PEROMYSCUS NEWSLETTER

Number Forty-Two

Fall 2007

Cover:

The 30,000th *Peromyscus maniculatus bairdii* (BW) born at the *Peromyscus* Genetic Stock Center since the stock was first acquired in 1963. Photograph by Clint Cook, University of South Carolina.

***Peromyscus Newsletter* Number 42**

Hello, All!

Many of you may have noticed there was no Spring 2007 issue of *Peromyscus Newsletter*. I have decided to reduce the frequency of *PN* to once per year for several reasons. First, I am no longer an official employee of the *Peromyscus* Genetic Stock Center, though I retain very close ties. I gave up my position at the end of June in order to be a full-time mom. Having a newborn in the house is quite demanding of my time as you might imagine. Primarily, however, I have made this change in order to have having larger, more interesting issues that will encourage people to download and read the Newsletter. I know all of you are extremely busy so I hope this change will be an efficient use of everyone's time.

As I mentioned in the last issue, many people are still not receiving emails from the peromyscusnewsletter@biol.sc.edu account. This, I believe, is caused by people's spam filters, so if you know of anyone having this problem please have them check their filters and specify this address as legitimate. I am limited in what I can do from this end.

Finally, as many of you already know, I got married last year and changed my last name to Glenn. My new email address is jglenn@biol.sc.edu, however, everything related to *PN* should be sent to peromyscusnewsletter@biol.sc.edu.

And as always, all suggestions to improve the Newsletter are appreciated! Take care and enjoy!

Julie

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News, Comments, and Announcements

A NEW *PEROMYSCUS* PHYLOGENY!!!!

"Toward a molecular phylogeny for *Peromyscus*: evidence from mitochondrial cytochrome-b sequences" by Robert D. Bradley, Nevin D. Durish, Duke S. Rogers, Jacqueline R. Miller, Mark D. Engstrom, and C. William Kilpatrick will be appearing in the next issue of Journal of Mammalogy, vol 88 no. 5.

♦♦♦

Dr. Knut Schmidt-Nielsen died January 25, 2007 of natural causes. A professor emeritus at Duke University, Dr. Schmidt-Nielsen revolutionized the study of animal physiology by comparing a wide variety of animals and linking their physiology to behavior and life history traits. He published 5 books and 270 papers. His *Animal Physiology* textbook is still widely used. He will be missed.

♦♦♦

Professor Donald Michie and **Dame Anne McLaren** were killed in a car accident on July 7, 2007. Dr. Michie was a researcher in artificial intelligence and the director of Edinburgh University's Department of Machine Intelligence and Perception. His ex-wife, Dr. McLaren, was a geneticist and the first female officer of the Royal Society.

♦♦♦

In May 2007, Joel Sartore, a photographer for **National Geographic** magazine visited the *Peromyscus* Genetic Stock Center to photograph the most endangered mammal in the United States, the Perdido Key Beach Mouse, *Peromyscus polionotus trissyllepsis*. National Geographic plans to publish an article about the Endangered Species Act in 2008 and the photograph may be included. Keep your fingers crossed that our mice get the publicity they deserve!

♦♦♦

Many of you may recall that the *Peromyscus* Genetic Stock Center aided in the rescue of 8 **Perdido Key Beach Mice**, *Peromyscus polionotus trissyllepsis*, from Hurricane Ivan in 2004. Notoriously difficult to breed in captivity, the Stock Center did manage to get a few pairs to produce offspring and their numbers increased to a little over 50 animals. These mice now have a new home at Santa Fe Community College Teaching Zoo in Gainesville, Florida.

♦♦♦

♦♦

Summer 2006 saw an **explosion of white-footed mice** (*Peromyscus leucopus*) in northern Michigan. Dr. Phil Myers has censused small mammals every year for 18 years and noted the sudden increase. He attributes the population explosion to two factors: a large crop of red oak mast in fall 2005 and an early spring, both of which perhaps decreased over-winter mortality.

P. leucopus, Otsego Co., Michigan
Photo by Dr. Phil Myers

♦♦

The 87th annual meeting of the **American Society of Mammalogists** took place Wednesday June 6 to Sunday June 10, 2007 at the University of New Mexico, Albuquerque. There were 29 presentations relating to *Peromyscus* research, some of which are included in the contributions section.

♦♦

The **Ecology and Conservation of Grassland Vertebrates** conference will be held April 15-19, 2008 at the University of Oklahoma, Norman. For more information go to: http://www.suttoncenter.org/ecgv.html

♦♦

The first report of **Tularemia** as a cause of death in wild deer mice has been documented in:

Wobeser G, M Ngeleka, G Appleyard, L Bryden, and MR Mulvey. 2007. Tularemia in deer mice (*Peromyscus maniculatus*) during a population irruption in Saskatchewan, Canada. Journal of Wildlife Diseases 43:23-31.

♦♦

♦♦

Did you donate blood 13 years ago to screen for hantavirus?

"Hantavirus and arenavirus antibodies in persons with occupational rodent exposure, North America" has recently been published by Charles F. Fulhorst, Mary Louise Milazzo, Lori R. Armstrong, James E. Childs, Pierre E. Rollin, Rima Khabbaz, C.J. Peters, and Thomas G. Ksiazek. The article is published in **EMERGING INFECTIOUS DISEASES 13 (4): 532-538 APR 2007** and can be viewed at http://www.cdc.gov/eid/content/13/4/532.htm

Samples were obtained from volunteers attending the following meetings: American Society of Mammalogists meeting (Washington, DC, 1994), Wildlife Disease Association meeting (Pacific Grove, California, 1994), Southwestern Association of Naturalists meeting (Emporia, Kansas, 1994), Wildlife Society meeting (Wenatchee, Washington, 1994), 16th Vertebrate Pest Conference (Santa Clara, California, 1994), and Colorado Pest Control Meeting (Denver, Colorado, 1994).

Antibodies against Sin Nombre Virus were detected in 4 (0.5%) of the 757 persons in the study.

♦♦

Also published by the Centers for Disease Control is **"Threat of Hantavirus Pulmonary Syndrome to Field Biologists Working with Small Mammals"** by DA Kelt, DH Van Vuren, MS Hafner, BJ Danielson, and MJ Kelly. The article is published in **EMERGING INFECTIOUS DISEASES 13 (9): 1285-1287 SEP 2007** and can be viewed at http://www.cdc.gov/eid/content/13/9/1285.htm

These authors determined the risk of acquiring hantavirus pulmonary syndrome for a biologist working with rodents in the field was very low and the recommendations for wearing personal protective equipment should be reviewed and revised.

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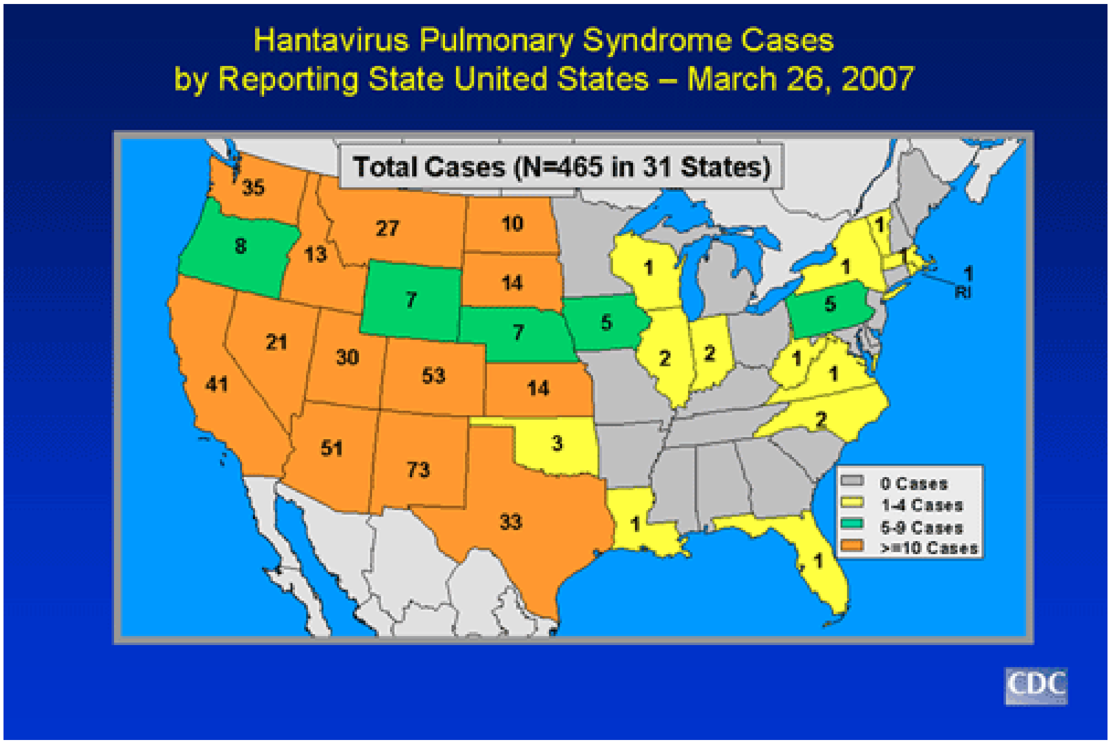
Hantavirus Hoax: The Centers for Disease Control and Prevention (CDC) has received several inquiries about an e-mail report of a stock clerk who became infected with hantavirus while working in a storeroom. According to the e-mail message, the infection resulted from exposure to dried rodent droppings that were contaminated with hantavirus. The e-mail message warns the reader to take precautions when handling items such as soda cans and grocery packages (for example, cereal boxes) because they may be contaminated with hantavirus.

The e-mail report is untrue. CDC could not substantiate this report of a hantavirus infection, nor has CDC been asked to participate in an investigation of the incident described in the e-mail.

♦♦

From May 1993 through March 26, 2007, a total of **465 cases of hantavirus pulmonary syndrome** have been reported in the United States. Thirty-five percent of all reported cases have resulted in death. Cases have been reported from 30 states, including most of the western half of the country and some eastern states. Over half of the confirmed cases have been reported from areas outside the Four Corners area.



