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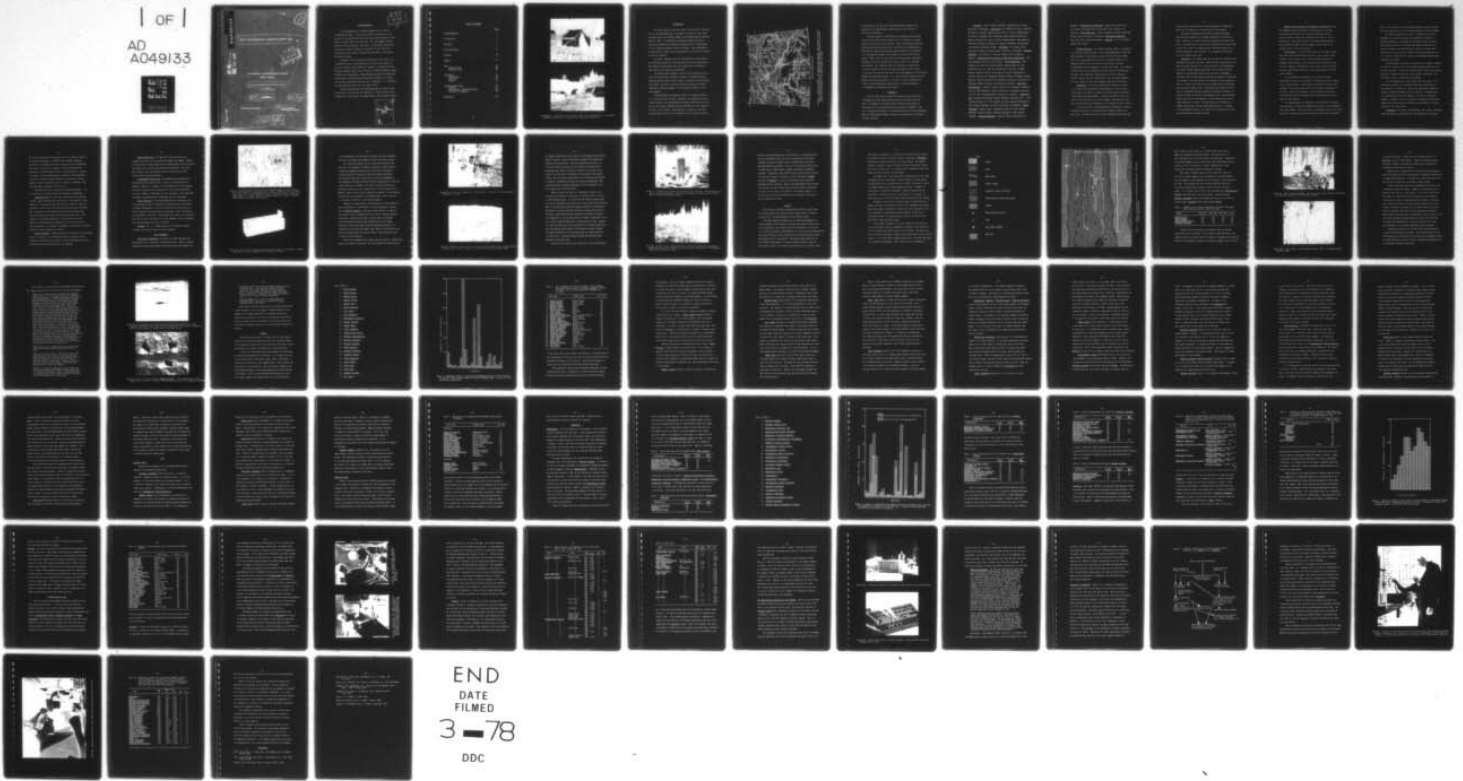
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6 REPORT OF FIELD COLLECTIONS and LABORATORY DIAGNOSTIC ASSAY.

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THE UNIVERSITY OF OKLAHOMA <sup>unit</sup> RESEARCH INSTITUTE  
NORMAN, OKLAHOMA

Ecology and Epidemiology Research Studies  
in Alaska

11 25 Apr 1966

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PRINCIPAL INVESTIGATOR:

10 Cluff E. Hopla  
Professor of Zoology

528279

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**ACKNOWLEDGEMENTS**

It seems appropriate to express appreciation to certain individuals who have, in one way or another, contributed to the success of the program this past year even though they may not have been an integral part of the activity. Mr. John Bushman, Project Officer, has been helpful in many ways. I have deeply appreciated his sense of values in working in a problem of this type and I believe his recent visits to Alaska have done much to help smooth difficulties there.

