Effects of fluorides on Peromyscus maniculatus in Glacier National Park
by Mark Leroy Fogelsong

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Zoology
Montana State University

Abstract:
Approximately 100 square miles of Glacier National Park receive atmospheric-borne fluorides from an aluminum plant located west of the park. A study was conducted on the west and southwest portion of Glacier National Park during the summers of 1972 and 1973 to determine what effects abnormal amounts of fluorides were having on a population of deer mice (Peromyscus maniculatus) located in the park. During the winter and spring of 1973, a laboratory study subjecting deer mice to three levels of fluoridated water was done to supplement information obtained from the field-trapped mice. Qualitative and quantitative vegetation analyses were conducted on five experimental sites located inside the contaminated region of the park and on four control sites outside of the contaminated area to determine similarity of the trapping sites. Eighty-two deer mice were collected from the experimental sites; 124 deer mice were collected from the control sites. Experimental sites had total relative indices of population size from 1972 and 1973 of 37 and 15 mice, respectively; control sites had total relative indices of 68 and 54, respectively for the same two years.

Deer mice from the control sites averaged 129.2 ppm of fluoride in their femurs, and the experimental mice averaged 699.2 ppm of fluoride in their femurs. Pregnant females from the control sites contained an average of 5.9 embryos and pregnant females from the experimental sites averaged 5.7 embryos per female. Overall sex ratio at the control sites was 1:1.07 females to males, and at the experimental sites the ratio was 1:1.22 females to males. Body weights were not significantly different between mice from the control and experimental sites. Fluoride concentration in the experimental mice was most directly correlated to body weight. Pregnant females from the experimental sites had significantly higher fluoride levels in their femurs than did experimental adult males. No fluorosis of the teeth was observed in any of the mice from the experimental sites. I observed no harmful effects in the mice from the experimental sites which could be ascribed to the abovenormal amounts of fluoride in their systems. In the laboratory experiment, three groups of deer mice, levels A, B and C were given water containing 1 ppm, 200 ppm and 600 ppm of sodium fluoride, respectively. All the mice were given food containing 30-35 ppm of fluoride. Mice in level A averaged 1262 ppm of fluoride in their femurs. Mice in level B averaged 7817 ppm of fluoride in their femurs, and mice in level C averaged 9164 ppm of fluoride in their femurs. Mice in levels B and C exhibited signs of dental fluorosis. No dental fluorosis was seen in mice from level A. One female in level B containing 7177 ppm of fluoride in her femur bore a healthy litter of four.

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A thesis submitted in partial fulfillment
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ABSTRACT

Approximately 100 square miles of Glacier National Park receive atmospheric-borne fluorides from an aluminum plant located west of the park. A study was conducted on the west and southwest portion of Glacier National Park during the summers of 1972 and 1973 to determine what effects abnormal amounts of fluorides were having on a population of deer mice *(Peromyscus maniculatus)* located in the park. During the winter and spring of 1973, a laboratory study subjecting deer mice to three levels of fluoridated water was done to supplement information obtained from the field-trapped mice. Qualitative and quantitative vegetation analyses were conducted on five experimental sites located inside the contaminated region of the park and on four control sites outside of the contaminated area to determine similarity of the trapping sites. Eighty-two deer mice were collected from the experimental sites; 124 deer mice were collected from the control sites. Experimental sites had total relative indices of population size from 1972 and 1973 of 37 and 15 mice, respectively; control sites had total relative indices of 68 and 54, respectively for the same two years. Deer mice from the control sites averaged 129.2 ppm of fluoride in their femurs, and the experimental mice averaged 699.2 ppm of fluoride in their femurs. Pregnant females from the control sites contained an average of 5.9 embryos and pregnant females from the experimental sites averaged 5.7 embryos per female. Overall sex ratio at the control sites was 1:1.07 females to males, and at the experimental sites the ratio was 1:1.22 females to males. Body weights were not significantly different between mice from the control and experimental sites. Fluoride concentration in the experimental mice was most directly correlated to body weight. Pregnant females from the experimental sites had significantly higher fluoride levels in their femurs than did experimental adult males. No fluorosis of the teeth was observed in any of the mice from the experimental sites. I observed no harmful effects in the mice from the experimental sites which could be ascribed to the above-normal amounts of fluoride in their systems. In the laboratory experiment, three groups of deer mice, levels A, B and C were given water containing 1 ppm, 200 ppm and 600 ppm of sodium fluoride, respectively. All the mice were given food containing 30-35 ppm of fluoride. Mice in level A averaged 1262 ppm of fluoride in their femurs. Mice in level B averaged 7817 ppm of fluoride in their femurs, and mice in level C averaged 9164 ppm of fluoride in their femurs. Mice in levels B and C exhibited signs of dental fluorosis. No dental fluorosis was seen in mice from level A. One female in level B containing 7177 ppm of fluoride in her femur bore a healthy litter of four.

INTRODUCTION

In recent years there has been a mounting interest expressed by biologists and conservationists concerning the environmental effects of various pollutants. Of these many pollutants, fluorides and their effects on biological systems have been of much concern. Fluorides are commonly found throughout nature in minerals, soils, natural waters and virtually all foods (Leech 1956, Robinson and Edgington 1946, Cholak 1959). Fluorides can be considered a pollutant when they create an "undesirable change in the physical, chemical or biological characteristics of our air, land and water that may or will harmfully affect human life or that of other desirable species, our industrial processes, living conditions and cultural assets; or that may or will waste or deteriorate our material resources" (National Research Council 1966).

Studies to determine the effects of excess fluorides on man and his environment are numerous. Early research on the subject dates back to 1883 with mention of damage done to vegetation near a copper smelter plant (Schroeder and Reuss 1883, cited in Thomas and Alther 1966). More current review papers, Suttie (1969), McCune and Weinstein (1971), and World Health Organization (1970), deal with the effects excess fluorides have had on livestock, vegetation and man, respectively. Jaroslav (1971) has summarized much of the research that has been done on all aspects of fluoride pollution. In spite of the copious amount of work on fluorides, I have found no studies which have considered the ecological effects of atmospheric fluoride pollution on an animal population in its natural habitat.

Near Columbia Falls, Montana, there is an aluminum plant owned by the Anaconda Aluminum Company. With the process of aluminum production at the plant, gaseous and particulate compounds are formed as by-products of reduction and emitted into the air. Among these emissions are the fluoride-containing compounds hydrogen fluoride, cryolite, aluminum fluoride, chiolite and others (Bolstad 1973). These emissions are subsequently airborne to the surrounding area and deposited. A part of Glacier National Park is in this area.

The contaminated portion of Glacier National Park is located six air miles northeast of the Anaconda Aluminum plant. Due to the distance from the plant, the park receives lower levels of fluorides than those present near the plant site (Carlson 1971). Carlson (1971) delineated approximately 100 square miles of the park as containing above-normal levels of fluoride in its vegetation (Figure 1). Femurs from animals present in this area reflect the higher levels of fluorides in their systems as compared to those collected from control sites having a low fluoride level in the vegetation (Gordon 1972).

The objectives of this study, conducted from June 1972 through August 1973, were to determine the effects of observed levels of fluoride on the teeth, body weight, fertility and population levels of

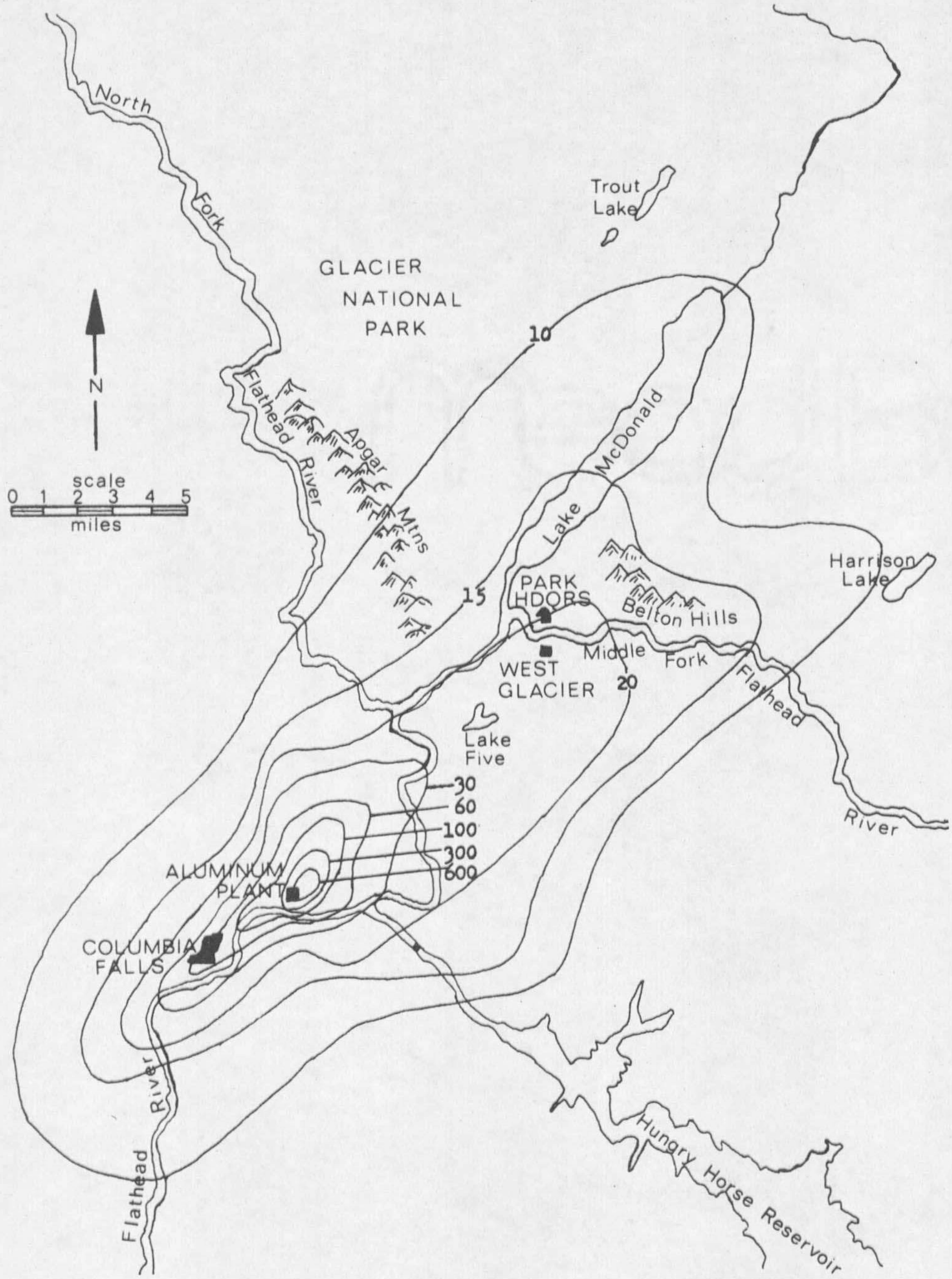

Figure 1. Isopols of fluoride pollution in parts per million at Columbia Falls, Montana, August 1971 (from Carlson 1971).

deer mice, *Peromyscus maniculatus*, in Glacier National Park. In conjunction with the field work, a laboratory experiment was conducted exposing deer mice to varying levels of fluorides under controlled conditions.

DESCRIPTION OF STUDY AREA

General Study Area

Glacier National Park is located in northwestern Montana. The study sites are located on the west slope of the Continental Divide, along the western and southern edges of the park. The sites range from the confluence of the North and Middle Forks of the Flathead River in the west, to a few miles east of the confluence of Bear Creek with the Middle Fork of the Flathead River in the south (Figure 2).

The western edge of the park is characterized by forest-covered mountains rising to approximately 2135 meters from U-shaped valleys with elevations near 1220 meters. This topography is largely due to the action of glaciers during the Pleistocene era. The exposed rocky ridges and underlying rock strata in this region are composed of sedimentary rock deposited during the Precambrian era (Fields 1971).

The average annual rainfall in the study area ranges from 28 inches at West Glacier on the west edge of the park, to 43 inches at Summit on the southern border of the park. Winters are characterized by cold weather and heavy snows. The summers are warm and moist (U. S. Weather Bureau 1972).