♦♦

THE *PEROMYSCUS* GENETIC STOCK CENTER

General

The University of South Carolina has maintained a genetic stock center for *Peromyscus* (deer mice and congeneric species) since 1985. The center was established under a grant from the Living Stocks Collection Program of the National Science Foundation and continues to be supported by NSF and the NIH Biological Models and Materials Research Program. It also receives support from the University and from user fees.

The major function of the Stock Center is to provide genetically characterized types of *Peromyscus* to scientific investigators and educators. Continuation of the center is dependent upon significant external utilization, therefore potential **users are encouraged to take advantage of this resource**.

Policies and Procedures

The Stock Center maintains several categories of stocks of living animals: 1) Closed colony random-bred[1] "wild-type" stocks of seven species of *Peromyscus*. 2) Two highly inbred[2] stocks of "wild-type" *P. leucopus*. 3) Stocks of fifeen coat color mutations, mostly in *P. maniculatus*. 4) Stocks of eight other monogenic traits. The Stock Center operates in strict compliance with the Animal Welfare Act and is located in an AAALAC approved facility. All animal care is performed by certified technicians. Stocks are monitored regularly for presence of disease and parasites and are free of hantavirus and 15 murine viruses.

The Stock Center also provides blood, organs, tissues, fetuses, skins and other biological materials from *Peromyscus.* The Stock Center operates a Molecular Bank where selected genomic libraries and probes are available. Other resources include a reference collection of more than 2,500 reprints of articles on peromyscine rodents, copies of which may be provided. The Stock Center is the primary sponsor of ***Pero*Base**, an on-line database dedicated to information regarding *Peromyscus* and closely related species.

Sufficient animals of the mutant types generally can be provided to initiate a breeding stock. Somewhat larger numbers, up to about 50 animals, can be provided from the wild-type stocks. Animals requested in greater numbers frequently require a "breed-up" charge and some delay in shipment.

Orders and Pricing

A user fee is charged for animals or materials provided by the Stock Center. A schedule of fees is shown on the next page. Fees vary with species and type of service provided. User assumes the cost of all shipment. Animals lost in transit are replaced without charge. Tissues, blood, skins, *etc*. are supplied at a modest fee that includes technician time. Arrangements for special orders will be negotiated. Billing will be submitted upon satisfactory delivery. **Write or call for details or special requirements.**

SCHEDULE OF USER FEES

Item	Academic and Government	Commercial
MATURE ANIMALS (each)		
Wild-type Stocks		
Smaller species (*P. maniculatus, P. polionotus, P. leucopus, P. eremicus*)	**$ 22.50**	**$35.00**
Larger species (*P. californicus, P. melanophrys, P. aztecus*)	**30.00**	**40.00**
Mutant and Inbred Stocks	**30.00**	**40.00**
Pregnant females (Smaller species)	**40.00**	**50.00**
(Larger species)	**55.00**	**65.00**
Special Attention (Diet, *etc*.)	**40.00**	**50.00**
F_1 Species Hybrids	**30.00**	**40.00**
TISSUE SAMPLES (Per sample)		
Solid	**25.00**	
Fluid (Blood, urine, saliva, etc.) per ml	**40.00**	
Flat skins (each)	**35.00**	
MOLECULAR MATERIALS		
Extracted DNA, 20 µg	**100.00**	
PCR Primers (500 µl @ 10 µM)	**10.00**	
Genomic & cDNA libraries	**300.00**	

OTHER CHARGES

Shipping costs = actual shipper's charges plus cost of mouse containers, packaging.

Lab fee for sample preparation.

Breed-up fees (for orders exceeding 50 animals) = *per diem* cage charges X cages required.

STOCKS AVAILABLE

WILD TYPE STOCKS	**ORIGIN**
P. maniculatus bairdii (BW Stock) Deer Mouse	Closed colony bred in captivity since 1948. Descended from 40 ancestors wild-caught near Ann Arbor MI.
P. maniculatus sonoriensis (SM2 Stock) Sonoran Deer Mouse	Derived from about 50 animals wild-caught by Jack Hayes in 1995 near White Mountain Research Station CA.
P. polionotus subgriseus (PO Stock) Oldfield Mouse	Closed colony since 1952. Derived from 21 ancestors wild-caught in Ocala Nat'l. Forest FL. High inbreeding coefficient.
P. polionotus leucocephalus (LS Stock) Beach Mouse	Derived from beach mice wild-caught on Santa Rosa Island FL between 1987-1988 and bred by R. Lacy.
P. leucopus (LL Stock) White-footed Mouse	Derived from 38 wild ancestors captured between 1982 and 1985 near Linville NC.
P. californicus insignis (IS Stock) California Mouse	Derived from about 60 ancestors collected between 1979 and 1987 in Santa Monica Mts. CA.
P. aztecus (AM Stock) Aztec Mouse	Derived from animals collected on Sierra Chincua Michoacan, Mexico in 1986.
P. melanophrys (XZ Stock) Plateau Mouse	Derived from animals collected between 1970 and 1978 from Zacatecas, Mexico and bred by R. Hill.
P. eremicus (EP Stock) Cactus Mouse	Originated from 10-12 animals collected at Tucson AZ in 1993.

INTERSPECIFIC HYBRIDS	
P. maniculatus X P. polionotus F_1 Hybrids	Bred by special order.
P. leucopus X P. gossypinus F_1 Hybrids	Sometimes available by special arrangement.

[3]**COAT COLORS**	**ORGINAL SOURCE**
Blonde *bln/bln*	Mich. State U. colony (Pratt and Robbins, 1982)
Albino *c/c*	Sumner's albino deer mice (Sumner, 1922)
Ashy *ahy/ahy*	Wild-caught in Oregon ~ 1960 (Teed et al., 1990)
Black (Non-agouti) *a/a*	Horner's black mutant (Horner et al., 1980)
[4]Brown *b/b*	Huestis stocks (Huestis and Barto, 1934)
California blonde *cfb/cfb*	Santa Cruz I., Calif., stock (Roth and Dawson, 1996)
Dominant spotting *S/+*	Wild caught in Illinois (Feldman, 1936)
Golden nugget b^{gn}/b^{gn}	Wild caught *P. leucopus* (Horner and Dawson, 1993)
Ivory *i/i*	Wild caught in Oregon (Huestis, 1938)
Platinum *plt/plt*	Barto stock at U. Mich. (Dodson et al., 1987)
[4]Silver *sil/sil*	Huestis stock (Huestis and Barto, 1934)
Tan streak *tns/tns*	Clemson U. stock from NC (Wang et al., 1993)
Variable white *Vw/+*	Mich. State U. colony (Cowling et al., 1994)
White-belly non-agouti a^w/a^w	Egoscue's "non-agouti" (Egoscue, 1971)
Wide-band agouti A^{Nb}/a	Natural polymorphism U. Mich. (McIntosh, 1954)

OTHER MUTATIONS AND VARIANTS

Alcohol dehydrogenase negative	Adh^0/Adh^0	South Carolina BW stock (Felder, 1975)
Alcohol dehydrogenase positive	Adh^f/Adh^f	South Carolina BW stock (Felder, 1975)
Boggler *bgl/bgl*		Blair's *P. m. blandus* stock (Barto, 1955)
Cataract-webbed *cwb/cwb*		From Huestis stocks (Anderson and Burns, 1979)
Epilepsy *epl/epl*		U. Michigan *P. m. artemisiae* stock (Dice, 1935)
Hairless-1 *hr-1/hr-1*		Sumner's hairless mutant (Sumner, 1924)
Hairless-2 *hr-2/hr-2*		Egoscue's hairless mutant (Egoscue, 1962)
Juvenile ataxia *ja/ja*		U. Michigan stock (Van Ooteghem, 1983)
Enzyme variants		Wild type stocks provide a reservoir of variants (Dawson, 1983)

[1] "Random bred" without deliberate selection, sib-sib matings avoided. [2] Inbred lines bred by sib-sib and/or parent-offspring mating for 21 generations or more. [3]Unless otherwise noted, mutations are in *P. maniculatus*.
[4]Available only as silver/brown double recessive.

Other Resources of the *Peromyscus* Stock Center

Highly inbred *P. leucopus* (I_{30+}) are available as live animals or as frozen tissues. Two lines developed by George Smith (UCLA) are currently maintained by the Stock Center.

Limited numbers of other stocks are on hand, but not currently available. Inquire.

Preserved or frozen specimens of types given in the above tables.

Flat skins of mutant or wild-type coat colors of any of the stocks listed above.

Reference library of more than 2500 reprints of research papers, articles and reports on *Peromyscus.* Single copies of individual articles can be photocopied and mailed. Please limit requests to not more than five articles at any given time. There will be a charge of 10 cents per photocopied page after the initial 20 pages.

Photocopies of back issues of *Peromyscus Newsletter* ($5 ea.) or single original back copies, when still available, without charge.

Materials are available through the *Peromyscus* Molecular Bank of the Stock Center. Allow two weeks for delivery. Included is purified DNA or frozen tissues of any of the stocks listed above. Several genomic libraries and a variety of molecular probes are available. (Inquire for more information)

For additional information or details about any of these mutants, stocks or other materials contact: Janet Crossland, Colony Manager,* Peromyscus *Stock Center, (803) 777-3107, e-mail crosslan@biol.sc.edu

PLEASE CALL WITH INQUIRIES

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New Molecular Resources for *Peromyscus*!!!!

100,000 Expressed Sequence Tags

In May 2007 the Stock Center was informed of a successful grant proposal to the Department of Energy's Joint Genome Institute to sequence 50,000 clones in both directions to generate 100,000 expressed sequence tags from the following 6 normalized libraries:

1. Brain and testes from *P. maniculatus bairdii* (BW) – 10,000 clones
2. Brain and testes from *P. polionotus subgriseus* (PO) – 10,000 clones
3. E12-14 embryos from *P. maniculatus bairdii* (BW) – 7,500 clones
4. Newborn *P. maniculatus bairdii* (BW) – 7,500 clones
5. Liver, kidney, and skin from *P. maniculatus bairdii* (BW) – 7,500 clones
6. Spleen from *P. maniculatus bairdii* (BW) – 7,500 clones

The ESTs are expected to be available by Spring 2008.

