79, 1131-1142.

Grahnab SE Ekhaga, Marieholm 330 33 Hillerstorp Sweden

Hahus, S.C. & Smith, K.G. (1990) Food habits of *Blarina, Peromyscus*, and *Microtus* in relation to an emergence of periodical cicadas (*Magicicada*). *Journal of Mammalogy*, 71, 249-252.

Hansen, L.P. & Batzli, G.O. (1979) Influence of supplemental food on local populations of *Peromyscus leucopus*. *Journal of Mammalogy*, 60, 335-342.

Hudson, P.J. & Bjornstad, O.N. 2003. Vole stranglers and lemming cycles. *Science*, 302, 797-798.

Jones, C.G., Ostfeld, R.S., Richard, M.P., Schauber, E.M. & Wolff, J.O. (1998) Chain reactions linking acorns to gypsy moth outbreaks and Lyme disease risk. *Science*, 279, 1023-1026.

Krohne, D.T., Couillard, T.J. & Riddle, J.C. (1991) Population responses of *Peromyscus leucopus* and *Blarina brevicauda* to emergence of periodical cicadas. *American Midland Naturalist*, 126, 317-321.

Marcello G. J., Wildner, S. M. & Meikle D.B. (2008) Population dynamics of a generalist rodent in relation to variability in pulsed food resources in a fragmented landscape. *Journal of Animal Ecology*, 77, 41-46.

May, R.M. & Anderson, R.M. (1978) Regulation and Stability of Host-Parasite Population Interactions: II. Destabilizing Processes. *The Journal of Animal Ecology*, 47, 249-267.

McAdam, A.G. & Millar, J.S. (1999a) The effects of dietary protein content on growth and maturation in deer mice. *Canadian Journal of Zoology*, 77, 1822-1828.

McAdam, A.G. & Millar, J.S. (1999b). Dietary protein constraint on age at maturity: an experimental test with wild deer mice. *Journal of Animal Ecology*, 68, 733-740.

McCracken, K.E., Witham, J.W. & Hunter, J.L. Jr. (1999) Relationships between seed fall of three tree species and *Peromyscus leucopus* and *Clethrionomys gapperi* during 10 years in an oak-pine forest. *Journal of Mammalogy*, 80, 1288-1296.

McShea, W. J. (2000) The influence of acorn crops on annual variation in rodent and bird populations. *Ecology*, 81, 228-238.

Ostfeld, R.S., Jones, C.G. & Wolff, J.O. (1996) Of mice and mast. *BioScience*, 46, 323-330.

Ostfeld, R. S., Richard, M.P., Schauber, E.M. & Wolff, J.O. (1998) Chain reactions linking acorns to gypsy moth outbreaks and Lyme disease risk. *Science* 279, 1023-1026.

Ostfeld, R.S. and Keeling, F. (2000) Pulsed resources and community dynamics of consumers in terrestrial ecosystems. *Trends in Ecology and Evolution*, 15, 232–237.

Pedersen, A.B. & Grieves, T.J. (2008) The interaction of parasites and resources cause crashes in a wild mouse population. *Journal of Animal Ecology*, 77, 370–377.

Pollock, K.H., Nichols, J. D., Brownie, C. & Hines, J.E. (1990) Statistical inference for capture-recapture experiments. *Wildlife Monographs* 107, 97 pp.

Pradel, R. (1996) Utilization of capture-mark-recapture for the study of recruitment and population growth rate. *Biometrics*, 52, 703-709.

Pucek, Z., Jedrzejewski, W., Jedrzejewska, B. & Pucek M. (1993) Rodent population dynamics in a primeval deciduous forest (Bialowieza National Park) in relation to weather, seed crop, and predation. *Acta Theriologica*, 38, 199-232.

R Development Core Team 2005. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN   3-900051-07-0, URL http://www.R-project.org

Rosenzweig, M. L. (1971) Paradox of enrichment: destabilization of exploitative ecosystem in ecological time. *Science*, 171, 385–387.

Roy, S. & Chattopadhyay, J. (2007) The stability of ecosystems: A brief overview of the paradox of enrichment. *Journal of Bioscience*, 32, 421–428.

Taitt, M.J. (1981) The Effect of Extra Food on Small Rodent Populations: I. Deer mice (*Peromyscus maniculatus*). *The Journal of Animal Ecology*, 50, 111-124.

Terman, C.R. (1998) Early-summer reproductive curtailment in wild white-footed mice and reproductive recovery in the laboratory. *Journal of Mammalogy*, 79, 320-325.

Vandegrift, K.J., Raffel, T.R. & Hudson, P.J. (2008) Parasites prevent summer breeding in white-footed mice, *Peromyscus leucopus*. *Ecology* (in press).

White, G.C. & Burnham, K.P. (1999) Program MARK: Survival estimation from populations of marked animals. *Bird Study* 46 Supplement, 120-138.

Wolff, J.O. (1985) Comparative population ecology of *Peromyscus leucopus* and *Peromyscus maniculatus*. *Canadian Journal of Zoology*, 63, 1548-1555.

Wolff, J.O. (1986) The effects of food on mid-summer breeding activity in *Peromyscus leucopus* in south-western Virginia. *Canadian Journal of Zoology*, 64, 855-858.

Wolff, J.O. (1996) Population fluctuations of mast-eating rodents are correlated with production of acorns. *Journal of Mammalogy*, 77, 850-856.

Yunger, J.A. (2002) Response of two low-density populations of *Peromyscus leucopus* to increased food availability. *Journal of Mammalogy*, 83, 267–279.

Chapter 2

Parasites prevent summer breeding in white-footed mice, *Peromyscus leucopus*.

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Abstract-

Food and parasites can independently play a role in destabilizing population fluctuations of animals and yet more than 50 years ago David Lack proposed that these two factors should act in concert. We examined the role of these factors on the vital rates of free-living white-footed mice (*Peromyscus leucopus)* over the summer and autumn months. We used a replicated factorial experiment in which deer exclosures doubled acorn availability and anthelmintic application reduced gastro-intestinal helminths. Specifically, we wanted to know if either factor or an interaction between the two accounted for the mid-summer breeding hiatus observed in this species. We found no influence of habitat quality on mouse breeding, vital rates, or demography; however, anthelmintic treatment resulted in mice continuing to reproduce during the hiatus at the same rate as previously and they also exhibited increased body condition, growth rate and survival. These results provide evidence that gastro-intestinal helminths reduce *P. leucopus* reproductive output in central Pennsylvania and these effects on reproduction could play a role in the unstable dynamics of small mammals.

Keywords- *Peromyscus*, nematode, parasite, mouse, habitat, acorn, reproduction, small mammal, body condition

 Introduction

In his classic book on the Natural Regulation of Animal Numbers, David Lack (1954) proposed that parasites would be unlikely to regulate animal populations unless they act in concert with food shortage. Lack considered only parasite-induced mortality and yet there is evidence that gastrointestinal helminths, particularly in moderate levels of infection, induce morbidity rather than mortality and tend to reduce host body condition (Tompkins et al. 2001; Stien et al. 2002) and fecundity (Hudson 1986). When parasite-induced reduction in fecundity is large relative to the impact on host mortality this can lead to instability and generate population cycles (May and Anderson 1978; Dobson and Hudson 1992). Studies on red grouse (*Lagopus lagopus scoticus*) have shown that the removal of the caecal nematode *Trichostrongylus tenuis* increased breeding production and reduced the instability of the population cycles (Hudson et al. 1999). Parasitism also interacts with other biological processes by increasing vulnerability to predators (Hudson et al. 1992; Yunger 2002; Kissui and Packer et al. 2004), reducing aggression (Fox and Hudson 2001), increasing transmission of secondary infections (Bentwich et al. 1995; Glass et al. 2002; Cattadori et al. 2005), and reducing access to food or other resources (Kristan 2002; Stein et al. 2002). In nature, many of these factors interact so that the observed dynamics are a tension between these stabilizing and destabilizing forces (Hudson et al. 2003). These studies identify the importance of focusing on the sub-lethal effects of parasitism and, in particular, the parasite induced reduction in host breeding production.

Small mammals provide a suitable system for investigating food and parasite interactions since they exhibit unstable dynamics, are infected with parasites that can be removed with

anthelmintics (Ferrari et al. 2004), and utilize resources which can be manipulated indirectly through habitat manipulation (e.g., excluding deer) (McShea 2000). Mast crops such as oak acorns provide an important winter food resource for mice and studies have shown that this food resource is not only associated with increased survival (Bendell 1959; Hansen and Batzli 1979; Pucek et al. 1993; Jones et al. 1998; McCracken et al. 1999; Falls et al. 2007) but also increased breeding during the following spring and summer (Smyth 1966; Hansen and Batzli 1979; Gashwiler 1979; Pucek et al. 1993; Wolff 1996; Ostfeld et al. 1996; Jones et al. 1998; McCracken et al. 1999; Elias et al. 2004; Falls et al. 2007).

If parasites are important to the unstable dynamics of small mammals, we would predict they would have a sub-lethal effect, reducing the reproductive output of their hosts or perhaps shaping the host's seasonal reproductive strategy (Raffel 2006). In this respect, the White-footed mouse, *Peromyscus leucopus,* is interesting because it exhibits a distinct bimodal breeding season in northeastern North America, when females cease breeding during a period in mid-summer, a pattern refereed to as the "mid-summer breeding hiatus" (Burt 1940; Brown 1964; Rintamaa et al. 1976; Cornish and Bradshaw 1978; Wolff 1985a; Terman 1998). Decreased resource abundance during this period is insufficient by itself to explain the pattern because food addition did not stop the hiatus (Wolff 1986) and many of the females during this period are adults and capable of reproducing (Terman 1998). To determine if parasites, food availability, or a combination of the two have a role in the mid-summer breeding hiatus, we used a factorial experiment and monitored the reproductive status and demography of 12 populations of *P. leucopus*. Our *a priori* predictions were that more acorns over the winter would produce females in better breeding condition that would have greater reproductive output and the removal of parasites would interact to increase production.

Materials and Methods

Experimental Design, Trapping, Habitat, and Density

Experimental grids were established in open hardwood forest, dominated by *Quercus* spp. and *Acer* spp., typical of the northeastern Appalachians. Twelve trapping grids were established, two in each of three deer exclosures, with two matching control grids located outside each exclosure. All grids were separated by at least 250 m. of woodland and each grid consisted of twelve 8 x 8 grids of multi-capture live traps (Ugglan, Graham, Sweden), set at 10 m. intervals. Grids were checked for two consecutive days, bi-weekly from May 12 through to August and then monthly until November 17th 2005. This produced 12 trapping sessions for each of the grids.

Deer exclosures were originally erected four years previous to the study. Shelter-wood cutting in the enclosures eliminated all but the largest, most prolific acorn producing trees and preliminary observations found this resulted in a dense understory of brambles (i.e., *Sambucus, Smilax,* and *Rubus* spp.) and increased both abundance and availability of acorns. Following techniques described by Wolff (1996) we undertook pre-mast counts of the number of acorns per branch on three randomly selected branches of seven trees per grid. During peak mast we also counted the number of acorns on the ground in seven random 1m x 1m plots on each grid in both 2005 and 2006. We undertook a survey of sapling density on each of the 12 grids by counting saplings (height > 0.2 m.) and seedlings (height < 0.2 m.) in five replicate transects (50 m.) inside and outside the exclosures. A measure of deer damage was also recorded by noting the proportion of seedlings which had their terminal buds browsed.

Anthelmintic Treatment, Vital Rate Estimates and Macroparasite Intensity

To obtain baseline demographic, and density levels, each site was trapped twice (n=321 captures) prior to applying anthelmintic. Distinguishing between *Peromyscus* species in the field is not easy, but based on morphometric data, the majority of animals in this study were classified as *P. leucopus*, though we cannot rule out that some mice may have been *Peromyscus maniculatus*. After the initial trapping, two grids (one inside and one outside) were selected at random from the 4 grids at each deer exclosure site, and all *Peromyscus* caught on these grids were treated with Levamisole Hydrochloride™ (15 mg/Kg) (Schering-Plough, New Jersey, USA) upon each capture. Body length, tail length, body mass, body condition, sex, and breeding condition were recorded for each capture. Animals were considered in breeding condition when males had scrotal testes and females were lactating, pregnant, or had a perforate vagina. Animals captured in more than one trapping session were considered residents.

Individual mice were identified using Trovan™ (EIDAP, Alberta, Canada) passive induced transponder tags inserted into the scruff of the neck. The gastrointestinal tract of incidental mortalities (n=34) was dissected and all worms were identified and counted to measure the prevalence and intensity of infection. These experiments were conducted with the approval of the Pennsylvania State Animal Care Committee (IACUC #16061, "Transmission Dynamics of Directly Transmitted Diseases in Wildlife Reservoir Hosts").

Statistical Analyses

Statistical analyses were carried out in R (www.r-project.org). The habitat data (acorns, seedlings, saplings and browse disturbance) were analyzed with nested linear mixed effect models (Plots were nested with grids and grids were nested within sites). Nested analyses of variance (Error term = grid) were used to analyze response variables such as body mass, body condition, days known alive, proportion of time in breeding condition (arcsine-transformed),

growth rate, and density, in order to ensure that grid was the unit of replication. When testing for the effects of anthelmintic treatment, we included only resident animals (caught in more than one trapping session) because newly captured individuals had yet to receive the first treatment. Growth rate was measured as the slope of a regression line drawn through the individuals mass versus days known alive plot for all individuals caught three or more times following treatment. Mass at first capture was included as a covariate in this analysis as it should influence growth rate. Density was estimated from Jolly-Seber population size estimates (see Pollock et al. 1990); when population size was the response variable, site and trapping session were included as covariates. Body condition was estimated from the residuals of a cubic spline fit of mass vs. body length. A generalized linear model (GLM) with binomial errors was used to determine differences in helminth prevalence among treatment groups and controls. The proportion of animals in breeding condition and the proportion pregnant were calculated for individual grids and arcsine square root transformed before analysis with Gaussian linear models. The best predictors of each response variable were selected using backwards stepwise selection, retaining variables with $P < 0.05$ based on F-statistics. To test for age-specific effects of treatment, we separated animals into mass classes such that juveniles were defined as less than 16 g, sub-adults as 16-20 g and adults as greater than 20 g.

RESULTS

Experimental Treatment, Trapping, and Habitat, and Density

A total of 1842 *P. leucopus* captures occurred during 12,500 trap nights. Of the 621 individually tagged animals, 415 were caught more than once (residents) and these individuals were caught an average of 5.0 times.

There were significantly more acorns inside exclosures (no deer) than in control areas (deer present) both on the tree (coef. = 0.50, F = 80.27, d.f. = [1,10], P<0.0001) and on the ground (coef. = 0.46, F = 37.62, d.f. = [1,10], P<0.0001). The same pattern was observed in 2006 as in 2005. There were also significantly greater densities of seedling (coef. = 1.35, F = 11.63, d.f. = [1,8], P = 0.009) and sapling trees (coef. = 1.22, F = 6.04, d.f. = [1,8], P = 0.04) inside the exclosures. The browse damage was lower (coef. = 0.91, F = 65.56, d.f. = [1,8], P < 0.0001) inside the exclosure. We refer to the habitat inside the exclosures as being good habitat and outside as poor habitat.

Based on the Jolly-Seber population size estimates, there was no influence of habitat or parasite treatment on the density of mice either before or after treatment (all P>0.05). The interaction term of the full model with parasites and habitat was also not significant (F = 0.016, d.f. = [1,10], P = 0.90). Overall, the population mean increased from about 8 individuals per grid to 16 in August and then returned to 8 by November.

The effect of treatment on parasites, mass, body condition and body growth rate

To determine if there were any pre-treatment differences in the response variables between experimental and control grids, we tested for the effects of parasite treatment and habitat type on density, mass, condition, and the proportion of the population breeding for the time period before anthelmintic treatment began. There were no significant initial differences between habitat types or treated and untreated grids for any of the response variables (all P > 0.05) except for mass (coef. = 2.29, F = 18.0, d.f. = [1, 10], P = 0.002) and body condition (coef. = 1.37, F = 5.32, d.f. = [1, 10], P = 0.04), which were higher in the anthelmintic treated grids when all individuals were included in the analysis. A closer examination of the data revealed this was due to a larger number of juvenile animals on the untreated grids during the pre-

treatment period as a consequence of a two recent litters on two of the grids. When juveniles animals were removed from the analysis, there was no difference between grids in either mass (F = 0.68, d.f. = [1, 10], P = 0.43) or condition (F = 1.69, d.f. = [1, 10], P = 0.22) and so we assume a comparison of adult mice between treatment and control is valid.

Lack's hypothesis predicts greater female mass inside the enclosures in the good quality habitat prior to the anthelmintic treatment. There was a tendency for adult females to be heavier within the enclosure (F = 2.87, d.f. = [1, 10], one-tailed P = 0.06) but this was not significant for all animals. To determine the number of grids needed for a significant result we undertook a power analysis (Power =0.8; P<0.05 one-tailed) and found 12 grids per treatment would be needed to determine significance.

After anthelmintic treatment, prevalence of all gastrointestinal nematodes was significantly lower on treated grids (5%) than untreated (58%) grids (χ^2 = 33.73, d.f. = [1,32], P = 0.001) although there was no significant difference between habitat types (χ^2 = 43.97, d.f. = [1,32], P = 0.67) and no significant interaction (χ^2 = 32.60, d.f. = [1,32], P = 0.54). The prevalence of the most common nematode, *Pterygodermatities peromysci* was also significantly lower (χ^2 = 30.0, d.f. = [1,32], P = 0.002) on treated grids (3%) as opposed to not treated grids (52%). Neither differences between habitat types (χ^2 = 39.28, d.f. = [1,32], P = 0.89) nor interactions were significant (χ^2 = 29.53, d.f. = [1,30], P = 0.56).

Following anthelmintic treatment, adult mice were significantly heavier (6.1%) than those on untreated grids (coef. = 1.57, F = 19.3, d.f. = [1, 9], P = 0.001) and this was true for both male (8.6% larger) and female (4.7% larger) mice (Table 2). There was no significant interaction between resident status (animals caught on more than one occasion) and treatment. An analysis of the mass of first captures (before their individual treatment but after the time

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others on the grid had been treated) identified significantly higher mass on treated grids (7.6% larger) (coef. = 1.70, F = 10.77, d.f. = [1,9], P = 0.008) (Table 2) (Fig. 5). These results indicate that the treatment of mice to remove parasites not only increased resident body mass, but also influenced the body mass of other individuals on the same grid. Mice on treated grids had significantly greater body condition (mass per body size) than those on untreated grids (coef. = 1.04, F = 18.48, d.f. = [1, 9], P = 0.002) and this held for both males and females (Table 2). There was no interaction between condition and resident status. Newly captured animals on treated grids had significantly greater body condition after treatment indicating that treatment improved mass in relation to body size (coef. = 1.16, F = 5.95, d.f. = [1,9], P = 0.03; Fig. 5d) but this did not hold for each sex independently (Table 2).