The coniferous forest which covers most of the general study area can be divided into two zones which Daubenmire (1943) calls the Douglas fir *(Pseudotsuga menziesii)* zone and spruce-fir *(Picea engelmannii-*

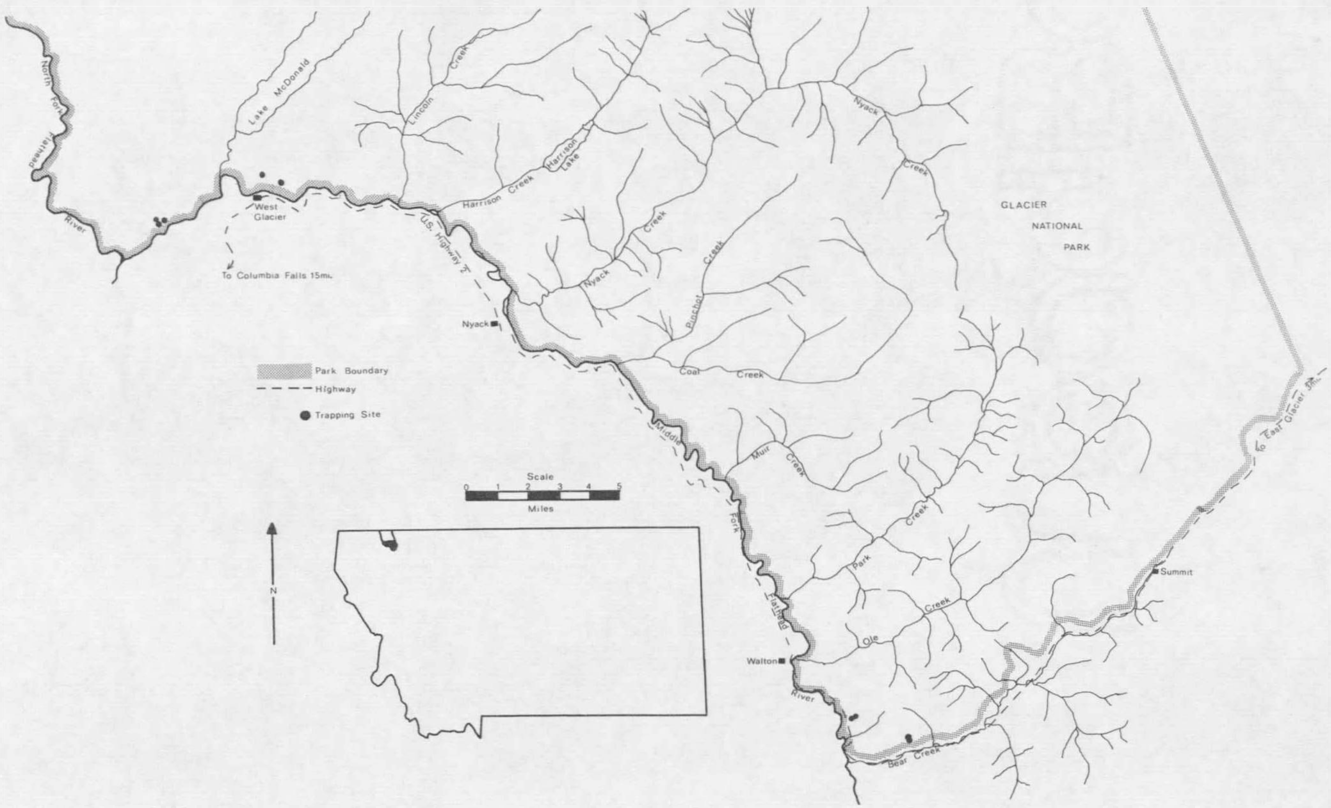

Figure 2. Map of study area and associated trapping sites.

Abies lasiocarpa) zone. Different seral stages of lodgepole pine *(Pinus contorta)* stands are found throughout the Douglas fir zone along with ponderosa pine *(Pinus ponderosa)* and Western larch *(Larix occidentalis)*. At elevations above 1525 meters the Douglas fir zone grades into the spruce-fir zone. Near the rivers of the valley floors, the black cottonwood *(Populus trichocarpa)* predominates and is the most conspicuous forest type. The contaminated portion of the park contains vegetation having 10 to 20 parts per million (ppm) of fluoride (Carlson 1971). Carlson states that vegetation located in control areas averaged 8.4 ppm of fluoride.

Collection Sites

Bear Creek: The two Bear Creek control sites are located 113° 31' 50" W and 48° 14' 24" N at an elevation of 1372 meters. The two sites lie one above the other on an open, south-facing slope about 122 meters north of U. S. Highway 2. The open slope has a few mature Douglas fir scattered over it. Shrubs, predominantly snowberry *(Symphoricarpos* spp.*)* and chokecherry *(Prunus virginiana)* provide about 24 percent of the ground cover. Grasses and forbs comprise 6 and 9 percent, respectively, of the ground cover. Trapping was conducted on the lower site from August 13 to August 16, 1972, and from June 21 through June 24, 1973, on the upper site.

Walton Goat Lick: The two Walton Goat Lick control sites are located 113° 35' 00" W and 48° 16' 15" N, at an elevation of 1250 meters. The two adjacent trapping sites, one 10 meters above the other, were located 100 meters from the highway on the southwest-facing slope. The site is fairly open with some small exposed rocky ridges. Shrubs cover about 32 percent of the area with mountain maple *(Acer glabrum)* and dog bane *(Apocynum* spp.*)* predominating. A few mature Douglas fir and other coniferous trees were dispersed over the area. Grasses and forbs represent 6 and 10 percent of the ground cover, respectively.

Deer mice were collected from July 29 through August 1, 1972, and from August 7 through August 11, 1973, on the lower site. The upper site was trapped from July 13 through July 16, 1973.

Belton Nob: The Belton Nob experimental site is located 113° 57' 40" W and 48° 30' 8" N, at an elevation of 1067 meters. The collection site lies on a small southwest-facing bench which has a few lodgepole pine scattered over it. Grasses compose 30 percent of the ground cover with orchard grass *(Dactylis glomerata)* being the predominate species. Shrubs and forbs make up 27 and 8 percent, respectively, of the ground cover. Mountain spray *(Holodiscus discolor)* is the most abundant shrub. Trapping on this site was conducted from July 5 through July 12, 1972.

Headquarters Hill: The Headquarters Hill experimental site lies northeast of Glacier Park's headquarters building. The southwest-facing slope is located 113° 58' 30" W and 48° 30' 22" N, at an elevation of 1098 meters. Shrubs provide 32 percent of the ground cover with nine bark *(Physocarpus malvaceus)* and dog bane making up the largest percentage of this group. Grasses provide 8 percent of the cover while forbs contribute 1 percent. The trapping site is fairly open but has a heavy Douglas fir canopy cover at its most southern edge. Trapping on this site was done from June 28 through July 3, 1973.

East Apgar Ridge: The East Apgar Ridge experimental site is located on the most southern edge of the Apgar Mountains at 114° 2' 40" W and 48° 29' 00" N. Its elevation is 1067 meters. The open site has a southeast exposure and is surrounded by lodgepole pine. Snowberry and rose *(Rosa* spp.*)* are the most abundant of the shrubs which provide 26 percent of the cover. Forbs and grasses contribute 14 and 6 percent, respectively, to the total cover. Collections of deer mice were made from July 14 to July 20, 1972, and from July 30 to August 5, 1973.

West Apgar Ridge: The Upper and Lower West Apgar Ridge experimental sites are located on a small, finger-like ridge 90 meters directly west of the East Apgar Ridge collection site. The lower trapping grid is located 114° 2' 49" W and 48° 28' 55" N. The site is 18 meters north of the dirt road which traverses this area. This site has a southwest exposure and an elevation of 1067 meters. This section of the ridge has forbs and shrubs each comprising 18 percent of the ground cover with grasses having 12 percent of the cover. The location is more level than the upper grid site and has less exposed ground. A few mature Douglas fir and ponderosa pine are present.

The upper grid site is 180 meters north of the lower site and is 20 meters higher in elevation. It is located 114° 2' 57" W and 48° 29' 00" N on a west-facing slope. Shrubs make up 19 percent of the vegetative cover. Grasses comprise 2 percent and forbs comprise 4 percent of the ground cover. The most abundant shrub is snowberry. An occasional paper birch *(Betula papyrifera)*, ponderosa pine and lodgepole pine are found on this portion of the ridge. Collections were made on the lower site from July 5 to July 10, 1973. The upper site was trapped from August 15 to August 21, 1972.

METHODS

Selection of Trapping Area

Three general criteria were considered in the selection of any one trapping site. The first was that of locating the site within the contaminated portion of the park if it was an experimental area, or locating the site well outside the contaminated area if it was to be a control site. Carlson (1971) was used as the guide to delineate the contaminated area of the park. Ease of accessibility was the second criterion used for selection. The third was that all sites be of relatively similar habitat type--that of open, shrub-covered slopes with little coniferous canopy coverage. From previous experience this type of habitat was found to have adequate populations of deer mice.

Trapping

Mice were collected from nine sites, four control and five experimental. At each of the nine sites, 100 Sherman live traps were placed in four parallel lines of 25 each. Individual traps in a line were approximately six meters apart. The four parallel lines were spaced at 22-meter intervals. This pattern encompassed a rectangular area of 9,054 square meters (Figure 3).

Traps were baited with rolled oats and left open at the site from three to seven days. Results from the first three days of trapping were expressed as a relative index of population size for each area.

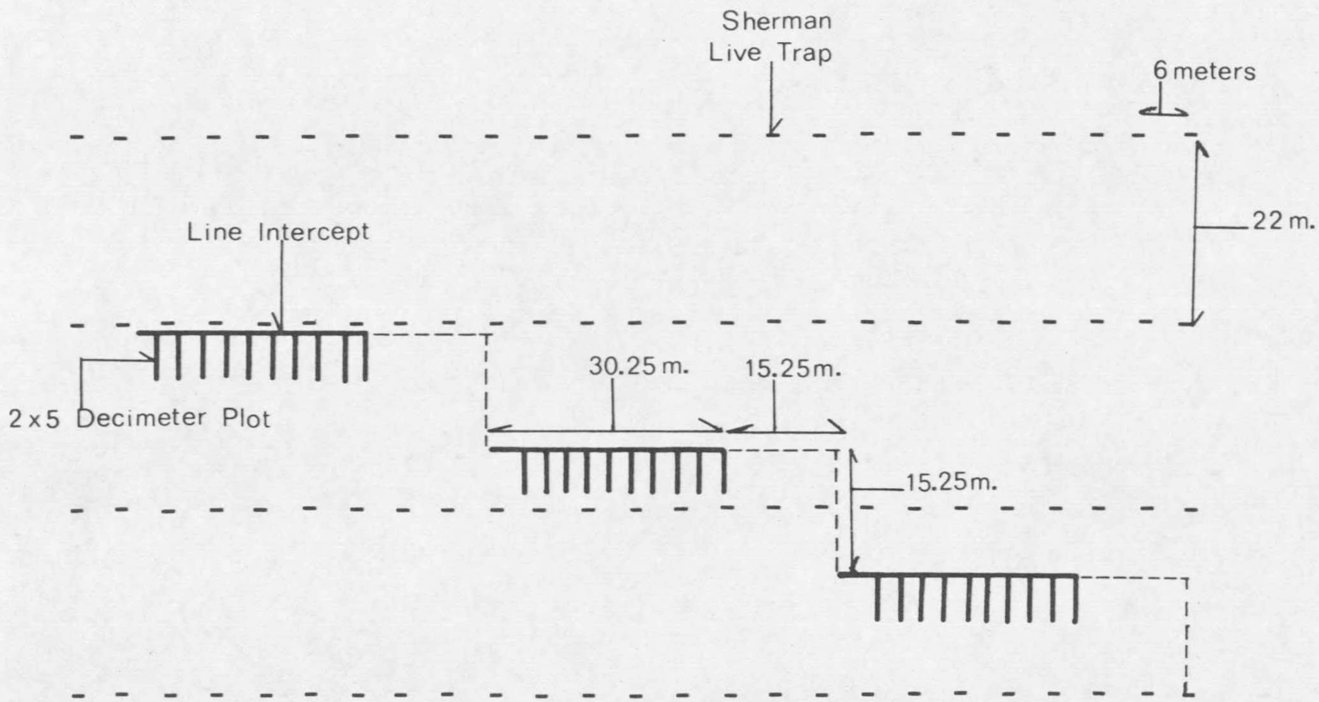

Figure 3. Pattern for trap placement, line intercept and 2 x 5 decimeter plots.

