

**Lyme Disease Survey at UNDERC:
Small Mammal Search for
Ixodes scapularis and
*Borrelia burgdorferi***

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1995

Abstract

From May 26 to July 24 small mammals were trapped in Gogebic County located in the upper peninsula of Michigan. These mammals were examined for various ectoparasites, with particular attention paid to locating *Ixodes scapularis*. The mammals were also surveyed for the Lyme disease spirochete *Borrelia burgdorferi*. 81 mammals of three species were trapped and examined. 23 ticks of 3 species, 19 fleas of the species *Orchopeas leucopus*, and 100+ lice of the species *Polypax auricularis* were found on the mammals. The tick species were *Dermacentor variabilis*, *Ixodes angustus*, and *Ixodes banksi*. Ear tissue biopsies taken to indicate the presence of the *Borrelia burgdorferi* spirochete were all found to be negative for the spirochete. Drag sampling was also used to survey for ticks in the area. 114 *Dermacentor variabilis* were found using this technique. 12 mouse nest boxes were set up throughout the area for the duration of the study. No ectoparasites were found using this method. No *Ixodes scapularis* were found throughout the course of the project, indicating that these ticks have not yet spread in detectable numbers to this particular part of the country. No *Borrelia burgdorferi* were present in the ear tissue of the mammals, indicating that the spirochete is not present in detectable amounts on the UNDERC properties.

Introduction

Ixodes scapularis is the tick vector of a number of human diseases, including Lyme Disease, ehrlichiosis, and babesiosis. *I. scapularis* exists in three forms throughout its two-year life cycle: larval, nymphal, and adult. Seasonal activity of *Ixodes scapularis* differs between each of the three stages. Peak activity of larvae occurs in late-July and early August, while peak activity of nymphs occurs in mid-June (Figure #2). Two peaks exist for the activity of adult *I. scapularis*; one occurring in late-May and the other occurring in mid-October (Fish.) *I. scapularis* needs a blood-meal from a host during each of the three stages of its life cycle. Larvae are not believed to be infectious, and therefore don't transmit the *Borrelia burgdorferi* spirochete. However, larvae are able to obtain the spirochete from the first blood meal, and transmit the spirochete as a nymph or an adult. Although the prevalence of spirochetes in nymphs (20-25%) is approximately half that found in adults, nymphs are responsible for nearly 90% of Lyme disease cases caused by *I. scapularis*. The small size of the nymph, and the coincidence of nymphal feeding activity with human outdoor activity are the basis for this trend.

Environmental factors, such as climate, soil type, and vegetation coverage affect the abundance and activity patterns of ticks and their vertebrate hosts (Mannelli, Kitron et. al.) The rate of development of *I. scapularis* is temperature dependent and areas of possible *I. scapularis* establishment must meet thermal requirements necessary for the maintenance of the population (Lindsay.) *Ixodes scapularis* is known or presumed to be endemic in 38 of the 70 counties of

Wisconsin(Herwaldt, Springs et.al.) Bayfield Co., the northern- most county in Wisconsin is the same latitude as Gogebic Co. , MI, which is the county in which the study sight is located. Bayfield Co. is endemic for *I. scapularis*. Price Co., WI, which is two counties to the west of Gogebic Co., and Marinette Co. WI, which is two counties to the east of Gogebic Co., are both endemic with *Ixodes scapularis* (Wis Epid Bull.)

The study was conducted on the 2,226 hectare UNDERC properties in Gogebic Co. MI, and in Langford campground of the Ottawa National Forest. The Notre Dame Vector Biology Lab began trapping small mammals on the UNDERC properties beginning in 1980. In 1984, the first *I. scapularis* was found at UNDERC, which was the first record of *I scapularis* in the upper peninsula. In 1992, the third specimen of *Ixodes scapularis* was collected at UNDERC (McCracken, et.al.) None have been collected since 1992, despite continued trapping No evidence of the *Borrelia burgdorferi* spirochete has been found at UNDERC, although a study in Menominee County, MI resulted in forty percent of the mice and sixty percent of the chipmunks testing positive for *B. burgdorferi*.