There was a weak tendency for treatment to increase growth rate in all mice (coef. = 0.02, F = 3.41, d.f. = [1,9], one-tailed P = 0.05; Fig 6a) but females had significantly higher growth rates on treated grids (coef. = 0.08, F = 6.31, d.f. = [1,9], P = 0.03; Fig. 6a), as did all sub-adults (coef. = 0.04, F = 7.39, d.f. = [1,9], P = 0.03; Fig. 6b). This is evidence that parasite removal increased mass, relative body condition and body growth rate of sub-adults and females.

The effects of treatment on vital rates

There was a significant effect of treatment on the proportion of animals in breeding condition (coef. = 0.10, F = 12.87, d.f. = [1,10], P = 0.005; Fig. 7c). This pattern holds for females (coef. = 1.36, F = 30.83, d.f. = [1,10], P = 0.0002; Fig.7c) but not males (F = 1.55, d.f. = [1,10], P = 0.24; Fig.7c) and was not significantly increased in nonresidents (F = 2.14, d.f. = [1,10], P = 0.17); Fig 7d). The time series indicates that these effects were greatest in mid-summer when the proportion of females breeding was increased from 5% to 40% although by the end of summer nearly 60% of females were breeding (Fig. 8).

The number of days known alive was greater for animals on treated than control grids (coef. = 0.32, F = 7.35, d.f. = [1,10], P = 0.02; Fig. 8a). However, when we look at the sexes independently, this pattern holds only for male animals (coef. = 0.35, F = 4.74, d.f. = [1,10], P = 0.05; Fig. 8a) and the influence on female survival is not significant (F = 1.75, d.f. = [1,10], P = 0.21; Fig. 8a). Thus parasite removal increased female breeding and increased male survival.

Amongst resident animals, the average proportion of time all individuals were in breeding condition was not significant at the 5% level but did show a tendency towards significance for the whole population (coef. = 0.17, F = 3.85, d.f. = [1,10], P = 0.08; Fig. 8b) and was significant for females (coef. = 0.17, F = 6.94, d.f. = [1,10], P = 0.02; Fig. 8b) and males (coef. = 0.23, F = 8.19, d.f. = [1,10], P = 0.02; Fig. 8b) when analyzed separately. The difference in proportion of time breeding was also significant for females when we considered pregnancy as the sole criterion for breeding status (coef. = 0.10, F = 5.04, d.f. = [1, 10], P < 0.05).

Discussion

Removal of parasites through anthelmintic treatment resulted in a reversal of the mid-summer breeding hiatus such that females continued to breed on grids with reduced parasites compared to control grids and at a rate similar to that observed in the first week of trapping. Anthelmintic treatment also led to higher body mass, body condition, growth rate, and survival of males consistent with the hypothesis that parasites had sub-lethal effects on individuals and affected vital rates of *P. leucopus*. In contrast, habitat quality had no significant direct effect or interaction with anthelmintic treatment on any indicator of mouse condition, survival, or reproduction intra-annually, despite both heightened resource availability and abundance of cover in the different habitat types. This does not refute the hypothesis that inter-annual dynamics are driven by food resources, or food coupled with parasites, but does refute the

hypothesis that food and parasites act in concert to stop mice breeding in mid summer, at least at this location and scale of study. These findings are significant at the population level since they identify that parasites reduce breeding production and this is a destabilizing process that may play a role in driving population cycles in mouse populations (Anderson & May 1978, Dobson & Hudson 1992). Previous work has focused on the role of food and predators in generating instability but these findings indicate that we should examine the hypothesis that parasites reduce fecundity and destabilize mouse populations.

The lack of any effect of habitat improvement on mouse vital rates was unexpected, given that the habitat manipulations doubled the availability of acorns and had obvious effects on cover and other food sources. Acorns are an important food source for *P. leucopus* during the winter months and are considered a primary determinant of over-winter survival, subsequent breeding condition, and have been shown to influence the intra-annual dynamics (Hansen and Batzli 1979; Pucek et al. 1993; Wolff 1996). High food availability over the winter prepares the mice for breeding and between year comparisons show that following heavy acorn production mice have greater mass and commence breeding earlier in the season and sometimes continue through the winter (Smyth 1966; Wolff and Durr 1986; Pucek et al. 1993; Elkington et al. 1996; Ostfeld et al. 1996; Shimada and Saitoh 2006). If food were to be important then we would predict greater body mass in females in the improved habitat. We did find some evidence that adult females had higher mass, but this was not statistically significant (one-tailed; p=0.06) and interestingly there was no effect on male body mass. One possible explanation is that the mice commenced breeding at an earlier date in the improved habitat and body mass of mice had fallen by the time we tested for differences.

An interesting finding was that some of these treatment effects were significant for the newly captured (i.e., not yet treated) mice, implying that there was a population level effect such that treatment of some individuals reduced transmission to other individuals in the same area. This has consequences for experimental design. If we had used individuals as the unit of replication within grids then we may not have identified this effect because we would have reduced infection levels in the neighboring control individuals, resulting in a type II error.

A possible weakness of this study is our limited knowledge of the efficacy of the anthelmintic against the parasite community. Levamisole hydrochloride™ has proven effective against nematodes in other mammals including mice (Ferrari et al. 2004) and birds (Hudson 1986), and is widely used as an agricultural anthelmintic. This anthelmintic does not clear cestode infections and these mouse populations were infected with a diverse parasite community including six nematodes (*Pterygodermatities peromysci, Syphacia peromysci, Heligmosomoides vandegrifti, Mastophorous muris, Capillaria americana, Aspicularis americana*), one trematode (*Brachylaima peromysci*)*,* and one cestode species (*Rodentalepsis* sp.). For the future, it will be interesting to tease apart which parasite species and interactions are having important effects.

Although both sexes responded to anthelmintic treatment with an overall increase in breeding effort, the effect was more pronounced in females. Indeed, females experienced a reversal of the mid-summer breeding hiatus observed in previous studies and which occurred on the control sites. Of course, comparisons of the sexes are difficult because male breeding status depends on descended testes and females have to be perforate, pregnant or lactating. Even so, the parasite-induced reduction in survival and reproductive effort in females should have a negative effect on host fitness. Since direct estimates of litter size and juvenile survival could not be made, we used the product of the number of days alive and the proportion of time in

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breeding condition to estimate a relative index of the lifetime reproductive potential within a year. Based on this measure, the lifetime reproductive potential was significantly greater on treated grids for all animals as well as for each sex when analyzed separately (Fig 8c). These estimates provide an integral of the findings of this study and indicate that the reproductive potential of females was approximately doubled by the application of anthelmintic.

There is growing evidence that parasites play an important role in shaping population dynamics, and that chronic parasite infection can influence host fitness by reducing breeding output (Hudson, 1986; Hudson et al. 1992; Stien et al. 2002; Telfer et al. 2002; Norris et al. 1994, Smith et al. 2008) or by shaping seasonal breeding strategies (Raffel 2006). We have shown that there are significant impacts of nematode worms on the body condition, mass, growth, and breeding of *P. leucopus*, and that parasites can account for the mid-summer breeding hiatus commonly observed in female mice. These results imply that parasites may play a more important role in the vital rates and temporal dynamics of *P. leucopus* than resource abundance. Further studies are needed to assess the importance of parasites to the long-term inter-annual dynamics of *P. leucopus*.

Table 2. Results of nested analysis of variance for the effects of anthelmintic treatment on mass and condition of male, female, and newly captured animals, using grid as the unit of replication (n = 12).

Response	Coef.	F	d.f.	P
Male Mass	1.40	7.1	1,10	0.02
Female Mass	1.36	30.83	1,10	0.0002
Male Condition	0.78	8.81	1,10	0.01
Female Condition	1.36	8.02	1,10	0.02
New Capture Mass	1.70	10.77	1,10	0.008
New Condition	1.16	5.95	1,10	0.03
New Male Mass	1.29	5.82	1,10	0.03
New Male Condition	0.58	1.32	1,10	0.28
New Female Mass	2.35	5.39	1,10	0.04
New Female Condition	2.28	3.51	1,10	0.09

Figure 5. The average mass and body condition of adult and sub-adult animals following anthelmintic treatment on treated (closed circles) and not treated (open circles) grids. (A) Mass differences for all, male and female animals. (B) Body condition differences and (C, D) the mass and body condition of newly captured individuals. Removal of parasites resulted in increased mass and body condition and this was also observed in neighboring animals from treated areas. Error bars are 1 standard error of the grid means (n=6)
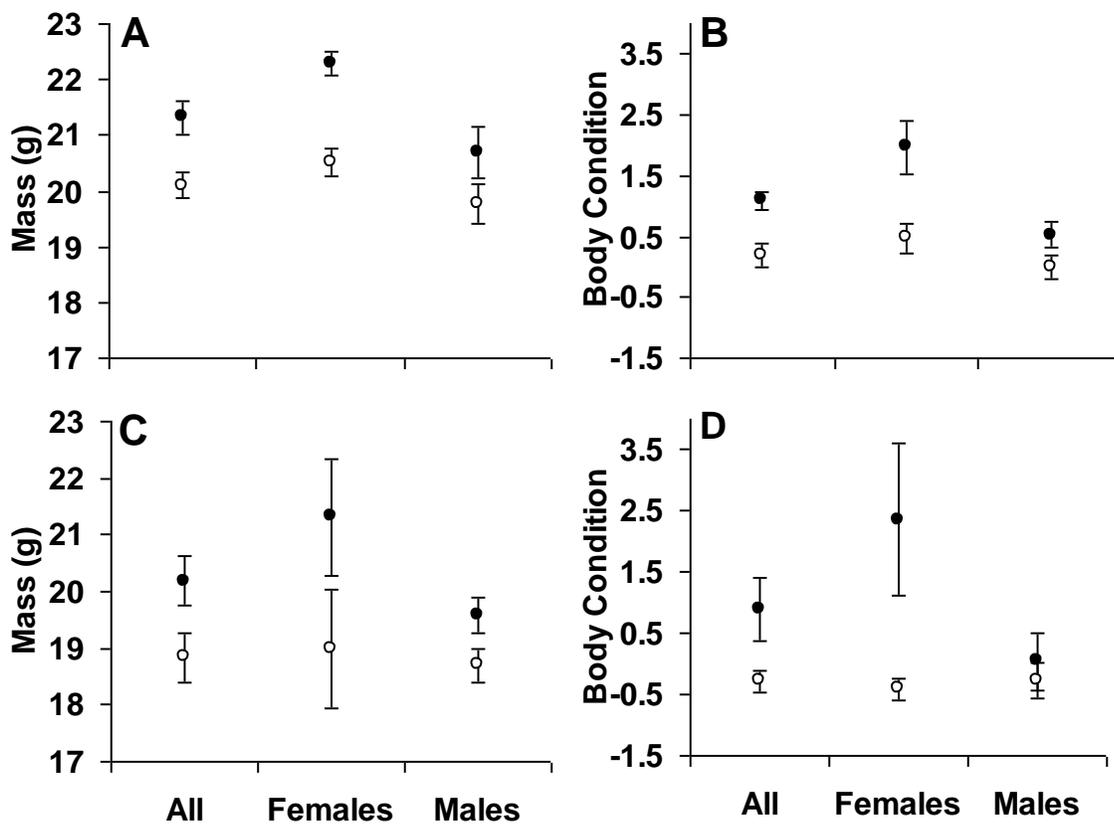
Figure 6. Experimental differences between grids in growth rate and breeding condition for anthelmintic treated resident mice (closed circle) and not treated controls (open circles). (A) The residuals of the growth rate vs. initial mass regression for all animals, females, and males. (B) The regression residuals for all juveniles, sub-adults, and adult animals. The proportion of (C) residents and (D) newly captured animals in breeding condition during the time period following anthelmintic treatment. Anthelmintic treatment significantly increased growth rate, especially in female and sub-adult animals. The treatment also significantly increases the proportion of animals in breeding condition but this pattern does not hold for not yet treated (newly captured) animals. Error bars are 1 standard error of the grid means (n=6).
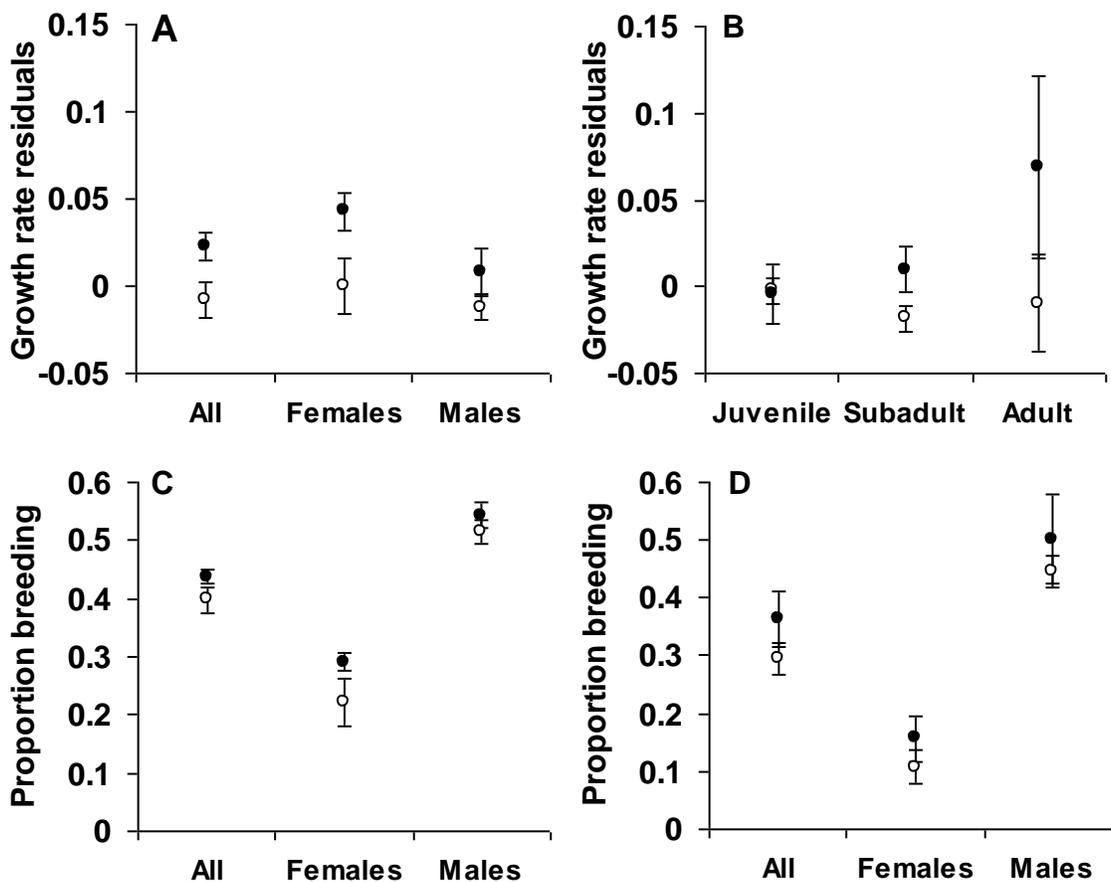
Figure 7. The proportion of resident animals breeding through time on treated (closed circles) and not treated (open circles) grids for (A) all animals (B) males and (C) females. Anthelmintic treatment increased the proportion of females breeding and reversed the mid summer breeding hiatus. Error bars are 1 standard error of the grid means (n=6) and the dotted vertical line represents the start of treatment.
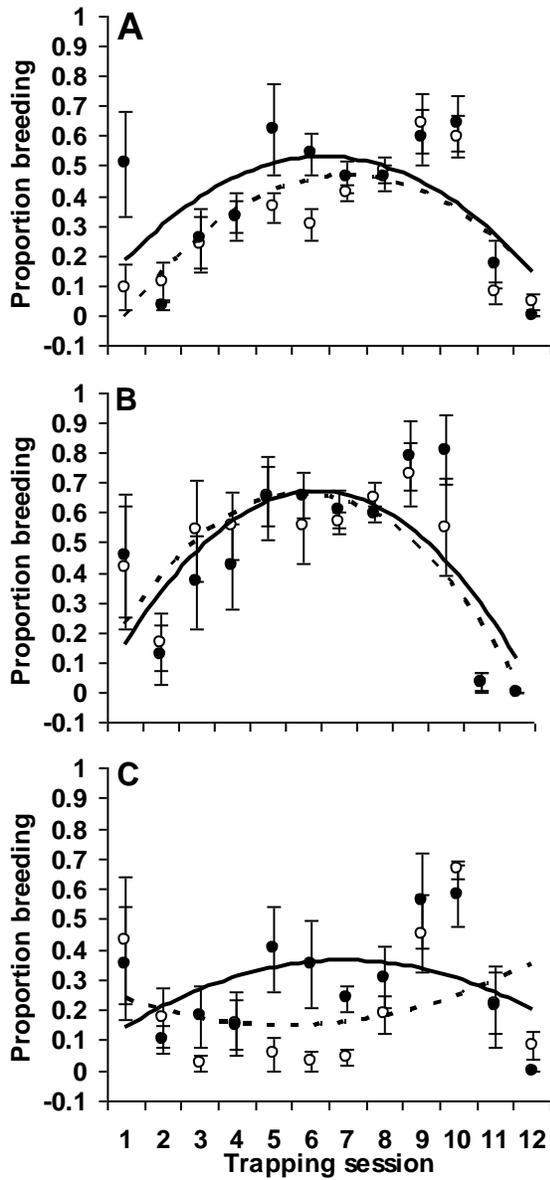
Figure 8. (A) Resident animal average days known alive for all animals, females, and males. (B) The proportion of time a resident individual was in breeding condition for all animals, females and, males. (C) The reproductive potential for all animals, females, and males, calculated as the number of days known alive times the proportion of time each resident individual was in breeding condition was significantly increased on treated grids for all animals (coef. = 35.94, F = 16.05, d.f. = [1,10], P = 0.002) and both sexes (male: coef. = 43.72, F = 5.45, d.f. = [1,10], P = 0.04; female: coef. = 25.49, F = 14.98, d.f. = [1,10], P = 0.003). Error bars are 1 standard error of the grid means (n=6).
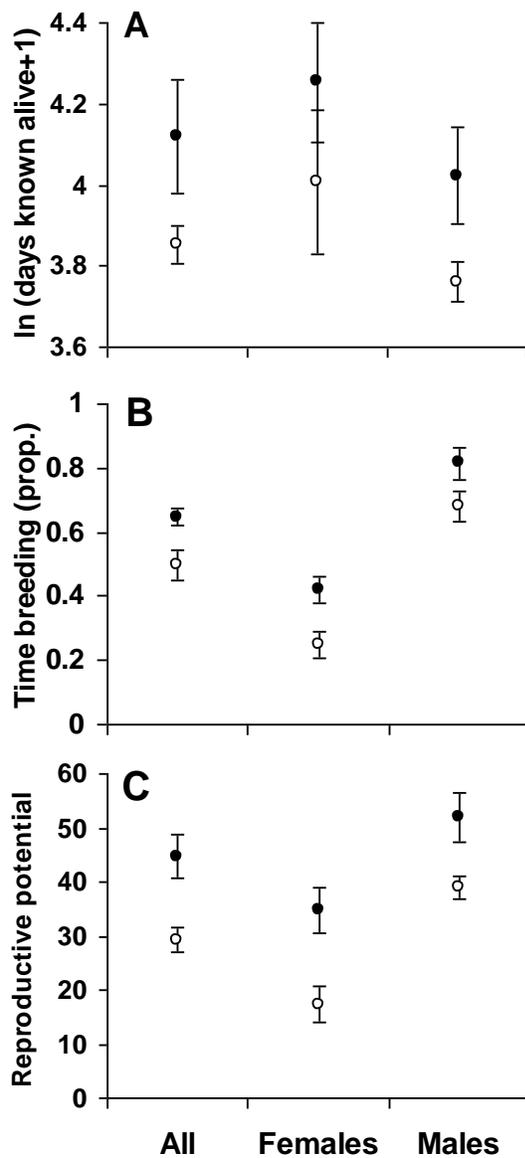
Chapter 2 References

Bendell, J.F. 1959. Food as a control of a population of white-footed mice, *Peromyscus leucopus* noveboracensis (Fisher). Canadian Journal of Zoology **37**:173-209.