Personnel of the United States Army Arctic Test Center have cooperated with us in many ways and are actually too numerous to mention. However, Colonel Walter F. Johnston, Lieutenant Colonel William S. Brophy, Major John A. Mojecki, and Master Sergeant Joseph P. Watson are acknowledged. Master Sergeant Watson has contributed significantly in coordinating and receiving shipments of freight, thus saving considerable time and loss of equipment. It is difficult to give sufficient credit for this type of help.

Various people within the Alaska Department of Fish and Game have given us of their time and information. Special mention must be made of Mr. Frank Jones, Mr. Robert Rausch, and Dr. Robert Weeden.

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Frontispiece. Field laboratory and camp. While not possessing most of the modern laboratory facilities, it does have the advantage of mobility.

## INTRODUCTION

In view of the fact that this study is essentially a continuation of the preceding year, the reader is directed to the rather extensive report for Contract Number DA 42-007-AMC-121(R) submitted March 30, 1965. In this work a deliberate attempt was made to provide the background information necessary for an understanding of the biological problems involved in Alaska. This undertaking was particularly needed in an investigation such as the one in which we are now involved.

This year a greater amount of our effort was concentrated in the Delta-Fort Greely area than any other within the Tanana Valley. Some regions visited previously were omitted from our schedule, particularly those along the Nenana Road.

Our study differs somewhat from that of the previous year in that one team has been retained in Alaska on a year-round basis. Knowledge gained by this team is useful in regards to the fur-bearing animals, and also by the fact that they were able to obtain information (although meager) on the population dynamics of the small mammals.

During November and December, almost the complete schedule of the field team was devoted to capturing live animals to send to another activity for microbiological studies. While this was not a part of the current proposed study, we entered into this request to the best of our efforts. Unfortunately, it came at a time when certain animals could not be furnished due to the peculiar habits



in hibernation, and also when trapping procedures needed to be changed for the small mammal population prior to the onset of real winter conditions.

A certain amount of psychological disturbance was maintained on the field crews this past summer due to an inability to secure laboratory facilities at Fort Greely. Many contradictory directives were received from various offices that had to be straightened out. Eventually we ended up moving from space assigned to us originally to a reconstructed Quonset type building in the old area of Fort Greely. While I have not seen these facilities myself, information furnished to me by the field crew indicates that it is minimally satisfactory. The tragedy of all this confusion at Greely need not have occurred at all for the space from which we were forced to move was not utilized this year by the other segments of the military command as previously planned. It is not intended to imply that this offered any serious deterrent to our work; it merely required that I had to spend considerable efforts while in Alaska attempting to straighten out needless small details.

#### PERSONNEL

The list that follows provides information concerning those individuals that have been associated with the project since May 1965. All members of the field crews, laboratory technologists, and certain of the secretarial staff have received clearance. The list is compiled according to the responsibility or area of performance. Only in those instances where a person was terminated is any mention of dates included.

Director: Cluff E. Hopla, Professor, Department of Zoology, University of Oklahoma. Consultants: Dr. William O. Pruitt, Associate Professor of Biology, Memorial University, St. John's, Newfoundland; Dr. David B. Lackman, Chief Serologist, Rocky Mountain Laboratory, Hamilton, Montana; Dr. Cora R. Owens, Microbiologist, Rocky Mountain Laboratory, Hamilton, Montana; Mr. Heinrich Springer, Ornithologist, P.O. Box 293, Fairbanks, Alaska. Serologist: Dr. George Cozad, Associate Professor of Microbiology, University of Oklahoma. Research Scientist: Dr. William L. Jellison, USPHS, retired, Hamilton, Montana. Administrative Secretary and Laboratory Technologist: Mrs. Joyce Markman, University of Oklahoma. Field Technicians: John Henshaw, P.O. Box 593, College, Alaska, appointed May 18, 1965, terminated July 31, 1965; Mr. Mike Molchan, P.O. Box 11, Eagle, Alaska, appointed July 6, 1965; Harold B. Ritter, Department of Zoology, University of Oklahoma; Thomas A. Schuck, Fairbanks, Alaska; Samuel W. Stoker, University of Oklahoma; John Taylor, University of Oklahoma; Chester Twitchell, University of Oklahoma. Laboratory Technologists: Donald J. Barras, University of Oklahoma, terminated July 31, 1965; Melinda H. Keezer, University of Oklahoma, appointed July 26, 1965; Arlene Robinson, University of Oklahoma. Laboratory Aids: Estelle Nora Howard, Norman, Oklahoma, appointed June 1, 1965, terminated August 31, 1965; Angela Cleveland, appointed September 9, 1965, Norman, Oklahoma; Jay Atkin, University of Oklahoma. Animal Caretaker: Douglas Jones, terminated October 31, 1965, Norman, Oklahoma; Vercey S. Davis, appointed November 9, 1965, Norman, Oklahoma. Research Assistant: Harold B. Ritter, University of