Materials and Methods

Trapping

From May 26 to July 24, 1995 small mammals were live trapped at the University of Notre Dame Environmental Research Center in Gogebic Co., MI, and at Langford Campground in the Ottawa National Forest. 3"x3"x9" Sherman live traps were operated. Traps were baited with peanut butter, and set out in the evenings about 1 hour before sunset, and checked in the morning about three hours after sunrise. Each site was trapped for five to seven days, with a line of five to ten traps each night. Three sites were trapped each night. The total

number of Sherman trap nights was 883 and the total success rate for the traps was 9.17%. 23 sites were trapped throughout the course of the project (Fig #1.).

Highest trapping success occurred in sites well covered with deciduous and coniferous trees on high, dry land. The presence of fallen trees and leaf litter were important factors that increased trapping success. Low, wet ground, areas with little tree canopy cover, and open grass lots yielded little or no trapping success. Placing the traps immediately next to fallen logs increased trapping success a large amount. Placing the peanut butter on the top of the inside of the trap also increased the success rate of the traps.

The traps containing mammals were collected every morning and taken to the Hank Research Laboratory on the UNDERC facility. Closed traps containing the mammals were placed in air tight plastic bags with two cotton-filled tubes soaked with Meto-fane anesthesia. The plastic bags remained closed for exactly 15 minutes, at which time the traps were removed, opened, and the mammal examined inside. More meto-fane was administered as needed in five minute increments using the same method as described. When the mammals "righting reflex" was no longer active, the mammal was placed into an enamel pan. A small veterinary ear punch was used to take an ear punch from each mammal captured. The ear punch was stored in a jar containing 95% ethanol, and flamed before and after every use. Ear punches were placed in a thirty percent glycerol/PBS cyroprotectant prepared by Glen Scoles of the Notre Dame VBL. The ear punches were then frozen in liquid nitrogen and stored for the summer.

After the ear punch was taken, the mammal was examined under low power using a stereo microscope. Special attention was paid to the ears, nose, eyes, vent, and to the area between the shoulder blades of each mammal. All ectoparasites were removed and placed in vials containing seventy percent

ethanol. A Meto-fane soaked cotton swab was placed by the nose of the mammal as needed during examination. Care was taken never to allow the Meto-fane to touch the mammal directly. After careful examination, the mammals were placed in individual shoe boxes, and allowed to revive for thirty to forty-five minutes. The mammals were then released at the site of original trapping.

The ear punches were taken to Ned Walker of Michigan State University, where they were unfrozen and placed in BSK II media and allowed to incubate. All tick identification was done by Dr. Walker.

Flagging

Flagging was done using a one meter x one meter square of flannel with weighted corners attached to a length of rope. The white flag was dragged by walking along roadsides with the flag dragging over vegetation. The flag was examined every 5 minutes, and ticks were removed and placed in ethanol for preservation.

Nest Boxes

The nest box design used is outlined in diagram #3. Twelve nest boxes were used for the summer. Six boxes were placed north of the UNDERC property in the Ottawa National Forest at Langford, Pomroy, and Moosehead campgrounds (Figure #4). Six boxes were placed on UNDERC property (Figure #1). All nest boxes were in place no later than June 12. Nest boxes were placed in areas with heavy tree cover, and pounded into the ground until the box was 12" above the ground. The nest boxes were checked every two weeks for signs of habitation of mice, and for ectoparasites.

Results and Discussion

Eighty-one mammals of three species were captured and examined for ectoparasites and for *B. burgdorferi*. The most abundant species captured was the Prairie Deer Mouse (*Peromyscus maniculatus*); sixty-eight of the eighty-one

mammals captured (84%) were *P. maniculatus*. Seven Eastern Chipmunks (*Tamias striatus*) were examined, and six White-footed Deer Mouse (*Peromyscus leucopus*) were examined.

Twenty-six ticks of three species were removed from the mammals. The most abundant tick species removed was *Dermacentor variabilis*. 21 of the 23 ticks were of this species. One *I. angustus* was found on July 5 at site 15 on a *P. maniculatus* (Figure #1). One *I. banksi* was found on June 6 at site 5 on a *P. maniculatus*. 19 fleas of the species *Orchopeas leucopus* were found on *Peromyscus maniculatus*. Over 100 specimens of lice were found on *P. maniculatus*, as well. All of the lice were of the species *Polypax auricularis*.

Approximately 40 *Collembola Sminthurade* were found on a *P. maniculatus* at site 2.