Full Genome Sequence

In September 2007 the National Human Genome Research Institute approved a proposal for 6x coverage of the *P. maniculatus bairdii* (BW) genome. In addition, NHGRI also approved 2x coverage of *P. polionotus subgriseus* (PO), *P. leucopus* (LL), and *P. californicus insignis* (IS).

The sequences are expected to be available by Summer 2008. To view a copy of the proposal go to:

http://stkctr.biol.sc.edu/Peromyscus_NHGRI_whitepaper.pdf

Beach Mouse Captive Population Feasibility Workshop
Topsail Hill Preserve State Park, FL
7 – 9 March 2007

Summary of:

Traylor-Holzer, K. and R.C. Lacy (eds.). 2007. *Beach Mouse Captive Population Feasibility Workshop Final Report*. IUCN/SSC Conservation Breeding Specialist Group, Apple Valley, MN.

Background

The US Fish and Wildlife Service and the IUCN/SSC Conservation Breeding Specialist Group, sponsored a meeting of Fish and Wildlife Service employees, researchers, and zoo personnel in March to discuss the current status of the seven beach mouse (*Peromyscus polionotus* spp.) populations and determine if any of them would benefit from a captive breeding program. Five subspecies occur along the coast of the Gulf of Mexico and two subspecies occur on Florida's Atlantic coast. All subspecies except for the Santa Rosa beach mouse (*P. polionotus leucocephalus*) are federally listed as endangered or threatened. Because they live in the dunes along beaches highly prized by real estate developers, their habitat is frequently destroyed by construction. Although they have suitable habitat on publicly held lands, these refuges are scattered and unconnected. Such fragmented habitat increases their vulnerability to domestic cats, hurricanes, and inbreeding. All subspecies have endured some local extinctions and several populations have been re-established with translocation efforts.

Meeting Format

After an overview of the status of each beach mouse subspecies, impacts of hurricanes on mice and habitat, summaries of population viability analyses, and the effects of previous translocation efforts, the participants identified the population threats and goals for each subspecies, and how various captive management options may reduce these threats. The group identified the following as potential goals of a captive population:

1. Provide an insurance policy against subspecies extinction.
2. Provide a source population for reintroduction into new habitat or habitat from which beach mouse populations have been extirpated.
3. Provide a source for demographic supplementation of small populations.
4. Provide a source for genetic supplementation of small (inbred) populations.
5. Preserve a genetic reservoir to guard against sudden population bottlenecks.
6. Preserve unique genetic lines to guard against loss of local genetically distinct populations.

7. Serve as ambassadors through education outreach to reduce threats associated with human activities.
8. Provide research opportunities to gain knowledge of the species and to improve the effectiveness of management actions.

The discussion then moved to captive management issues including inbreeding effects and adaptation to captivity, as well as an overview of the Key Largo wood rat (*Neotoma floridana smalli*) breeding and reintroduction program as a potential model for a similar program for beach mice. The participants then split into two smaller working groups, one discussing short-term and the other discussing long-term captive strategies. Three short-term and six long-term strategies were identified, and for each strategy considered, the working groups summarized:

1. Roles and benefits
2. Risks
3. Challenges/Obstacles
4. Knowledge/Data Gaps
5. Resources Needed

After the two groups reconvened, they also discussed cryopreservation and other assisted reproductive techniques, as well as the pros and cons of their suggested strategies vs. no captive management. Finally, they made their subspecies-specific recommendations.

Short-Term Management Strategies

1. Seasonal Holding: Holding program in which mice are captured on *an annual basis* in anticipation of possible catastrophes (e.g., hurricanes or extremely low summer numbers). Mice would not be bred and would be released if no catastrophe occurred. If a catastrophe occurs, these mice would be used to initiate a long-term breeding program.

2. Rescue/Emergency: Capture and holding of mice *immediately before* an anticipated catastrophe or when population numbers are low or face high risk of extinction. Similar to seasonal holding, mice would not be bred and would be released if feasible. If catastrophe events prevent release, this would evolve into a long-term breeding program.

3. Short-Term Colony: Planned, structured approach in which mice would be captured, held, and bred *in anticipation of an upcoming reintroduction opportunity*. This approach would entail an established end date and might be used to offset some of the impacts from large developments and beach nourishment projects.

Working group recommendation: For all subspecies, evaluate the need for a rescue response and develop rescue plans as appropriate in the event of

impending hurricanes and other foreseeable catastrophes expected to significantly impact or decimate beach mouse populations. Other short-term strategies were thought to be too costly or risky compared to their anticipated benefits and were not recommended for implementation. For these reasons, the group preferred *in situ* (in the wild) management over development of a seasonal holding (#1 above) or short-term colony (#3 above) strategy.

Long-Term Management Strategies

1. Supplementation Colony: Breeding population maintained in a semi-natural setting, likely managed with periodic transfers to and from the wild population. Used to *supplement declining wild populations* and possibly for outreach.

2. Traditional Colony: Large breeding population of mice in a controlled laboratory environment. Would be intensively *managed to retain genetic diversity*, either as a closed colony (no exchange with the wild) or open colony (periodic exchange with the wild or other captive populations).

3. Semi-Natural Enclosure: Breeding population in a semi-natural enclosure in an attempt to mimic the natural environment as close as possible to *minimize adaptation to captivity*. May be managed fairly rigorously, or with a more hands-off approach, and carefully monitored.

4. Experimental Population: Breeding population placed in unoccupied habitat outside the current range. Would provide *invaluable research opportunities* and potentially could be used for reintroduction. More likely used for less threatened subspecies, as the removal of mice is less likely to negatively affect the donor population.

5. Long-Term Holding: Population established as a *protective measure* in the face of an impending natural disaster (e.g., hurricane) or human-related threat (e.g., extensive development) that may destroy known habitat. Mice could be held up to 2 years, and would be released when suitable habitat is again available. May be used for outreach.

6. Education / Exhibit: Ambassador mice to be used in *public outreach programs*, particularly in zoological institutions. Comprised of mice of non-endangered subspecies or surplus animals from other programs. Facilities could also be used as an emergency resource for holding rescued mice prior to and following a catastrophic event.

Working group recommendations:
For the most endangered subspecies, the Perdido Key, Choctawhatchee and Alabama beach mice – the establishment of a supplemental or semi-natural colony may be desirable but only if the wild population can withstand the removal

of individuals to establish a captive population. Such removals might need to be opportunistic (e.g., rescued mice that cannot be returned to the wild).

For less threatened subspecies – supplemental colonies, traditional laboratory colonies, and/or semi-natural colonies were recommended in varying degrees and combinations to provide backup secure populations and maximize research opportunities.

Although *in situ* (in the wild) conservation efforts such as minimizing habitat loss and fragmentation, reducing threats to mouse populations and habitat, and using management strategies such as habitat restoration and translocation are critical to endangered species such as the beach mouse, these *in situ* management actions were not addressed by this meeting, which focused solely on captive options. This does not imply that they are not considered important to beach mouse management or should not be implemented. Furthermore, any recommendations for captive management do not imply that these strategies should replace *in situ* conservation efforts, but rather should be viewed as an additional potential management tool.

Copies of *Beach Mouse Captive Population Feasibility Workshop Final Report* can be ordered through the IUCN/SSC CBSG office (office@cbsg.org or www.cbsg.org).

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NOTICE

***PEROMYSCUS NEWSLETTER* IS NOT A FORMAL SCIENTIFIC PUBLICATION.**

THEREFORE…

INFORMATION AND DATA IN THE CONTRIBUTIONS SECTION SHOULD NOT BE CITED OR USED WITHOUT PERMISSION OF THE CONTRIBUTOR.

THANK YOU!

◄►

Peromyscus maniculatus As an Animal Model of Restricted Repetitive Behavior in Autism

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Restricted, repetitive behavior is one of three core features of autism along with deficits in social behavior and language and communication (DSM-IV, American Psychiatric Association, 1994). Such behavior includes stereotyped motor movements (e.g., body rocking, hand flapping), repetitive manipulation of objects, compulsions, rituals, and insistence on sameness (Lewis & Bodfish, 1998). These behaviors frequently dominate the daily activity of children with autism and can significantly interfere with opportunities to develop functional behaviors. Repetitive behaviors are also commonly observed in other neurodevelopmental, psychiatric, neurological, and genetic conditions such as mental retardation, Tourette's syndrome, Fragile-X syndrome, Rett syndrome, Parkinson's disease, and schizophrenia (Frith & Done, 1983; Turner,1999; Lewis & Bodfish, 1998).

Little is known about the neurobiological basis of abnormal repetitive behaviors in children with autism and related neurodevelopmental disorders. This lack of knowledge precludes effective early intervention and prevention strategies. Appropriate animal models could provide a wealth of information about the neurobiological mechanisms mediating the development and expression of such persistent, fixed, and habitual behavior (Lewis et al., 2007). Animal models relevant to abnormal repetitive behavior in humans generally fall into three classes: repetitive behavior associated with targeted insults to the CNS; repetitive behavior induced by pharmacological agents; and repetitive behavior associated with restricted environments and experience (Lewis et al., 2007).

Our lab has employed *Peromyscus maniculatus* as a model of restricted repetitive behaviors. In this model, deer mice exhibit repetitive hindlimb jumping and backward somersaulting as a consequence of environmental restriction operationalized as being reared in standard laboratory caging. These behaviors do not require social isolation, specific cues or contexts, or a pharmacological agent for induction. These behaviors occur at a high rate, appear relatively early in development, persist across much of the life of the animal, and show considerable heterogeneity in individual levels of expression. These features make *Peromyscus* an appealing model of the repetitive sensory motor behaviors characteristic of autism and related neurodevelopmental disorders. Moreover, animal models focused on the sequelae of experiential restriction are relevant to

the clinical disorder given that the early occurrence of social, communicative and adaptive behavior deficits in very young children with autism likely markedly attenuate experience-dependent behavioral and brain development. Of interest is the fact that repetitive motor behavior appears to be an invariant consequence of experiential deprivation or restriction of most species tested.