Bentwich, Z. A., Kalinkovich, and Z. Weisman. 1995. Immune activation is a dominant factor in the pathogenesis of African AIDS. Immunology Today **16**: 187–19.

Brown, L. N. 1964. Reproduction of the brush mouse and white-footed mouse in the central United States. The American Midland Naturalist **72**:226-240.

Burt,W.H. 1940. Territorial behavior and populations of some small mammals in southern Michigan. Misc. Publication of the Museum of Zoology, University of Michigan, **45**: 1-58.

Cattadori, I.M., D.T. Haydon, and P.J. Hudson. 2005. Parasites and climate synchronize red grouse populations. Nature **433**: 737-741.

Cornish, L.M. and W.N. Bradshaw. 1978. Patterns in twelve reproductive parameters for the white-footed mouse (*Peromyscus leucopus*). Journal of Mammology **59** (4):731-739.

Dobson, A.P. and P.J. Hudson. 1992. Regulation and stability of a free-living host-parasite system, *Trichostrongylus tenuis* in red grouse. II. Population models. Journal on Animal Ecology **61**:487-500.

EIDAP Inc. of Sherwood Park, Alberta, Canada. http://www.eidap.com/.

Elias, S. P., J. W. Witham, and M. L. Hunter. 2004. *Peromyscus leucopus* abundance and acorn mast: population fluctuation patterns over 20 years. Journal of Mammal **85**:743-747.

Elkinton, J. S., W. M. Healy, J. P. Buonaccorsi, G.H. Boettner A.M. Hazzard, H. R. Smith, and A.M.Liebhold. 1996. Interactions among gypsy moths, white-footed mice, and acorns. Ecology **77**:2332–2342.

Falls, J.B., A.E. Falls and J.M. Fryxell 2007. Fluctuations of deer mice in Ontario in relation to seed crops. Ecological Monographs, **77**(1):19–32.

Fox, A.and P.J. Hudson. 2001. Parasites reduce territorial behavior in red grouse (*Lagopus lagopus scoticus*) Ecology Letters **4**(2):139–143.

Ferrari, N., I.M. Cattadori , J. Nespereira, A. Rizzoli, and P.J. Hudson. 2004. The role of host sex in parasite dynamics: field experiments on the yellow-necked mouse *Apodemus flavicollis*. Ecology Letters **7**(2): 88–94.

Glass, G.E., T.L.Yates, J.B. Fine, T.M. Shields, J.B. Kendall, A.G. Hope, C.A. Parmenter, C.J. Peters, T.G. Ksiazek, C.S. Li, J.A. Patz, and J.N. Mills. 2002. Satellite imagery characterizes local animal reservoir populations of Sin Nombre virus in the southwestern United States. Proceedings of the National Academy of Science USA **99**(26):16817-16822.

Gashwiler, J.S. 1979. Deer mouse reproduction and its relationship to the tree seed crop. American Midland Naturalist **102**(1):95-104.

Glazier, D. 1985. Energetics of litter size in five species of *Peromyscus* with generalizations for other mammals. Journal of Mammalogy **66**:629-642.

Grahnab SE Ekhaga, Marieholm 330 33 Hillerstorp Sweden

Hansen, L.P. and G. O. Batzli. 1979. Influence of supplemental food on local populations of *Peromyscus leucopus*. Journal of Mammalogy **60**(2):335-342.

Holmstad, P., Hudson, P.J. and A. Skorping. 2005. The influence of a parasite community on the dynamics of a host population: a longitudinal study on willow ptarmigan and their parasites. Oikos **111**:377-391.

Hudson, P.J. 1986. The effect of a parasitic nematode on the breeding production of red grouse. Journal of Animal Ecology **55** 85-94.

Hudson, P.J., A.P. Dobson, and D. Newborn. 1992. Do parasites make prey vulnerable to predation? Red grouse and parasites. Journal of Animal Ecology **61**:681-692.

Hudson, P.J., A.P. Dobson, and D. Newborn. 1999. Population cycles and parasitism. Science **282:** 2256-2258.

Hudson, P.J., A. P. Dobson, I.M. Cattadori, D. Newborn, D.T. Haydon, D.J. Shaw, T.G. Benton, and B.T. Grenfell. 2003. Trophic interactions and population growth rates: describing patterns and identifying mechanisms. In <u>Wildlife Population Growth Rates</u>. Edited by R.M.Sibly, J.Hone and T.H. Clutton Brock. pp 198-224. Cambridge University Press.

Lack, D. 1954. The Natural Regulation of Animal Numbers. Oxford University Press, Oxford.

Lello, J. B. Boag, A. Fenton, I. R. Stevenson, & P. J. Hudson. (2004). Competition and mutualism among the gut helminths of a mammalian host. Nature **428:** 840-844.

Jones, C.G., R.S. Ostfeld, M.P. Richard, E.M. Schauber, and J.O.Wolff. 1998. Chain reactions linking acorns to gypsy moth outbreaks and Lyme disease risk. Science **279**:1023-1026.

Kristan, D.M. 2002. Maternal and direct effects of the intestinal nematode *Heligmosomoides polygyrus* on offspring growth and susceptibility to infection. The Journal of Experimental Biology **205**:3967–3977.

Kissui, B.M. and C. Packer. 2004. Top-down population regulation of a top predator: Lions in the Ngorongoro Crater. Proceedings of the Royal Society London Series B **271**: 1867-1874.

May, R.M. and R.M. Anderson, 1978. Regulation and Stability of Host-Parasite Population Interactions: II. Destabilizing Processes. The Journal of Animal Ecology 47(1):1 249-267.

McCracken, K.E., J.W. Witham, M.L. Hunter, Jr. 1999. Relationships between seed fall of three tree species and *Peromyscus leucopus* and *Clethrionomys gapperi* during 10 years in an oak-pine forest. Journal of Mammalogy **80**(4):1288-1296.

McShea, W. J. 2000. The influence of acorn crops on annual variation in rodent and bird populations. Ecology **81**:228-238.

Millar, J.S. 1989. Reproduction and development. pp 169-232, in Advances in the study of *Peromyscus* (Rodentia) (G.L. Kirkland, Jr. and J. N. Layne, eds.) Texas Tech University Press, Lubbock, Texas, 367 pp.

Norris, K., Anwar M., and A.F. Read, 1994. Reproductive effort influences hematozoan parasites in great tits. Journal of Animal Ecology **63**:601-610.

Ostfeld R.S., C.G. Jones and J.O. Wolff. 1996. Of mice and mast. BioScience 46 (5):323-330.

Pollock, K.H., J. D. Nichols, C. Brownie, and J.E. Hines 1990. Statistical inference for capture-recapture experiments. Wildlife Monographs 107. 97 pp.

Pucek, Z., W. Jedrzejewski, B. Jedrzejewska and M. Pucek. 1993. Rodent population dynamics in a primeval deciduous forest (Bialowieza National Park) in relation to weather, seed crop, and predation. Acta Theriologica **38**: 199-232.

R Development Core Team 2005. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org

Raffel, T. R**.** 2006. Drivers of seasonal infection dynamics in the parasite community of red-spotted newts *(Notophthalmus viridescens).* Ph. D. Thesis. The Pennsylvania State University, University Park. 160 p.

Rintamaa, D.L., P.A. Mazur, and S.H. Vessey. 1976. Reproduction during two annual cycles in a population of *Peromyscus leucopus noveboracensis*. Journal of Mammology **57**:593-595.

Schering-Plough Corporation World Headquarters 2000 Galloping Hill Road Kenilworth, N.J. 07033-0530.

Shimadaa, T. and T. Saitoh 2006. Re-evaluation of the relationship between rodent populations and acorn masting: a review from the aspect of nutrients and defensive chemicals in acorns. Population Ecology *48*:341-352.

Smith, M. J., A.White, J. A. Sherratt, S. Telfer, M. Begon, and X. Lambin 2008. Disease effects on reproduction can cause population cycles in seasonal environments. Journal of Animal Ecology **77**(2): 378–389.

Smyth, M. 1966. Winter breeding in woodland mice, *Apodemus sylvaticus*, and voles, *Clethrionomys glareolus* and *Microtus agrestis*, near Oxford. Journal of Animal Ecology **35**:471–485.

Stien, A., R.J. Irvine, E. Ropstad, O. Halvorsen, R. Langvatn, and S.D. Albon 2002. The impact of gastrointestinal nematodes on wild reindeer: Experimental and cross-sectional studies. Journal of Animal Ecology **71**:937-945.

Telfer, S., M. Bennett, K. Bown, R. Cavanagh, L. Crespin, S. Hazel, T. Jones, and M. Begon. 2002. The effect of cowpox virus on survival in natural rodent populations: increases and decreases. Journal of Animal Ecology **71**:558-568.

Terman, C.R. 1998. Early-summer reproductive curtailment in wild white-footed mice and reproductive recovery in the laboratory. Journal of Mammalogy **79**(1):320-325.

Tompkins, D.M., A.P. Dobson, P. Arneberg, M.E. Begon, I.M. Cattadori, J.V. Greenman, J.A. Heesetrbeek, P.J. Hudson, D. Newborn, A. Pugliese, A.P. Rizzoli, R. Rosa, F. Rosso and K. Wilson. 2001. In: *Ecology of wildlife diseases*. Ed. by Hudson, P.J., A. Rizzoli, B.T. Grenfell, H. Heesterbeek, and A.P. Dobson. Oxford University Press.

Wolff, J.O. 1985a. Comparative population ecology of *Peromyscus leucopus* and *Peromyscus maniculatus*. Canadian Journal of Zoology **63**:1548-1555.

Wolff, J.O. and D.S. Durr. 1986. Winter nesting behavior of *Peromyscus leucopus* and *Peromyscus maniculatus*. Journal of Mammalogy **67**: 409-411.

Wolff, J.O. 1986. The effects of food on mid-summer breeding activity in *Peromyscus leucopus* in south-western Virginia. Canadian Journal of Zoology **64**:855-858.

Wolff, J.O. 1996. Population fluctuations of mast-eating rodents are correlated with production of acorns. Journal of Mammalogy **77**(3):850-856.

Yunger, J.A. 2002. Response of Two Low-Density Populations of *Peromyscus leucopus* to increased food availability. Journal of Mammalogy **83** (1):267-279.

Chapter 3

The gut macroparasites of Pennsylvania white-footed mice,

Peromyscus leucopus.

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ABSTRACT-

Peromyscus leucopus (the white-footed mouse) exhibits unstable dynamics and we examined the hypothesis that this instability is generated by parasitism with the nematode Pterygodermatities peromysci. Mathematical models predict that instability will be generated by chronic infections of parasites that reduce host fecundity when the distribution of parasites within the host population is close to random. We examined the patterns of infection of P. leucopus with P. peromysci from both intensive and extensive study sites in central Pennsylvania. There were 7 gastrointestinal worms infecting the mice and seasonal variation in prevalence of P. peromysci ranged from 12.3% to 60.1%. Prevalence in adult animals was 30.8% while it was 4.6% in juveniles. Males and females did not differ significantly in terms of prevalence. When we compared the log variance to log mean relationship of P. peromysci between sites, the slope was not significantly different from unity, indicating a random distribution of parasites within the host population. Generalized linear models found the likelihood of infection in adults was influenced by body mass, co-infections with other worms, and breeding condition. Likewise, the intensity of P. peromysci infection was also positively related to these co-infections and body mass. Syphacia peromysci infection status was related to co-infection with P. peromysci and was lower in females than males. In males, co-infection was the only significant parameter in the S. peromysci intensity model. However, in the female model, intensity increases with body length but decreases with mass and breeding animals have higher levels of infection than non-breeders. The results of our co-infection models provide a more clear explanation for the above pattern because they indicate the breeding condition is the only significant parameter for females and amongst breeding females, lactating animals have co-infections 7.3% of the time while others breeding but not lactating only harbor both parasites

1.6% of the time. *P. peromysci* exhibits an age intensity relationship that rises to a peak and turns over such that older individuals, of greater mass, have lower infections. As expected we found relationships with proxies of age (mass and length) and breeding and note the patterns of infection suggest the parasites may well have a significant impact upon female fecundity. We discuss the hypothesis that *P. peromysci* may play and important role in generating oscillations in mouse abundance because the worm exhibits a *Poisson* distribution and reduces breeding productivity of females during the summer months.

Introduction

White-footed mice in North America, including *Peromyscus maniculatus* and *P. leucopus* are known to be reservoirs of a range of zoonotic pathogens that spillover into humans. These include Hanta virus, arboviruses, Lymphocytic Choriomeningitis Virus, bacterial pathogens such as *Borrelia burgdofferi*, *Anaplasma phagocytophilum*, *Francisella tularensis*, and *Yersinia pestis*. Spillovers of these zoonotic infections are relatively rare but tend to occur when mice populations reach high densities and the likelihood of contact with humans is increased (Glass et al. 2002). Much of the literature on the dynamics of *P. leucopus* indicates that the eruptive dynamics of mouse populations are correlated with food availability in the form of acorn mast which results in greater over winter survival and subsequent breeding (Wolff 1986; Jones et al. 1998; Elias et al. 2004; Falls et al. 2007). However, there is now evidence that the parasitic helminth *Pterygodermatities peromysci* may play a role in destabilizing mice populations by decreasing body condition and breeding production in the summer months (Vandegrift et al. 2008) and by interacting with food during the winter months to reduce population growth rate (Pedersen & Grieves 2007). These observations indicate that parasites may play a key role in influencing the dynamics of mouse populations and in so doing, the spillover of other pathogens to humans. We investigated the population biology of *Pterygodermatities peromysci* and examined the interaction between *P. peromysci* and other component species of the parasite community.

Parasites can play an important role in shaping host population dynamics, particularly when parasite infections are chronic and decrease host body condition and breeding production (Hudson, 1986; Hudson et al. 1992; Norris et al. 1994; Stien et al. 2002). The models of Anderson & May (1978; May & Anderson 1978) identify the conditions necessary for a parasite

to regulate a host population and the conditions that will generate instability. In essence, the models predict that parasitic nematodes will regulate the host population if the growth rate of the parasite is faster than the growth rate of the host and instability occurs when the relative impact of the parasite on the breeding production of the mice is greater than the impact on host survival, relative to its distribution in the host population (Dobson & Hudson 1992). There is field evidence that the parasitic nematode *Trichostrongylus tenuis* in Red grouse (*Lagopus lagopus scoticus*) reduces fecundity, exhibits a low degree of aggregation and destabilizes the dynamics of grouse populations and so generates the observed instability (Hudson et al. 1992; Hudson et al. 1999). In this paper, we examined the population biology of the dominant helminth of mice, *P. peromysci,* with the objective of providing details on the parasite distribution within the host population and how this varies in time and space. *P. leucopus* is infected with a diverse community of parasites and so we also asked general questions about the population biology of these species and how they interact with *P. peromysci.*

Methods and Materials

Extensive Sampling

Mice were trapped from 2003 to 2007 using snap traps set out in 20 squares with three traps at each corner and each square separated by 50 meters, following the procedures described by Liukko (1990). In general, we set approximately 250 traps at each site and then checked traps for four days until we had generated 1000 trap nights for each site in both spring and fall until spring 2007. Traps were baited with peanut butter, set in the evening just before dark and subsequently checked the following dawn. If trap success was very low, we would extend the trapping period and set additional traps in an attempt to obtain a sample of 30 mice.

Intensive Sampling

Mice were caught and individually tagged in live capture multiple-capture Ugglan special #2 traps from June of 2003 through to August 2007 and we generated catch-mark-recapture data which were used to estimate population size and follow the fate of individuals. Traps were baited with sunflower seeds and potato and we provided hay as an insulated bedding material. Traps were set in the evening and checked for two or three consecutive days. In 2003, we had one large 17x14 grid containing 238 traps in 15 meter intervals and we monitored this grid weekly from June 5 to Nov. 11. We did not monitor this population over the winter months when snow and frosts prevented trapping. Trapping resumed on Apr 5th 2004 and occurred biweekly until Oct 1st. During 2004, we established three control grids which remained for the next three years and incidental mortalities on these grids were also dissected. All animal experiments were conducted with the approval of the Pennsylvania State University Animal Care Committee (IACUC #16061, "Transmission Dynamics of Directly Transmitted Diseases in Wildlife Reservoir Hosts").

Small mammal processing

On capture we recorded body length, tail length, body mass, sex, body condition, and breeding status. Body condition was estimated from the residuals of a cubic spline fit of mass vs. body length. Animals were considered in breeding condition if they had descended testes (males), or if they were lactating, pregnant, or had a perforate vagina (females).