Oklahoma. Entomological Technicians: Megan Young, half-time, University of Oklahoma; Robin Young, half-time, University of Oklahoma. Hourly Employees: Ermona McGoodwin, Bethesda, Maryland; Marjorie Orr, University of Oklahoma. Secretarial Assistant: Mrs. Gilda Olive, Hamilton, Montana. Trappers: Dr. L. L. Huffman, Paxson Lake, Alaska.

Literature Survey: Dr. Jellison and Mrs. Olive, in collaboration with Mr. Kenneth Neiland of the Alaska Department of Fish and Game, prepared a host-parasite index of Alaska this past summer. While not a large publication it was an extremely tedious one to prepare because of the very nature of the entries involved. Considerable time was expended in editing this report. Dr. Jellison and Mrs. Olive are now preparing a review of the literature pertaining to the organisms of tularemia and Q fever in Eurasia. Letters from Dr. Jellison indicate that they are making progress but that completion will come somewhat later than anticipated.

Consultants: It will be noted that Dr. William O. Pruitt is no longer with the University of Oklahoma but still serves a vital role as an advisor in studies involving population dynamics of the small and large mammals. He spent the month of July, full time, in the field with the crews in Alaska. His departure from the active scene was anticipated but is a difficult loss to resolve.

A visit was paid to the Rocky Mountain Laboratory to discuss problems involved in our work with Drs. Lackman and Owens this past July. The pros and cons of various techniques were mentioned

and particularly valuable were those pertaining to the RIP test which will be reported in a later section of this report.

Mr. Heinrich Springer was located at Delta this summer where he was an engineer for bridge construction on the Tanana River. However, his after-hours were reasonably free and he was able to contribute much in the way of identification of birds taken in mist nets as well as showing various individuals some of the subtle techniques of mist netting. It is hoped we will be able to use him further next year.

Serologist: Dr. George Cozad has been with the University of Oklahoma for the past six years. While his Ph.D. thesis was in the area of medical mycology, he is well-trained in the fundamentals of medical bacteriology and immunology. He has spent previous summers at the Oak Ridge Laboratories and thus is thoroughly conversent with the radioisotope techniques as applied to immunological studies. For this reason he was brought into the program to work on a quarter time basis during the summer and as he had free time during the academic year. Arrangements were made for him to travel to the Rocky Mountain Laboratory at Hamilton, Montana, to discuss the RIP test with Dr. David Lackman and to gain additional insight into some of the vagaries of this technique that are at times difficult to control. He also serves as a consultant as needed, particularly for Miss Arlene Robinson who is in charge of the serological survey. Dr. Cozad will be available on a full time basis during the summer of 1966.

Administrative Secretary and Laboratory Technologist: Mrs. Joyce Markman serves principally as administrative secretary, coordinating the activities of the hourly employees and the routine financial aspect that otherwise would take the time of the Director. Her proficiency has increased immeasurably as she has gained insight into the program. She has been an invaluable asset to me this past year.

Field Survey: It will be noted that Mr. Sam Stoker and Mr. Ritter are carry-overs from the last year, thus, giving a basic foundation to each field crew. Both are highly skilled and competent field workers. They have gained the necessary insight into the problems in working with other people, dealing with the military, and understanding some of the unique aspects of field work in Alaska.