Our studies indicate that starting at or shortly after weaning (PND21), approximately 80% of deer mice proceed to develop high rates of stereotypy over the next 60 days when housed under standard laboratory conditions. Such development can be largely attenuated by housing these mice in larger, more complex environments over that same time period (Powell et al., 2000; Hadley et al., 2006). As we have shown, older adult deer mice do not benefit as much as younger mice by such an enriched environment (Hadley et al., 2006), suggesting that there is a sensitive period for the prevention or attenuation of stereotypy after which long-term experience-dependent neurobiological alterations may result in the behavior being relatively irreversible.

The efficacy of environmental complexity in attenuating or preventing stereotyped behavior in deer mice behavior led to the question of relevant neurobiological mechanisms. Environmental enrichment has been reported to be associated with a wide range of CNS effects including dendritic branching, spine density, synaptogenesis, angiogenesis, gliogenesis, gene expression, apoptosis, and neurogenesis (Lewis, 2004). Our results (Turner & Lewis, 2003; Turner et al., 2002; 2003) indicated that enrichment-related brain differences were observed only in deer mice that “benefited” from enrichment as defined by prevention/attenuation of stereotypy and that these differences were regionally selective for motor cortex and basal ganglia. These results point to the importance of cortical-basal ganglia circuitry in the development and expression of repetitive behavior in deer mice.

The primacy of cortical-basal ganglia circuitry in the mediation of stereotypy in *Peromyscus* was supported by more direct pharmacological and biochemical studies conducted in our lab. We have shown that stereotypy in deer mice was attenuated selectively via intrastriatal administration of either the D1 dopamine receptor selective antagonist SCH23390 or the NMDA receptor-selective glutamate antagonist MK-801 (Presti et al., 2003). These results show that interruption of glutamatergic cortical projections to striatum or dopaminergic projections to striatum from substantia nigra can selectively reduce spontaneous stereotypy. Furthermore, we showed that repetitive behavior in deer mice was associated with an imbalance in the activity of the direct and indirect pathways of the basal ganglia, favoring overactivity of the direct pathway (Presti & Lewis, 2005).

Our current research is focused on neuroadaptive changes in specific striatal cell types associated with the development of stereotypy in deer mice. Although the deer mouse model has a number of advantages, it is not an optimal model for

addressing questions about the genetics of abnormal repetitive behavior. We await further progress in assembling the *Peromyscus maniculatus* genome.

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Regulatory T cell-like responses in deer mice persistently-infected with Sin Nombre virus

Summary of:

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We initiated this project in 1998 when it was becoming apparent that the immune response was, in part, responsible for the pathogenesis of hantavirus cardiopulmonary syndrome (HCPS) in humans. At the time, substantial progress had been made in understanding the ecology of Sin Nombre virus (SNV) infection of deer mice, the virology of human and deer mouse infections, and the pathology associated with fatal HCPS cases. However, virtually no work was being conducted to understand the immunology of hantavirus infections in rodent reservoirs. This was largely because very little was known about deer mouse immune systems (a couple of papers that described gamma and mu immunoglobulins), let alone their responses to infectious agents, thus such examination seemed intractable.

Because so little was known about deer mouse immune systems, we had to develop methodologies for propagating deer mouse T cells, which must have been involved in SNV responses since high-titered antiviral IgG was produced in deer mice, and to clone cytokine and related genes for the development of detection assays. Because several mammalian genomes had been sequenced, we were able to used degenerate PCR to amplify and clone relevant orthologous sequences from deer mice. It took us about 7 years to develop these methodologies, but once they were in place we were able to address the important questions in rather short order.

Initially, we suspected that deer mouse immune responses at persistence might be mediated by T helper type 2 (Th2) cells and a type II immune response. However, we did not observe a clear polarization of such cells; IFNg, a type I cytokine produced by Th1 cells, was expressed by some virus-specific deer mouse T cells, and IL-5, produced by Th2 cells, was also expressed by some of the T cells. At about this time, the first papers were being published that

described a new group of CD4+ T cells that were referred to as regulatory T cells (Treg cells). Although several subsets of Treg cells are now recognized, they all have one common feature; they suppress inflammatory responses in a cytokine- and cell contact- dependent manner. With a lack of apparent immune polarization, we began to focus on genes associated with Treg cells, including transforming growth factor beta and the Forkhead box P3 (FoxP3) transcription factor. Fortunately, these genes are highly-conserved among mammalian species and were relatively easy to clone.

Once we had these genes, we were in a position to profile CD4+ T cell subsets based upon gene expression of IFNg, tumor necrosis factor and lymphotoxin (Th1 cells), IL-4 and IL-5 (Th2 cells), or TGFb or IL-10 (Treg cells) by real-time PCR. We then generated polyclonal virus-specific T cell lines from persistently infected deer mice and all expressed TGFb, a cytokine produced by Treg cells and a potent anti-inflammatory cytokine. Treg cell responses are associated with persistent infection with many other viruses, including human pathogens. However, since hantaviruses cause apparently innocuous infections in their rodent reservoirs, there may be little consequence for such infections, which may result in the natural maintenance of the virus.

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What affects survival of deer mice? Investigating the effects of environmental factors, individual characteristics and infection with Sin Nombre virus

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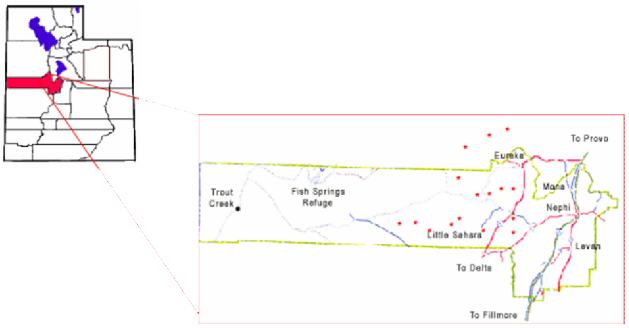
Until recently, the role played by endemic pathogens in the dynamics of their host has been overlooked. There is a growing amount of evidence showing that parasites can affect life-history parameters of their host and influence their population fluctuations (e.g., Hudson et al. 1998, Telfer et al. 2002).

Hantavirus strains are endemic in a number of rodent and shrew species. In general, these viruses are perceived to be asymptomatic and cause chronic infections in their host (Bernshtein et al. 1999, Botten et al. 2000). However, some studies have found pathologic changes due to infection with hantaviruses, including edema in the lungs and periportal hepatitis (Lyubsky et al. 1996, Netski et al. 1999). Others have found reduced body mass gain in infected individuals (Douglas et al. 2007, Childs et al. 1989) or lethargic behavior suggestive of illness caused by hantavirus (Fulhorst et al. 2002).

The deer mouse (*Peromyscus maniculatus*) is the primary reservoir of Sin Nombre virus (SNV), a hantavirus that has been extensively studied since a human outbreak in the four-corners region of the United States. SNV infection in deer mice is chronic (Netski et al. 1999) and the virus is transmitted horizontally, apparently through aggressive interactions (Calisher et al. 2007). With the goal of contributing to resolving the controversy about the effects of hantavirus infections on their host, we asked whether deer mice infected with SNV had lower survival than uninfected individuals. We addressed this question in the context of other factors that can also affect survival probabilities of the studied deer mice populations. More specifically we investigated the role of human disturbance, seasonality, climate, density and individual characteristics.

We conducted seasonal (spring & fall) live-trapping in 18 different sites located in the Tintic valley of central Utah. All sites are dominated by sagebrush and juniper trees with different degrees of disturbance caused by the use of off-road vehicles (ORV). A web of 148 live-traps was set and checked for 3 nights at each site. Animals were marked, identified, weighted and sexed. Before releasing the animals, a blood sample was collected to test for antibodies to SNV using an enzyme linked immunosorbent assay (ELISA).



We used generalized linear mixed models (GLMM) to estimate the probability of surviving to the following season, based on several fixed factors (sex, weight, infection status, season, density, degree of disturbance, and dry or wet climatic conditions) and a random factor (site). We tested the effects of two-way interactions thought to be important and the main effects of each factor.

During the five years of this study, we captured a total of 3104 unique adult individuals with 6567 total captures. Among females, 13% were infected, whereas infected males represented 20% of the total males captured. More than 90 percent of the captured individuals did not survive to the following season. The factors that were found to affect the probability of surviving to the next season were season, deer mice density, and the interactions between the degree of disturbance and climatic conditions, and sex and infection status.

Here we want to comment about the highly significant effect ($p << 0.01$) of the interaction between sex and infection status. Survival probabilities for males were twice that of females (Fig. 1). Infection with SNV had a greater effect on females than males. Infected males had 10% lower survival probabilities than uninfected males; whereas for females, SNV infection reduced their survival by half (Fig. 1).

Our results agree with an observed decreased survival of young deer mice naturally infected with SNV in Montana (Douglas et al. 2001), and a decreased winter survival of bank voles infected with Puumala hantavirus (Kallio et al. 2007). In conclusion, infection with SNV appears to negatively affect survival of deer mice, and of females in particular, in combination with an array of different environmental factors.

Acknowledgements

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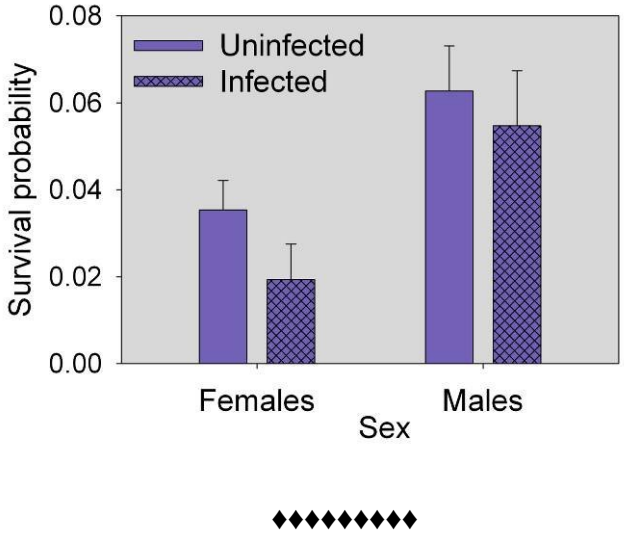
Figure 1. Least square means estimates for the interaction between sex and SNV infection status, a significant term in the GLMM model for the survival probabilities of deer mice.