The remaining time was used to collect additional mice if less than 10 mice were caught during the first three days. The traps were checked each morning. All trapped mice were killed and taken to a laboratory for postmortum analysis. Trapping was conducted from June 20 through September 12, 1972, and from June 21 through August 11, 1973.

Vegetation

A plant species list was made for each rectangular trapping site. In places where two trapping sites were adjacent, one list was made for the two sites. The common and scientific names of the plants follow those in Nelson (1970), Booth (1950) and Booth and Wright (1959). In addition to the species list, three 30.5-meter line intercepts were used to sample the canopy cover above one meter in height. The intercept lines were placed inside each rectangular trapping site in a stairstep manner (Figure 3). Along each 30.5-meter line, ten 2 x 5 decimeter plots were spaced at 3.05-meter intervals to sample the canopy coverages and frequencies of the low-growing taxa. This method of measuring low-growing species is similar to that described by Daubenmire (1959).

Laboratory Studies

From January 8 through June 12, 1973, 60 deer mice were kept as male-female pairs in an animal room at Montana State University at room temperature and on a natural photoperiod. These mice were subjected to varying levels of fluorides in the form of sodium fluoride dissolved in the drinking water and provided ad libitum. Level A had 21 mice which received water containing 0.9-1.1 ppm of fluoride. Level B had 20 mice which were subjected to water containing 200 ppm of fluoride, and the remaining 19 mice in level C were given 600 ppm of fluoride in the water. Excess food containing 30-34 ppm of fluoride was kept in the cages at all times. Water bottles from the individual cages were weighed weekly to observe any changes in consumption. At the end of the experiment surviving mice were killed and prepared for analysis.

Postmortum Preparation and Analysis

All deer mice from the field and laboratory were weighed as soon after death as possible. It was noted at this time whether females were pregnant or nursing, and the number of embryos present were counted. The eyeballs, adrenal glands, and female reproductive tracts were removed and preserved in a 10 percent formalin solution. Both femurs from each mouse were removed and frozen. The bodies were also frozen.

Two to three months later, the fixed lenses were dried at 100° C for three weeks and then weighed to the nearest tenth of a milligram. The lenses were categorized according to weight. Lens weights were used to determine the relative age of the mice. The female reproductive tracts and adrenal glands were not used for analysis.

In 1972, femurs were selected from mice of each trapping site to give an equal distribution between males and females in juvenile, subadult and adult age groups. One femur from each of the 81 selected mice was sent to Dr. C. C. Gordon, University of Montana, where an Orion Fluoride Specific Probe was used for their analysis. In 1973, however, single femurs from all 82 trapped mice were sent to Dr. Gordon for analysis, together with femurs selected at random from mice used in the laboratory experiment. Fluoride concentrations are expressed as ppm dry weight. The duplicate femurs are kept at Montana State University.

Gross observations with a binocular scope were made of the teeth of all mice to record any dental fluorosis. Histological sagittal- and cross-sections were made from the teeth of 14 representative mice skulls. These were selected from both field-trapped and laboratory mice which had high levels of fluorides in their femurs. The statistical methods follow those in Snedecor and Cochram (1967) and Tate and Clelland (1957). The probability (P) values calculated from (t) tests were considered significant if P was equal to or less than .050.

RESULTS

Vegetation

Shrubs and Trees Above One Meter in Height

Results from the line intercept data for both the control and experimental sites are found in Table 1.

In general, canopy coverage above one meter in height in the control sites is denser than that in the experimental sites, with the exception of Lower Bear Creek control site and Headquarters Hill experimental site.

In the control sites of Upper Bear Creek and the two Walton Goat Lick sites, mountain maple and serviceberry together provide 59 to 93 percent of the upper canopy coverage. In the Lower Bear Creek site chokecherry provides 87 percent of the upper story coverage.

In the experimental sites there is no one taxon which contributes heavily to the upper story in more than two of the sites. However, more species are represented in the upper canopy strata here than in the control sites. Serviceberry is the most common species in the upper story, being recorded in three of the five sites.

Vegetation Under One Meter in Height

Table 2 indicates the results from the 2 x 5 decimeter plots in the various trapping sites.

TABLE 1. PERCENT COVERAGE AND RELATIVE DOMINANCE OF PLANT SPECIES ABOVE ONE METER IN HEIGHT AS MEASURED BY THREE 30.5 METER LINE INTERCEPTS PER SITE.

	Controls				Experimental				
Taxa	Lower Bear Creek (8/19/73)[1]	Upper Bear Creek (7/25/73)	Lower Walton Goat Lick (8/20/73)	Upper Walton Goat Lick (8/6/73)	Belton Nob (8/22/73)	Head-quarters Hill (8/21/73)	East Apgar Ridge (7/30/73)	Lower West Apgar Ridge (7/23/73)	Upper West Apgar Ridge (8/21/73)
SHRUBS AND TREES:									
Acer glabrum	---	5/45[2]	15/64	7/82	---	---	---	---	---
Amelanchier alnifolia	---	2/14	2/9	1/11	1/25	9/57	---	T/78[3]	---
Ceanothus sanguineus	---	---	---	---	---	---	T/1	---	---
Holodiscus discolor	---	---	---	---	3/58	---	---	---	---
Physocarpus malvaceus	---	---	---	---	---	9/43	---	---	---
Prunus pennsylvanica	---	---	2/7	---	---	---	---	---	---
Prunus virginiana	1/87	1/12	---	---	---	---	1/31	---	---
Pseudotsuga menziesii	T/13	4/29	5/20	---	---	---	2/56	---	---
Rosa spp.	---	---	---	---	---	---	T/12	T/22	---
Salix spp.	---	---	---	---	1/17	---	---	---	---
Sorbus scopulina	---	---	---	T/7	---	---	---	---	---
Symphoricarpos spp.	---	---	---	---	---	T/-	---	---	---
Total	1/100	12/100	24/100	8/100	4/100	18/100	3/100	1/100	0/0

[1]Date of vegetation sampling

[2]Percent coverage/relative dominance

[3]Trace (T)-Less than 1%

TABLE 2. CANOPY COVERAGE AND FREQUENCY OF TAXA FOR GRASSES, FORBS, AND SHRUBS OCCURRING IN NINE TRAPPING SITES AS INDICATED BY MEASUREMENTS FROM 30, 2X5 DECIMETER PLOTS ON EACH SITE.

Taxa	Controls				Experimental				
	Lower Bear Creek (8/19/73)[1]	Upper Bear Creek (7/25/73)	Lower Walton Goat Lick (8/20/73)	Upper Walton Goat Lick (8/6/73)	Belton Nob (8/22/73)	Head-quarters Hill (8/21/73)	East Apgar Ridge (7/30/73)	Lower West Apgar Ridge (7/23/73)	Upper West Apgar Ridge (8/21/73)
GRASS AND GRASS-LIKE PLANTS:									
Agropyron bakeri	---	3.6/30[2]	---	---	---	---	---	---	---
Agropyron spicatum	5.0/40	3.3/23	2.3/10	4.0/26	---	2.3/10	1.3/20	---	.1/3
Agropyron trachycaulum	.5/3	---	.1/3	---	---	1.3/3	3.0/10	---	.5/3
Agrostis scabra	---	---	---	---	---	---	.5/3	.5/3	---
Bromus tectorum	---	---	---	---	---	---	.6/13	---	---
Carex spp.	4.6/36	2.4/16	4.7/26	1.3/20	.2/6	.7/13	4.2/40	---	.4/6
Dactylis glomerata	---	---	---	---	10.5/36	---	---	---	---
Danthonia intermedia	---	---	---	---	---	---	---	.9/20	---
Festuca idahoensis	---	---	---	---	---	---	.5/6	10.3/70	.5/3
Phleum pratense	---	---	1.8/10	---	5.5/40	---	.1/3	---	---
Poa compressa	---	---	---	---	1.2/13	---	.1/3	---	---
Unidentified grasses	.2/6	---	3.0/23	.3/13	13.3/56	4.3/43	.3/13	.2/6	.7/26
Total Grasses	5.7/47	6.9/50	7.2/40	4.3/40	30.5/97	7.9/57	6.4/60	11.9/83	1.8/37
Total Sedges	4.6/36	2.4/16	4.7/26	1.3/20	.2/6	.7/13	4.2/40	0	.4/13
FORBS:									
Achillea millefolium	1.8/20	1.2/13	1.5/20	2.2/20	2.3/23	---	4.3/40	5.9/56	---
Allium cernuum	.2/6	---	---	---	---	.09/3	.1/3	.3/10	---
Arabis hoboellii	---	.2/6	.1/3	---	---	.09/3	---	.2/6	.1/3
Arabis sparsiflora	---	---	---	---	---	---	.7/6	---	---
Asclepias spp.	---	---	---	---	.2/6	---	.1/3	---	---
Aster conspicuus	2.2/20	---	1.2/13	.6/6	---	---	---	---	---
Aster intergrifolius	---	.5/3	.5/3	---	.08/3	.09/3	---	---	---
Balsamorrhiza sagittata	---	---	---	---	---	---	---	1.0/6	---
Campanula rotundifolia	.1/3	---	---	---	---	---	---	---	---
Castilleja spp.	---	.5/3	---	.7/10	---	---	---	---	---
Clematis columbiana	---	---	1.3/3	---	---	---	---	---	---
Epilobium angustifolium	---	2.8/16	---	.1/3	---	---	---	---	.5/3
Fragaria virginiana	.1/3	1.3/20	1.7/16	---	---	.09/3	---	.7/10	---
Galium boreale	1.2/13	.1/3	---	---	---	---	---	.5/3	---
Heuchera cylindrica	.1/3	---	---	---	---	---	---	---	---
Hypericum perforatum	---	---	---	---	---	---	---	2.7/43	---
Linaria vulgaris	---	---	---	---	---	---	2.0/3	---	.5/3
Lupinus sericeus	---	---	---	.5/3	---	---	---	---	---
Monarda fistulosa	---	---	---	---	---	.09/3	.7/6	2.3/13	1.8/23
Penstemon confertus	---	---	---	.2/6	---	---	2.5/20	---	---
Penstemon virens	3.8/40	2.2/20	1.3/16	2.3/23	---	---	.5/3	---	1.7/20
Potentilla gracilis	---	---	.6/6	1.7/6	.03/3	.3/10	1.8/23	2.3/26	.1/3
Sedum stenopetalum	---	---	.7/13	---	---	---	---	.7/26	---
Senecio canus	---	---	---	---	.6/6	---	.1/3	1.3/20	---
Smilacina racemosa	---	.1/3	---	---	---	---	---	---	---
Solidago occidentalis	---	---	.6/6	---	---	---	---	---	---
Trifolium agrarium	---	---	---	---	4.4/33	---	1.3/3	---	---
Trifolium repens	---	---	1.4/10	---	---	---	---	---	---
Unidentified forbs	---	.3/10	.3/10	1./3	---	.09/3	.3/13	---	---
Total Forbs	9.5/70	9.2/50	11.2/40	9.3/47	7.6/37	.9/27	14.4/66	17.9/80	4.7/57
SHRUBS AND TREES:									
Acer glabrum	1.3/3	1.8/10	13.6/23	6.3/10	---	.5/3	---	---	---
Amelanchier alnifolia	2.8/3	2.0/6	3.3/6	2.1/3	4.4/16	4.9/20	2.8/3	5.4/10	8.8/36
Apocynum spp.	---	.7/10	---	10.6/43	3.8/23	7.0/43	---	.7/10	1.7/13
Aralia nudicaulis	---	---	---	---	---	---	---	---	1.3/3
Arctostaphylos uva-ursi	---	.5/3	---	---	---	---	---	---	---
Berberis repens	.7/13	.2/6	1.1/10	1.5/26	.4/6	.8/16	.5/3	1.3/3	---
Ceanothus spp.	1.3/3	.1/3	---	.1/3	.7/10	1.3/3	3.2/10	---	1.5/10
Holodiscus discolor	---	---	.5/3	---	9.8/13	---	---	---	---
Physocarpus malvaceus	---	---	---	---	---	9.0/30	---	---	---
Prunus virginiana	7.3/46	8.4/33	2.6/6	1.2/13	---	---	3.0/3	2.0/3	1.7/16
Pseudotsuga menziesii	---	---	2.0/3	---	---	6.6/10	---	---	---
Rosa spp.	.7/10	.5/3	1.4/10	5.2/30	4.5/20	3.6/30	5.8/26	.6/6	---
Rubus parviflorus	2.3/10	2.7/23	1.0/6	1.0/6	---	.5/3	---	---	---
Salix spp.	---	---	---	---	3.3/3	---	---	---	---
Sorbus scopulina	---	---	---	2.8/3	---	---	---	---	---
Spiraea betulifolia	2.6/23	2.8/33	.5/3	2.7/26	2.5/33	.6/6	1.5/26	1.3/16	.5/3
Symphoricarpos spp.	3.5/43	4.5/50	6.6/36	3.1/26	3.8/23	3.3/33	9.3/36	6.8/33	3.2/43
Vaccinium spp.	---	---	.1/3						
Total Shrubs & Trees	22.5/87	24.2/93	32.7/66	35.6/93	33.2/87	38.1/87	26.1/70	18.1/80	18.7/87
BARE ROCK:	28.7/57	17.4/47	15.4/43	33.4/57	2.4/17	2.6/67	23.6/60	13.0/80	39.6/66
BARE GROUND:	27.7/63	2.4/20	17.8/40	7.0/37	11.3/50	21.2/53	5.3/33	4.2/73	20.2/43
LITTER:	8.7/57	24.2/83	18.7/77	12.8/80	15.4/87	34.4/80	30.3/87	34.3/93	13.1/80