Mr. John Taylor was brought with us this past summer specifically because of his interest in obtaining practical experience in boreal botany. Large numbers of botanical specimens were secured in the various study areas and are being classified as he has time. Later in the summer it is anticipated the botanical identifications will be completed and a report submitted on this aspect of the problem.

Mr. Thomas Schuck is a graduate of the University of Alaska, having majored in wildlife management and has been a very dependable and steady worker. This past winter a considerable part of his effort went into a study on the activities of the beaver and he performed

most of the autopsies on the fur-bearing mammal carcasses brought into the regional office of the Alaska Department of Fish and Game.

Mr. Chester Twitchell was taken to Alaska because of his background in vertebrate ecology and expressed an interest in population dynamics. Originally it was hoped that Mr. Twitchell would become involved in a problem that would be suitable for a doctorate thesis, thus requiring him to remain on a year-round basis for at least two years. However, he decided Alaska was not to his liking; although to his credit, he performed admirably well throughout the summer.

Mr. Mike Molchan has been a professional fur trapper in Alaska for the past fifteen years and furnished occasional material to us over the past year. He has been associated with some of my previous programs in Alaska and has been a very reliable individual. He was hired in July to replace Mr. John Henshaw.

Mr. John Henshaw had a brief tenure with the project. Unfortunately, because of an extremely inflexible personality, it was impossible to retain him. This was an unfortunate experience because considerable emphasis had been placed on recommendations that indicated he would be an expert in the area of population dynamics. However, he had no interest in anything but large game mammals and simply could not conform to the multiplicity of tasks required of our field crews.

Laboratory: Mr. Barras, Miss Robinson, Mrs. Keezer, Professors Cozad and Hopla are responsible for this segment of the program.

Mr. Barras resigned from the project July 31 to accept a position as Assistant Professor at Nicholls State College, Thibodaux, Louisiana. Mrs. Melinda Keezer was appointed as his replacement and comes to us with a B.S. degree in microbiology from the University of Indiana where she had a strong interest in studies of viruses. She had approximately a year and a half experience working in a medical research laboratory, dealing with various phases of virus investigation prior to coming to Oklahoma. She has been highly competent in her position.

Miss Robinson gives a reliable and steady performance. She is regarded as the leader among the laboratory technologists.

Laboratory Aids: Mr. Jay Atkin, Miss Estelle Nora Howard, and Mrs. Angela Cleveland have functioned in this capacity. Miss Howard was a highly superior young lady but decided to enter the university life full time and thus was replaced by Mrs. Cleveland. Mrs. Cleveland has been remarkably good help and certainly has saved her salary with regards to handling and caring for teflon grinders in the washing processes. Mr. Jay Atkin has a B.S. degree in microbiology and ultimately will leave us to become a full time student in the school of pharmacy. He has been a pleasant, dedicated worker throughout his tenure.

Animal Caretaker: Douglas Jones, who had been with this program since its inception, was terminated October 31 because outside interests conflicted with his duties. He was replaced by Mr. Davis who has been distinctly superior in all respects.

Research Assistant: Mr. Harold B. Ritter was hired as a research assistant upon his return from Alaska this summer. However, he decided that he would rather work in Alaska rather than to continue with school and since Mr. Mike Molchan was asking for a two month leave (without pay), Mr. Ritter was sent to fill his slot and continue work during this spring.

Entomological Technicians: Miss Megan Young and Miss Robin Young work half-time while going to school at the University of Oklahoma. Robin is in charge of all illustrations for this program as well as helping with the preparation of the various arthropods for study. Megan is responsible for the cataloging of all materials and shares the preparation of arthropods with her counterpart.

Hourly Employees: Miss Ermona McGoodwin has completed a few of the drawings needed to complete the illustrations for the study of Alaskan Siphonaptera. It will be recalled that she served in a similar capacity the previous year and had been a full time employee in my program for some time. Miss Marjorie Orr works as a secretarial assistant and gives editorial aid to Mrs. Markman, as well as helping with the cataloging and filing of records.

Trappers: Dr. L. L. Huffman aids in the securing of mammal specimens, particularly those of the fur-bearers.