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Hantavirus Pulmonary Syndrome Case at the U.S. Air Force Academy

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In July 2006, a 58-year-old man camping at a recreational facility at the U.S. Air Force Academy (USAFA) contracted a fatal case of hantavirus pulmonary syndrome (HPS). This was the first documented HPS case in El Paso County, and the seventeenth HPS-related fatality in Colorado since 1993 (www.cdphe.state.co.us/dc/zoonosis/hanta/index.html). In this note, we review the circumstances associated with this HPS case, and describe the USAFA and public response to this tragic incident.

A variety of ecosystems and a great diversity of wildlife are found on the 18,455 acres that comprise the USAFA reservation. Along this part of the Colorado Front Range, the majority of the USAFA land is unique, as it remains relatively undisturbed by human intervention and development. The base residential complexes, academic and training facilities, and recreational sites are proximate to wildlife habitat, and a variety of mammalian species are regularly observed around or even within these areas and the associated human structures. Our live-trapping activities over the last several years reveal that the deer mouse (*Peromyscus maniculatus*) is the most common and ubiquitous small mammal on the USAFA; individuals captured at higher (western) and lower elevations (eastern) compare favorably with *P. m. rufinus* and *P. m. nebrascensis*, respectively.

Various natural circumstances impacted the local deer-mouse populations and may have contributed to an increased risk of hantavirus infection in 2006. Although the Sin Nombre virus (the pathogenic hantavirus in the western U.S.) is presumably present in deer-mouse populations throughout Colorado, the geographic and temporal prevalence of seropositive deer mice is surprisingly variable (Calisher et al. 2002; Yates et al., 2002). The ecological and demographic factors affecting the incidence of infected individuals are undoubtedly complex; however, the mild winters and wet springs of 2005 and 2006 are consistent with high population density, which is generally associated with higher prevalence of seropositive deer mice (Calisher et al. 2002; Yates et al., 2002). Additionally, the possible negative impact of West Nile virus on local raptor and corvid populations (LaDeau et al. 2007) would have reduced avian predation on rodents and other small mammals, and thereby indirectly contributed to increased numbers of deer mice. Indeed, our USAFA trapping

activities revealed that the deer-mouse population was exceptionally high during 2005 and 2006. With this unusually high population of deer mice, the potential for human contact and hantavirus exposure was greatly increased.

The duration and location of the victim's camping activities, and the consequent infestation of his motor home, unfortunately exposed him to large numbers of deer mice. The victim and his wife were long-term residents (approximately three months at the time of infection) of the Peregrine Pines FamCamp, which is situated in prime deer-mice habitat (Fig. 1). The general area of this large recreational site comprises Ponderosa pine (*Pinus ponderosa*) with a thin Gambel [scrub] oak (*Quercus gambelii*) understory, interspersed with small open fields. The ready accessibility to and food availability within the interior of the victim's motor home resulted in significant deer-mouse infestation; the USAFA's pest-management contractor later trapped 27 deer mice from the vehicle. The hantavirus infection likely occurred while the victim was cleaning deer-mouse nests and droppings from the enclosed storage compartments beneath his motor home.

In his efforts to sanitize the motor home, the victim experienced the classic risk factors for HPS: contact with aerosolized deer-mouse urine and droppings in a relatively confined space. The victim noticed the onset of symptoms (fever and aches) on 11 July 2006 (Tuesday). His condition worsened later in the week, with respiratory involvement developing over the weekend. He was admitted to the USAFA Hospital that weekend or early the second week, and placed on respiratory support. HPS was subsequently confirmed by serology. The victim was transferred to the ICU of a Colorado Springs hospital as his condition further deteriorated. He died 19 July (Wednesday), eight days after the onset of symptoms. [Note: HIPAA rules preclude our access to the details of his admittance to and transfer from the USAFA Hospital.]

Although this HPS case was an isolated incident, USAFA officials moved quickly to ensure public safety and to address public concerns. An *ad hoc* "Hantavirus Working Group," composed of personnel from multiple mission elements (USAFA Hospital, Safety, Bioenvironmental, Public Health, Public Affairs, Biology, Legal, Civil Engineering, and Natural Resources), was convened on 20 July to explore and implement the appropriate measures in risk mitigation and public information. Through press releases, base-wide emailings, flyers (prepared by Public Health and the Department of Biology, and distributed at the FamCamp and other outdoor facilities), briefings, and newspaper articles, the USAFA increased its efforts to inform base residents, outdoor-training leaders, and visiting campers of hantavirus, HPS, deer mice, and risk-mitigation measures. Because the newly arrived basic cadets would soon begin a two-week outdoor-training program, letters were sent to assure their parents of the appropriate efforts to ensure safety and to minimize risk. The victim's motor home was isolated at the FamCamp, and the pest-management contractor

removed the deer mice infesting the vehicle; automobile insurance covered the cost of professional decontamination.

In the aftermath of this tragic event, the reaction of USAFA personnel and residents was completely rational and appropriate. Colorado residents already have a general awareness of hantavirus risk, and the prompt dissemination of information by USAFA officials further precluded unreasonable alarm within the community. With the onset of cold weather last year, *The Academy Spirit* (the USAFA weekly newspaper) published a brief article reminding base residents to guard against deer mice taking up residence in their homes. A positive long-term response is the increased level of awareness and vigilance of facilities managers and base residents. For example, on request the following week, Public Health and Department of Biology personnel conducted an assessment at the USAFA Stables; our inspection revealed large quantities of unsecured and spilled horse feed that, not surprisingly, attracted hundreds of rodents to the barn room. Soon after our assessment, the Stables acquired a stilted grain silo that excludes mice and that dispenses feed with minimal spillage. More recently, our assessment of the USAFA Observatory revealed considerable deer-mouse activity; in preparation for increased utilization of this facility, a contract was issued for professional cleaning, decontamination, and "mouseproofing."

Because the situation of facilities and homes on the USAFA and the ongoing development of various areas in the county are conducive to contact between humans and deer mice, this increased awareness of hantavirus is clearly beneficial in promoting vigilance and risk mitigation. However, this positive consequence of the HPS case should not obscure the great personal tragedy for the victim and his family and friends. It is somewhat ironic that the victim contracted HPS on a simple camping trip, while those of us who have closely worked with hundreds of *Peromyscus maniculatus* over multiple decades have apparently not been infected by hantavirus.

Figure 1. The FamCamp site at which the victim and his wife were long-term campers.

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First report of Hantavirus occurrence in the nimble-footed mouse, *Peromyscus levipes*, in northern Mexico

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Hantavirus pulmonary syndrome (HPS) is a zoonosis caused by members of the genus *Hantavirus*, family *Bunyaviridae*. Humans usually become infected with hantaviruses by inhalation of aerosolized droplets of urine, saliva, or respiratory secretions from infected rodents. Sin Nombre virus is the major cause of HPS in the United States and Canada, and other hantaviral species have been associated with HPS in North America. In Mexico, hantaviruses are disregarded due to limited knowledge about reservoir ecology, epidemiology, and epizootiology. Nevertheless, surveys in states that border with Mexico (Arizona, New Mexico, Texas) have shown the presence of hantavirus in several rodent species that also occur in Mexico (Mantooth et al. 2001; Mills et al. 1999). No survey aimed to detect these kinds of viruses has been done in the corresponding states in Mexico (Chihuahua, Coahuila, Nuevo Leon and Tamaulipas).

We investigated the prevalence of hantavirus at a protected area in NE Mexico, which has a unique mix of Neartic and Neotropical small mammal species (Vargas-Contreras and Hernandez-Huerta 2001). We conducted fieldwork in El Cielo Biosphere Reserve (ECBR) which is located ca. 300 miles from the border with Texas (23 03'42" N and 99 12'18" W). High habitat heterogeneity and diversity characterize the eastern facing slopes of ECBR with a steep elevational gradient from 100 to 2000 m where three major vegetation types occur: Tropical Subdeciduous Forest (TSDF), Cloud Forest (CF) and Pine-Oak Forest (POF) (Puig and Bracho 1987). Our work was done at TSDF and CF localities. At each locality we sampled all major microhabitats using Sherman live trap transects of 200 traps each. Blood samples were collected with Nobuto strips, air-dried and stored in separate plastic bags until analyzed in the laboratory (Mills et al. 1995). Whole blood samples from rodents were tested for IgG antibodies by using an inactivated SNV recombinant nucleocapsid protein

antigen by an indirect enzyme-linked immunosorbent assay (ELISA) according to a standardized protocol (Feldman et al. 1993; CDC 1994). Serologic tests detected the presence of an active immune response to a hantavirus, and positive findings with SNV antigens in the IgG ELISA indicate infections with North American hantaviruses.

Trapping effort consisted of 6,032 trapnights that resulted in 568 capture events (9.42% success rate) of 248 individuals. We obtained blood samples from 199 individuals that represent all the 15 species we captured (*Peromyscus pectoralis*, *P. levipes*, *P. ochraventer*, *Sigmodon toltecus*, *O. couesi*, *O. chapmani*, *O. rostratus*, *L. irroratus*, *B. taylori*, *Oligoryzomys fulvescens*, *Rattus rattus*, *Reithrodontomys fulvescens*, *Mus musculus* and *Cryptotis obscura*). Only one species, *P. levipes*, had 8 individuals (ca. 26%) that were positive for the presence of hantavirus antibodies. Many of the small mammal species in the communities at ECBR have been mentioned as potential reservoirs of hantaviruses in Mexico (Sanchez-Cordero et al. 2005) but only the dominant species in the CF, the nimble footed mouse (*P. levipes)*, had individuals seropositive to hantavirus. This is the first time hantavirus antibodies have been reported in this species and this is the first evidence for the state of Tamaulipas. Although no association to human disease from rodents in this state has been reported further research in this area is needed.