[1]Date of vegetation sampling

[2]Canopy coverage (percent area covered)/average frequency (percent occurrence among plots)

The total coverages and frequencies provided by grasses in the control sites are similar. Total coverage values for the grasses range from 4.3 to 7.2 percent and frequencies range from 40 to 50 percent. Bluebunch wheatgrass *(Agropyron spicatum)* is common to all four control sites, and is the most abundant grass in all sites except Upper Bear Creek. Values for sedge coverage range from 1.3 to 4.7 percent with frequencies ranging from 16 to 36 percent.

In the experimental sites, the total grass coverage varies greatly from a high of 30.5 percent at the Belton Nob site to a low of 1.8 percent in the Upper West Apgar Ridge site. Frequency of occurrence of the grasses ranges from 97 percent on Belton Nob to 37 percent on Upper West Apgar Ridge. Bluebunch wheatgrass, slender wheatgrass *(Agropyron trachycaulum)*, and Idaho fescue *(Festuca idahoensis)* are the most common species present. Sedge coverage in the experimental sites ranges from 0 to 4.2 percent and frequency values range from 0 to 40 percent.

Total vegetative coverage of forbs among the control sites is very similar with coverage percentages ranging from 9.2 to 11.2 percent and frequency values ranging from 40 to 70 percent. Yarrow *(Achillea millefolium)* and blue penstemon *(Penstemon virens)* are common to all four sites. These two species combined contribute one-third to three-fifths of the forb coverage in all control sites with the exception of the Lower Walton Goat Lick site where they provide only one-fourth of the forb coverage.

In the experimental sites there is again a large range of coverage and frequency values for forbs. The values vary from 0.9 to 17.9 percent for total coverage and from 27 to 80 percent for total frequency. Much of this difference can be explained by the time of year the site was sampled and the arid condition existing at that time. The three experimental sites with the lowest values for coverage and frequency were sampled in the latter part of August, 1973, whereas the other two experimental sites which have the higher forb coverage were sampled in the latter part of July, 1973. Many forbs in the August sampling were dried beyond recognition.

Northwest cinquefoil *(Potentilla gracilis)* is the only forb common to all experimental sites; horse mint *(Monarda fistulosa)* is common to all but Belton Nob. Neither of the two forbs contribute over 33 percent to the ground coverage in any site.

The total low-growing shrub coverages for the experimental and control sites are similar except for the Lower and Upper West Apgar sites, which have the lowest coverage and frequency values. Snowberry, *Spiraea betulifolia* and serviceberry are the only shrubs common to all the trapping sites.

Species List

Table 3 lists the species found at all the trapping sites. Grass species vary from site to site with no single one being common to all

TABLE 3. PLANT SPECIES FOUND AT THE TRAPPING SITES DURING THE PERIOD SAMPLED.

Taxa	Control Sites		Experimental Sites				
	Bear Creek 7/25/73*	Walton Goat Lick 8/6/73	Belton Nob 8/22/73	Head-quarters Hill 8/21/73	East Apgar Ridge 7/30/73	Lower West Apgar Ridge 7/23/73	Upper West Apgar Ridge 8/21/73
GRASS AND GRASS-LIKE PLANTS:							
Agropyron bakeri	+						
Agropyron inerme	+						
Agropyron spicatum	+	+		+	+		+
Agropyron trachycaulum	+	+		+	+	+	+
Agropyron trachycaulum var. *unilaterale*						+	
Agrostis scabra					+	+	
Avena byzantina						+	
Bromus tectorum					+	+	+
Calamagrostis montanensis				+		+	+
Calamagrostis purpurascens	+						
Carex spp.	+	+		+			+
Dactylis glomerata			+				
Danthonia intermedia						+	
Danthonia unispicata						+	
Elymus canadensis				+			
Festuca idahoensis			+		+	+	+
Festuca rubra						+	
Hordeum spp.				+			
Phleum pratense		+	+		+	+	+
Poa compressa			+		+	+	+
Poa nervosa	+						
Poa palustris			+			+	
Sporobolus airoides						+	
Stipa lettermani					+		
Stipa richardsonii						+	
Trisetum wolfii	+						
Total Grasses	8	4	5	6	8	15	8
FORBS:							
Achillea millefolium	+	+	+		+	+	+
Allium cernuum	+	+		+	+	+	+
Anaphalis margaritacea					+		
Antennaria rosea	+	+					
Arabis holboellii	+	+	+	+		+	+
Arabis sparsiflora					+	+	
Asclepias syriaca	+		+		+	+	+
Aster conspicuus	+	+	+	+			
Aster ericoides					+	+	
Aster ericoides var. *prostratus*					+	+	
Aster intergrifolius	+	+	+	+			
Balsamorrhiza sagittata			+		+	+	+
Calochortus elegans	+			+		+	
Campanula rotundifolia	+	+		+	+	+	
Castilleja spp.	+	+		+		+	
Clematis columbiana		+					
Epilobium angustifolium	+	+		+		+	+
Epilobium latifolium	+						
Erigeron speciosus					+	+	
Erigeron subalpinum						+	
Fragaria virginiana	+	+		+	+	+	
Galium boreale	+		+		+	+	
Geranium viscosissimum					+	+	
Heuchera cylindrica	+				+	+	
Hypericum perforatum	+				+	+	+
Iliamna rivularis	+	+					
Linaria vulgaris					+		+
Lupinus sericeus		+				+	
Melilotus alba	+	+					
Melilotus officinalis						+	
Monarda fistulosa				+	+	+	+
Penstemon confertus	+	+	+	+	+	+	
Penstemon lyallii	+						
Penstemon virens	+	+	+		+	+	+
Potentilla gracilis		+	+	+	+	+	+
Prunella vulgaris						+	
Sedum stenopetalum	+	+				+	
Senecio canus	+	+	+		+	+	+
Senecio foetidus	+						
Smilacina racemosa	+					+	
Solidago canadensis	+						
Solidago occidentalis		+			+		
Trifolium agrarium			+		+	+	
Trifolium repens		+					
Verbascum thapsus	+	+	+	+	+	+	+
Total Forbs	27	22	13	13	24	31	13
SHRUBS AND TREES:							
Acer glabrum	+	+	+	+	+	+	+
Amelanchier alnifolia	+	+	+	+	+	+	+
Apocynum spp.	+	+	+	+	+	+	+
Aralia nudicaulis				+			+
Arctostaphylos uva-ursi	+					+	
Berberis repens	+	+	+	+	+	+	+
Betula papyrifera			+	+			+
Ceanothus spp.	+	+	+	+	+		+
Ceanothus sanguineus						+	
Crataegus douglasii							+
Holodiscus discolor		+	+	+	+		+
Juniperus communis					+		
Juniperus scopulorum	+				+		
Larix occidentalis		+	+	+			
Philadelphus lewisii				+			
Physocarpus malvaceus				+			
Pinus contorta			+	+	+	+	+
Pinus flexilis	+						
Pinus ponderosa				+	+	+	+
Populus tremuloides	+						
Prunus pennsylvanica		+	+				
Prunus virginiana	+	+	+		+	+	+
Pseudotsuga menziesii	+	+	+	+		+	
Rosa spp.	+	+	+	+	+	+	+
Rubus parviflorus	+	+		+	+	+	
Salix spp.		+	+	+		+	+
Shepherdia canadensis	+	+		+	+	+	
Sorbus scopulina		+					
Spiraea betulifolia	+	+	+	+	+	+	+
Symphoricarpos spp.	+	+	+	+	+	+	+
Vaccinium spp.		+					
Total Shrubs & Trees	16	18	16	20	16	16	16
FERNS:							
Gymnocarpium robertianum						+	+

*Date site was surveyed

areas. The number of grass species per site varies from four to eight with the exception of Lower West Apgar Ridge which has 15 species.

Forb species number about the same from site to site except for Belton Nob, Headquarters Hill and Upper West Apgar Ridge. These sites with the lower number of species were sampled during the latter part of a dry summer which could account for the difference.

The number of shrub and tree species found in all sites varied from 16 to 20. Of the 16 to 20 species of shrubs and trees, six species--mountain maple, serviceberry, dog bane, Oregon grape holly, rose and snowberry--are common to all the sites. Ferns were found only on the Upper and Lower West Apgar Ridge sites.

Field Populations of Deer Mice

A total of 206 deer mice were collected from both control and experimental sites during the two summers. One hundred twenty-four mice were from control sites; 82 were from experimental sites (Table 4).

Relative Index

The control sites had higher relative index values than the experimental sites for both trapping seasons (Table 4). A (t) test indicated the difference between the relative index numbers for the control and experimental sites was not significant.

TABLE 4. TRAPPING RESULTS AND RELATIVE INDICES FOR COLLECTION SITES.

	Total Number Mice Trapped		Relative Index*	
	1972	1973	1972	1973
CONTROL SITES:				
Lower Bear Creek	30		30	
Upper Bear Creek		28		28
Lower Walton Goat Lick	38	15	38	13
Upper Walton Goat Lick		13		13
Total	68	56	68	54
EXPERIMENTAL SITES:				
Belton Nob	26		15	
Headquarters Hill		9		8
East Apgar Ridge	21	8	15	2
Lower West Apgar Ridge		9		5
Upper West Apgar Ridge	9		7	
Total	56	26	37	15

*Number trapped during first three days of trapping period (300 trap days).

There was also a difference seen in relative indices for sites which were trapped both years. The control site of Lower Walton Goat Lick indicated a 67 percent decrease from 38 to 13 trapped mice per relative index period from 1972 to 1973. The experimental site of East Apgar Ridge indicated an 87 percent decrease from 15 to two mice per relative index period from 1972 to 1973.

Fertility

Of the 124 deer mice caught on the control sites, nine were nursing young and three were pregnant. The pregnant females contained an average of 5.7 embryos with a range of five to seven embryos per female. In the experimental sites, there was a total of 82 mice trapped, of which five were nursing and seven were pregnant. Pregnant females from the experimental sites contained an average of 5.9 embryos with a range of five to seven embryos per female.

Age Distribution and Sex Ratio

Table 5 indicates the lens weight, lens weight category and the number of mice in each category from the control and experimental sites.

TABLE 5. DISTRIBUTION OF MICE IN LENS WEIGHT CATEGORIES.