Results of flagging were similar to results found by Patrick Keaney in the summer of 1994. The highest distribution of ticks were found on the road next to Morris Lake. 47 *D. variabilis* were found during 3.5 hours of dragging this area throughout the summer. The road running from the North gate to Tenderfoot Creek was also well populated. 33 *D. variables* were found during 3 hours of dragging throughout the summer. One area that did not correlate with Keaney's data from last year was the road to Moccasin Lake. 1.5 hours of dragging this area produced no ticks of any kind. Also the west property road leading to Cranberry Lake produced no ticks of any kind (Figure 1.)

Results from the mouse nest boxes were disappointing. The boxes were distributed throughout different habitats and in different areas throughout the properties. The boxes were in use for seven weeks, and checked every two weeks. No evidence of habitation by *any* species was observed. The seven week period should have allowed ample time for habitation by some type of species. The habitats in which the boxes were placed ranged from low, wet ground, to

high, dry ridges. No conclusions have been drawn at this time to explain the lack of success of the nest boxes.

Seven of the eighty-one mammals caught with the Sherman traps were from Langford campground in the Ottawa National Forest (figure #4.) These mammals were of interest because no trapping had been done in this area before, and the area provided a different habitat than that of the UNDERC properties. No ectoparasites of any kind were found on the seven mammals, and all of the seven mammals appeared to be very healthy.

81 ear-tissue samples were tested for the *Borrelia burgdorferi* spirochete. All 81 samples tested negative for the presence of the spirochete, indicating that *B. burgdorferi* is not present in detectable amounts on the UNDERC property and the Langford Lake campground in the Ottawa National Forest.

The UNDERC properties and the properties immediately to the north of UNDERC were well scoured for ticks using these three techniques. The fact that no *I. scapularis* were found by any of the methods suggests that the UNDERC properties are relatively free from any population of *I. scapularis*. The fact that *I. scapularis* have not been found in any substantial number in the last ten years of searching on UNDERC suggests strongly that this assumption is true at this point in time. Furthermore, the lack of *B. burgdorferi* in any of the ear-samples suggests that the spirochete is not present in this area of the country.

Acknowledgments

I would like to thank Dr. George B. Craig, Jr. Without Dr. Craig's encouragement and guidance this project would not have been possible. Valuable trapping and personal safety advice was provided by Kay Stewart and Bob Spence of the Freimann Life Science Center. Dr. Ned Walker provided expertise in the field of tick identification and incubation of ear biopsies. Special thanks to Jeff Runde and to other members of the 1995 Practica in Aquatic Biology. Living allowances and tuition were provided by the Bernard J. Hank Family Endowment.

Literature Cited

- Anonymous, 1994. Lyme disease update-1993 *Wis Epidemiol Bull* 15: 1-3
- Fish, Durland. Environmental Risk and Prevention of Lyme Disease. *The American Journal of Medicine* 98: 2S-9S
- Ginsburg, Howard S. Ecology and Environmental Management of Lyme Disease. Rutgers University Press. New Brunswick, New Jersey.1993
- Herwaldt, Barbara, Fern E. Springs, Paul P. Roberts et. al. Babesiosis in Wisconsin: a Potentially Fatal Disease. *The American Journal of Tropical Medicine & Hygiene* 53:146-151
- Keaney, Patrick. 1994 The effectiveness of Various Trapping Techniques in Collecting *Ixodes scapularis* and Other Species of Ticks. unpublished
- Lindsay, L. Robbin, Ian Barker, et. al. Survival and Development of *Ixodes scapularis* Under various Climatic Conditions in Ontario, Canada. *Journal of Medical Entomology* 32(2) 142-151
- Mannelli, Alessandro, Uriel Kitron, Carl J. Jones, and Timothy L. Slajchert. Influence of Season and Habitat on *Ixodes scapularis* Infestation on White-footed Mice in Northwestern Illinois. *The Journal of Parasitology.* 80: 1038-1042
- McCracken, Kevin, David Amrol, Ned Walker, and George B. Craig, Jr., Ectoparasites from mammals of Michigan's Upper Peninsula. *Vector Control Bulletin of the North Central States.* 4(1):42-53
- Walker, Ned. Michigan State University. personal communication