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3800 m elevation slows growth in *Peromyscus maniculatus*

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Understanding how organisms partition energy when resources become limiting is an instructive first step towards appreciating both the physiological ecology of a species and the evolution of energy budgets. When faced with a hypoxic challenge (high altitude), young growing mammals may respond by using energy that otherwise would have been allocated to growth for maintenance of existing tissues. Conversely, an organism may accelerate growth of those organs or organ systems involved with oxygen acquisition at the cost of decreased whole animal growth rate or individual organ size. As another alternative, growth rate or adult size may not be compromised at high altitude.

As a first step towards understanding differences in growth, and thus energy partitioning across altitudes, we measured post-natal growth rates in two laboratory colonies of *Peromyscus maniculatus sonoriensis*. This subspecies is particularly well suited for characterizing life at high altitude because it inhabits a wide altitudinal range, from below sea level to above 4000 m.

We measured body mass, snout-rump length (SR), and hindfoot length (HF) from 3-42 days of age in two groups of deer mice: the first group underwent gestation and growth at 340 m elevation (low-born, LB); the second developed at 3800 m (high-born, HB). We fitted each character to a Gompertz growth curve (Figure 1). Additionally, using the derivative of the Gompertz equation, we calculated the maximum growth rate of each character, each animal's age when it achieved the maximum growth rate of each character as well as the size of the character during its maximum growth rate (Figure 1). We compared overall curve shape and maximum growth rate characteristics separately by gender using an analysis of covariance, with litter size, parity, and family as covariates

In both males and females, LB mice were heavier at birth, and older when they achieved their maximum rate of mass gain compared to HB mice. LB females also did not achieve as high a rate of mass gain as those born at high altitude. However, in other traits, it appears that males show more differences across altitudes than females: for all three traits (body mass, SR, and HF), male mice at high altitude are younger when they reach their maximum rate of growth, while there is no difference in SR or HF in female mice between altitudes. Additionally, LB males achieve a higher final body mass than HB males.

Our models predict no difference in HF, but they do predict larger body size (as measured by SR) and faster mass gain in LB mice. This, taken with our finding that HB mice are younger when they achieve maximum growth rates of both SR and body mass, suggests that (a) differences in energy partitioning may exist across altitudes in *P. m. sonoriensis*, (b) mice at high altitude are likely doing the majority of growth early in life, presumably to ensure the ability to reproduce in an "uncertain" environment, and (c) growth in males and females may be affected differently by high altitude; female mice may be better buffered against hypoxia because of the need to support pregnancy. Here, we also note that neither mothers nor offspring were resource-limited at either altitude; all were given *ad libitum* food, water, and bedding.

P. maniculatus sonoriensis
Photo by Gregory A. Russell

Acknowledgments

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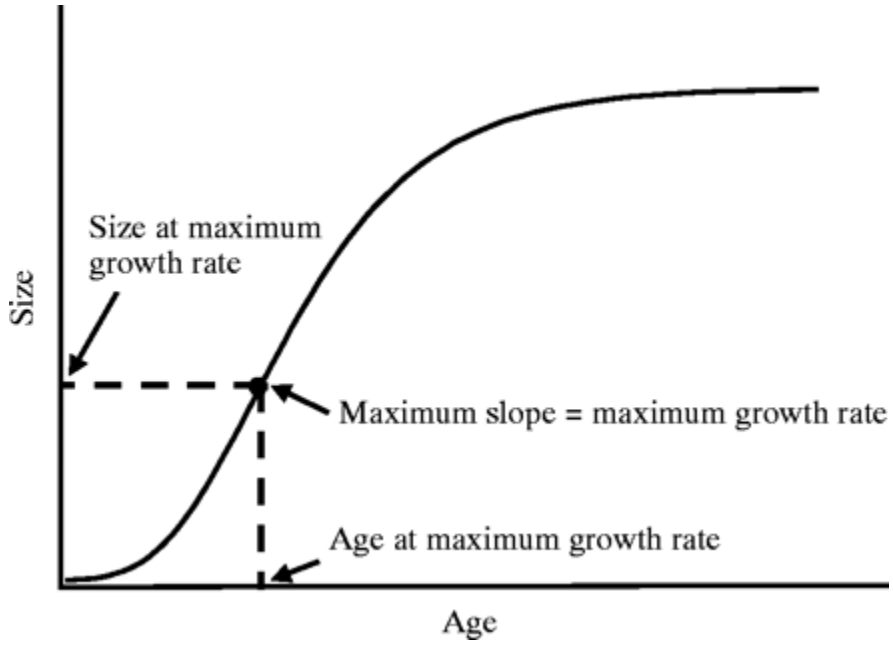
Figure 1. Gompertz growth curve showing the three calculated variables used in analyses of offspring growth (after Kristan, D.M. (2002) *J. Exp. Biol.* **205**:3697).



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The Effects of Diet and Social Stress on Humoral and Cell-mediated Immunity in *Peromyscus leucopus*

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As human disturbance creates habitats that are increasingly fragmented and unstable, wild animal populations can face greater levels of physical, nutritional, and social stress than they would normally experience. Our previous research showed that white-footed mice (*Peromyscus leucopus*) from disturbed habitat patches exhibited decreased humoral and increased cell-mediated immune responses compared with mice from undisturbed patches. Our objective was to determine which stressor, or which combination of nutritional and social stressors, has a greater effect on the immune system in order to better understand the effects of anthropogenic disturbance on white-footed mouse populations. We hypothesized that social stress, in the form of high density, and dietary stress, in the form of low protein, have similar effects on immune function.

Forty-eight adult male *P. leucopus* were trapped in Calloway County, Kentucky. Mice were kept in the lab for a 30-d acclimation period and maintained under standard laboratory conditions with a 14L:10D photoperiod. Mice were then randomly assigned to one of four experimental groups. A 2x2 experimental design was used with two density levels and two levels of dietary protein. High density was defined as two mice per cage while low density was defined as one mouse per cage. Mice in the high protein group were fed a diet containing 30% protein by weight and mice in low density groups were fed a diet containing 5% protein by weight. Caloric content of both diets was held constant and the diets differed only in the amount of protein contained. Mice were fed their assigned diet beginning on day 0 of the experiment. On day 2, blood was collected from each mouse while anesthetized using retro-orbital bleeding. Blood was then used for initial measurements of hemagglutination. Serum was also collected and used for initial measurements of corticosterone. Mice in high density groups were paired on day 5. On day 12, the humoral immune system of each mouse was stimulated using an IP injection of a 10% sheep RBC (SRBC) solution. In order to stimulate the cell-mediated immune system, pokeweed was injected into a randomly selected hind foot on day 15. The other foot was given an injection of an equal amount of sterile saline. Foot thickness was measured immediately before injection and 24 h after injection using a digital caliper. On day 17, blood was again collected from mice while anesthetized via retro-orbital bleeding and used for the same tests as the initial measurements.

We rejected our null hypothesis. By examining the difference between initial and final corticosterone levels, we found that the mice did, in fact, experience at least acute stress. In terms of immunity, low dietary protein had a significant negative effect on the cell-mediated immune response in white-footed mice compared with their counterparts fed a high protein diet (Fig. 1). In contrast, high density was associated with a significant negative effect on the humoral immune response (Fig. 2).

White-footed mice, known vectors of Lyme disease and Hantavirus, occur in close proximity to humans in residential and agricultural areas. Increased social stress and decreased nutritional stress have been associated with white-footed mice living in habitats exposed to these types of anthropogenic disturbances. Based on our results, we predict that social stress alone is sufficient to reduce humoral immune function in the wild, thereby increasing the risk of contracting and transmitting infectious diseases from white-footed mice populations to adjacent animal and human populations.

Acknowledgements

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Figure 1. High density had a significant negative effect (± 1 SE) on the humoral immune response (two-way ANOVA, P=0.002).

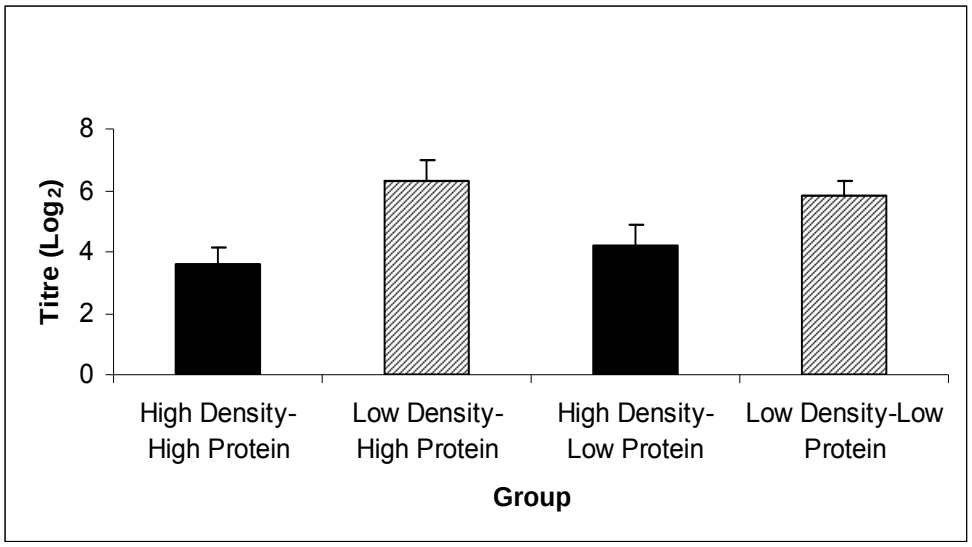
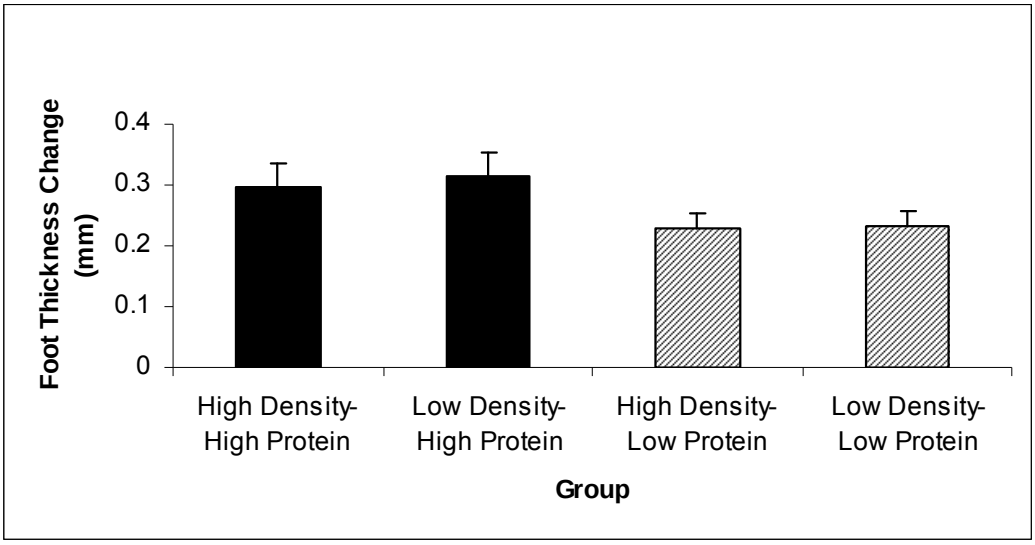


Figure 2. Low protein had a significant negative effect (± 1 SE) on the cell-mediated immune response (two-way ANOVA, *P*=0.0413).