Dissections

Mice trapped in the extensive study were pinned to a dissection tray and lightly sprayed with 70% ethyl alcohol, the body cavity was opened with scissors, and the gut (from just above the stomach to the anus) was placed in a water bath. The gut was cut into 5 cm portions and the tract opened longitudinally and carefully examined under a dissecting microscope and all

gastrointestinal worms removed, collected, and preserved. Worm identification to the level of Genus was accomplished with use of <u>The CIH Keys to the Nematode Parasites of Vertebrates</u> (Anderson et al. 1974). Identification to the species level was done with Lichtenfels (1970). The species, sex, and location within the tract of each of the worms was recorded and each gut was checked twice.

Statistics

Statistical analyses were carried out in R (www.r-project.org). Generalized linear models (GLM) with binomial errors were used to select the best model parameters for the parasite infection and co-infection status (co-infection status is if the individual has both worms or not). Model parameters included the categorical variables; year, month, sex, field site, juvenile status (whether mass less than 16 grams or not) breeding condition (lactation, perforate vagina, pregnancy, descended testes), and each other parasite (absence or presence). The numerical variables were body mass and body length. The function GLM.nb (for negative binomially distributed data) was used to select the best models for parasite intensity data. We used negative binomial errors for the intensity models because when we aggregate all the data from different times and sites the resultant distribution is negative binomial. The best predictors of each response variable were selected using backwards stepwise selection, retaining variables with $P < 0.05$ based on F-statistics. Age-specific effects were tested by separating animals into mass classes such that juveniles were defined as less than 16g. We also created age prevalence and age intensity curves with estimates of age based on five mass classes of; less than 16g, 16-18g, 18.1-21g, 21.1-23g, and greater 23g. We then used GLMs to test if the relationships for each of the four most prevalent nematodes were type 1 (linear), type 2 (asymptotic), or type 3 (convex). Binomial errors were used for the age prevalence model while negative binomial errors were

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used for the age intensity (log transformed) analysis. Model comparisons were made using AIC values.

To examine the distribution of the parasites with the host populations, we calculated the variance to mean ratio for each of 17 sample populations obtained during the extensive sampling and examined Taylor's Power Law (Taylor 1961) by plotting log variance against log mean. We also used a linear model to obtain confidence intervals for the slope of the relationship between log variance and log mean *P. peromysci* per host.

Results

We identified seven species of gastrointestinal helminths infecting *P. leucopus*: four nematode species: *P. peromysci* (Lichtenfels), *Mastophorus muris* (Gmelin), *Capillaria americana* (Read), *S. peromysci* (Harkema), and *Heligmosomoides vandegrifti* (Durette-Desset & Kinsella); one cestode species, *Hymenolepis s. str.* and one trematode species, *Brachylaima peromysci*, (Reynolds). Overall prevalence of *P. peromysci* was 22.7% (n=866. s.e.=0.01) but varied seasonally from a low of 12.3% in November (n=130, s.e.=0.009) to 36.0% in July (n=38, s.e.=0.38) and by site from 0% (n=25 combined 5 sites, s.e.=0) to 60.7% (n=28 s.e.=0.04). The prevalence among juveniles (animals less than 16g) was only 4.6% (n=241, s.e.=0.008) and 30.0% in adults (n=614, s.e.=0.003); adult male prevalence was 32% (n=354, s.e.=0.01) and 27% in females (n=259, s.e.=0.01). The prevalence of *P. peromysci* was 30.8% (n=315, s.e.=0.01) in adult animals that were in breeding condition, while in non-breeding adults it was 28.8% (n=293, s.e.=0.01; Table 3). Amongst the extensively sampled animals (n=644, s.e.=0.006), *P. peromysci* prevalence was 18.3% (n=644, s.e.=0.006), significantly less than those animals captured during the intensive sampling 34.9% (n=233, s.e.=0.01; (coef.=0.87, z=5.05, p<0.0001). We also found

that amongst intensively trapped animals, the *P. peromysci* prevalence of those found dead in trap was lower than those caught live and euthanized (coef.=0.80, z=2.12, p=0.03)).

P. peromysci distribution among the host population

We examined the distribution of *P. peromysci* within the adult mouse population sampled during the extensive trapping and found that within each site and in each year, the variance to mean ratio was equal to or less than unity in 10 out of 17 cases and ranged from 0.56 to 8 with a mean of 1.52 ± 0.4. The single distribution with a high variance to mean ratio was generated by one heavily infected individual, and when this outlier was ignored the range fell to 0.56-1.98 and the mean to 1.12 ±0.1 (Table 4). We examined this further using Taylor's Power law and found the linear model, forced through the origin had a slope not significantly different from unity (m=.94; 95% C.I. [0.77-1.11]; Fig 9a&b). We also calculated k of the negative binomial for each distribution and found this distribution only passed the goodness of fit test for only 4 of the 17 distributions (one being the outlier; k=0.05) and k ranged from 0.05 to 3.1 in these populations (Table 4). However, when we combine the data from different sites and years, the distribution is aggregated (k=0.69) and the negative binomial provides a better fit (AIC=553.55) than the *Poisson* (AIC=581.18) distribution. In subsequent analyses of intensity we use negative binomial GLMs.

Infection status and the intensity of P. peromysci

The minimal GLM with binomial errors that explained whether a mouse was infected with *P. peromysci* or not (defined as infection status) identified the likelihood increased significantly when the animals were co-infected with either *S. peromysci* (coef. =1.33, z=5.57, p<0.0001) and *B. peromysci* (coef. =1.21, z=2.71, p=0.007), and also increased with body length (coef. =0.04, z=2.87, p=0.004). Juvenile status was negatively related to likelihood of infection

(coef. =-1.56, z=-4.21, p<0.0001). Since the importance of age is reflected in both body length and the binomial juvenile term, we examined adults and juveniles separately. Juvenile infection status was positively related to breeding condition (coef. =1.60, z=2.41, p=0.02) such that breeding juveniles were more likely to be infected 14.9% (n=27, s.e.=0.02) than non-breeders 3.4% (n=206, s.e. 0.002). This would indicate animals get infected when they breed, but the reproductive juveniles also had higher mass on average than the non-breeders (coef. =-0.96, z=-2.74, p=0.006), and so we cannot rule out increased exposure. In the model with adults, the likelihood of *P. peromysci* infection increased with significantly with four parameters including, mass (coef. =0.09, z=3.32, p=0.0009), pregnancy (coef. =-0.96, z=-2.74, p=0.006), and co-infections with both *S. peromysci* (coef. =1.38, z=5.51, p<0.0001) and *B. peromysci* (coef. =1.09, z=2.28, p=0.02). The negative association with pregnancy indicates pregnant adult females 22.4% (n=67, s.e.=0.02) were less likely to be infected than non-pregnant adult females 28.7% (n=187 s.e.=0.01). When we removed pregnant animals from the data set, mass (coef. =0.11, z=3.64, p=0.0003), *S. peromysci* (coef. =1.32, z=4.95, p<0.0001), and *B. peromysci* (coef. =1.17, z=2.27, p=0.02) remained significant.

   P. peromysci intensity (including zero counts) increased with the presence of *S. peromysci* (coef. =0.91, z=4.65, p<0.0001) and *B. peromysci* (coef. =1.24, z=3.76, p=0.0002), body length (coef. =0.04, z=3.16, p=0.002) and mass (coef. =0.07, z=3.76, p=0.0002). When we analyzed the age and breeding cohorts separately, the intensity in juveniles was positively associated with breeding condition (coef. =1.94, z=2.00, p=0.04) as with likelihood of infection. Intensity in adults increased positively with mass (coef. =0.09, z=4.85, p<0.0001) and co-infections with *B. peromysci* (coef. =1.07, z=3.30, p<0.0001) and *S. peromysci* (coef. =0.93, z=4.97, p<0.0001).

Infection status and the intensity of S. peromysci infection

Mice were more likely to be infected with *S. peromysci* when they are also infected with *P. peromysci* (coef. =1.28, z=5.53, p<0.0001) and they are less likely to be infected with *S. peromysci* in the month of October (coef. =-2.13, z=-2.75, p=0.006). Since the prevalence of *S. peromysci* in adult mice (13.7%; n=614; 0.005) is double that of juveniles (6.3%; n=241; s.e.=0.004), we excluded juveniles and looked at likelihood in adults. Adult mice were more likely to be infected with *S. peromysci* when *P. peromysci* was present (coef.=1.39, z=5.79, p<0.0001).

S. *peromysci* intensity was significantly lower in females than males (coef. =-1.02, z=-2.29, p=0.02) and increased with body mass (coef. =1.19, z=2.26, p=0.02), probably as a function of age. Interestingly, when we examine the sexes independently we find *P. peromysci* infection status was the only significant parameter associated with increased *S. peromysci* infection of males (coef. =0.60, z=2.73, p=0.006), while in females, mass (coef. =-0.35, z=-3.35, p=0.0008) has a negative effect while body length had a significant positive effect (coef. =0.17, z=2.92, p=0.003). We again divided females into juveniles and adults. In the adult model, intensity again increased with body length (coef. =0.17, z=2.72, p=0.006) and fell with mass (coef. =-0.67, z=-4.65, p<0.0001). Intensity was also higher in breeding females (coef. =2.41, z=2.65, p=0.008) while in non-breeding adult females the intensity falls with mass (coef. =-0.35, z=-2.86, p=0.004). These results imply that non-breeding females in good condition have low parasite intensities but the intensity of infection increases once they are breeding.

Co-infection between P. peromysci and S. peromysci

Since both *S. peromysci* and *P. peromysci* predict the infection status of the other, we investigated this co-infection association further. The minimal model for co-infection status

(infected with both worms or not) indicated body length (coef. =0.07, z=3.32, p=0.0009) and lactation (coef. =1.94, z=3.29, p=0.001) had significant positive effects, while sex (coef. =-3.09, z=3.34, p=0.0008) and the months of October (coef. =-3.38, z=-2.42, p=0.02) and November (coef. =-3.09, z=-2.56, p=0.01) all had a negative influence. In the male model, increased body length led to increased likelihood of the co-infection (coef. =0.07, z=-3.27, p=0.003) and the months of May (coef. =-1.94, z=-1.60, p=0.02), June (coef. =-2.59, z=-2.10, p=0.03), October (coef. =-2.25, z=-2.24, p=0.02), and November (coef. =-3.10, z=-2.53, p=0.01) all were significant negative parameters, as they represent low points in the seasonality of these co-infections. In females, breeding condition (coef. =1.54, z=2.57, p=0.01) was the only significant parameter with 7.3% (n= 139, s.e.=0.006) of reproductive females infected by both parasites while only 1.6% (n= 243, s.e.=0.001) of non-breeders were infected. Amongst non-breeding females there were no significant parameters in the model although in breeding females, lactation proved to be the important breeding criteria and was the sole positive significant parameter (coef. =2.24, z=2.75, p=0.0006) in the model. Of lactating females, 17.4% (n= 47, s.e.=0.02) had co-infections while those not lactating had co-infections only 1.8% (n= 335, s.e.=0.001) of the time. So in males the co-infection appears seasonal and is related to age whereas in females it is to be associated with breeding and specifically lactation.

Host body mass and body condition in relation to parasitism

We examined how body mass and body condition (as estimated from the residuals of a cubic spline fit of mass vs. body length were related to parasitism. In the mass model, the GLM identified a positive relationship with body length (coef.=0.20, t=13.73, p<0.0001) and breeding condition (coef.=1.86, t=8.13, p<0.0001) and negative relationship with juvenile status (coef.=-5.27, t=-16.93, p<0.0001). Juvenile body mass fell with *S. peromysci* presence (coef.=-1.22, t=-

2.27, p=0.02) but had a positive relationship with body length (coef.=0.14, t=8.08, p<0.0001) and) breeding condition (coef.=1.49, t=3.60, p=0.0004) while . Non-reproductive juvenile mass was negatively related to *S. peromysci* (coef.=-1.24, t=-2.01, p=0.04) infection status and positively related to body length (coef.=0.16, t=7.83, p<0.0001). Juvenile body mass amongst breeding juveniles exhibited had an interaction between body length and *P. peromysci* infection status with a negative coefficient (coef.=-0.34, t=-3.77, p=0.001). The prevalence of both *S. peromysci* and *P. peromysci* was higher in breeding juveniles (15%, 11%) than in non-breeding juveniles 11% and 6%, respectively; but this difference was only significant for *P. peromysci* (coef.=1.60, z=2.41, p=0.02).

Mass in adult mice increased with *P. peromysci* infection status (coef.=1.47, t=2.56, p=0.01) but interacted with sex so we examined sexes independently. Adult male mass was greater amongst mice with descended testes (coef.=1.39, t=4.07, p<0.0001) and with *P. peromysci* infections (coef.=1.09, t=2.97, p=0.003). Adult female body mass fell with *S. peromysci* infection status (coef.=-1.23, t=-2.06, p=0.04) and was positively related to breeding condition (coef.=3.02, t=7.09, p<0.0001). Mass of breeding males increased with *P. peromysci* infection status (coef.=1.47, t=2.82, p=0.005). In females, the mass of breeders fell with *S. peromysci* status (coef.=-1.92, t=-2.11, p=0.04).

The GLM results for body condition were much less complex because sex, breeding condition, and juvenile status fell out of the model and *P. peromysci* infection status (coef. =-.006, t=1.92, p=0.04) was the only significant parameter and had a negative impact on body condition.

In sum, infected animals were larger and this is probably associated with age and exposure history; however, it is interesting that for both *S. peromysci* and *P. peromysci* this

pattern is reversed when we examined body condition and this suggests the parasite may be reducing body condition (Fig. 10F). It is noteworthy that parasite infection status remains significant in the models of breeding animals as opposed to non-breeders. Juveniles as we have defined here (<16g) and not typically found to be in breeding condition (27 of 235), but when they are, their prevalence (15%) is 5x higher than non-breeding juveniles (3%). Average mass of these juvenile breeders was significantly higher then the non-breeders (coef.=0.37, z=3.05, p=0.002) indicating again that even when we separate animals into mass classes there is still an age effect.

Age prevalence and intensity curves

The relationship between age and prevalence of infection with *P. peromysci* rose to a maximum in middle aged animals and then fell in older animals (Figure 11A) while the age intensity best fit model indicated a slight turn over (age + age^2 model AIC=1302.3) this was not significantly different from a basic model of a simple rise to an asymptote (Figure 12A) as expected for a parasite with constant mortality and no acquired immunity (Hudson & Dobson 1995, Hudson et al 2006). The age intensity curves for *P. peromysci* from each of the four extensive trapping show a similar pattern (Figure 13).

The age prevalence curves for the other three parasites (*S. peromysci*, *B. peromysci* and *H. vandegrifti)* were not clear because the AICs for the different models were indistinguishable and so we assume that these were simple curves rose to an asymptote (Figure 11B-D; Table 5). *P. peromysci* exhibited an increase in prevalence to a peak in July followed by a decline (Fig. 14a) and this could also be related to the decrease in prevalence in older individuals (Fig. 11A).

Discussion

We found that *P. peromysci* exhibited a frequency distribution not different from random in the host population. Indeed examination of Taylor's power law where we plotted log variance against mean identified a slope of almost 1 and unlike the majority of parasite populations *P. peromysci* exhibited a random distribution (Shaw & Dobson 1995). We know that the mean of infection changes over time within a population and between populations, so we took particular care not to sum distributions from different times or places and so inadvertently generating an aggregated distribution (Wilson et al. 2001). This random distribution coupled with the parasite induced reduction in fecundity observed in this system (Vandegrift et al. 2008) leads us to expect instability in the system, unless there are other strong density dependent effects, such as in worm establishment or survival which we have yet to identify (May & Anderson 1978). These findings do provide sufficient evidence to suppose that parasites, perhaps interacting with food conditions (Pedersen & Grieves 2008) may play a significant role in generating the unstable dynamics observed in *P. leucopus* (Wolff 1986).

The finding that mass and body length played important roles in explaining the variance in infection status and intensity for both *P. peromysci* and *S. peromysci* was expected because these variables were both correlated with age are thus likely to be related to exposure. However, we tried to address these effects by removing juveniles from the analyses and by examining the age-intensity and age-prevalence data. An interesting finding in the infection status and intensity models was the importance of co-infections. In the co-infection model for reproductive females, lactation was the only significant parameter and this provides further (although only correlative) evidence that the worms impact female breeding. It will be a priority in the future to identify how the parasites interact with each other because there is accumulating evidence that there are significant competitive interactions between parasites and with pathogens that can be either

direct or host mediated (e.g. cross immunity and immunosuppression) and these may alter the dynamics of each individual parasite (Cattadori et al. 2005; Holmstad et al. 2004; Lello et al. 2004).

The type three convex shapes of the *P. peromysci* age prevalence curve can be generated by a number of mechanisms and they can be difficult to disentangle. Frailty of the dataset due to inadequate sampling of the older age classes will lead to a type three relationship in the age intensity curve but not the age prevalence curve, in particular when the frequency distribution of the parasite is aggregated. In this case the distribution is close to random and it would seem unlikely that this could explain the turn over in the curve. A second explanation is an age related variation in exposure or susceptibility to infective stages. We have no evidence to refute this, although the results from co-infection imply that susceptibility does not change with age. The transmission of *P. peromysci* involves ingestion of the intermediate host, the camel cricket (*Ceuthophilus* spp., Scudder); there may well be seasonal variation in the availability of these intermediate hosts that would be reflected as age exposure. The convex age prevalence curve could also be generated by parasite induced mortality, and this maybe a possible explanation given that there is evidence that males may suffer reduced survival from infection (Vandegrift et al. 2008). Finally, the turn over could be a signal of acquired immunity although this would appear unlikely in this instance since the parasite distribution is random. The random distribution of parasites within the host population implies there is likely some density dependent constraint in the system. The alternative is that there no variation between hosts in their susceptibility and any acquired immunity would generate variation in susceptibility and lead to an aggregated distribution.

There are many problems associated with collecting parasite data including, but not limited to, biased sampling, appropriate sample sizes, obtaining representative samples from each age class, and accounting for seasonal and spatial variation. We strived to satisfy these criteria but fell short in at least two ways. First, low density of mice in the fall and spring sometimes made it difficult to obtain our target sample size of 30 individuals, and the effective sample size was decreased further when we parsed the data into cohorts of animals for analysis (e.g. males and females). Second, we collected only detailed seasonal data from one year and never during the winter months and there could be important changes in parasite dynamics during these time periods. Nevertheless these longitudinal and vertical samplings of parasites have provided us an initial insight into various aspects of the population biology of *P. peromysci* and will provide the foundation to produce some basic models that could help explain the parasite-host dynamics.