Lens Weight (mg.)	Category	No. Mice From Control Sites	No. Mice From Experimental Sites
2.0-2.9	I	0	1
3.0-3.9	II	3	1
4.0-4.9	III	12	7
5.0-5.9	IV	23	15
6.0-6.9	V	23	12
7.0-7.9	VI	28	9
8.0-8.9	VII	10	10
9.0-9.9	VIII	14	14
10.0-10.9	IX	6	9
11.0-11.9	X	1	0
	Total	120	78

Figure 4 illustrates the distribution of males and females from both control and experimental sites over the range of lens weight categories. Mice with a lens weight in categories I-III are probably juveniles, those in categories IV-VI are sub-adults, and those in categories VII-X are adults. Lenses from all but one female that were

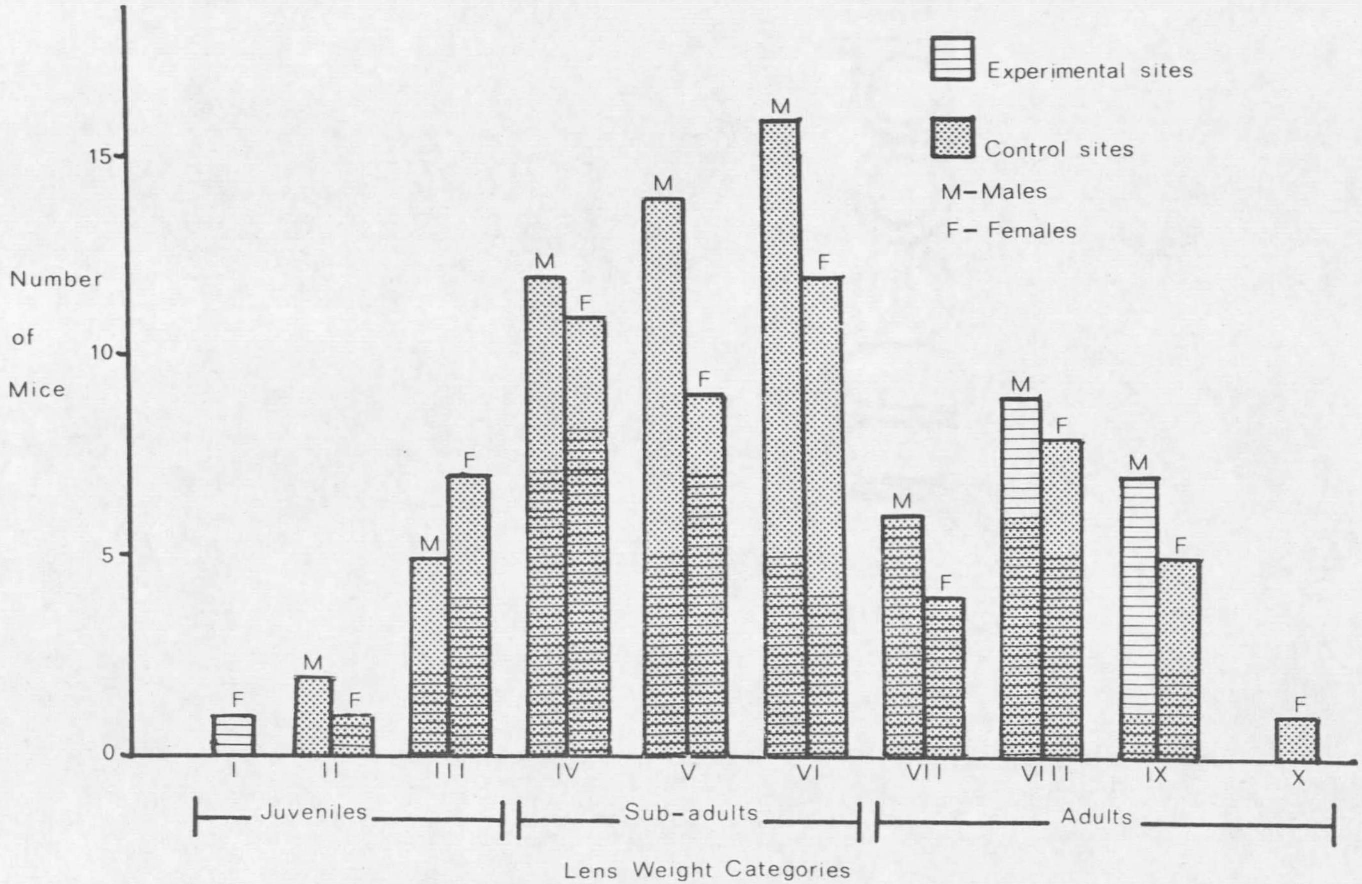

Figure 4. Distribution of mice from the control and experimental sites according to sex and lens weight category.

pregnant or nursing fell into categories VII-X. Lenses from sexually active males, as measured by testicular development, were also in categories VII-X. In the control sites 60 females and 64 males were caught for a ratio of 1:1.07 females to males. At the experimental sites 37 females and 45 males were caught for a ratio of 1:1.22 females to males. A Chi Square contingency test indicated that there is no significant difference between the sex ratios from the control and experimental sites.

There is a general trend in the control sites of a higher ratio of males to females through category VII, with the exception of category III. In categories VIII-X, there is a shift to a higher female to male ratio.

In the experimental sites just the opposite trend is seen. In lens categories I-V there are more females than males; in categories VI-X there are more males than females. Contingency tests were calculated with sex ratios among the juveniles, sub-adults and adults from Figure 4 to determine if any one of these groups exhibited a significantly different sex ratio from the expected. The contingency test indicated the shifting in sex ratios as seen in Figure 4 to be due to sampling error.

Body Weight

Mice were classified into three age classes, juvenile, sub-adult, and adult, on the basis of lens weights. Group 1 contained mice with lens weights in categories I-III. Group 2 contained mice with lenses in categories IV-VI, and Group 3 had mice with lenses in categories VII-X. Table 6 shows the average body weights of the males and females in the three groups from the control and experimental sites. There were significant differences in mean body weights of males and females only in group 2 from the control sites and group 3 from the experimental sites ($P<.025$; d.f. 61 and $P<.005$; d.f. 12, respectively). A (t) test was used to compare the average body weights of control and experimental mice within each sex and age class. There were no significant differences between the mean body weights of control and experimental males or females within any age class.

TABLE 6. AVERAGE BODY WEIGHT OF MICE IN GRAMS FROM CONTROL AND EXPERIMENTAL SITES.

	Group 1 Males	(Juveniles) Females	Group 2 Males	(Sub-adults) Females	Group 3 Males	(Adults) Females
Control Sites	11.31	11.71	15.78	14.86	21.28	21.32
Sample Size	7	8	42	32	13	18
Experimental Sites	13.56	12.71	15.27	15.38	19.70	22.51
Sample Size	3	6	17	19	22	11

Fluoride Concentration in Femurs

A summary of the results from the fluoride analysis of femurs of trapped mice for the various sample sites is found in Table 7. The mean, range, standard deviation and 95% confidence limits (±2 standard

TABLE 7. FLUORIDE CONCENTRATION IN FEMURS OF MICE COLLECTED FROM CONTROL AND EXPERIMENTAL SITES. FLUORIDE CONCENTRATIONS ARE EXPRESSED AS PPM DRY WEIGHT.

Site	No. Samples Submitted For F-Analysis	Mean F-Concentration (ppm)	Range F-Concentration (ppm)	Standard Deviation (ppm)	2 X Standard Error of Mean (ppm)
Lower Bear Creek	18	81.62	41.8-198.4	40.69	19.18
Upper Bear Creek	28	194.41	69.9-691.7	118.18	44.68
Lower Walton Goat Lick	33	105.4	37.6-575.2	93.62	32.62
Upper Walton Goat Lick	13	135.2	59.8-239.4	52.32	29.06
Belton Nob	18	448.13	239.5-1605.5	311.58	146.96
Headquarters Hill	9	1181.51	297.8-2332.3	699.14	466.08
East Apgar Ridge	26	510.78	62.4-1161.3	305.10	119.88
Lower West Apgar Ridge	9	977.22	506.1-1556.6	298.01	198.66
Upper West Apgar Ridge	9	378.38	252.5-950.1	203.53	135.68

errors of the mean) are graphically shown in Figure 5.

Mice from the control sites contained an average of 129.2 ppm of fluoride in their femurs. Individuals from the experimental sites averaged 699.2 ppm in their femurs. There is a significant difference ($P<.001$; d. f. 10) between the average amount of fluorides present in the femurs of mice from the control sites and that found in mice from

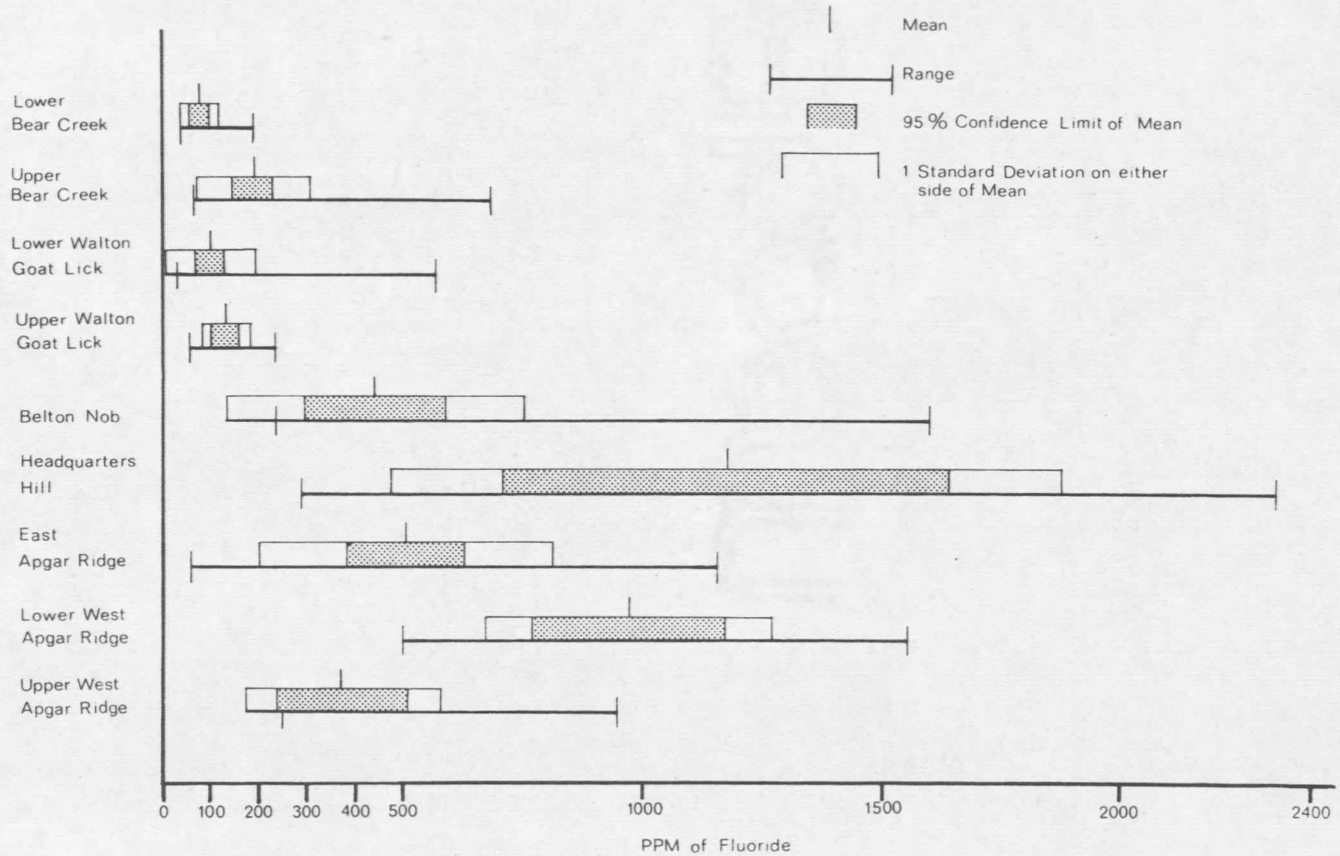

Figure 5. Fluoride concentrations in femurs of mice from nine trapping sites.

the experimental sites. Table 8 shows the average fluoride concentration in the femurs of juvenile, sub-adult, and adult males and females from the control and experimental sites.