#### FIELD PROCEDURES

Collection of Specimens: Collection of field specimens was essentially that as reported for the previous year. Again, a greater emphasis was placed upon mammals rather than upon birds because most

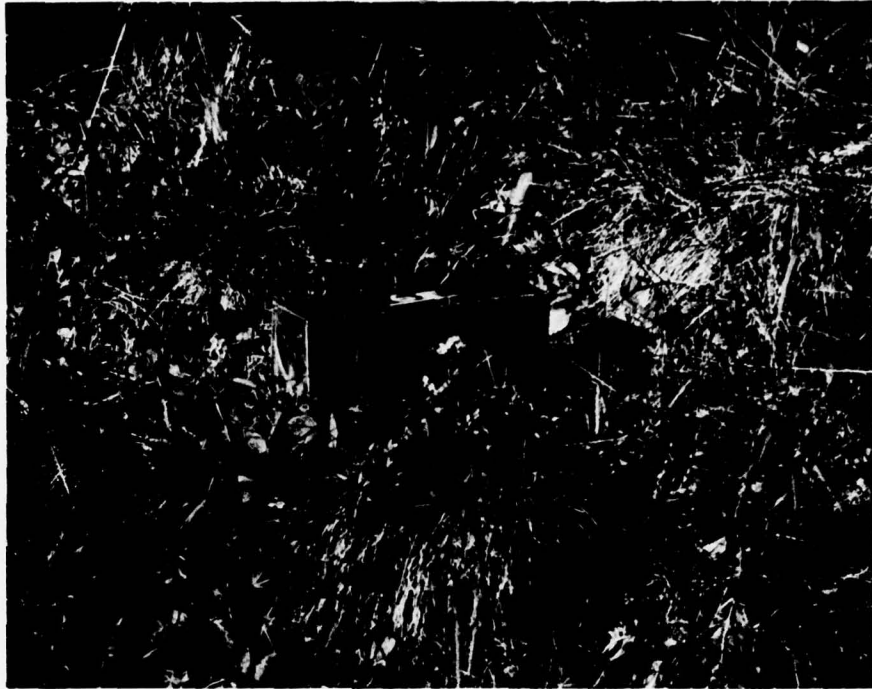


Plate I. Folding Sherman live trap used for summer trapping. Placed within a No. 2 nail sack, it is satisfactory for retaining voles alive for 4-6 hours late in the fall if the temperature does not drop below  $-10^{\circ}$  F. This is an ideal trap for the warmer seasons in Alaska because it is light in weight and collapses for storage and transport.



Plate II. Pruitt trap, constructed of masonite and used in cold weather. Usually this trap is used in conjunction with a plywood "chimney".

of our knowledge in the New World concerning tularemia organisms and Q fever are based upon mammalian rather than avian studies.

Our collecting efforts did not differ significantly from last year. We attempted to acquire a relatively large series of animals for biomedical assay work but also were interested in obtaining indicies of population densities whenever possible. For the latter purposes, we continued to use the standardized plot sampling techniques modified by Pruitt especially for taiga and tundra conditions of Alaska. This year study plots were put in the Gerstle River area and at Delta Creek. We continued with the SUM Plot (Plate IV) and those of Manley Hot Springs, but essentially deleted Circle Hot Springs and the Nenana areas. Plots were set in a variety of habitats in the Fairbanks Region.

During the summer months, folded Sherman live traps (Plate I) were used whenever it was feasible to obtain specimens. Species such as Microtus gregalis were again found difficult to trap by this method so that frequently we had to resort to Museum Special snap traps. Because of the ease in using these, the Schuyler traps (Preferred by Pruitt) were abandoned by us this summer. Sometimes a likely area was literally saturated with traps. We checked the live traps four times a day (early in the morning, mid-morning, mid-afternoon, and late at night). Snap traps were used as often as time permitted.

Once the live mammals were caught they were placed in appropriate sized cloth bags and returned to the field laboratory (Frontispiece)



Plate III. Setting up a mammal grid in Delta Creek. One acre plots are used with 100 traps to a plot.



Plate IV. SUM Plot, upland tundra. In this area brown lemmings and various species of voles are obtained. This is the mammal grid we have in upland tundra.

and lightly anesthetized, after which a blood sample was secured by cardiac puncture. Once this had been obtained, the animals were brushed for ectoparasites. The blood samples were placed in a "cool box" which consisted of a pit dug into the ground until permafrost was reached, usually at a depth of 2-1/2 feet in the taiga to not more than a few inches in some areas of the upland tundra. The clots were ringed after two to four hours and allowed to contract overnight after which the serum was harvested. The serum was pooled, consisting maximumly of five specimens of the same species from the same habitat.