The final dynamics of parasite and host are determined by the relative growth rates of host and parasite, the impact the parasite has on its host, and the tensions between the stabilizing and destabilizing processes as explored in the models by Anderson & May (1978) and Dobson & Hudson (1992). Both the age intensity relationship and the frequency distribution are little more than an integral of the underlying parasite-host interactions (virulence, transmission, susceptibility) but they do provide a signal of which processes are dominating within the system. The frequency distribution being close to random, coupled with parasite induced reduction in host fecundity (Vandegrift et al. 2008) provide good evidence to suppose that these parasites may well play a role in destabilizing the host-parasite system. The distribution also implies that there may be little variation in susceptibility and exposure to infection but this study has focused on the interaction within the host and we have ignored the dynamics in the intermediate host.

The seasonal behavior of the intermediate host coupled with the patterns of infection and transmission could well have a significant influence on the system. An interesting feature of the system is that the female worms are very large (up to 38mm) and the worms can carry large numbers of eggs *in utero* (up to 237,000) implying a highly fecund parasite but one that has the energy resources to grow to a large size rapidly before the host is likely to die. A feature that we suspect is uncommon amongst rodents. For the future, we plan to examine the dynamics in the intermediate host and to construct a model to determine under what conditions *P. peromysci* might influence *P. leucopus* dynamics.

The study of the how and why animal populations fluctuate has been a central question in ecological literature for decades yet only recently have we pursued these questions rigorous empiricism via large scale replicated population level manipulations. If these types of experiments allow us to parameterize models that can predict future dynamics of rodent populations then we may be able apply this knowledge with a population intervention strategy. Moreover, if these parasites turn out to be important drivers of the dynamics then may be able to use the worms as a form of bio-control, of course using our model predictions to target and develop our intervention strategies. In the event that an emergent or pre-existing rodent borne pathogen arises and becomes a threat to human health, this could be particularly useful information. One example of a rodent associated pathogen as discussed above is the bubonic plague and it killed between one-half and one-third of the European population in a span of five years (Keeling & Gilligan, 2000).

References

Anderson, R.C., A.G. Chabaud, and S. Willmott. 1974. <u>CIH Keys to the Nematode Parasites of Vertebrates.</u> Commonwealth Agricultural Bureaux, Central Sales Branch, Farnham Royal, Slough SL2 3BN, England. 8vo, iv + 17 to 58 pp.

Anderson, R.M., and R.M. May. 1978. Population biology of infectious disease. Part I. Nature 280:361-367

Cattadori, I.M., D.T. Haydon, and P.J. Hudson. 2005. Parasites and climate synchronize red grouse populations. Nature **433**: 737-741.

Dobson, A.P. and P.J. Hudson. 1992. Regulation and stability of a free-living host-parasite system, *Trichostrongylus tenuis* in red grouse. II. Population models. Journal on Animal Ecology **61**:487-500.

Elias, S. P., J. W. Witham, and M. L. Hunter. 2004. *Peromyscus leucopus* abundance and acorn mast: population fluctuation patterns over 20 years. Journal of Mammal **85**:743-747.

Falls, J.B., A.E. Falls and J.M. Fryxell 2007. Fluctuations of deer mice in Ontario in relation to seed crops. Ecological Monographs, **77**(1):19–32.

Glass, G.E., T.L.Yates, J.B. Fine, T.M. Shields, J.B. Kendall, A.G. Hope, C.A. Parmenter, C.J. Peters, T.G. Ksiazek, C.S. Li, J.A. Patz, and J.N. Mills. 2002. Satellite imagery characterizes local animal reservoir populations of Sin Nombre virus in the southwestern United States. Proceedings of the National Academy of Science USA **99**(26):16817-16822.

Holmstad, P., Hudson, P.J. and A. Skorping. 2005. The influence of a parasite community on the dynamics of a host population: a longitudinal study on willow ptarmigan and their parasites. Oikos **111**:377-391.

Hudson, P.J. 1986. The effect of a parasitic nematode on the breeding production of red grouse. Journal of Animal Ecology **55** 85-94.

Hudson, P.J., A.P. Dobson, and D. Newborn. 1992. Do parasites make prey vulnerable to predation? Red grouse and parasites. Journal of Animal Ecology **61**:681-692.

Hudson, P.J., A.P. Dobson, and D. Newborn. 1999. Population cycles and parasitism. Science **282:** 2256-2258.

Lichtenfels, J.R., 1970. Two new species of Pterygodermatities (Paucipectines) Quentin, 1969 (Nematoda: Rictulariidae) with a key to the species from North American rodents. Proceedings of the North American Helminthological Society 30(1):94-101.

Liukko U.M. 1990. <u>Pikkunisakasharrastajan</u> <u>opas</u>. Tammi, Helsinki. 111 pp.

Lello, J. B. Boag, A. Fenton, I. R. Stevenson, & P. J. Hudson. (2004). Competition and mutualism among the gut helminths of a mammalian host. Nature **428:** 840-844.

Jones, C.G., R.S. Ostfeld, M.P. Richard, E.M. Schauber, and J.O.Wolff. 1998. Chain reactions linking acorns to gypsy moth outbreaks and Lyme disease risk. Science **279**:1023-1026.

Keeling, M.J. and C. A. Gilligan, 2000. Bubonic Plague: A Metapopulation Model of a Zoonosis. Proceedings of the Royal Society Series B Biological Sciences 267 (1458):2219-2230.

May, R.M. and R.M. Anderson, 1978. Regulation and Stability of Host-Parasite Population Interactions: II. Destabilizing Processes. The Journal of Animal Ecology 47(1):1 249-267.

Norris, K., Anwar M., and A.F. Read, 1994. Reproductive effort influences hematozoan parasites in great tits. Journal of Animal Ecology **63**:601-610.

Pedersen A.B. and T.J. Grieves 2007. The interaction of parasites and resources can cause population crashes in a wild mouse population. Journal of Animal Ecology 77(2):370-377.

R Development Core Team 2005. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org

Shaw, D.J, and A.P. Dobson 1995. Patterns of macroparasite abundance and aggregation in wildlife populations: a quantitative review. Parasitology 111: S111-S133

Stien, A., R.J. Irvine, E. Ropstad, O. Halvorsen, R. Langvatn, and S.D. Albon 2002. The impact of gastrointestinal nematodes on wild reindeer: Experimental and cross-sectional studies. Journal of Animal Ecology **71**:937-945.

Taylor, L.R. 1961. Aggregation, variance and the mean. Nature **189**: 732-735.

Tompkins, D.M., A.P. Dobson, P. Arneberg, M.E. Begon, I.M. Cattadori, J.V. Greenman, J.A. Heesetrbeek, P.J. Hudson, D. Newborn, A. Pugliese, A.P. Rizzoli, R. Rosa, F. Rosso and K. Wilson. 2001. In: *Ecology of wildlife diseases*. Ed. by Hudson, P.J., A. Rizzoli, B.T. Grenfell, H. Heesterbeek, and A.P. Dobson. Oxford University Press.

Vandegrift, K.J. T.R. Raffel, and P.J. Hudson. 2008. Parasites prevent summer breeding in white-footed mice, *Peromyscus leucopus*. Ecology (in press)

Wolff, J.O. 1996. Population fluctuations of mast-eating rodents are correlated with production of acorns. Journal of Mammalogy **77**(3):850-856.

Figure 9. The relationship between log variance and log mean *P. peromysci* intensity for adult animals on the four extensive trapping sites for each year from 2003 to 2007. (A) The dotted line in the top graph represents the 1:1 relationship where the variance equals the mean, as expected for a random distribution and the solid line is the fitted regression line weighted for sample size. (B) year to year changes in the variance to mean ratio between sites (mean = 1.55 st. err. = 0.41). One site (DP) had a variance to mean ratio of 8 in 2007, when this outlier was removed the mean= 1.15 $\pm$ 0.1. The dotted line in the bottom panel also represents a 1:1 variance to mean ratio.
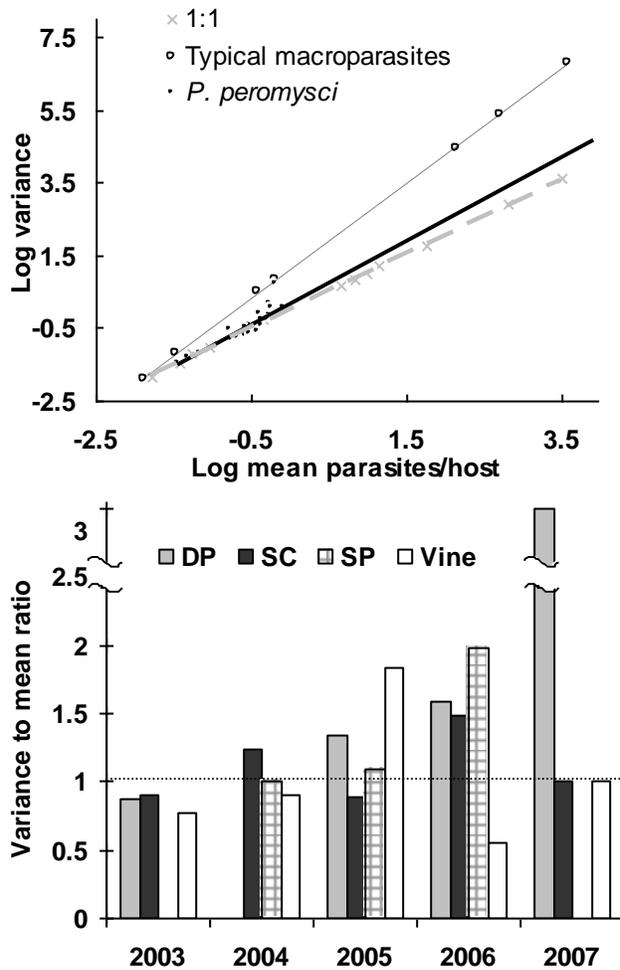
Figure 10. The mass, body length, and body condition of *P. leucopus* infected and not infected with *P. peromysci* (A,C,E) and *S. peromysci*. (B,D,F) Error bars are one standard error of the mean.
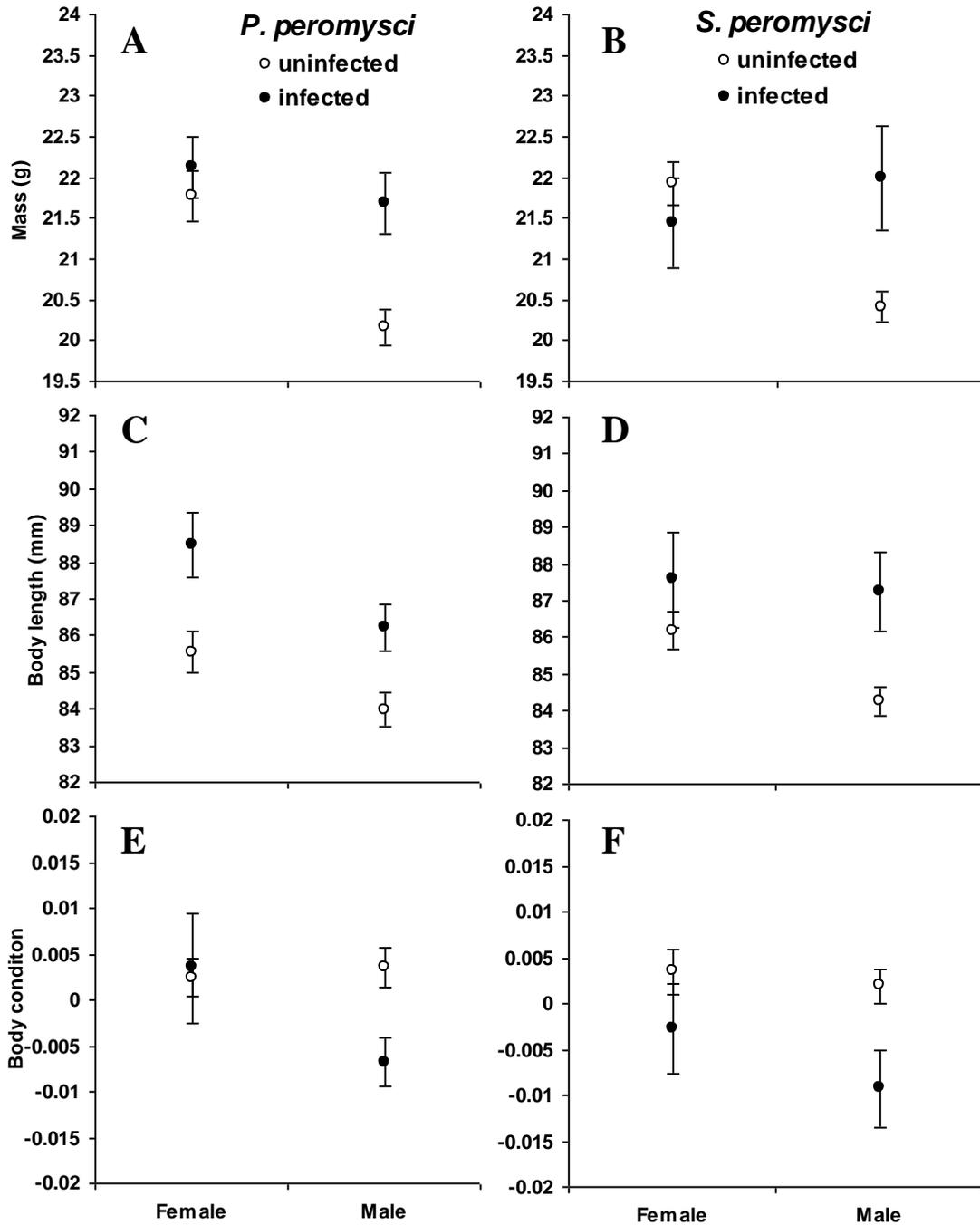
Figure 11. Age prevalence curves for the four most common nematodes infecting *P. leucopus.* Mice were categorized into mass classes: 1 (4-15.9g), 2(16-18.9g), 3(19-21g), 4(21.1-23g), 5(23.1 and up). The line is a second order polynomial spline curve weighted by sample size. Error bars are one standard error of the mean.
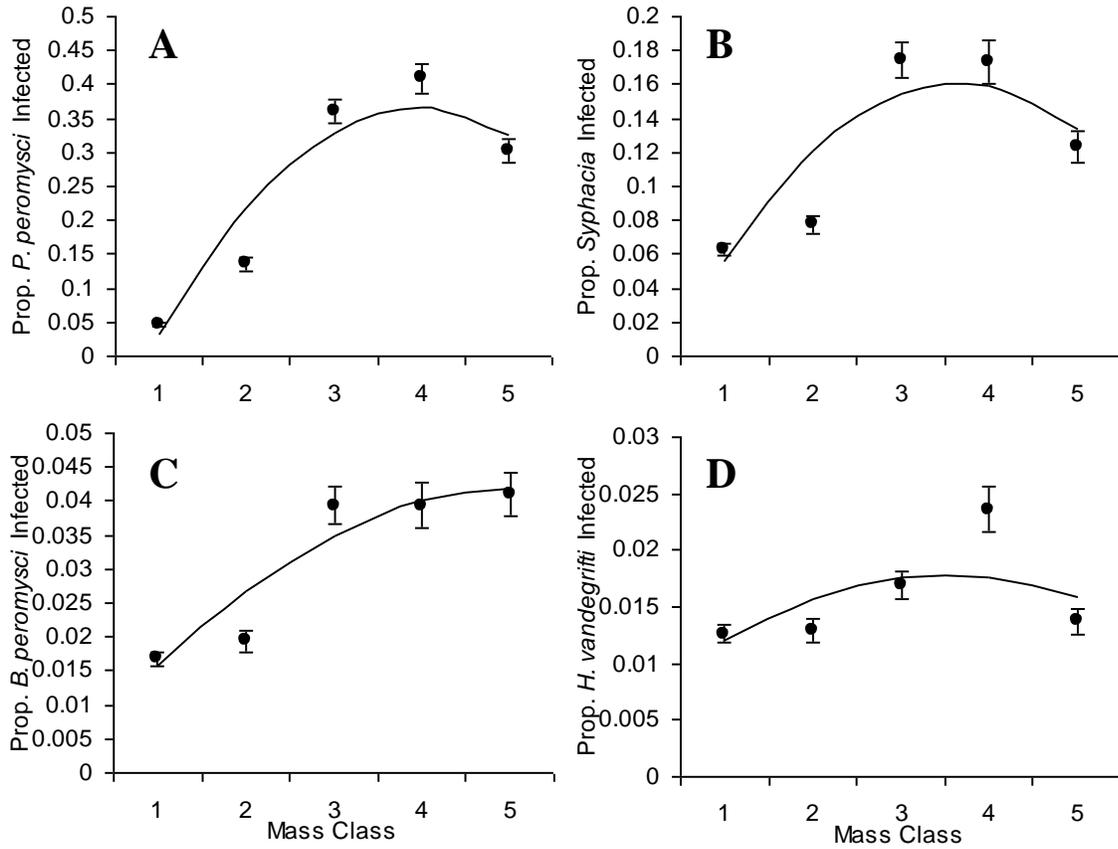
Figure 12. Age intensity curves for the four most common nematodes infecting *P. leucopus.* (A) *P. peromysci*, (B) *S. peromysci*, (C) *B. peromysci*, and (D*) H. Vandegrifti.* Mass classes are: 1 (4-15.9g), 2(16-18.9g), 3(19-21g), 4(21.1-23g), 5(23.1 and up). The fitted line is a second order polynomial spline curve weighted by sample size. Error bars are one standard error of the mean.
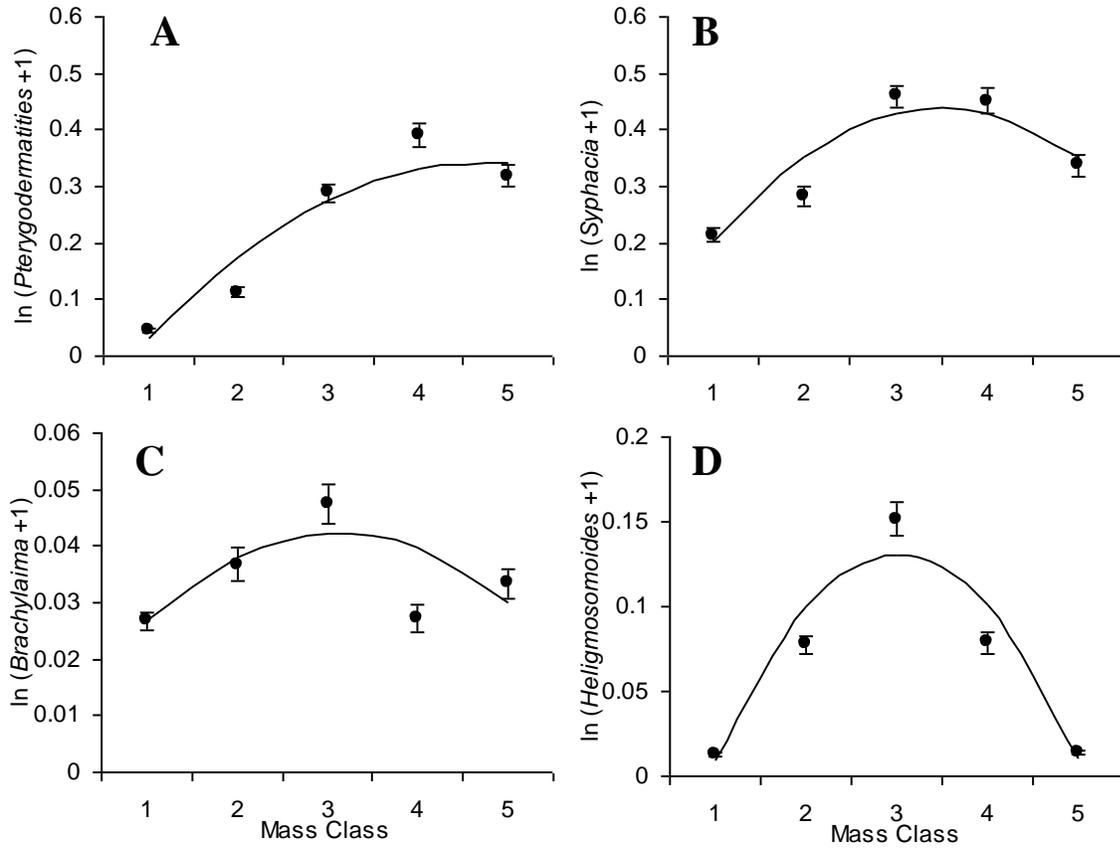
Figure 13. Age intensity curves for *P. peromysci* for both males and females from four extensive sampling sites where autumn and springtime sampling occurred yearly from 2003 to 2006. The line is a second order polynomial spline curve weighted by sample size. Error bars are one standard error of the mean.
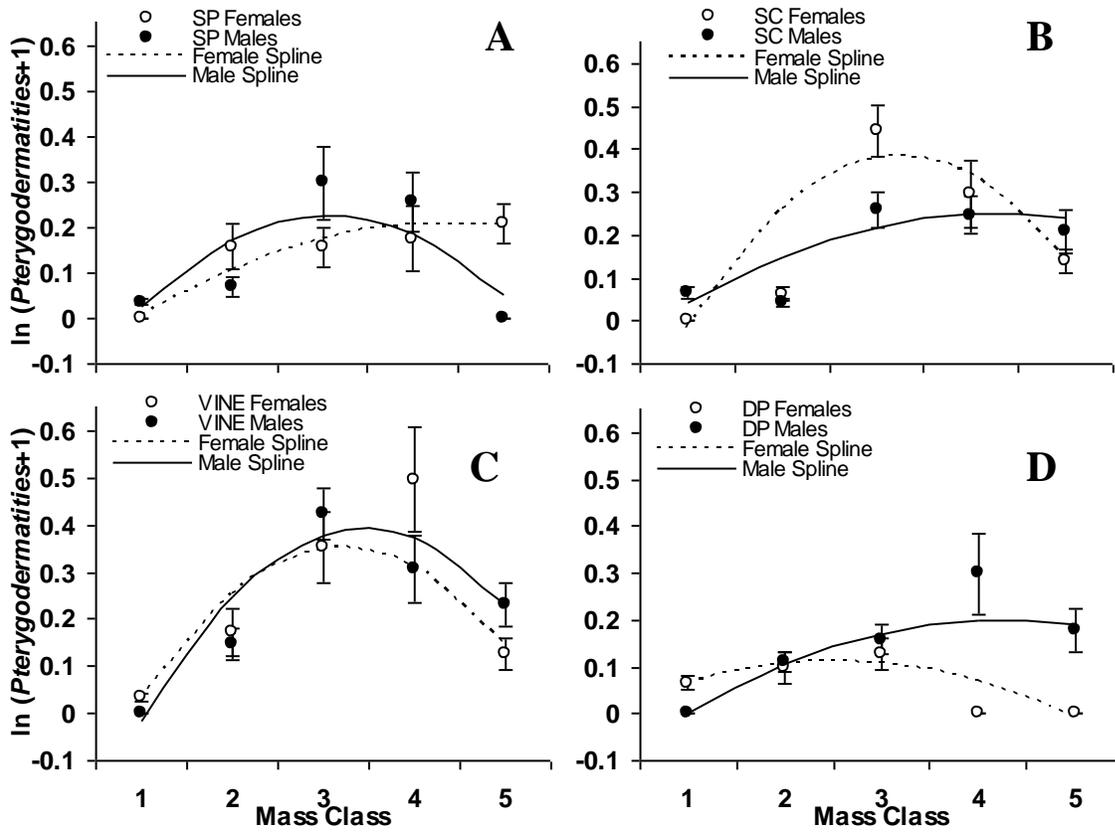
Figure 14. The monthly prevalence of the six most common gut macroparasites infecting male and female *P. leucopus* in central Pennsylvania. The month of October (p=0.006) was a significant predictor in the GLM for *S. peromysci* for all animals and both October (p=0.01) and November (p=0.03) were significant predictors in the *S. peromysci* model for males only. Error bars are one standard error of the mean.
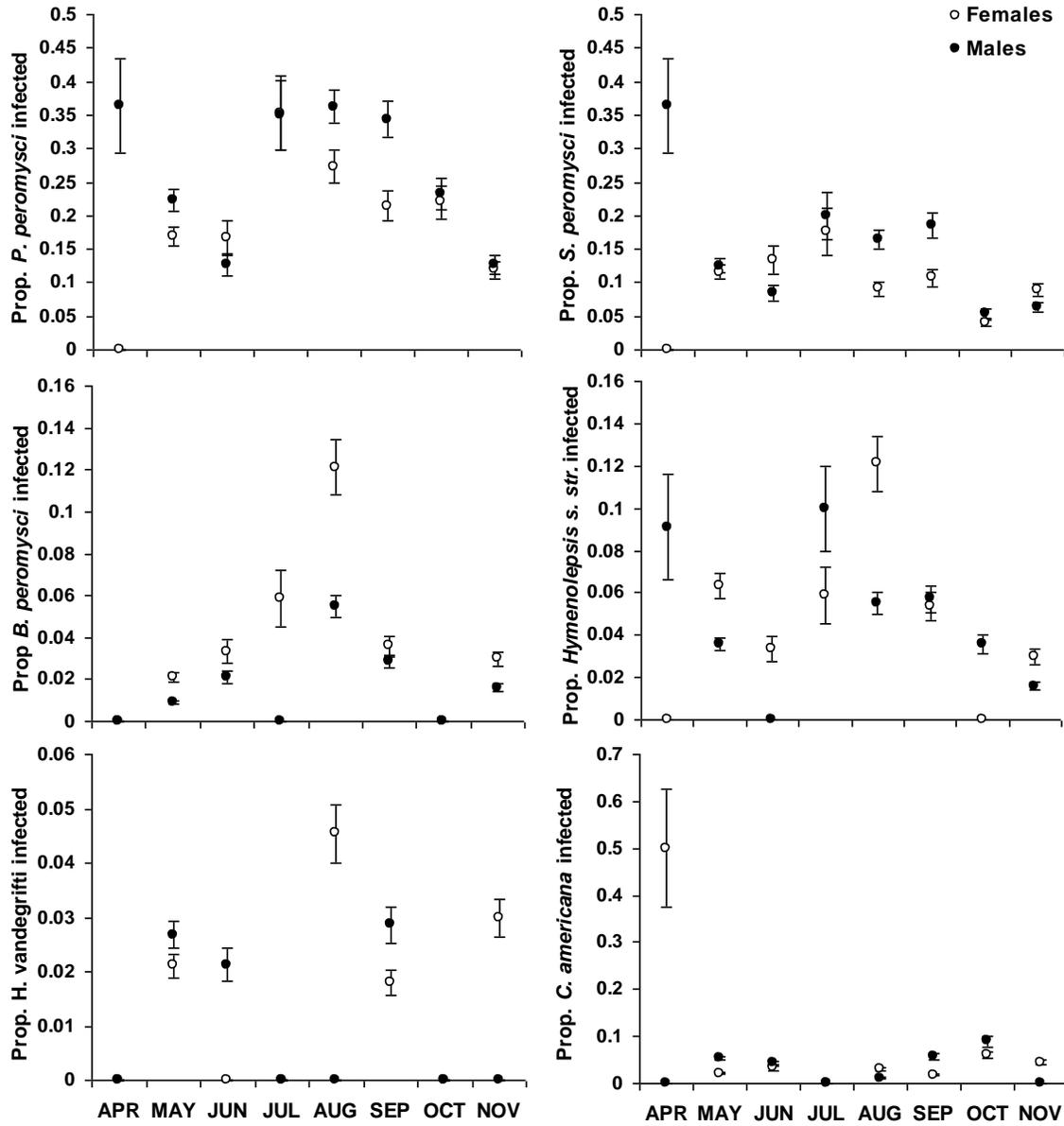
Figure 15. The patterns of co-infection between *P. peromysci* and *S. peromysci*. (A) The *S. peromysci* were clustered into seven categories based on the intensity of infection. The classes were: 0 (766), 1-3 (16), 4-8 (15), 9-12 (17), 15-25 (12), 26-35 (15), 37-68 (10), 73-10600 (15). The number given in parentheses following the range of worms per host is the sample size for each *S. peromysci* class. (B) The bottom portion of the figure requires no intensity classes; instead the number of *P. Peromysci* per host is given on the x-axis. Error bars are one standard error of the mean.
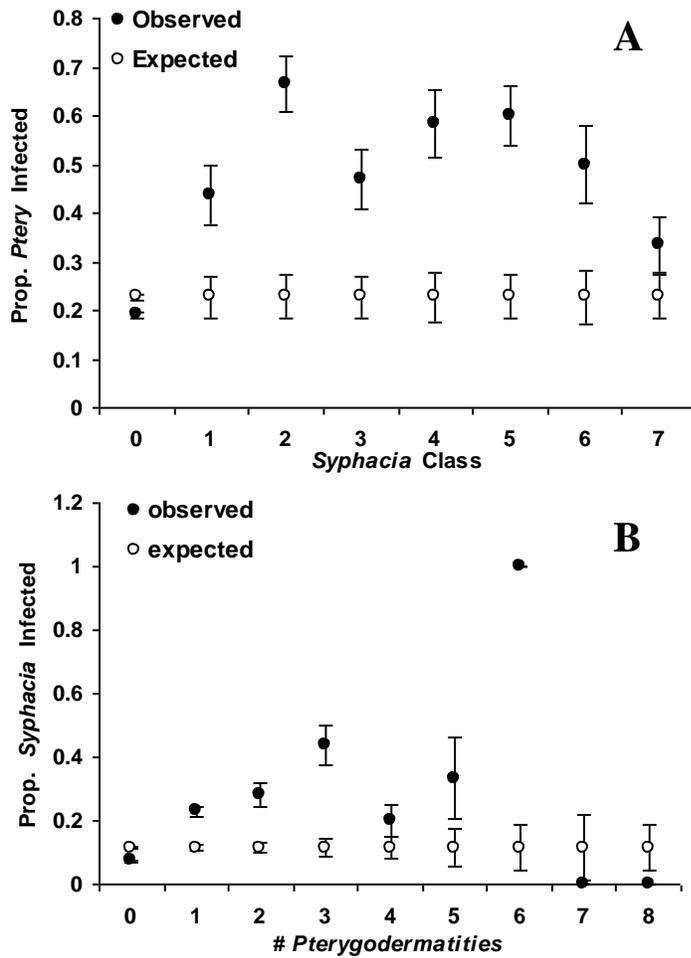
Table 3. The prevalence of *P. peromysci* in male and female *P. leucopus* caught on the four extensive trapping sites between autumn 2003 and spring 2007.