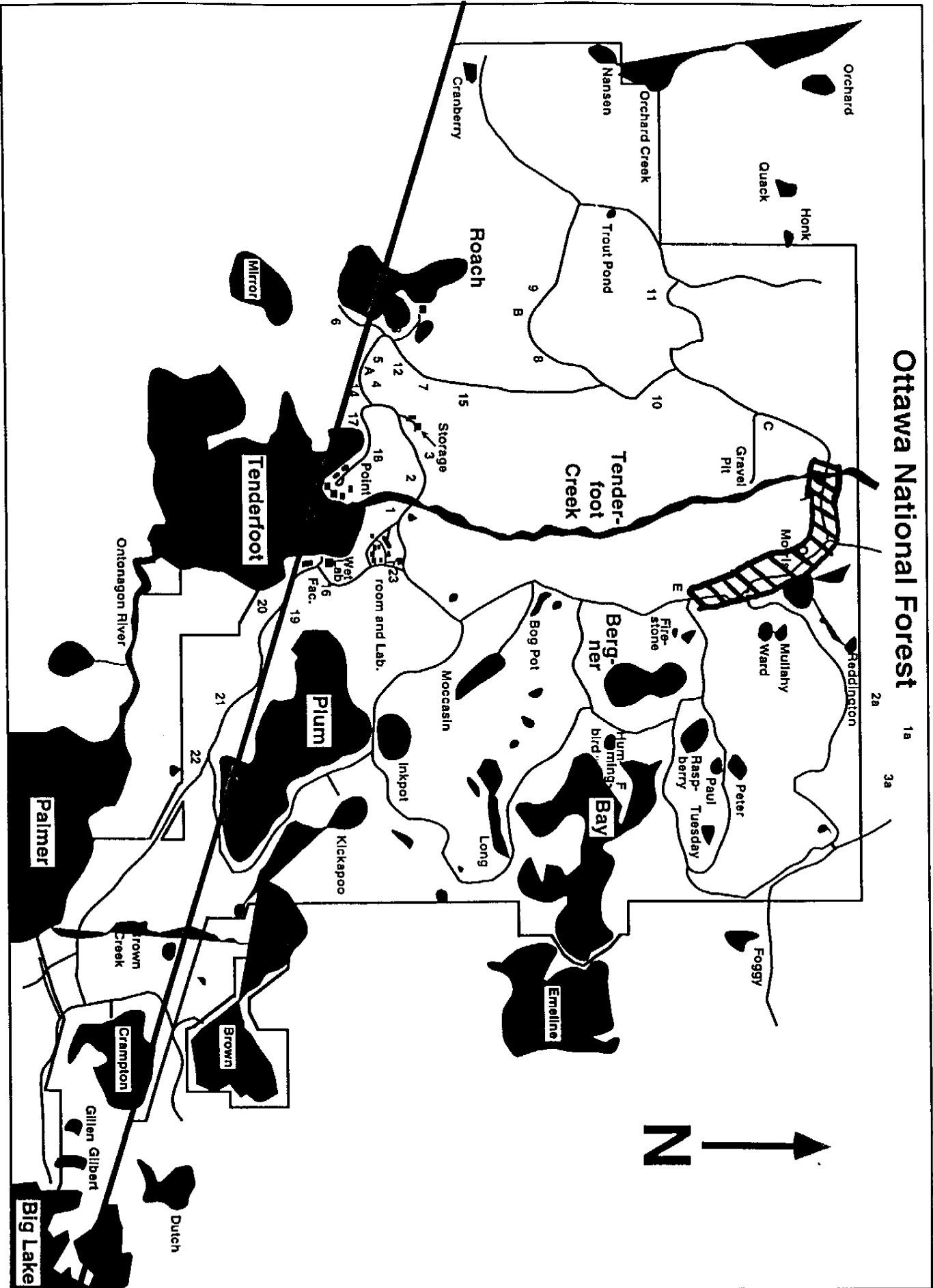


Figure 1. Trap sites on UNDERC properties

A,B,C,D,E,F = Mouse