Liquid nitrogen was used as a refrigerant because of the difficulty and expense of obtaining a reliable source of dry ice in the Fairbanks region. We found that the liquid nitrogen chest did not need recharging more than once a month under field conditions. Some of the new models came with plastic handles on the canisters. According to the Union Carbide Corporation, these were equally as durable as those with the metal extensions and would not conduct the temperature to the outside nearly so rapidly. Supposedly, this was to make liquid nitrogen last a longer period of time. However, we found that under the traveling conditions in Alaska (rough highways and equally rough flights on helicopters and small planes) the plastic handles for the canisters were not nearly as good as the metal ones. A special request was made and canisters were supplied of the metal type.

Arthropods (including ticks, mosquitoes, other haematophagous



Plate V. "Chimney" box in which the masonite trap is lowered. These boxes are set out in the fall and baited. As the snow accumulates in winter, one is still able to continue population studies.



Plate VI. Western edge of Delta Creek area. The arctic avens and cottonwoods predominate the gravel outwash in the foreground. Black spruce is the dominant plant of the study area proper.

Diptera, and Siphonaptera) were collected as in the previous year, the only difference being that with the mosquitoes we did some sweeping of vegetation where certain animals such as moose and ground squirrels were known to occur on a relatively regular basis in hopes that we might obtain a higher percentage of engorged specimens. Apparently these efforts were not particularly successful from these aspects. Be that as it may, 303 pools of Aedes, 85 pools of Culiseta, 11 pools of blackflies, and 44 pools of Siphonaptera were obtained. Some of the Siphonaptera were retained for taxonomic studies, as were lice and mites because essentially the fauna of the latter is not known in the boreal regions. In another year the mites and Anoplura can be included in the pools for microbiological assay.

#### ECOLOGY

The ecology of Alaska was thoroughly discussed in the report of last year and at this time only will special mention be made of the new areas. The most germane is that of Delta Creek.

The Delta Creek Site (Figure 2) is located approximately 35 miles southwest of Fort Greely, along the east slope of Delta Creek. It is surrounded in all directions by the tributaries of the Creek and is about one-half to three-quarters of a mile wide and approximately four and a half miles long, enveloped by a relatively barren outwash which gives rise to steep bluffs. The western edge (Plate VI) of this island is populated by a stand of mixed deciduous trees and black spruce forest. The former (consisting mostly of poplar, alder,

and aspens) is arranged in a narrow band around the spruce forests. The undercover here is relatively sparse, consisting of Equisetum (horsetail), high-bush cranberry, and some lichens. The eastern boundary, formed by a small tributary of Delta Creek about 20 feet wide and 2-1/2 feet deep, is virtually free of deciduous forest and almost entirely consists of black spruce.

The larger part of the study area, specifically the site where the mammal plot was established, consists largely of black spruce interspersed with an occasional birch or aspen. The stand of spruce is somewhat sparse and of medium height and girth. Inasmuch as black spruce is generally considered to grow in areas where the permafrost is relatively close to the surface of the ground, one would judge that it would be found a short distance from the surface.

The ground cover within the spruce forest is essentially of a muskeg type with a deep hummock cover of moss, sphagnum, and lichens which also support blueberries, crowberries, and a profusion of low-bush cranberries. Some dwarf birch are present as well as an occasional willow. The plateau above the bluff on the east soon gives way to typical upland tundra.

In the upper southeast end of the island the tributary has, in the last year, flooded approximately one-third of the island and left it covered with a thick layer of silt and mud. The spruce are still alive but John Taylor thought they would probably die out within a year. It is likely that flooding would occur in the same area again next spring during breakup. With a dense cover of sphagnum on a

Key:



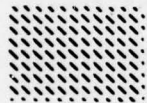
Spruce



Bluff



Delta Creek



Gravel outwash



Islands of alder and willow



Black spruce and deciduous forest



Tundra



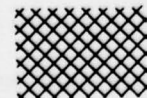
Meteorological station



Camp



Plot, small mammals



Mud flow

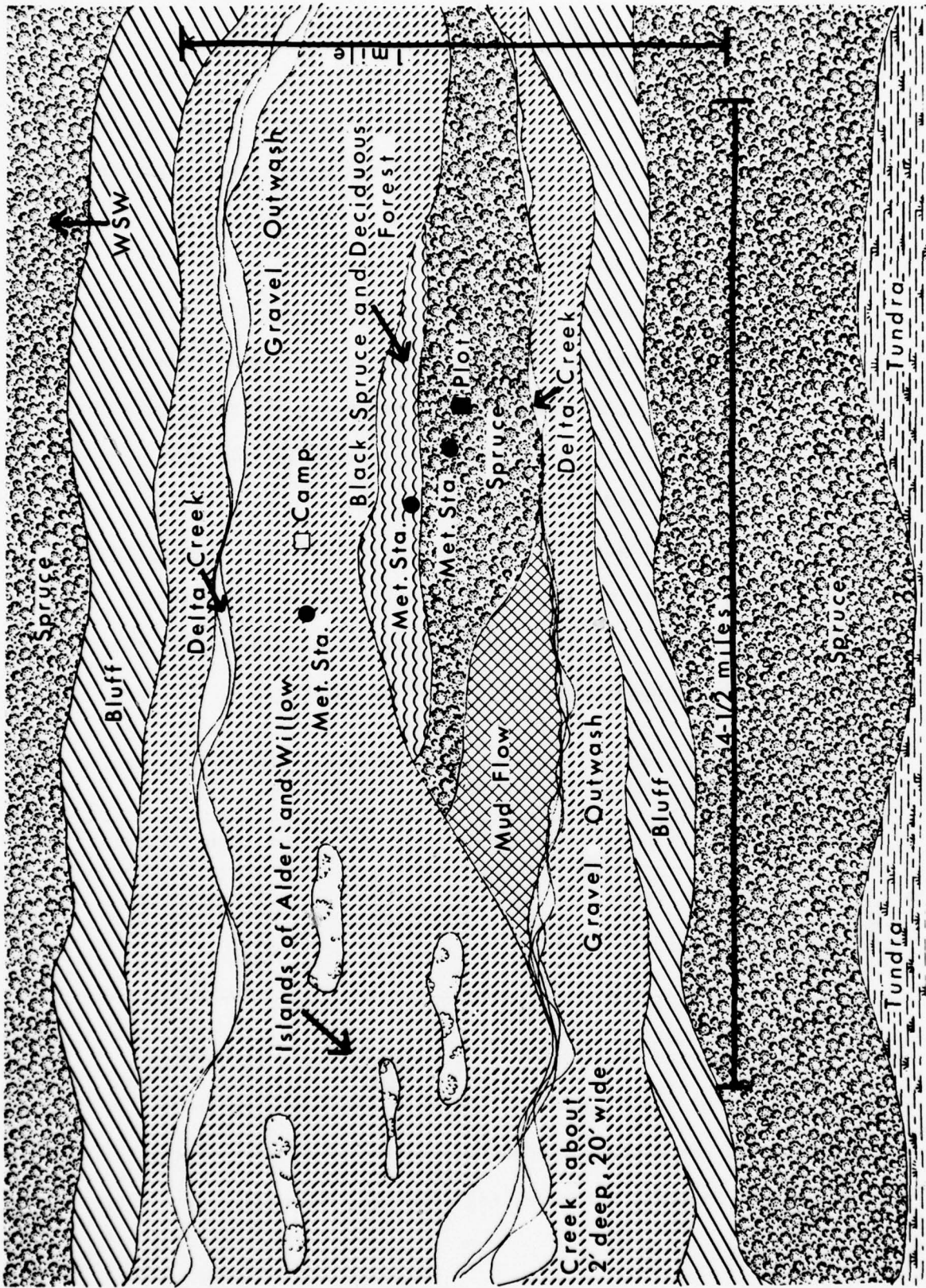


Figure 2. Schematic representation of the Delta Creek Study Site. Distances are only approximations.

major portion of the island, it is doubted that there is any danger of flooding here during breakup. The ground cover is a good indication that it has been stable for many years. Ordinarily to have the sphagnum type of ground cover well established requires as much as 25 to 30 years and it cannot withstand the violent actions that flooding achieves during spring breakup.