site	sex	n	*P. peromysci*	*S. peromysci*	*H. s. str.*	*C. americana*	*B. peromysci*	*H. vandegrifti*
Deer Pens	♀	58	6.9	1.7	5.2	6.9	1.7	5.2
	♂	69	13.0	2.9	1.4	2.9	2.9	2.9
Scotia	♀	76	21.6	20.3	2.7	2.7	4.1	2.7
	♂	90	23.8	23.8	1.2	0.0	0.0	3.6
Spray Fields	♀	52	13.5	7.7	0.0	1.9	1.9	0.0
	♂	55	12.7	10.9	0.0	3.6	0.0	0.0
Vineyard	♀	53	20.8	9.4	9.4	5.7	0.0	0.0
	♂	80	30.0	13.8	8.8	15.0	1.3	1.3

Table 4. We calculated the variance to mean ratio of *P. peromysci* infecting *P. leucopus* on our extensive sampling plots during each year. The ratio of variance to mean ratio was equal to or less than unity in 10 out of the 17 cases and ranged from 0.56 to 8 with a mean of 1.52 ± 0.4; the one distribution with a high variance to mean ratio (DP07) was generated by a single individual with 8 worms. When this outlier is removed the range fell to 0.56-1.98 and the mean to 1.12 ± 0.1.

Site and year	Variance to Mean Ratio	Number of Adults
DP03	0.88	25
DP04	NA	19
DP05	1.35	25
DP06	1.58	17
DP07	8.00	7
SC03	0.90	11
SC04	1.24	50
SC05	0.89	19
SC06	0.94	31
SC07	1.00	15
SP04	1.00	14
SP05	1.09	30
SP06	1.98	19
SP07	NA	7
VINE03	0.77	12
VINE04	0.91	26
VINE05	1.84	21
VINE06	0.56	19
VINE07	1.00	17

Table 5. AIC values for the GLMs of the age intensity and age prevalence data. These results indicate the relationship for *P. peromysci* is convex and type 3 because the age + age^2 model is a better fit of the data than both the linear and the logistic model. The patterns for the other three worms are not as clear and the model fits cannot be distinguished from one another.

	AIC values		
	age	log age	age +age^2
P. peromysci prevalence	560.3	546.4	523.3
S. peromysci prevalence	419.8	417.0	415.3
B. peromysci prevalence	97.7	97.7	99.7
H. vandegrifti prevalence	106.3	106.3	108.3
P. peromysci intensity	1315.9	1309.7	1302.3
S. peromysci intensity	5578.1	5577.8	5578.3
B. peromysci intensity	2500.7	2501.0	2501.9
H. vandegrifti intensity	2528.8	2530.0	2528.2

Chapter 3 References

Anderson, R.C., A.G. Chabaud, and S. Willmott. 1974. <u>CIH Keys to the Nematode Parasites of Vertebrates.</u> Commonwealth Agricultural Bureaux, Central Sales Branch, Farnham Royal, Slough SL2 3BN, England. 8vo, iv + 17 to 58 pp.

Anderson, R.M., and R.M. May. 1978. Population biology of infectious disease. Part I. Nature 280:361-367

Cattadori, I.M., D.T. Haydon, and P.J. Hudson. 2005. Parasites and climate synchronize red grouse populations. Nature **433**: 737-741.

Dobson, A.P. and P.J. Hudson. 1992. Regulation and stability of a free-living host-parasite system, *Trichostrongylus tenuis* in red grouse. II. Population models. Journal on Animal Ecology **61**:487-500.

Elias, S. P., J. W. Witham, and M. L. Hunter. 2004. *Peromyscus leucopus* abundance and acorn mast: population fluctuation patterns over 20 years. Journal of Mammal **85**:743-747.

Falls, J.B., A.E. Falls and J.M. Fryxell 2007. Fluctuations of deer mice in Ontario in relation to seed crops. Ecological Monographs, **77**(1):19–32.

Glass, G.E., T.L.Yates, J.B. Fine, T.M. Shields, J.B. Kendall, A.G. Hope, C.A. Parmenter, C.J. Peters, T.G. Ksiazek, C.S. Li, J.A. Patz, and J.N. Mills. 2002. Satellite imagery characterizes

local animal reservoir populations of Sin Nombre virus in the southwestern United States. Proceedings of the National Academy of Science USA **99**(26):16817-16822.

Holmstad, P., Hudson, P.J. and A. Skorping. 2005. The influence of a parasite community on the dynamics of a host population: a longitudinal study on willow ptarmigan and their parasites. Oikos **111**:377-391.

Hudson, P.J. 1986. The effect of a parasitic nematode on the breeding production of red grouse. Journal of Animal Ecology **55** 85-94.

Hudson, P.J., A.P. Dobson, and D. Newborn. 1992. Do parasites make prey vulnerable to predation? Red grouse and parasites. Journal of Animal Ecology **61**:681-692.

Hudson, P.J., A.P. Dobson, and D. Newborn. 1999. Population cycles and parasitism. Science **282:** 2256-2258.

Lichtenfels, J.R., 1970. Two new species of Pterygodermatities (Paucipectines) Quentin, 1969 (Nematoda: Rictulariidae) with a key to the species from North American rodents. Proceedings of the North American Helminthological Society 30(1):94-101.

Liukko U.M. 1990. Pikkunisakasharrastajan opas. Tammi, Helsinki. 111 pp.

Lello, J. B. Boag, A. Fenton, I. R. Stevenson, & P. J. Hudson. (2004). Competition and mutualism among the gut helminths of a mammalian host. Nature **428:** 840-844.

Jones, C.G., R.S. Ostfeld, M.P. Richard, E.M. Schauber, and J.O.Wolff. 1998. Chain reactions linking acorns to gypsy moth outbreaks and Lyme disease risk. Science **279**:1023-1026.

Keeling, M.J. and C. A. Gilligan, 2000. Bubonic Plague: A Metapopulation Model of a Zoonosis. Proceedings of the Royal Society Series B Biological Sciences 267 (1458):2219-2230.

May, R.M. and R.M. Anderson, 1978. Regulation and Stability of Host-Parasite Population Interactions: II. Destabilizing Processes. The Journal of Animal Ecology 47(1):1 249-267.

Norris, K., Anwar M., and A.F. Read, 1994. Reproductive effort influences hematozoan parasites in great tits. Journal of Animal Ecology **63**:601-610.

Pedersen A.B. and T.J. Grieves 2007. The interaction of parasites and resources can cause population crashes in a wild mouse population. Journal of Animal Ecology 77(2):370-377.

R Development Core Team 2005. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org

Shaw, D.J, and A.P. Dobson 1995. Patterns of macroparasite abundance and aggregation in wildlife populations: a quantitative review. Parasitology 111: S111-S133

Stien, A., R.J. Irvine, E. Ropstad, O. Halvorsen, R. Langvatn, and S.D. Albon 2002. The impact of gastrointestinal nematodes on wild reindeer: Experimental and cross-sectional studies. Journal of Animal Ecology **71**:937-945.

Taylor, L.R. 1961. Aggregation, variance and the mean. Nature **189**: 732-735.