Nest-box locations



= High success areas in dragging for ticks

SEASONAL ACTIVITY OF *Ixodes dammini* WESTCHESTER CO., NY 1987-1989

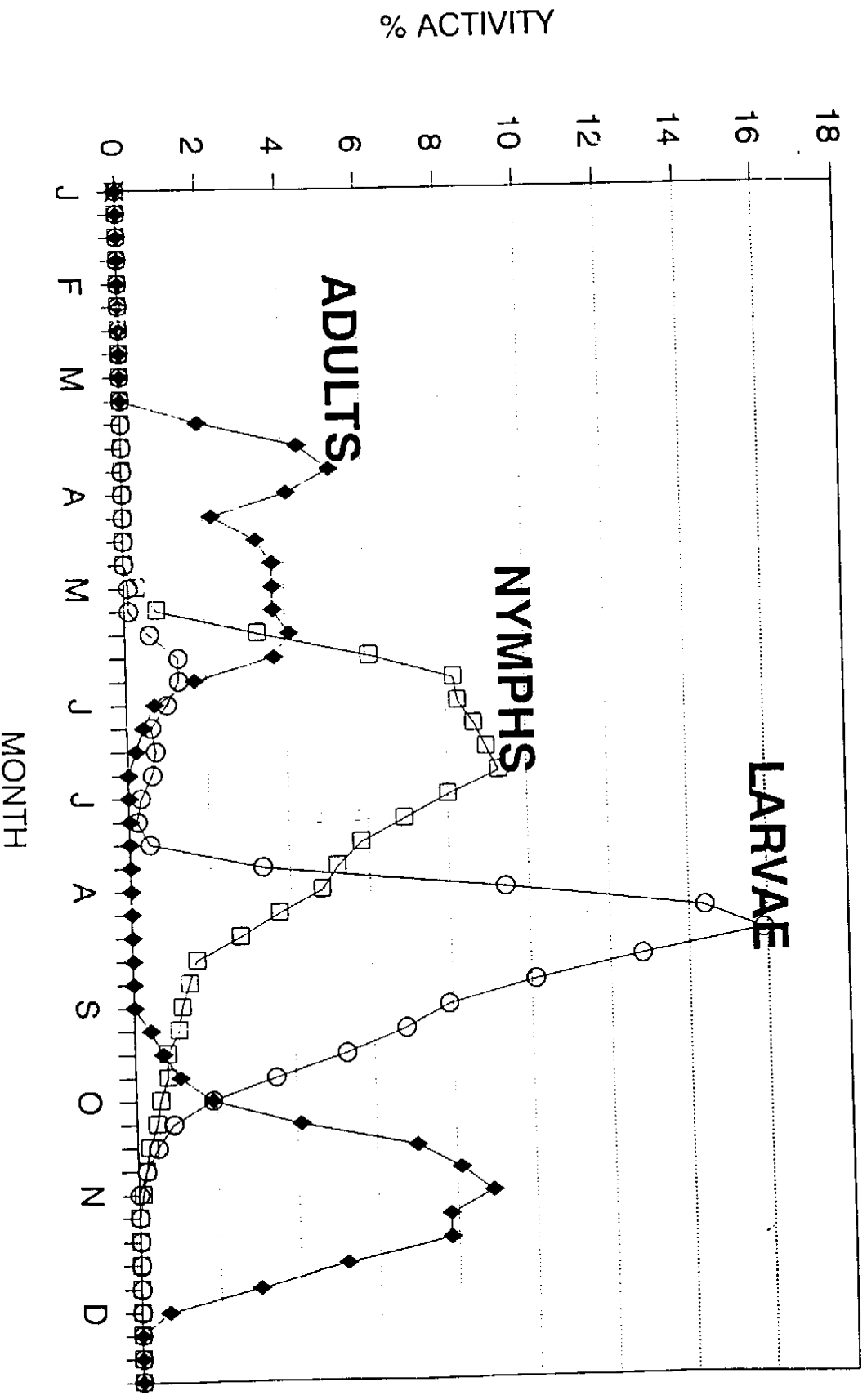
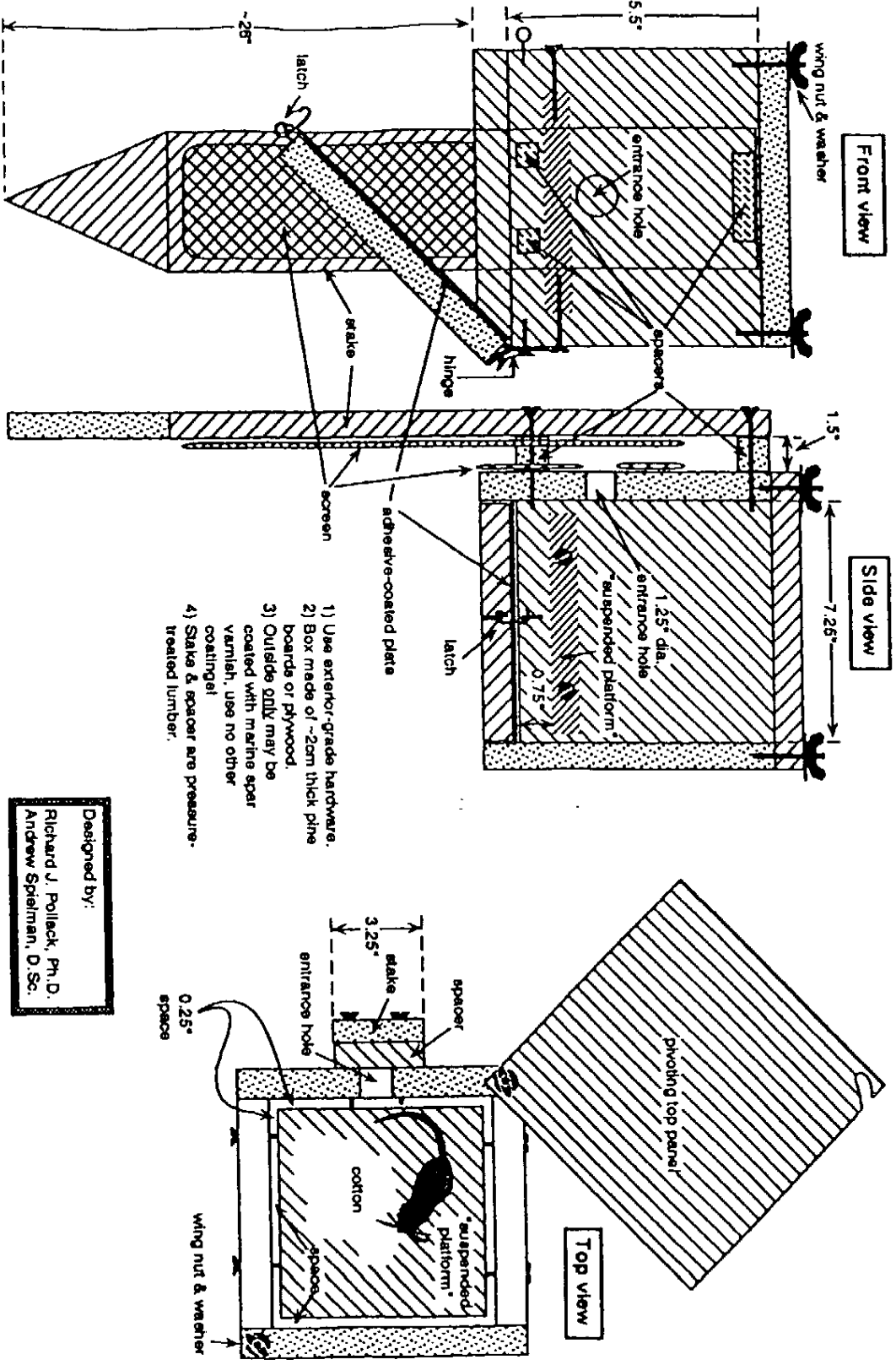