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Apoptosis and Proliferation levels during *Peromyscus* Placental Development

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Two different species of the Rodent genus *Peromyscus* produce interspecies hybrids when in captivity. When bred, *P. maniculatus* (strain – BW) and *P. polionotus* (strain- PO) produce parent – of – origin specific reciprocal embryos and placentas. Hybrid dysgenesis is seen in the large offspring (PO x BW – female is always presented first) while the small offspring (bw x po – lower case to denote size difference) is viable and fertile. Investigations regarding *Peromyscus* hybrids suggest that the hybrid dysplasia is particularly pronounced in the placenta.

The placenta acts as an interface for nutrient and waste exchange between the mother and fetus. Disconcerted development of the placenta can have deleterious effects even if there are no inherent perturbations in the embryo or mother (Wake 1978; Cross 2000; Fisher 2000). The extra-embryonic tissue can survive and grow without an embryo (molar pregnancy) while an embryo cannot survive and grow without extra-embryonic tissue (Wake 1978). The study of placental development in normal and growth - affected litters highlights how little we know about its development across species and genus.

The earliest stages of *Mus* extraembryonic tissue formation occur at the 16 cell stage. At this time the outer cell layer is separate from the inner cell layer and gives rise to the trophectoderm. At E 8.0 the chorioallantoic attachment occurs. Labyrinth development takes place by E10.5, and a placenta is considered mature at E14.5 (Cross 2005). Normal placental growth shows increasing proliferation and organization of the placenta until E14.5 when it is considered mature (Watson 2005). Sporadic apoptosis, on the other hand, starts as early as initial embryonic gene expression (2 cell stage) and increases until birth (Huppertz 2005).

Apoptotic and proliferation levels were used to study the rapid weight gain seen in the PO x BW placenta and the small size of the bw x po placenta previously investigated (Vrana 2000). BrdU labeling identified proliferating cells while TUNEL labeling identified apoptotic cells (Figure 1 a, b, and c). PO/BW displayed high proliferation and low apoptosis at E10.5. Proliferation all but

stopped at E13.5, and no proliferation was seen at E16. At the same time, the amount of apoptosis in the placenta continued to increase.

Both the PO x BW and bw x po placenta's had shifted proliferation curves. Their placentas reached almost 100 units more proliferation than PO/BW, although at different times. At E10.5, the PO x BW apoptosis and proliferation are near zero, thereafter, proliferation increases sharply, reaching its highest level at E13.5. The proliferation curve has a slight descent, equalizing to the level of apoptosis. The apoptosis curve increases steadily across development.

Initially (E10.5), bw x po placenta has apoptotic features 7 times higher than PO/BW. This apoptotic level is accompanied by high levels of proliferation. Soon, at E13.5, both apoptosis and proliferation are drastically reduced to near zero units. A slight increase in both curves is seen at E16.

The locations of apoptosis and proliferation in PO/BW and PO x BW placentas were as expected when compared to *Mus* species (Huppertz 2005; Jurisicova 2005). The highest levels of proliferation in the bw x po were centered at the spongiotrophoblast layer and maternal deciduas with apoptotic cell locations found throughout the placenta (Data not shown).

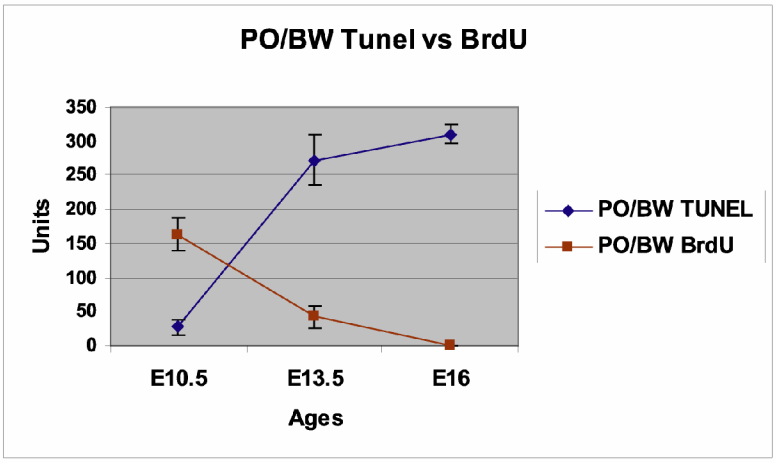
PO x BW and bw x po placenta have opposite placental size, differences in proliferation, and drastic differences in levels of apoptosis. PO x BW placentas has been shown to proliferate past the stage of PO/BW, while apoptosis was at normal levels. The increased proliferation rate corresponds to investigations of increased placental size, possibly linking these two events.

The bw x po has a smaller yet viable placenta. The small placenta, small embryonic size, and reduced spongiotrophoblast layer (Vrana 2000; Vrana 2001) could be linked to timing and levels of apoptosis, among other factors. These events result in embryo survival, albeit, at the cost of size to both embryo and placenta.

The placenta is a diverse and effective organ that allows for proper transfer of nutrient and waste between the embryo and mother. Improper timing and level of proliferation and apoptosis of the placenta can have a wide range of effects as demonstrated by *Peromyscus* hybrids.

Figure 1: Number of BrdU vs. TUNEL expressing cells in each placenta. A) PO/BW placentas, B) PO x BW placentas, C) bw x po placentas. The number of expressing cells were counted in each group and averaged. Error bars are equal to one standard deviation. All units are comparable across graphs. N ≥ 3 placentas were used for each group.

A.



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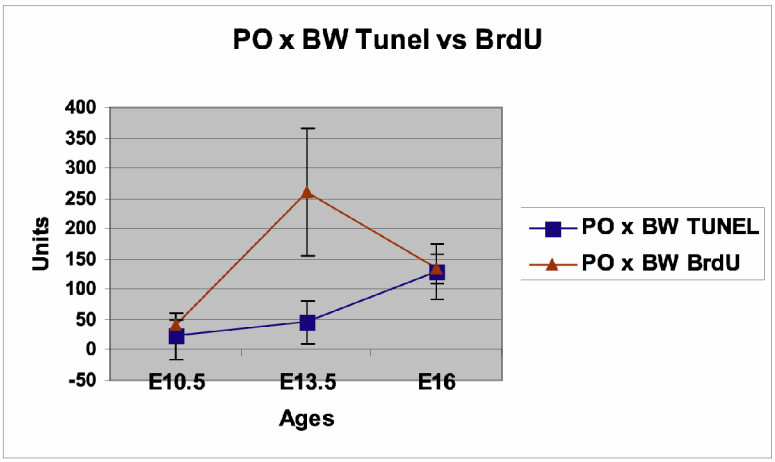

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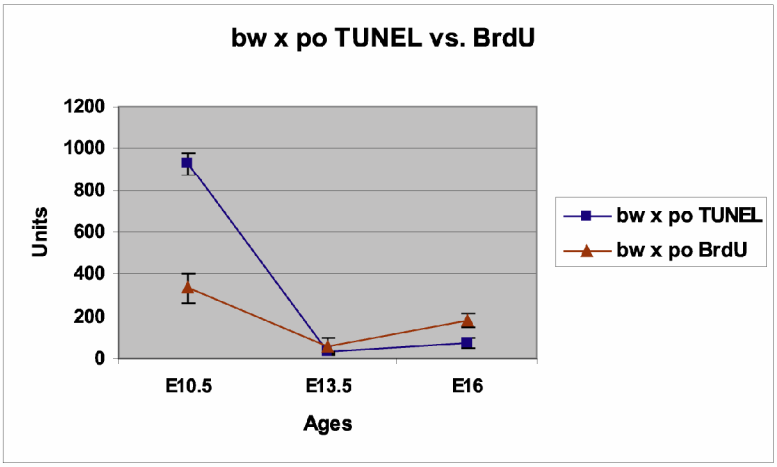

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Responses of *Peromyscus polionotus niveiventris* and *P. gossypinus* to prescribed fire and mechanical thinning of Florida scrub

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Responses of small mammals to habitat restoration techniques are species specific. Small mammal species can respond positively, negatively or neutrally to important habitat components changed by restoration treatments. For example, deer mouse (*Peromyscus maniculatus*) abundance increases immediately after prescribed burns and thinning of forest stands. Presumably, deer mice are able to exploit the habitat mosaic of vegetation patches and open spaces created by fire (Kaufman et al. 1983, 1990) and the complex microhabitats created as a result of forest thinning (Sullivan et al. 1999; Converse et al. 2006). Negative small mammal responses to prescribed fire restoration treatments stem from direct mortality of small mammal individuals (Erwin and Stasiak 1979; Haty et al. 1991). Therefore, restoration programs designed to improve degraded habitats may influence small mammal population dynamics.