Tompkins, D.M., A.P. Dobson, P. Arneberg, M.E. Begon, I.M. Cattadori, J.V. Greenman, J.A. Heesetrbeek, P.J. Hudson, D. Newborn, A. Pugliese, A.P. Rizzoli, R. Rosa, F. Rosso and K. Wilson. 2001. In: *Ecology of wildlife diseases*. Ed. by Hudson, P.J., A. Rizzoli, B.T. Grenfell, H. Heesterbeek, and A.P. Dobson. Oxford University Press.

Vandegrift, K.J. T.R. Raffel, and P.J. Hudson. 2008. Parasites prevent summer breeding in white-footed mice, *Peromyscus leucopus*. Ecology (in press)

Wolff, J.O. 1996. Population fluctuations of mast-eating rodents are correlated with production of acorns. Journal of Mammalogy **77**(3):850-856.

Chapter 4

Conclusions and Direction for Future Study

Chapter 1

Much research on population dynamics has focused on resource availability and in our small mammal system this resource consists of oak mast in the form of acorns. We investigated how the small mammal community inhabiting Central Pennsylvania would respond to a spring-time pulsed resource rather than a typical autumnal mast. The two pulses we studied differed in their nutritional values, specifically the amounts of protein and carbohydrate. We compared the influence of a naturally occurring pulse of periodical cicadas (high protein) with an experimental spring pulse of carbohydrate rich seeds. We used a controlled and replicated population level field experiment and capture-mark-recapture techniques to record the vital rates, demographics, and abundance of *P. leucopus*, as well as other small mammals and their parasites.

The density of *P. leucopus* on grids where cicadas emerged was 55% higher than controls as a consequence of early breeding while grids with additional sunflower seeds remained at control levels. The increase in density on cicada grids was coupled with a doubling of the parasite prevalence and a 41% reduction in breeding which reduced the initially high recruitment rates and brought the population back down to control levels soon after the cicadas perish.

Other small mammals including *T. striatus* and *B. brevicauda*, increased in density but there was no effect on *S. cinereus*. In contrast to the presence of cicadas, there was no influence of sunflower seed supplementation on small mammal density, vital rates, or reproduction with the exception of an increase in *B. brevicauda* density. There is no clear explanation for the

increase in *B. brevicauda* unless maybe one of its prey items (arthropods) responded to the sunflower seeds.

These finding provide support for the notion that *P. leucopus* may be limited by protein rather than carbohydrates in the spring time. It is well known that protein and especially the sulphur amino acids are very important for the nutrition of pregnant animals (McAdam & Millar, 1999). Thus, it makes sense that a protein-rich pulse given just at the start of the breeding season would kick start the population, while extra carbohydrates have no noticeable impact. In contrast to this, the nutritional requirements of animals in the autumn are different because rather than reproducing, these animals must put on fat and store energy to increase their chances of over-wintering and recruiting into the next year's spring breeding population. We conclude that the response of small mammals to seasonal pulses depends on timing, food type, and species.

For the future it will be important to test a recent hypothesis, that small mammals can and do predict when a pulsed resource such as a banner oak masting year will occur. There is some evidence for this hypothesis in red squirrels (*Tamiasciurus hudsonicus*) which drastically increase their reproductive effort, in heavy mast years, just prior to mast of their favored foodstuff, White Spruce (*Picea glauca*) seeds. This places their young on the ground with abundant food and affords a better chance to survive and reproduce (Boutin et al. 2006). In other words, rather than basing reproductive effort on current resources, the reproductive rates are driven by future fitness payoffs. There is also a bit of evidence that *P. leucopus* can do this in anticipation of periodical cicadas (Marcello et al. 2007). Our cicada experiment was not designed to answer this question; however, it could have done, if we had started trapping earlier. Our current idea is that *P. leucopus* simply eat the cicada nymphs when they emerge near the surface about a month before the actual emergence and molting and when field workers record

them. The situation could be nearly the same with the *T. hudsonicus* in that they also may simply eat un-ripened pine cones (which are high in protein) in banner mast years. In a typical mast year, White Spruce (*Picea glauca*) trees generate cones only on the crown of the tree and it is only during banner masting years that the entire tree bears cones. Since *T. hudsonicus* home ranges do not typically extend into the tops of the trees or at least the squirrels do not spend much time there, the cones are only readily accessible to the squirrel only during heavy mast years. A simple test of these hypotheses would be to use stable isotopes to determine if, when, and how much of these food items are being consumed. In addition, one could use polymerase chain reaction amplification of prey remains in the guts of animals sampled through time to determine the identity of the food stuffs.

Chapter 2

In 1954, David Lack postulated parasites would be unlikely to regulate animal populations unless they were acting in conjunction with poor food supply (Lack, 1954). Lack considered only parasite induced mortality, and we now know that gut macroparasites typically have sub-lethal impacts on their hosts such as reduced body condition (Tompkins et al. 2001; Stein et al. 2002) and/or decreased breeding production (Hudson 1986) rather than causing direct mortality. When the impact of the parasite on host fecundity is large relative to the impact on host survival, then this can lead to instability and generate population cycles (Anderson and May 1978, May and Anderson 1978; Dobson and Hudson 1992). There is now field evidence in wild populations of *L. scoticus* that nematodes can play a role in host dynamics because experimental removal of the caecal nematode *T. tenuis* increased breeding production and reduced the instability in the population dynamics of *L. scoticus* (Hudson et al. 1999).

The focus of Chapter 2 was to determine the impacts nematodes in our *P. leucopus* populations as well as to determine if there was a significant interaction between acorn availability and the parasites. To accomplish this, we used a replicated factorial experiment in which deer exclosures doubled acorn availability and anthelmintic application reduced gastro-intestinal helminths. Our *a priori* predictions were that more acorns over the winter would produce females in better breeding condition that would have a greater reproductive output and that worm removal would interact to increase production. More specifically, we wanted to know if either factor or an interaction between the two accounted for the mid-summer breeding hiatus observed in this species.

We found there was little influence of habitat quality (increased acorn availability) on the density and demographic variables; however, the influence of the nematode removal was profound. Both male and female animals showed highly significant increases in body weight, body length, and body condition. A greater proportion of the animals were in breeding condition and the duration of individuals breeding was also increased. Removal of parasites through anthelmintic treatment resulted in a reversal of the mid-summer breeding hiatus such that females continued to breed on grids with reduced parasites compared to control grids and at a rate similar to that observed in the first week of trapping. We were unable to detect any significant differences in density but we may argue that the increase in body size led to a competitive advantage and thus a correspondingly larger home range and this was significantly increased in the parasite free animals.

There is growing evidence that parasites play an important role in shaping population dynamics, and that chronic parasite infection can influence host fitness by reducing breeding output (Hudson, 1986; Hudson et al. 1992; Stien et al. 2002; Telfer et al. 2002; Norris et al.

1994) or by shaping seasonal breeding strategies (Raffel 2006). We have shown that there are significant impacts of nematode worms on the body condition, mass, growth, and breeding of *P. leucopus*, and that parasites can account for the mid-summer breeding hiatus commonly observed in female mice. These results imply that parasites may play a more important role in the vital rates and temporal dynamics of *P. leucopus* than resource abundance. Further studies are needed to assess the importance of parasites to the long-term inter-annual dynamics of *P. leucopus*.

Chapter 3

In Chapter 3 we examined the population biology of the dominant parasitic helminth *P. peromysci* as well as the other helminths of *P. leucopus* with the objective of providing details on the parasite distribution within the host population and how this varies in time and space. *P. leucopus* is infected with a diverse community of parasites and so we also asked general questions about the population biology of these other species and how they interact with *P. peromysci*.

The distribution of the parasites among the host population is a critical factor in the stability of the relationship between parasite and host. In the models of Anderson and May (1978), aggregation has a stabilizing influence, mainly because of the assumption that the parasites' impact on the host increases linearly with increasing parasite intensity. The highly infected individuals in the tail of the distribution who are responsible for the majority of the transmission are also incurring a disproportionate amount of parasite induced damage. At some threshold of infection, parasite induced mortality occurs and this acts to cut off the tail of the distribution, which in turn decreases transmission and results in stabilization. In contrast,

parasites can be randomly distributed where the variance to mean ratio is approximately equal. This type of distribution is destabilizing because with this orientation, a greater proportion of the host population experiences sub-lethal effects caused by moderate levels of infection (e.g. decreased fecundity). Furthermore, light levels of infection result in low rates of parasite induced mortality and therefore transmission of the parasites is not compromised, as long as the population is not so under-dispersed as to preclude breeding opportunities.

We were able to identify seven of the eight worms to the species level and we also discovered new species of nematode (*Heligmosomoides vandegrifti;* see Appendix B). Generalized linear models indicated body mass, body length, breeding condition, and co-infections with other worm species can all significantly influence the infection status and/or intensity of infection within an individual. We separated the data into age cohorts to examine age prevalence and intensity patterns and we show that *P. peromysci* exhibits a type three response in that the curves turn over to form a convex shape. We also explored patterns of co-infection between the two dominant parasites in the system, *P. peromysci* and *Syphacia peromysci*. However, the most important finding in this chapter is that *P. peromysci* exhibits a near random distribution among the host population in that the variance to mean ratio is near unity. Random distributions are not typical of macroparasites and this characteristic coupled with the impact on female breeding would lead us to expect the unstable dynamics exhibited by *P. leucopus*.

The study of the how and why animal populations fluctuate has been a central question in ecological literature for decades yet only recently have we pursued these questions with rigorous empiricism via large scale replicated population level manipulations. If these types of experiments allow us to parameterize models that can predict future dynamics of rodent

populations then we may be able apply this knowledge with a population intervention strategy. Moreover, if these parasites turn out to be important drivers of the dynamics then we may be able to use the worms as a form of bio-control, of course using our model predictions to target and develop our intervention strategies. In the event that an emergent or pre-existing rodent borne pathogen arises and becomes a threat to human health, this could be particularly useful information. The bubonic plague killed between one-half and one-third of the European population in a span of five years, and it is still responsible for between 1000 and 3000 human deaths per year (Keeling & Gilligan 2000).

Introduction and Conclusion References

Bendell, J.F. 1959. Food as a control of a population of white-footed mice, *Peromyscus leucopus noveboracensis* (Fisher). Canadian Journal of Zoology 37:173-209.

Bentwich, Z. A., Kalinkovich, and Z. Weisman. 1995. Immune activation is a dominant factor in the pathogenesis of African AIDS. Immunology Today 16: 187–19.

S. Boutin, L.A. Wauters, A.G. McAdam, M. M. Humphries, G. Tosi, A.A. Dhondt 2007. Anticipatory Reproduction and Population Growth in Seed Predators. Science (314):1928 -1930.

Cattadori, I.M., D.T. Haydon, and P.J. Hudson. 2005. Parasites and climate synchronize red grouse populations. Nature 433: 737-741.

Dobson, A.P. and P.J. Hudson. 1992. Regulation and stability of a free-living host-parasite system, *Trichostrongylus tenuis* in red grouse. II. Population models. Journal on Animal Ecology 61:487-500.

Elias, S. P., J. W. Witham, and M. L. Hunter. 2004. *Peromyscus leucopus* abundance and acorn mast: population fluctuation patterns over 20 years. Journal of Mammal 85:743-747.

Elton C.S. 1924. Periodic fluctuations in the numbers of animals: their causes and effects. Journal of Experimental Biology 2:119-163.

Falls, J.B., A.E. Falls and J.M. Fryxell 2007. Fluctuations of deer mice in Ontario in relation to seed crops. Ecological Monographs, 77(1):19–32.

Ferrari, N., I.M. Cattadori , J. Nespereira, A. Rizzoli, and P.J. Hudson. 2004. The role of host sex in parasite dynamics: field experiments on the yellow-necked mouse *Apodemus flavicollis*. Ecology Letters 7(2): 88–94.

Fox, A.and P.J. Hudson. 2001. Parasites reduce territorial behavior in red grouse (*Lagopus lagopus scoticus*) Ecology Letters 4(2):139–143.

Gashwiler, J.S. 1979. Deer mouse reproduction and its relationship to the tree seed crop. American Midland Naturalist 102(1):95-104.

Glass, G.E., T.L.Yates, J.B. Fine, T.M. Shields, J.B. Kendall, A.G. Hope, C.A. Parmenter, C.J. Peters, T.G. Ksiazek, C.S. Li, J.A. Patz, and J.N. Mills. 2002. Satellite imagery characterizes local animal reservoir populations of Sin Nombre virus in the southwestern United States. Proceedings of the National Academy of Science USA 99(26):16817-16822.

Gilg, O., Hanski, I. and B. Sittler 2003. Cyclic dynamics in a simple vertebrate predator-prey community. Science 302:866-868.

Hansen, L.P. & Batzli, G.O. (1979) Influence of supplemental food on local populations of *Peromyscus leucopus*. Journal of Mammalogy, 60, 335-342.

Holmstad, P., Hudson, P.J. and A. Skorping. 2005. The influence of a parasite community on the dynamics of a host population: a longitudinal study on willow ptarmigan and their parasites. Oikos 111:377-391.

Hudson, P.J. & Bjornstad, O.N. 2003. Vole stranglers and lemming cycles. Science, 302, 797-798.

Hudson, P.J. 1986. The effect of a parasitic nematode on the breeding production of red grouse. Journal of Animal Ecology 55 85-94.

Hudson, P.J., A.P. Dobson, and D. Newborn. 1992. Do parasites make prey vulnerable to predation? Red grouse and parasites. Journal of Animal Ecology 61:681-692.

Hudson, P.J. Dobson, A.P. & Newborn, D. (1998). Prevention of population cycles by parasite removal. Science. 282, 2256-2258.

Hudson, P.J., A.P. Dobson, and D. Newborn. 1999. Population cycles and parasitism. Science 282: 2256-2258.

Hudson, P.J. Dobson, A.P., Cattadori, I.M.C., Newborn, D., Haydon, D.T., Shaw, D.J., Benton, T.G. and Grenfell, B.T. (2003) Trophic interactions and population growth rates: describing patterns and identifying mechanisms. In Wildlife population growth rates. Edited by R.M.Sibly, J.Hone & T.H. Clutton Brock. pp 198-224. Cambridge University Press

Jones, C.G., R.S. Ostfeld, M.P. Richard, E.M. Schauber, and J.O.Wolff. 1998. Chain reactions linking acorns to gypsy moth outbreaks and Lyme disease risk. Science 279:1023-1026.

Keeling, M.J. and C.A. Gilligan 2000. Metapopulation dynamics of bubonic plague. Nature 407: 903-906.

Krohne, D.T., Couillard, T.J. & Riddle, J.C. (1991) Population responses of Peromyscus leucopus and Blarina brevicauda to emergence of periodical cicadas. American Midland Naturalist, 126:317-321.

Kissui, B.M. and C. Packer. 2004. Top-down population regulation of a top predator:

Krebs, C.J., S. Boutin, R. Boonstra, A.R.E. Sinclair, J.N.M. Smith, M.R.T. Dale, K. Martin, and R. Turkington. 1995. Impact of food and predation on the snowshoe hare cycle. Science 269: 1112-1115.

Lack, D. 1954. The Natural Regulation of Animal Numbers. Oxford University Press, Oxford.

Lafferty, K. D. and A. K. Morris. 1996. Altered behavior of parasitized killifish increases susceptibility to predation by bird final hosts. Ecology 77:1390-1397.

Lello, J. B. Boag, A. Fenton, I. R. Stevenson, and P. J. Hudson 2004. Competition and mutualism among the gut helminths of a mammalian host. Nature 428: 840-844.

Marcello G. J., Wildner, S. M. & Meikle D.B. (2008) Population dynamics of a generalist rodent in relation to variability in pulsed food resources in a fragmented landscape. Journal of Animal Ecology, 77, 41-46.

May, R.M. and R.M. Anderson, 1978. Regulation and Stability of Host-Parasite Population Interactions: II. Destabilizing Processes. The Journal of Animal Ecology 47(1):1 249-267.

McAdam, A.G. & Millar, J.S. (1999a) The effects of dietary protein content on growth and maturation in deer mice. Canadian Journal of Zoology, 77, 1822-1828.

McCracken, K.E., J.W. Witham, M.L. Hunter, Jr. 1999. Relationships between seed fall of three tree species and *Peromyscus leucopus* and *Clethrionomys gapperi* during 10 years in an oak-pine forest. Journal of Mammalogy 80(4):1288-1296.

Norris, K., Anwar M., and A.F. Read, 1994. Reproductive effort influences hematozoan parasites in great tits. Journal of Animal Ecology 63:601-610.

Ostfeld R.S., C.G. Jones and J.O. Wolff. 1996. Of mice and mast. BioScience 46 (5):323-330.

Park T. 1948. Experimental studies of interspecies competition. 1. Competition between populations of the flour beetles, *Tribolium confusum* Duval and *Tribolium castaneum* Herbst. Ecological Monographs 18:267-307.

Pucek, Z., Jedrzejewski, W., Jedrzejewska, B. & Pucek M. 1993 Rodent population dynamics in a primeval deciduous forest (Bialowieza National Park) in relation to weather, seed crop, and predation. Acta Theriologica, 38, 199-232.

Shaw, D.J, and A.P. Dobson 1995. Patterns of macroparasite abundance and aggregation in wildlife populations: a quantitative review. Parasitology 111: S111-S133.

Smyth, M. 1966. Winter breeding in woodland mice, *Apodemus sylvaticus*, and voles, *Clethrionomys glareolus* and *Microtus agrestis*, near Oxford. Journal of Animal Ecology 35:471–485.

Stien, A., R.J. Irvine, E. Ropstad, O. Halvorsen, R. Langvatn, and S.D. Albon 2002. The impact of gastrointestinal nematodes on wild reindeer: Experimental and cross-sectional studies. Journal of Animal Ecology 71:937-945.

Telfer, S., M. Bennett, K. Bown, R. Cavanagh, L. Crespin, S. Hazel, T. Jones, and M. Begon. 2002. The effect of cowpox virus on survival in natural rodent populations: increases and decreases. Journal of Animal Ecology 71:558-568.

Tompkins, D.M., A.P. Dobson, P. Arneberg, M.E. Begon, I.M. Cattadori, J.V. Greenman, J.A. Heesetrbeek, P.J. Hudson, D. Newborn, A. Pugliese, A.P. Rizzoli, R. Rosa, F. Rosso and K. Wilson. 2001. In: Ecology of wildlife diseases. Ed. by Hudson, P.J., A. Rizzoli, B.T. Grenfell, H. Heesterbeek, and A.P. Dobson. Oxford University Press.

Vandegrift, K.J. T.R. Raffel, and P.J. Hudson. 2008. Parasites prevent summer breeding in white-footed mice, *Peromyscus leucopus*. Ecology (in press)

Vessey, S. H. and K.B. Vessey, 2007. Linking behavior, life history and food supply with the population dynamics of white-footed mice (Peromyscus leucopus). Integrative Zoology 2:123-130.