TABLE 8. AVERAGE FLUORIDE CONCENTRATIONS IN THE FEMURS OF JUVENILE, SUB-ADULT, AND ADULT MALES AND FEMALES FROM CONTROL AND EXPERIMENTAL SITES.

	Control			Experimental		
Juvenile	Males 6[3]	221.1[1]	(90.0-691.7)[2]	Males 3	673.7	(313.6-1140.8)
	Females 7	118.5	(69.9-177.3)	Females 5	511.2	(297.8-1073.2)
Sub-Adult	Males 30	103.5	(37.6-575.2)	Males 15	428.2	(220.5-1556.6)
	Females 20	87.8	(50.2-222.7)	Females 17	336.9	(199.6-1032.4)
Adult	Males 10	193.1	(72.2-331.9)	Males 16	719.9	(266.1-1354.8)
	Females 12	199.3	(178.6-218.9)	Females 0	0	0
	Pregnant and Nursing Females 3	186.6	(115.1-295.9)	Pregnant and Nursing Females 12	1161.3	(62.4-2332.3)

[1]Average in ppm of fluoride.

[2]Ranges of fluoride levels.

[3]Sample size.

Among the control sites there are differences in the average fluoride concentration in mice femurs. Fluoride concentrations in mice from the Upper Bear Creek site differ significantly ($P<.005$; d. f. 51) from mice taken at the Lower Walton Goat Lick site and from the Lower Bear Creek site ($P<.001$; d. f. 36), but fluoride concentrations in femurs from the Upper Bear Creek site do not differ significantly from those of the Upper Walton Goat Lick site (Table 7). Femur fluoride levels from Upper Walton Goat Lick differ significantly only from those of the Lower Bear Creek site ($P<.010$; d. f. 22). The average fluoride levels in mice femurs from the two control sites of Upper and Lower Walton Goat Lick do not differ significantly, nor do those levels found at the Lower Walton Goat Lick and Lower Bear Creek sites differ significantly (Table 7).

There are differences in the average fluoride concentrations in femurs of mice from different experimental sites also. No significant differences were measured in the average fluoride concentrations among mice femurs from Belton Nob, East Apgar Ridge, and Upper West Apgar Ridge sites (Table 7). There are no significant differences between the average fluoride levels found in femurs at Lower West Apgar Ridge and Headquarters Hill, but both Headquarters Hill and Lower West Apgar Ridge do differ significantly ($P<.010$) from the other three experimental sites (Table 7).

Some of the differences in fluoride concentration of femurs from the sites is due to observed variation between years. Collections made from experimental sites in 1973 show higher concentrations of fluoride in the femurs and differ significantly from those made in 1972. The East Apgar Ridge site which was trapped in both 1972 and 1973 demonstrates this effect. In 1972, mice trapped from this site averaged

441.3 ppm of fluoride in their femurs and mice trapped in 1973 averaged 667.2 ppm of fluoride in their femurs. In 1973 fluoride concentrations from East Apgar Ridge do not differ significantly from the 1973 averages from Headquarters Hill and Lower West Apgar Ridge. The 1972 fluoride averages from East Apgar Ridge are not significantly different than the averages of Upper West Apgar Ridge and Belton Nob, sampled in 1972.

Except for Lower Walton Goat Lick, the control sites trapped in 1973 had significantly higher fluoride averages than those obtained during 1972 (Table 7). Upper Bear Creek and Upper Walton Goat Lick, trapped in 1973, had higher fluoride averages than did Lower Bear Creek and Lower Walton Goat Lick. Lower Walton Goat Lick, trapped both years, did not show this trend.

Simple correlation coefficients (R) were calculated for the relationship of lens weight to fluoride content in femurs, and for body weight to fluoride concentration in femurs for mice from both the control and experimental sites. Lens weights in the above correlation were used as an indicator of relative age. There was no significant correlation between the lens weight and fluoride concentration in femurs of mice from the control sites ($R = .10$, $P<.100$; d. f. 86, one-tailed test). There was a significant correlation between body weight and fluoride concentration in the femurs among the control mice ($R = .20$, $P<.05$; d. f. 90). Among mice from experimental sites, there was a significant correlation found between lens weight and femur fluoride concentration (R = .49, P<.005; d. f. 68) and between body weight and fluoride concentration in the femur (R = .55, P<.0005; d. f. 69).

Since lens weight and body weight were both significantly correlated with fluoride concentration in the femurs in the mice from the experimental sites, a multiple linear regression was calculated using lens weight and body weight as the independent variables and fluoride concentration in the femur as the dependent variable. Results from this regression would indicate which dependent variable contributed most to the fluoride concentration in the femurs of the mice. The results of the regression are: (R) squared[1] was calculated to be .3210. Body weight had a partial correlation coefficient (r)[2] of 3218; (P<.003). Lens weight had a partial correlation coefficient (r) of .1579; (P<.101). The (F) value obtained from an analysis of variance for this regression was 15.365; (P<.00003).

A (t) test was used to determine if males concentrated fluoride in their femurs at significantly different rates than did females. The

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[1]The proportion of the variation of the dependent variable measurements removed by the multiple regression.

[2]Partial correlation coefficient which is the correlation between the dependent variable and the named independent variable while the other independent variables are held constant.

control and experimental mice were divided into three general age groups based on lens weight. Males were compared to females for each group. There were no significant differences found in the fluoride accumulation between males and females of the same relative age group in either the control or the experimental sites, with one exception. Pregnant or nursing mice in age group 3 from the experimental sites showed a significant difference ($P<.050$; d. f. 13) in the fluoride accumulation than did males of the same age group. Pregnant and nursing females from the control sites, when compared to the control males of the same age group, did not exhibit this trend (Table 8).

Femurs from pregnant or nursing females in the control sites did not differ significantly in fluoride content from femurs of non-pregnant, non-nursing females in the same age group. All females in group 3 from the experimental sites were either pregnant or nursing so this comparison between females could not be done. Sexually active males from the experimental and control sites did not show any significant difference in fluoride concentration than did other males of the same age group and area, control or experimental.

Characteristics of Teeth

An examination of the teeth of all trapped mice from the experimental sites revealed no dental fluorosis. All incisors exhibited the characteristic yellow pigmentation on the anterior surfaces. No abnormal coloration, spotting, or mottled effects were seen on the enamel.

Growth and wear appeared normal (Figure 6). The representative cross-sections of incisors of animals from control and experimental sites showed no abnormal mottling in the dentin layer (Figures 10, 11).

Laboratory Experiment

Fluoride Content in Femurs

Average water consumption in grams of water per day per mouse was 4.64 grams in level A, 4.66 grams in level B, and 4.28 grams in level C. This small variation in average water consumption can be considered insignificant and not indicating a differential consumption rate for a particular level.

Six adult mice sampled from level A contained an average of 1262.0 ppm of fluoride in their femurs. Six adult mice sampled from level B averaged 7817.2 ppm and six adult mice from level C had an average of 9164.0 ppm of fluoride in their femurs.

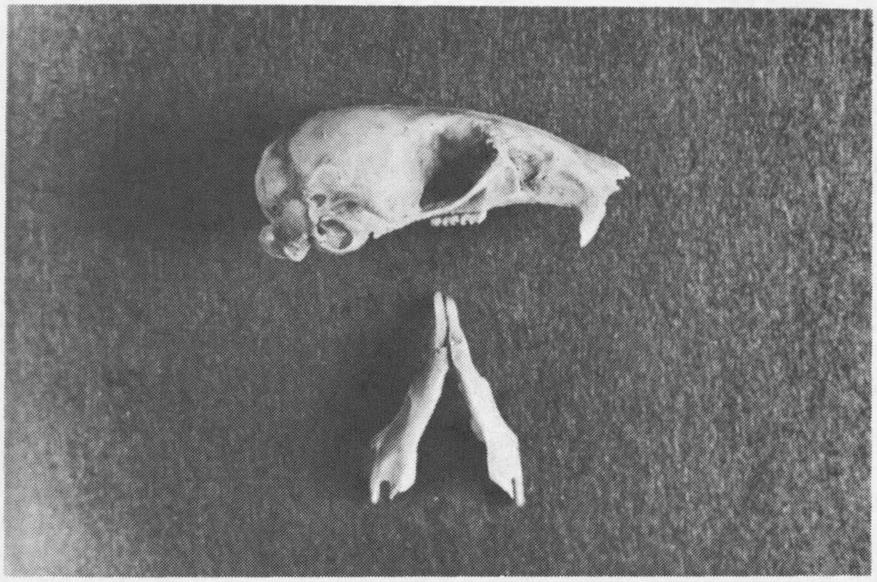
Two male mice from level A died during the experiment. There were four deaths in level B, one male and three females. Thirteen mice in level C died, eight males and five females. Ten mice from level C died during the 28-day period from March 22 to April 22, 1973, approximately $2\frac{1}{2}$ months after treatment had begun.

In level B one female produced a litter of four young on May 22, 1973. The young appeared healthy and normal. The mother and her young were killed 20 days later. The mother's femur contained 7177 ppm of fluoride and the two juveniles whose femurs were analyzed contained 3071 ppm and 5333 ppm of fluoride.

Characteristics of Teeth

No abnormal spotting or growth was seen in the enamel layer in mice from level A (Figure 7). The histological sections of the level A teeth revealed no abnormalities in the dentin layer (Figure 12). There was noticeable dental fluorosis in the incisors of the mice from levels B and C (Figures 8, 9). Incisors from mice in level B had lost the characteristic yellow pigmentation and appeared chalky-white with dull gray or brown spots. The incisors were slightly larger than those mice from level A (Figure 8). Histological sections showed dark, mottled areas throughout the dentin layer (Figure 13).

The mice incisors from level C exhibited more dental fluorosis than those in level B. Not only were the teeth chalky-white and spotted, but abnormal growth and wear patterns were quite evident (Figure 9). The histological sections of these teeth indicated a heavier mottling effect in the dentin layer than in level B (Figure 14).



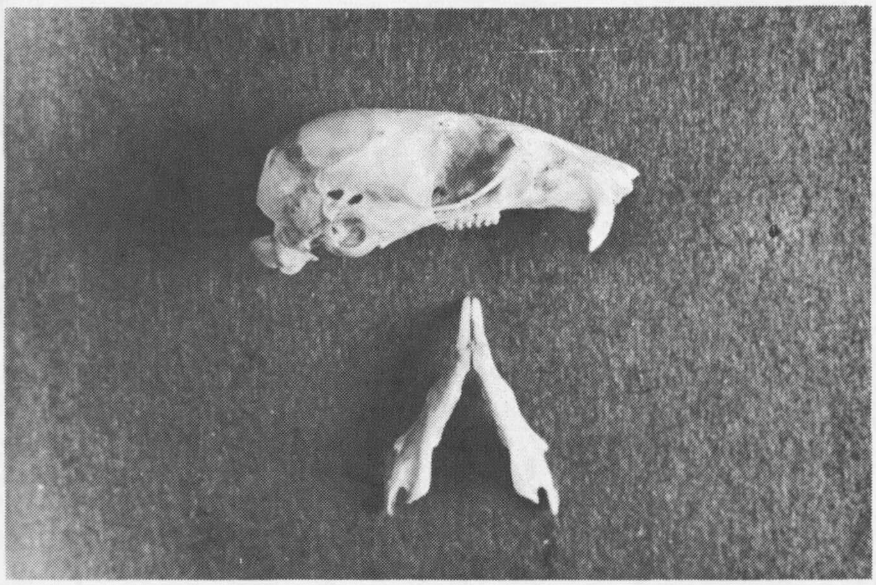


Figure 6. Photographs of mice incisors from experimental sites.