My objective was to evaluate the responses of *P. p. niveiventris*, a federally listed small mammal, and *P. gossypinus* to prescribed fires, mechanical cutting, and a combination of fire and cutting treatments of coastal scrub at Cape Canaveral Air Force Station, Florida (Fig. 1). I compared relative abundance and demographic parameters from 18 land management compartments. Small mammals were live trapped in Sherman live traps, and mark / recapture techniques were used to study the populations.

I trapped 146 individual *P. p. niveiventris* (315 total captures), 130 *P. gossypinus* (300 total captures). The two species were captured in compartments under different management treatments. Number of first time captures of *P. p. niveiventris* appeared to be higher in compartments that were burned relative to other treatments, whereas the number of *P. gossypinus* appeared to be greater in compartments that were cut (Fig. 2). Repeated measure analysis of variance (RM-ANOVA) showed that the mean number of *P. p. niveiventris* captured at least once differed significantly among treatments (Table 1) with the mean number trapped in burned and cut and burned compartments significantly greater than fire suppressed, i.e., control (Table 2). Analyses of body mass and reproductive condition were not different among management treatments for *P. p. niveiventris*. There were no significant differences in the number of new captures, reproductive condition, and body mass for *P. gossypinus* among treatments (Table 1).

In summary, *P. p. niveiventris* and *P. gossypinus* had specific responses to land management treatments. *Peromyscus polionotus niveiventris* responded positively to compartments that had been prescribed burn; the number of first time captures was higher relative to other land management treatments. Although not statistically significant, there was a numeric response by *P. gossypinus* to mechanical cutting of scrub. My results suggest that the application of fire as a management strategy will favor populations of *P. p. niveiventris*, while mechanical cutting may benefit populations of *P. gossypinus*. However, longer term studies should be conducted to verify the patterns I have observed.

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I thank the small mammal field crew for helping with the rigorous demands of small mammal trapping. Their enthusiasm and hard work made this project possible. Personnel at the 45th CES / CEVR Wing at Cape Canaveral Air Force Station provided logistical support. This study was funded by the US Department of Defense.

Table 1. RM-ANOVA results on effects of land management treatments on the number of first time captures of two small rodent species. The *F* values of significant results are in bold. *P = 0.02, **P = 0.01

Small rodents	Treatment (*d . f.* = 3, 13)	Season (*d. f.* = 3, 39)	Season x Treatment (*d. f.* = 3, 39)
P. p. niveiventris	**4.33***	0.91	**2.81****
P. gossypinus	1.50	0.16	1.59

Table 2. Contrast analyses comparing the number of first time captures of *P. p. niveiventris* in scrub habitat under different land management treatments. The *F* values of significant results are in bold. *P = 0.01, **P = 0.04

Source	Contrast		
	Burned vs. Fire suppressed	Cut vs. Fire suppressed	Cut + burned vs. Fire suppressed
Seasons (*d. f.* = 1, 13)	2.70	1.51	4.07
Season x Treatment (*d. f.* = 3, 13)	**7.15***	2.27	**3.50****

Fig 1. Map of Cape Canaveral Air Force Station, FL USA, and location of land management compartments used to investigate small mammal responses to management treatments i.e., burned, cut, cut and burned, and fire suppressed.

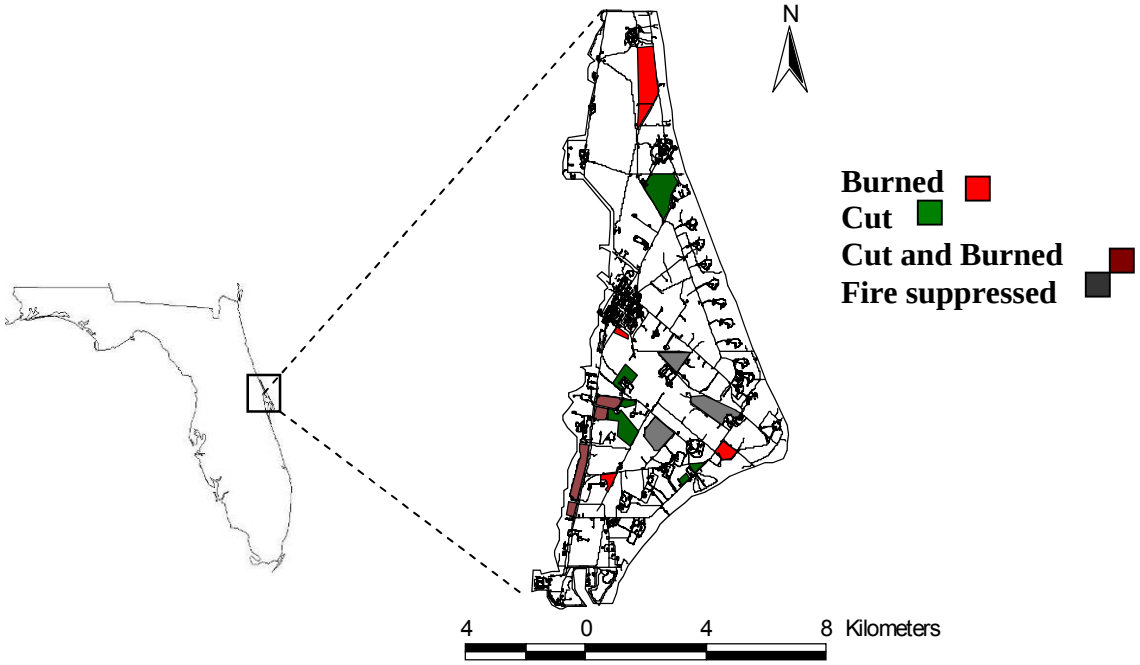
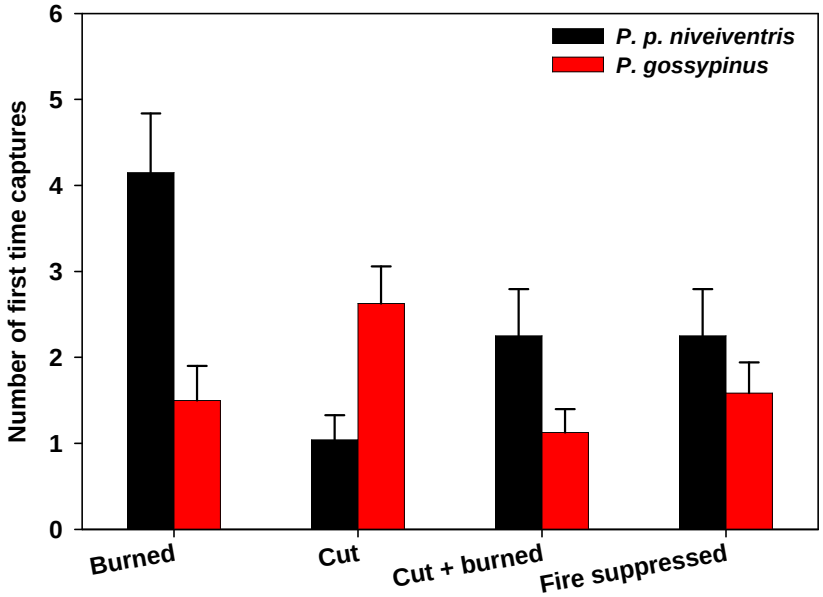


Fig 2. Mean (± 1 SE) number of *P. p. niveiventris* and *P. gossypinus* captured in coastal Florida scrub under different management strategies. Rodent populations were sampled during 2004-2005 field season.



Foraging behavior of beach mouse affected by moonlight and extreme cold

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The Alabama Beach Mouse (*Peromyscus polionotus ammobates*) is one of five federally endangered subspecies of beach mice endemic to the coasts of Alabama and Florida. Human encroachment on beach mouse habitat underscores the importance of understanding how natural and anthropogenic nocturnal light intensity affect foraging behavior. If increased nocturnal light intensity increases perceived risk of predation, then foraging activities should be constrained. This can lead to habitat degradation in areas surrounding the "footprint" of human developments. Also, if foraging activities are indeed a function of nocturnal light intensity, then rapid population censuses (like those conducted following hurricanes) may be biased by the particular moon phase during which the census occurred. Although we were primarily interested studying the effects of nocturnal light on foraging behavior, our experimental design easily allowed us to assess the effects of temperature on foraging behavior.

We quantified feeding behaviors of *P.p. ammobates* in experimental feeders containing fixed mixtures of sand and seeds. If higher light intensity increases the perceived risk of predation, then we expect foraging mice to require higher consumption rates to make this elevated risk worthwhile. As feeding reduces seed density, consumption rates will eventually fall below some threshold level and mice should give up on the feeders, leaving behind a measurable density of seeds (the giving up density, or GUD). GUD can also be influenced by temperature. On extremely cold nights, mice will require higher rates of consumption to offset the costs of exposure. Thus, GUD should be greater on nights that are relatively very cold.

We measured GUD in six feeders every day from February 6 to April 19, 2007 in a wildlife refuge unaffected by artificial light. We used a nocturnal light sensor sensitive to 0.01 mLux in order to quantify variation in light intensity resulting from each night's particular combination of moon phase, moon transit, and cloudcover. Minimum daily temperatures were obtained from weatherunderground.com for the nearby city of Gulf Shores, AL.

We created a multiple regression model of GUD using light intensity, minimum daily temperature, and time (duration of the study). Although our model

explained a small proportion of total variation in GUD (R^2 = 0.23), we found a positive and highly significant (P < 0.0001) relationship with light intensity, a negative and highly significant relationship with temperature (P < 0.0001), and no relationship with time (P = 0.97).

These results are consistent with our predictions that light and extreme cold degrade foraging conditions. No effect of time on GUD indicates that there was no seasonal variation in foraging conditions and that animals did not exhibit a behavioral response to prolonged exposure to the feeding stations. Bird et al. (2004) found that *P.polionotus leucocephalus* exploited fewer patches and consumed fewer seeds in patches located near light fixtures than those located farther away. More work is needed to quantify the effects of light on carrying capacities for beach mice.

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