Figure 2.

Figure 3. Nest -box design

An artificial nest box for collecting ectoparasites from small mammals.



- 1) Use exterior-grade hardware.
- 2) Box made of ~2cm thick pine boards or plywood.
- 3) Outside ONLY may be coated with marine spar varnish; use no other coating!
- 4) Slake & spacer are pressure-treated lumber.

Designed by:
 Richard J. Pollack, Ph.D.
 Andrew Spielman, D.Sc.

Figure 4. Ottawa Nest-box and trap locations

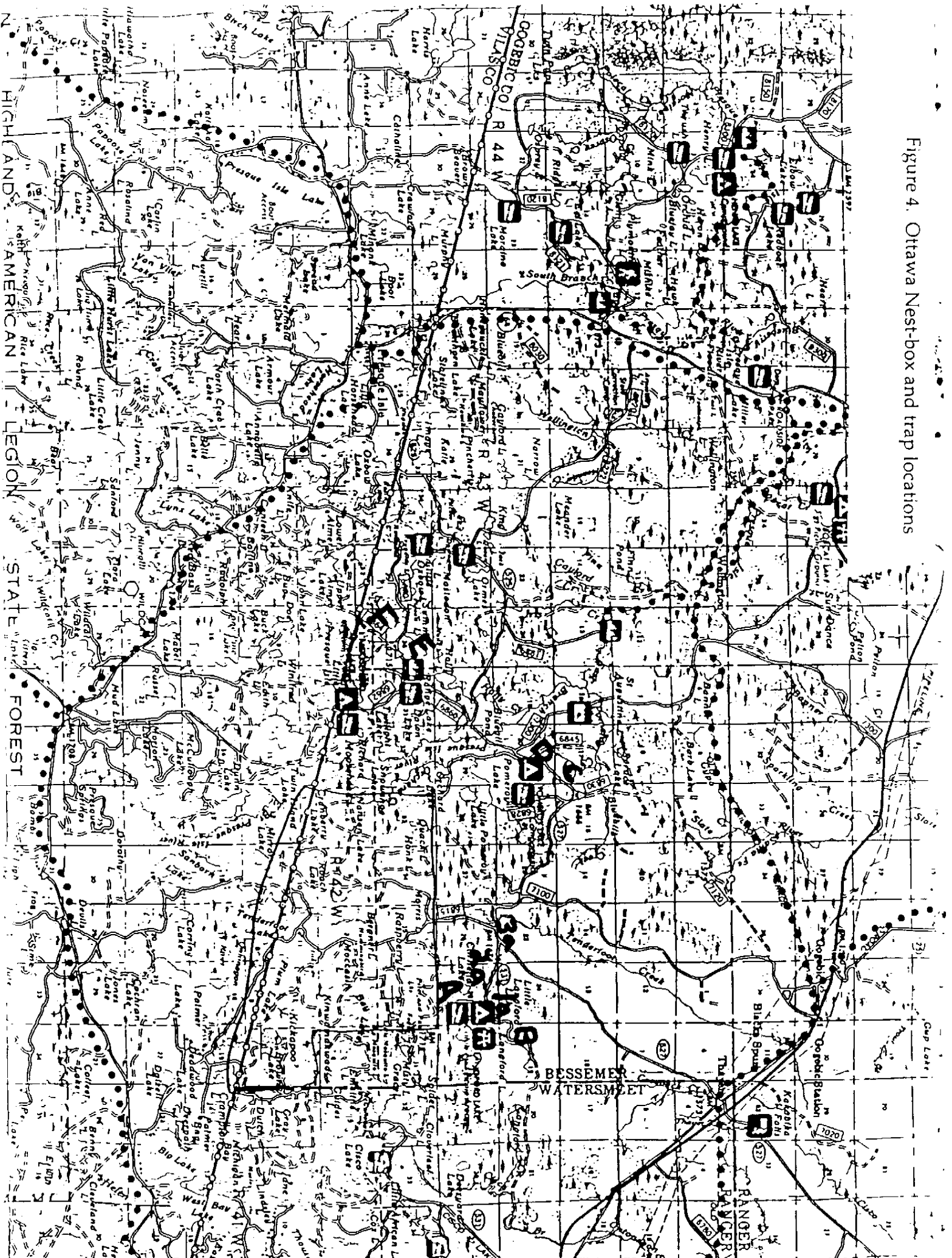


Figure 5. Collection data for the 1995 trapping season

Mammals	Species	3
	Individuals	81
Ear Biopsies	Total	81
	Positive	0
Ticks	Species	3
	Individual	23
Fleas	Species	1
	Individual	19
Lice	Species	1
	Individual	100+

Figure 6. Ectoparasites collected

<u>Ectoparasite Species</u>	<u>Host Species</u>	<u>Site #</u>
<i>Dermacentor variabilis</i>	<i>Peromyscus maniculatus</i>	1,7,13,22
<i>Ixodes angustus</i>	<i>Peromyscus maniculatus</i>	15
<i>Ixodes banksi</i>	<i>Peromyscus maniculatus</i>	5
<i>Columbola sminthurade</i>	<i>Peromyscus maniculatus</i>	2
<i>Polypax auricularis</i>	<i>Peromyscus maniculatus</i>	6, 11, 12, 15, 17, 18, 20, 21
<i>Orchopeas leucopus</i>	<i>Peromyscus maniculatus</i>	13, 15, 22

Figure 7. Ottawa Data

Species	# collected	ectoparasites	site #
<i>Tamias striatus</i>	3	0	2a
<i>Peromyscus maniculatus</i>	4	0	3a, 2a