Wolff, J.O. 1996. Population fluctuations of mast-eating rodents are correlated with production of acorns. Journal of Mammalogy 77(3):850-856.

Wolff, J.O. (1986) The effects of food on mid-summer breeding activity in *Peromyscus leucopus* in south-western Virginia. Canadian Journal of Zoology, 64, 855-858.

Yunger, J.A. (2002) Response of two low-density populations of *Peromyscus leucopus* to increased food availability. Journal of Mammalogy, 83, 267–279.

Appendix A

Gastrointestinal Helminths of the Masked Shrew, *Sorex cinereus*, from Pennsylvania

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ABSTRACT: Eight helminth species (6 cestodes, 2 nematodes) were recovered from 30 *Sorex cinereus* (Mammalia: Soricidae) collected in the Pennsylvania State University Experimental Forest in Huntingdon County, Pennsylvania. All 8 helminths were new records for Pennsylvania and 3 tapeworms (*Lineolepis lineola*, *Staphylocystoides serrula, Soricinia pulchra*) were new records for *S. cinereus*. There appears to be little evidence for host specificity among tapeworms of *Sorex* spp. in North America, but no species are shared with *Blarina* spp.

Shrews of the genus *Sorex* (Mammalia: Soricidae) are among the most common mammals of North America, but because of their extremely small size, are seldom seen, and their helminths have been comparatively poorly studied. The masked shrew, *Sorex cinereus* Kerr, 1792, is perhaps the most widely distributed member of the genus in North America, occurring throughout Alaska and most of mainland Canada, and in the northern tier of the United States from Washington east to Maine and south in the Appalachian Mountains as far as Georgia. It is found in a great range of habitats from tundra to arid grasslands, and occurs in both coniferous and hardwood forests (Whitaker, 2004).

Gastrointestinal helminths from *S. cinereus* have been reported from Alaska (Voge and Rausch, 1955), Montana (Senger, 1955), Colorado (Leiby, 1961), Wisconsin (Read, 1949; Rausch and Kuns, 1950), Iowa (Wittrock and Hendrickson, 1979), Alberta (Vaucher and Durette-Desset, 1973), and Ontario (Vaucher and Durette-Desset, 1973), but no records have been published from the United States east of the Mississippi. This study reports on the gastrointestinal helminths of a sample of *S. cinereus* from Pennsylvania.

During the course of a small mammal study in the Pennsylvania State University Experimental Forest (40˚37' 09.9" N, 77˚54'29.3" W) between May and September, 2006, a number of shrews were victims of incidental trap mortality. The area is a typical northeastern Appalachian forest dominated by hardwoods such as oaks (*Quercus* spp.) and maples (*Acer* spp.). The gastrointestinal tracts of 30 *S. cinereus* were examined for helminths within a few hours after death. Cestodes were preserved in 90% ethanol, stained with Ehrlich's hematoxylin or Semichon's carmine, and mounted in Canada balsam. Nematodes were preserved in 70% ethanol with 5% glycerine, studied in temporary mounts of lactophenol, and then returned to the preservative. Voucher specimens were deposited in the U. S. National Parasite Collection, Beltsville, Maryland under accession numbers 00000-00000.

Cestoda

Lineolepis lineola

(Oswald, 1951)

Prevalence: Hosts infected, 26 of 30 (87%).

Site of infection: small intestine.

Type host and locality: Sorex fumeus (Miller, 1895), Revenge, Hocking County, Ohio.

Geographic range: United States: Ohio.

Specimens deposited: USNPC 00000 (1 slide).

Remarks: After examining the type material of both species, Senger (1955) found no difference in shape or size between the hooks of *L. lineola* and *Lineolepis parva* (Rausch and Kuns, 1950), and declared *L. lineola* a synonym of *L. parva.* Olsen (1969) re-examined the paratypes of both species and felt that Senger (1955) was mistaken in his interpretation of the shape of the hooks of *L. lineola.* In addition, Olsen felt that the 2 species could be differentiated on the basis of the

arrangement of the testes, the size of mature strobila, and the shape of the extended cirri, and restored *L. lineola* to a valid species. This is the first record of *L. lineola* since the original description and the first record from *S. cinereus.*

Mathevolepis macyi

(Locker and Rausch, 1952)

Prevalence: Hosts infected, 17 of 30 (57%).

Site of infection: small intestine.

Type host and locality: Sorex vagrans Baird, 1857, Portland, Oregon.

Other reported hosts: Sorex bendirii (Merriam, 1884), Oregon (Neiland, 1953); *Sorex cinereus*, Iowa (Wittrock and Henderson, 1979); *Sorex ornatus* (Merriam, 1895), California (Davis and Voge, 1957); *Sorex trowbridgii* Baird, 1857, California (Voge, 1955a); *Sorex vagrans*, Montana (Senger, 1955; Kinsella, in press).

Geographic range: United States: California, Iowa, Montana, Oregon.

Specimens deposited: USNPC 00000 (1 slide).

Remarks: Although the original description of this tapeworm by Locker and Rausch (1952) did not mention a rostellum, Voge (1955a) re-examined the type material and found a small unarmed rostellum. This structure was present in all specimens examined here.

Soricinia kenki

(Locker and Rausch, 1952)

Prevalence: Hosts infected, 2 of 30 (7%).

Site of infection: small intestine.

Type host and locality: Sorex vagrans, Portland, Oregon.

Other reported hosts: Sorex bendirii, Oregon (Neiland, 1953); *Sorex obscurus* Merriam, 1895, Oregon (Neiland, 1953); *Sorex cinereus*, Montana (Senger, 1955); *Sorex pacificus* Coues, 1877, California (Voge, 1955a); *Sorex vagrans*, Montana (Kinsella, in press).

Geographic range: United States: California, Montana, Oregon.

Specimens deposited: USNPC 00000 (1 slide).

Remarks: This is the only unarmed tapeworm without a rostellum found in *Sorex* spp. in North America. The large, densely spined cirrus is also characteristic. This is the second record from *S. cinereus* and extends the known range of this tapeworm from Montana to Pennsylvania.

Soricinia pulchra

(Voge, 1955)

Prevalence: Hosts infected, 6 of 30 (20%).

Site of infection: small intestine.

Type host and locality: Sorex trowbridgii, California.

Other reported hosts: Sorex pacificus, California (Voge, 1955b).

Geographic range: United States: California.

Specimens deposited: USNPC 00000 (1 slide).

Remarks: This is the first record of this species since the original description from California shrews by Voge (1955b) and a new host record for *S. cinereus*. It is characterized by a very large scolex in comparison to the strobila, an unarmed rostellum, and a small spherical uterus.

Staphylocystoides serrula

(Oswald, 1951)

Prevalence: Hosts infected, 20 of 30 (67%).

Site of infection: small intestine.

Type host and locality: Sorex fumeus, Revenge, Hocking County, Ohio.

Other reported hosts: Sorex fumeus, Tennessee (Cox et al. 1956); *Sorex palustris* Richardson, 1828, Montana (Senger, 1955).

Geographic range: United States: Montana, Ohio, Tennessee.

Specimens deposited: USNPC 00000 (1 slide)

Remarks: Cox et al. (1956) redescribed *S. serrula*, based on specimens from *S. fumeus* in Tennessee, and found considerable variation in some morphological characteristics. In particular, hook number varied from 8 to 11. There are substantial similarities between this species and *Staphylocystoides sphenomorphus* (Locker and Rausch, 1952) in hook size, hook shape, and overall shape of the strobila and gravid proglottids. The only reliable character to separate them appears to be the shape of the cirrus, which is bulbous in *S. sphenomorphus*, and thin in *S. serrula.* The presence of *S. serrula* in Montana, based on the identification of a single specimen from *S. palustris* by Oswald (see Senger, 1955), needs to be confirmed by further collecting, since *S. sphenomorphus* is quite common in Montana shrews (Senger, 1955; Kinsella, in press). This is a new host record for *S. cinereus.*

Staphylocystoides longi

(Oswald, 1951)

Prevalence: Hosts infected, 4 of 30 (13%).

Site of infection: small intestine.

Type host and locality: Sorex fumeus, Revenge, Hocking County, Ohio.

Other reported hosts: Sorex bendirii, Oregon (Neiland, 1953); *Sorex cinereus*, Iowa (Wittrock and Hendrickson, 1979); *Sorex vagrans*, Montana (Kinsella, in press).

Geographic range: United States: Iowa, Montana, Ohio, Oregon.

Specimens deposited: USNPC 00000 (1 slide).

Remarks: In the original description of *S. longi*, Oswald (1951) reported 8 hooks on the rostellum. Neiland (1953) found cestodes in *S. benderii* from Oregon that were identical in all respects to *S. longi*, except for having 10 hooks on the rostellum. Senger (1955) thought that Neiland's specimens were probably *Staphylocystoides parvissima* (Voge, 1953), although Olsen (1969) pointed out that the shape of the hooks of *S. longi* and *S. parvissima* is quite different. Kinsella (unpubl. data) found specimens in *S. vagrans* from Montana similar in hook shape to *S. longi*, but again with 10 hooks instead of 8. Genetic studies may be necessary to determine whether these eastern and western forms are different species.

Nematoda

Baruscapillaria rauschi

(Read, 1949)

Prevalence: Hosts infected, 9 of 30 (30%).

Site of infection: stomach, small intestine.

Type host and locality: Sorex cinereus, Madison, Wisconsin.

Other reported hosts: S. cinereus, Iowa (Wittrock and Henderson, 1979; *Sorex palustris*, Montana (Conaway, 1952); *Sorex vagrans*, Montana (Kinsella, in press).

Geographic range: United States: Iowa, Montana, Wisconsin.

Specimens deposited: USNPC 00000 (1 vial).

Longistriata alainchabaudi

Vaucher and Durette-Desset, 1973

Prevalence: Hosts infected, 23 of 30 (77%).

Site of infection: small intestine.

Type host and type locality: Sorex cinereus, Algonquin Park, Ontario, Canada (Vaucher and Durette-Desset, 1973).

Other reported hosts: Sorex fumeus, Canada (Vaucher and Durette-Desset, 1973), *Sorex arcticus* Kerr, 1792, Canada (Vaucher and Durette-Desset, 1973).

Geographic range: Canada: Ontario.

Specimens deposited: USNPC 00000 (1 vial).

Remarks: This is the first record of this nematode since the original description and the first record from the United States.

Although there are no species of tapeworms shared between *Blarina* spp. and *Sorex* spp. in North America (Kinsella, in press), there seems to be little host specificity among tapeworms within *Sorex* spp., and many tapeworm species appear to be widely distributed in North America. For example, this paper extends the known range of *S. pulchra* from California to Pennsylvania, *S. kenki* from Montana to Pennsylvania, and *M. macyi* from Iowa to Pennsylvania. The latter species has now been reported from 6 species of *Sorex* in North America. However, no species of tapeworm has yet been reported from both Nearctic and Palearctic species of *Sorex*. A number of shrew species in eastern North America, notably *Sorex hoyi* and *Sorex longirostris*, have yet to be examined for helminths, so there is much yet to be learned.

Appendix A References

Conaway, C. H. 1952. Life history of the water shrew (*Sorex palustris navigator*).American Midland Naturalist 48:219-248.

Cox, D. D., H. Ciordia, and A. W. Jones. 1956. Variations in *Hymenolepis serrula*Oswald, 1951, (Cestoda: Hymenolepididae), a cestode from the smoky shrew, *Sorex fumeus* Miller, 1895, with special reference to three geographic areas. Journal of the Tennessee Academy of Science 31:287-299.

Davis, B. S., and M. Voge. 1957. Observations on *Hymenolepis macyi* Locker and Rausch, with a revised diagnosis of this cestode. Proceedings of the Helminthological Society of Washington 24:1-4.

Kinsella, J. M. 2007. Helminth parasites of the vagrant shrew, *Sorex vagrans*, from western Montana, USA. Acta Parasitologica: in press.

Leiby, P.D. 1961. Intestinal helminths of some Colorado mammals. Journal of Parasitology 47:311.

Locker, B., and R. Rausch. 1952. Some cestodes from Oregon shrews, with descriptions of four new species of *Hymenolepis* Weinland, 1858. Journal of the Washington Academy of Sciences 42:26-31.

Neiland K.A. 1953. Helminths of northwestern mammals, Part V. Observations on cestodes of shrews with the descriptions of new species of *Liga* Weinland, 1857, and *Hymenolepis* Weinland, 1858. Journal of Parasitology 39:487-495.

Olsen, O. W. 1969. *Hymenolepis pribilofensis*. n. sp. (Cestoda: Hymenolepididae) from the Pribilof shrew (*Sorex pribilofensis* Merriam) from the Pribilof Islands, Alaska. Canadian Journal of Zoology 47:449-454.

Oswald, V. H. 1951. Three new hymenolepidid cestodes from the smoky shrew, *Sorex fumeus* Miller. Journal of Parasitology 37:573-576

Rausch, R., and M. L. Kuns. 1950. Studies on some North American shrew cestodes Journal of Parasitology 36:433-438.

Read, C. P. 1949. Studies on North American helminths of the genus *Capillaria* Zeder, 1800 ((Nematoda): I. Capillarids from mammals. Journal of Parasitology 35:223-230.

Senger, C. M. 1955. Observations on cestodes of the genus *Hymenolepis* in North American shrews. Journal of Parasitology 41:167-170.

Vaucher, C., and M. C. Durette-Desset. 1973. Nématodes Héligmosomes parasites d'Insectivores Soricidés de la region holoarctique. Annales de Parasitologie Humaine et Comparée 48:135-167.

Voge, M. 1955a. Notes on four hymenolepidid cestodes from shrews. Journal of Parasitology 41:74-76.

Voge, M. 1955b. *Hymenolepis pulchra*. n. sp., a cestode from the shrew *Sorex trowbridgei* in California. Proceedings of the Helminthological Society of Washington 22:90-92.

Voge, M., and R. Rausch. 1955. Occurrence and distribution of hymenolepidid cestodes in shrews. Journal of Parasitology 41:566-574.

Whitaker, J. O. 2004. *Sorex cinereus*. Mammalian Species No. 743, 9 p.

Wittrock, D. D., and G. L.Hendrickson. 1979. Helminths of shrews, *Blarina brevicauda* and *Sorex cinereus*, in Iowa. Journal of Parasitology 65:985-986.

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Education

Pennsylvania State University, University Park PA
-M.S. of Animal Science. May 2002
-PhD. Of Biology (pending Summer 2008)
Juniata College, Huntingdon PA, B.S.
-Biology and Psychology, May 1999

Professional Work Experience

Centre for Infectious Disease Dynamics Research assistant May '03-Present
PSU Eberly College of Science, Peter J. Hudson, Supervisor. Teaching and research assistantships. Trapping of wild small mammals and development of a lab colony of *Peromyscus*.
3 years of field experience with small mammals. Developed a knowledge of statistical procedures (GLM, LME, LM, AOV, LMER) in S Plus and R .

Poultry Science/Operations Research Lab Assistant May 99- May 02
PSU Department of Dairy and Animal Science, William B. Roush, Supervisor. Research graduate assistantship. Artificial neural network and genetic algorithm research. Attended conferences. Math Modeling in Nutrition and Health Sciences. 2001 Southeastern Poultry and Egg (PSA) Conference. ADSA, ASAS, AMSA, PSA Joint Meeting. 2001 Joint Conference of the Animal Sciences. PSA. 2002 Southeastern Poultry and Egg (PSA).

Animal Diagnostic Laboratory Research Assistant May '96-August '96
PSU Department of Veterinary Science. Dr. Anthony Castro and Dr. Barrett Cowen, Supervisors. Treated and cared for virus isolation poultry flocks in the bio-containment facility.
Lab work included cell culture, necropsy, slide staining, ELISA, ouchterlony immunodiffusion assay and other serology. Attended Northeastern Conference for Avian Disease (NECAD '96)

Publications

Wilson, L.L., P.A Nordstrom, M.J. Richards, D.L. Smith and **K.J. Vandegrift** 1998. Dairy and veal producer assessment of changes in care, handling, and transportation. The Professional Animal Scientist. 15:24-33

Wilson, L.L., P.A. Nordstrom, M.J. Richards, D.L. Smith, and **K.J. Vandegrift** 1998. Veal producer perceptions of handling and transportation factors. The Producers Connection. 7 (9): 6-8 (Reprinted in WVGJ, August 1998).

Wilson, L.L., P.A. Nordstrom, J.L. Smith, D.L. Swanson, D.L. Smith, M.J. Richards, and **K.J. Vandegrift** 1998. The Producers Connection. 7(10): 11-12. (Reprinted in WVGJ, August 1998. Presented at Northeast Animal and Dairy Science meetings.

Vandegrift, K.J., T.L. Cravener, and W.B. Roush 2001. Examination of the relationship between daily growth and feed intake velocities of individual broilers. Poultry Science. Vol. 91S:S136 SPSS Conference

Vandegrift, K.J., T.L. Cravener, and W.B. Roush, 2002. An analysis of the nonlinear dynamics of daily broiler growth and feed intake. Poultry Science. Vol. 103S. SPSS Conference.

Vandegrift, K.J., T.L. Cravener, R.M. Hulet, and W.B. Roush, 2003. Analysis of the nonlinear dynamics of daily broiler growth and feed intake. Poulty Science. 2003 Jul;82(7):1091-9.

Vandegrift, K.J., T.R. Raffel, and P.J. Hudson. 2008. Parasites prevent summer breeding in white-footed mice, *Peromyscus leucopus. Ecology* (in press).

Vandegrift, K.J. and P.J. Hudson. 2008. Responses to enrichment: Small mammals vary in their response to a springtime cicada emergence. Journal of Animal Ecology (in press).

Kinsella, J.M., **K. J. Vandegrift**, L.K. Grant, B.A. Bozick, and P.J. Hudson. 2008. Gastrointestinal Helminths of the Masked Shrew, *Sorex cinereus*, from Pennsylvania. Comparative Parasitology 75(1) 141–144.

Teaching Experience

Introductory Biology -1year, Animal Physiology-1 year, Experimental Field Biology- 3 years

Miscellaneous achievements

I had the honor of having a new species named after me (*Heligmosomoides vandegrifti*). This nematode infects *Peromsycus maniculatus* in our system and is described in the following reference: Marie-Claude Durette-Desset and John M. Kinsella, 2008. A new species of *Heligmosomoides* (Nematoda, *Heligmosomidae*) parasitic in *Peromyscus maniculatus* (Rodentia, Cricetidae) from Pennsylvania, USA. Acta Parasitologica 52(4):342-345.