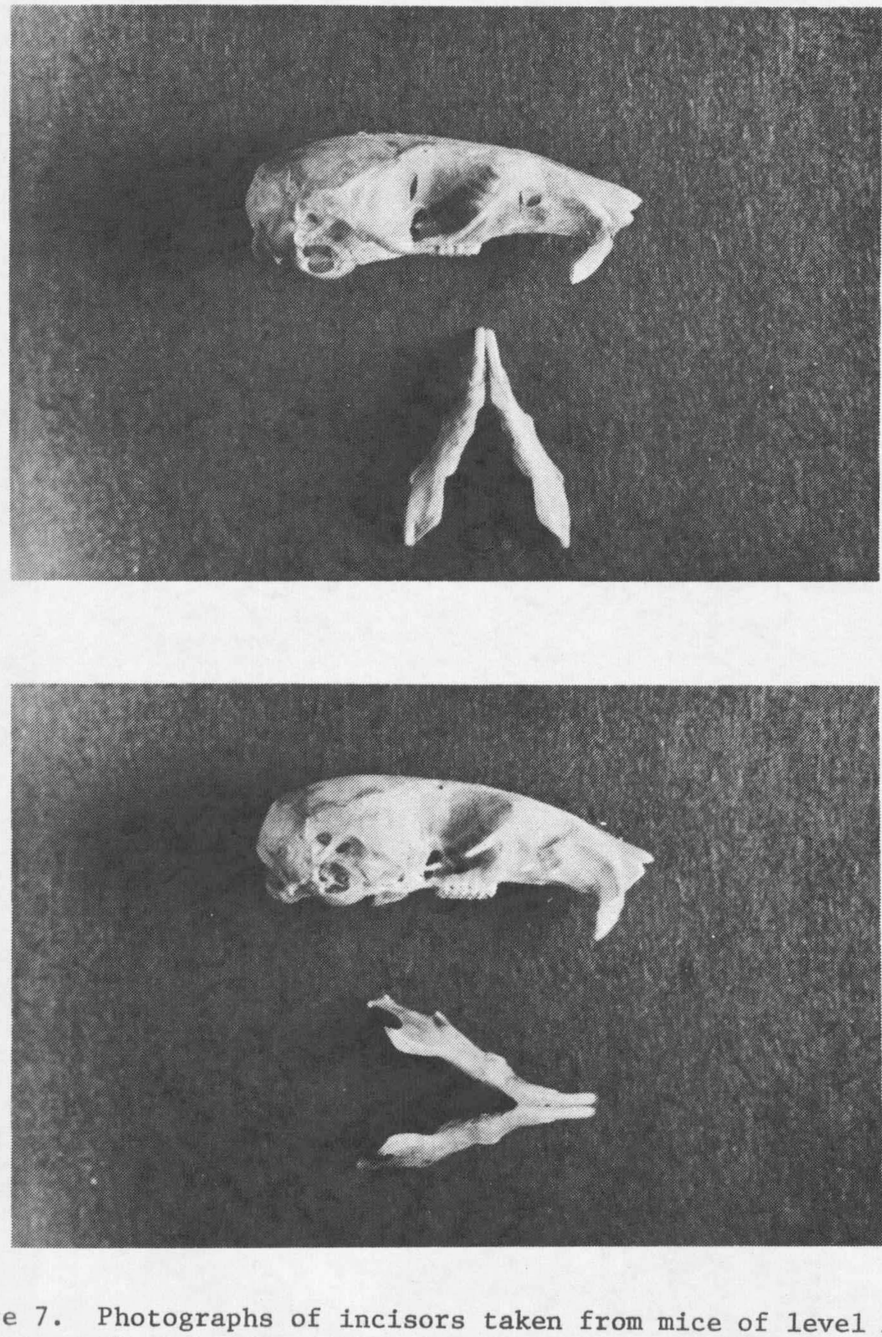


Figure 7. Photographs of incisors taken from mice of level A in the laboratory experiment.

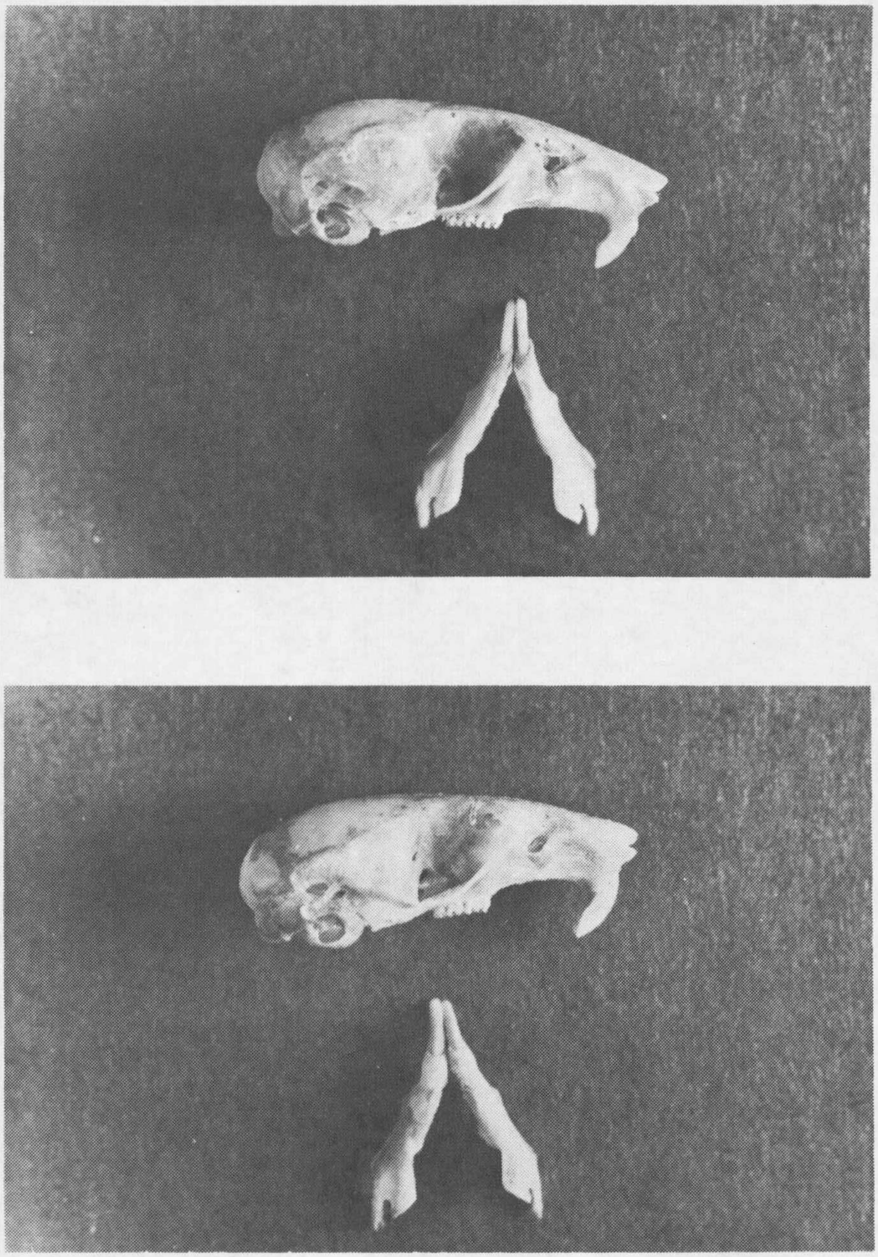


Figure 8. Photographs of mice incisors from level B, laboratory experiment. Note enlarged upper incisors.

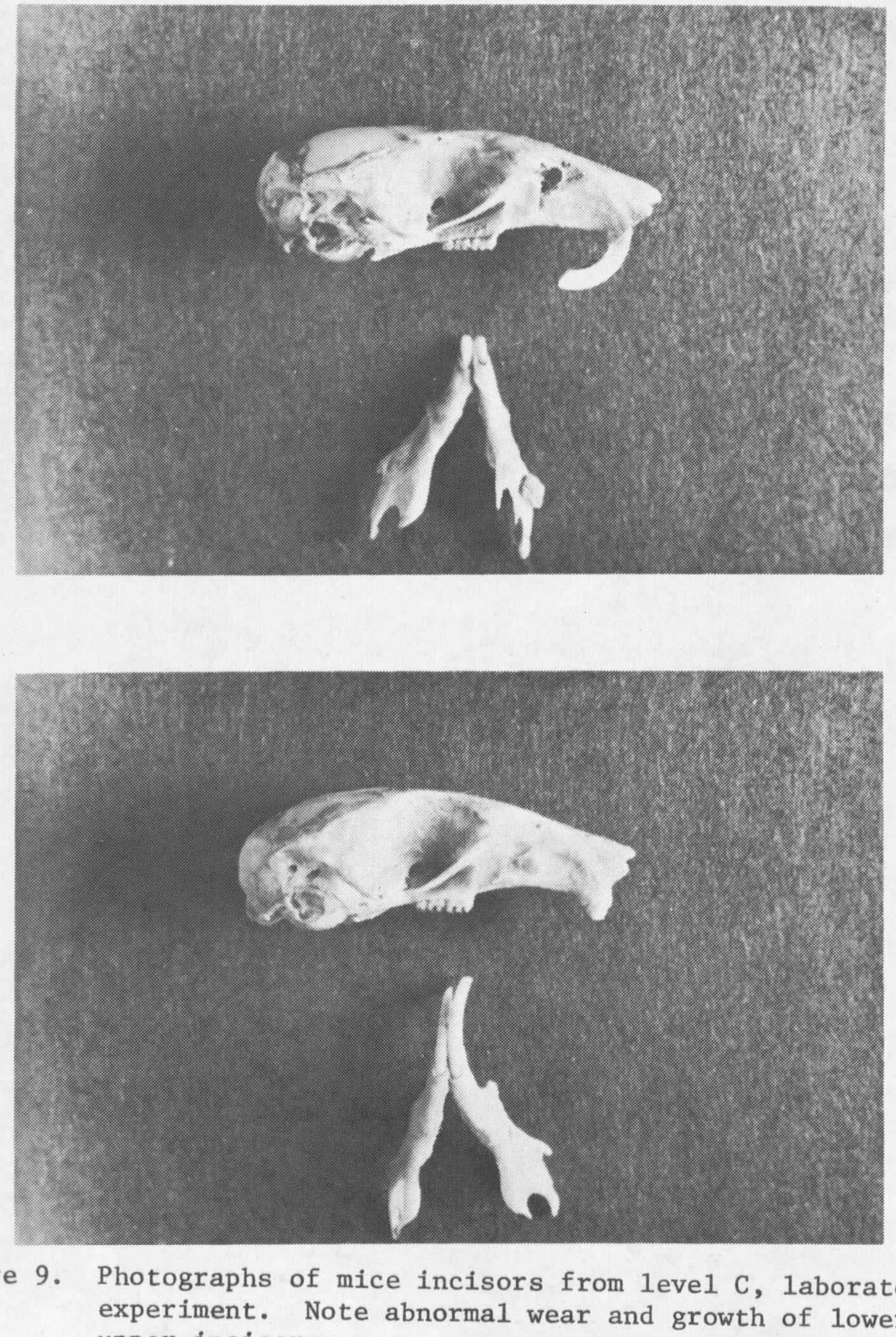


Figure 9. Photographs of mice incisors from level C, laboratory experiment. Note abnormal wear and growth of lower and upper incisors.

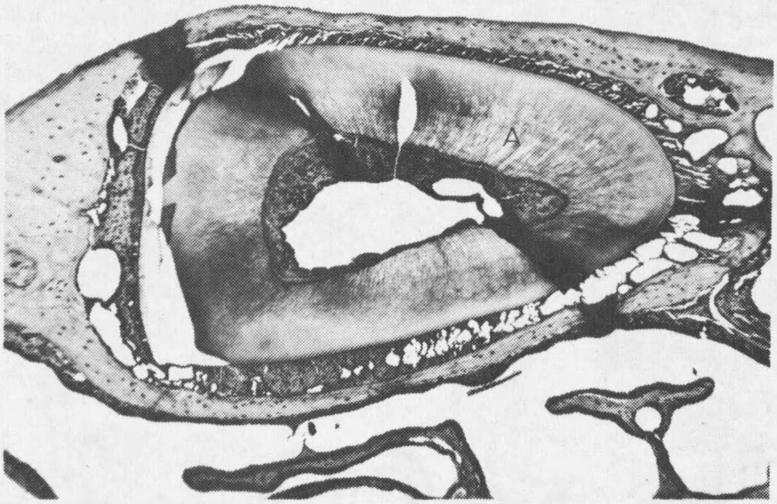


Figure 10. Cross-section of an upper incisor from a mouse collected at a control site. (A) denotes dentin layer. Magnification 64X.

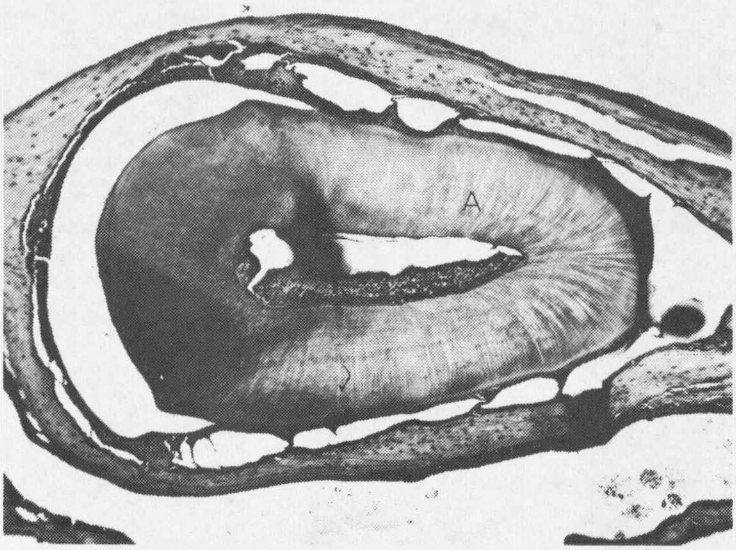


Figure 11. Cross-section of an upper incisor from a mouse collected at an experimental site. (A) denotes dentin layer. Magnification 64X.

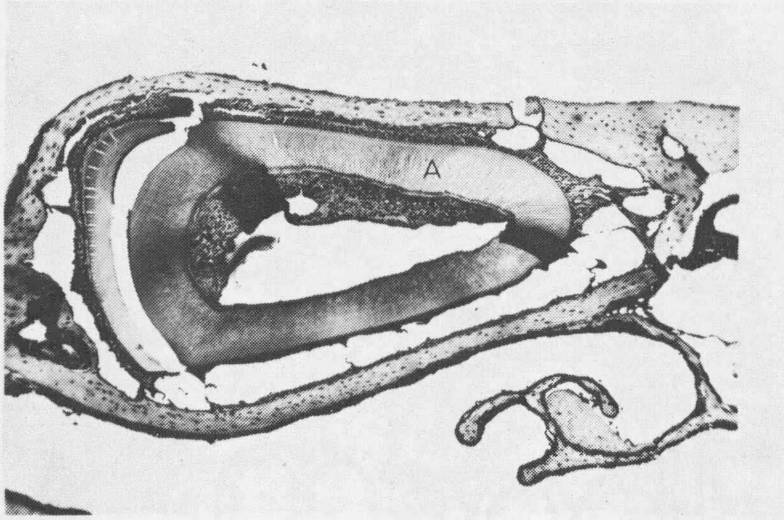


Figure 12. Cross-section of an upper incisor from a mouse in level A of the laboratory experiment. (A) denotes dentin layer. Magnification 64X.

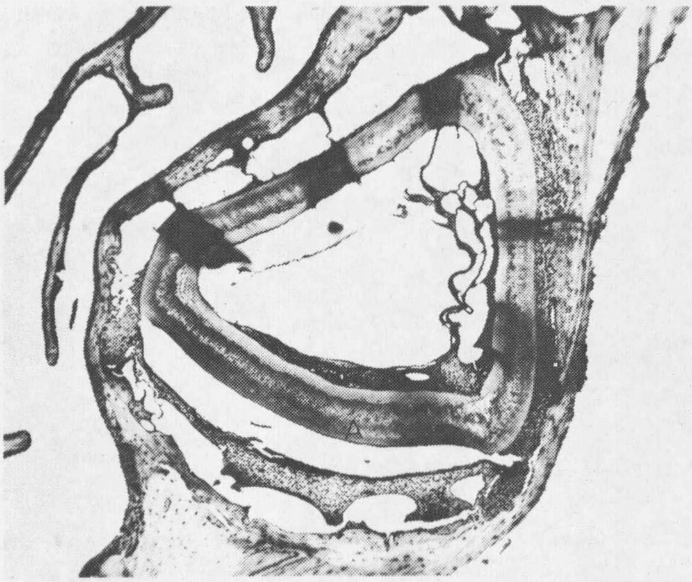


Figure 13. Cross-section of an upper incisor from a mouse in level B of the laboratory experiment. (A) denotes the dentin layer. Note the mottling which has occurred in the dentin. Magnification 64X.

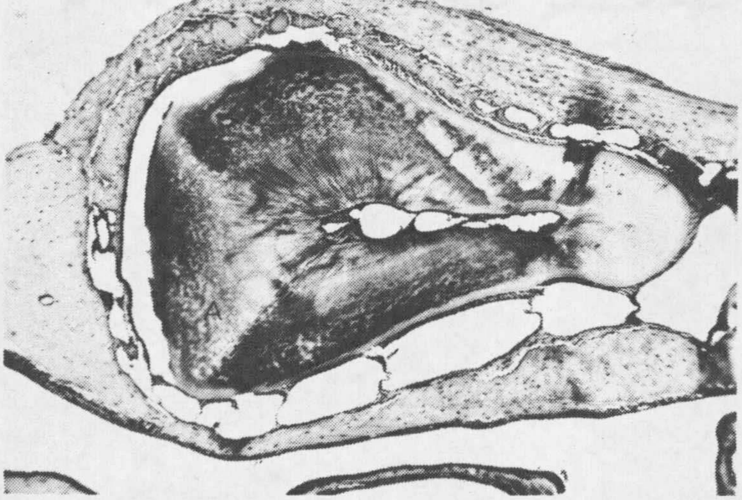


Figure 14. Cross-section of an upper incisor from a mouse in level C of the laboratory experiment. (A) denotes the dentin layer. Note the heavy mottling which has occurred. Magnification 64X.

DISCUSSION

The results indicate that the vegetative coverage and composition are different in each of the control and experimental sites. This difference in vegetation is most pronounced in the Upper West Apgar Ridge site, Belton Nob and Lower West Apgar Ridge in comparison of these three sites to the control sites (Table 2). Belton Nob tends to have more grass coverage and not as much open ground as do the control sites. Lower West Apgar Ridge does not have the shrub coverage the control sites do, and Upper West Apgar Ridge, though having similar shrub coverage as the control sites is barer in the under story with fewer grasses and forbs and has much more bare ground. In other comparisons, such as East Apgar Ridge and Headquarters Hill with any of the control sites, the values for grass and shrub coverages are very similar. But even in sites which are similar, it is difficult to determine how each site's vegetation influences a population of animals living there.

Variation in vegetative coverages and composition likely influences to a large degree the deer mice population size in these sites. As my trapping results show, there is a difference in population sizes between control and experimental sites and, on the basis of my data, this difference cannot be attributed to the high levels of fluoride found in the experimental sites. The following points support this conclusion.

First, although the sample size was small, the fertility of pregnant females from experimental and control mice, as measured by the average number of embryos present in the uterus, was approximately the same. Scheffer (1924) found that field-trapped deer mice average 5.10 embryos per pregnant female. Since the mean embryo count for females from the control and experimental sites was 5.7 and 5.9, respectively, it indicates that normal reproduction and fertility were not hampered to any observable degree in the mice from the experimental sites. In addition, a female from the laboratory experiment contained four times as much fluoride in her femur as most of the pregnant females from the experimental sites, and she bore a healthy-appearing litter of four. This suggests that reproduction and fertility are not sensitive to these levels of fluoride. Shupe *et al.* (1963) support this in that they found 12-100 ppm of fluoride in the diet of feeder cattle had no effect on fertility or reproduction. Other researchers have found low levels of fluorides to be essential to normal fertility, but this has not been definitely established (Messer, Armstrong, Singer 1972; Jaroslav 1971).

Second, aside from the differences in numbers of mice collected from the experimental and control sites, there is no significant difference in the sex ratios or age structures between experimental and control sites. The sex ratios I found at the control sites, 1:1.07 females to males, and at the experimental sites, 1:1.22 females to males, are not significantly different and are very nearly those found by Hays (1958). These findings suggest that neither the males nor the females from the experimental sites are being differentially subjected to any stress.

The population age structures in the control and experimental areas are nearly the same as both have similarly-shaped histograms (Figure 4). The trend of more adult males than adult females observed in the control population, and the opposite of that, more adult females than adult males in the experimental population, are not statistically significant. In view of this, I consider the populations similar.

Third, the comparison of body weights between mice from the control and experimental sites indicates no significant differences in growth gains of individuals from the experimental sites (Table 6). Data are lacking for deer mice, but other investigators using feeder sheep found that the sheep can ingest up to 100 ppm of fluoride in their diet without adverse effects on their growth (Harris *et al.* 1963; Hobbs *et al.* 1954). The mice from the experimental sites were exposed to vegetative food sources containing only 15-20 ppm of fluoride (Carlson 1971). Other sources of fluoride uptake by the mice could be in the form of moisture with particulate fluorides in it, insects, inhalation of fluoride compounds in the air, and through the grooming of their fur coat which may have picked up deposited fluoride compounds.

The fourth point is that no dental fluorosis was observed in the mice from the control or experimental sites. Jaroslav (1971) states that "developing teeth are extremely sensitive to fluorides, and abnormalities in permanent dentition are the most obvious signs of the ingestion of increased amounts of fluorides in the ration." Since the deer mouse incisors are continually growing the teeth would be one of the first organs to manifest any observable damage due to excess fluorides.

Finally the mean levels of fluoride occurring in the apparently healthy control mice of level A in the laboratory experiment were higher than those fluoride levels seen in the experimental mice from the field. This also seems to indicate that the fluoride levels present in the field-trapped mice from the experimental sites are not detrimental to their health.

For these reasons I conclude that no adverse effects of fluorosis were seen in the experimental population in the biological aspects I measured. This is not to say that other areas of the deer mouse biology might not be affected. Bone strength, enzyme activity or subtle ecological changes in the population could be areas affected by abnormal fluoride accumulation.

Incidental Findings

A few incidental findings have been observed throughout the study which may not have a direct bearing on the question of whether excess fluorides in the park are affecting the deer mice. First, there were higher fluoride levels measured in the femurs of mice from both control and experimental sites in 1973 than in 1972. This trend was most evident at the experimental site of East Apgar Ridge which was trapped both years. The control site of Lower Walton Goat Lick, also trapped both years, did not show this trend, but the overall fluoride average in mice from control sites trapped in 1973 is greater than the average of mice trapped in 1972 (Figure 5).

I also observed a high fluoride concentration in pregnant and nursing females as compared to other adults from the same site in the experimental areas (Table 8). Similar data have been reported for pregnant women and this increase could possibly be accounted for by the increased availability of the skeleton because of the hormone-induced bone changes in the normal cycle of preparturition (Hodge, Smith and Gedalia 1970). This trend was not observed in the laboratory mice due to the small sample of pregnant females.

The observed fluorosis of the teeth of laboratory mice in level B and C is similar to results reported by Bhussry (1970). Bhussry also states that drinking water with 500 ppm of fluoride was toxic and only a few of the experimental rats lived. These findings are very similar to the marked increase in deaths I observed in the laboratory mice which were given 600 ppm of fluoride as compared to mice receiving

200 ppm and 1 ppm of fluoride in their water.

An interesting finding from the laboratory experiment was the very high level of fluoride in the femurs of the juveniles born in level B. Layne (1968) indicates that weaning generally occurs 21-28 days after birth. Since the juveniles were killed at an age of 20 days, the high concentration of fluoride must have been deposited during gestation through the placenta or obtained later during nursing. Studies of fetal fluoride concentrations support the placenta-transfer method of fluoride to the fetus (Gedalia 1970). Suttie, Miller and Phillips (1957) suggest that even with relatively large increases in dietary fluoride consumption there is little increase in milk fluoride content. This suggests that the majority of fluoride in the juvenile femurs was probably deposited during gestation.

Although I found no adverse effects due to excess fluorides in the mice from the experimental sites, future investigators may want to look at those areas of the deer mouse biology I did not study, or possibly select another study animal more sensitive to fluorides. In any case, monitoring the fluoride levels in Glacier National Park should continue with both plant and animal species. Continued observations may provide information concerning the long-range effects of fluorides on the contaminated region of Glacier National Park's ecosystem which would in turn add information to various biological aspects of pollution by atmospheric-borne fluorides.

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