The number of mammals taken from the study plot (Table 1) and also by random sampling, has indicated that the small mammals are not present in large numbers; on the contrary, a light population was seen. The sampling thus far conforms relatively well with the visual estimates made during our first visit to the area in August. In a habitat such as this, the red-backed vole (Clethrionomys rutilus) should always be the most abundant. Around the edges Microtus oeconomus likely would predominate and as you go into the upland tundra, oeconomus should again exceed rutilus.

Table 1. Mammals taken at Delta Creek Study Site; 300 trap nights were used each month to obtain this data.

Mammal	Aug 1965	Sept 1965	Mar 1966	Total
<u>Sorex arcticus</u>	02	09	01	12
<u>Sorex cinereus</u>	00	04	00	04
<u>Clethrionomys rutilus</u>	16	60	07	83
<u>Microtus oeconomus</u>	00	06	01	07

Insofar as the avian fauna is concerned, there is nothing distinctive about the island. At the times we have been there, the population was not unusual insofar as density of numbers were concerned. Such birds as Swainson's thrush, fox sparrows, redpolls, and a variety



Plate VII. Delta Creek in November. The snow on the trees indicate little wind activity has taken place since snowfall started.



Plate VIII. Birch forest, near Fairbanks, Alaska. This is secondary growth nearing climax.

of warblers were seen. Likely the pine grosbeak would be a Rara avis in such a small habitat. Almost no sign was seen for ptarmigan. However, observations in March have indicated that occasionally these birds are present.

Summer sign of snowshoe hare and red squirrel were virtually absent and there has been nothing gained in subsequent observations to change this impression. A few trails of these hares have been seen during the winter but no animals actually sighted.

As for large game, there was an abundance of old bison sign on the gravel outwash in August. However, no animals have been observed there during or since August. In a recent letter from Sam Stoker, he reports that a bison census has been completed indicating that there are approximately 169 animals in the Delta area. This is nearly half of what had been reported to us last summer. Caribou are variable and at certain times of the year much more abundant than any of us had originally anticipated. During August three were seen in the general vicinity by one of the field crew although two weeks previous to their trip, 200 head of caribou were reported only a few miles away by one of the game biologists of the Alaska Department of Fish and Game.

Apparently the bison (in the past) have used the Creek as a source of food during past winters. Such long stretches of open land generally are blown free of snow and various leguminous plants are made available to them for forage. Evidently the buffalo have not followed this pattern this year.

The following is a quote from correspondence obtained from Mr. Stoker concerning their last trip to the Delta Creek Site.

"Ritter and I went to Delta Creek the 28th of March through April 2. The trip was not what it might have been due to weather. A sudden thaw caught us, which made trapping difficult and tracks hard to interpret. We flew a helicopter survey of the area first then camped on the site proper for five days and trapped and walked over the area extensively. There are caribou in the area and probably will be for some time. There is a more or less resident band of six caribou that seem to hang about and feed in and out of the site itself and scattered herds amounting up to 500 animals feed on the plateau to the east and west of the site, crossing back and forth from time to time, directly through our study area. At the time, these herds were about evenly divided between the east and west plateau, above the creek valley. I would say at the time we saw them, that there were as many as 500 caribou within a ten mile radius of our camp. These are migrant herds, however, and apparently move in and out of the area at no fixed schedule with the exception of the one resident band of six. These herds cross generally at the extreme upper and lower ends of the island, usually in an east to west direction with little movement up and down the creek valley. There are well defined descending and ascending trails up and down the bluffs to both sides, but through the valley itself, the trails seem to fan out and the caribou feed and rest for a short time.

"There are also two resident moose and probably some additional specimens passing through from time to time.

"There is no sign of bison. The Fish and Game just finished their bison census in the Delta area and came in with the count of 169 animals which is much lower than expected. This was the first really thorough count in recent years. They saw no sign of bison in the Delta Creek though I think some will probably move in later this spring.

"There are at least two resident coyotes working up and down the creek-bed and wolves crossing from time to time with the caribou. There is some sparse sign of snowshoe hares; mostly along the bluff to the east and around the upper end of the island.



Plate IX. The vanishing taiga, the cleared areas are man made and that in the immediate foreground is no longer used for agriculture. The latter is characteristic of most attempts at farming within the Tanana Valley.

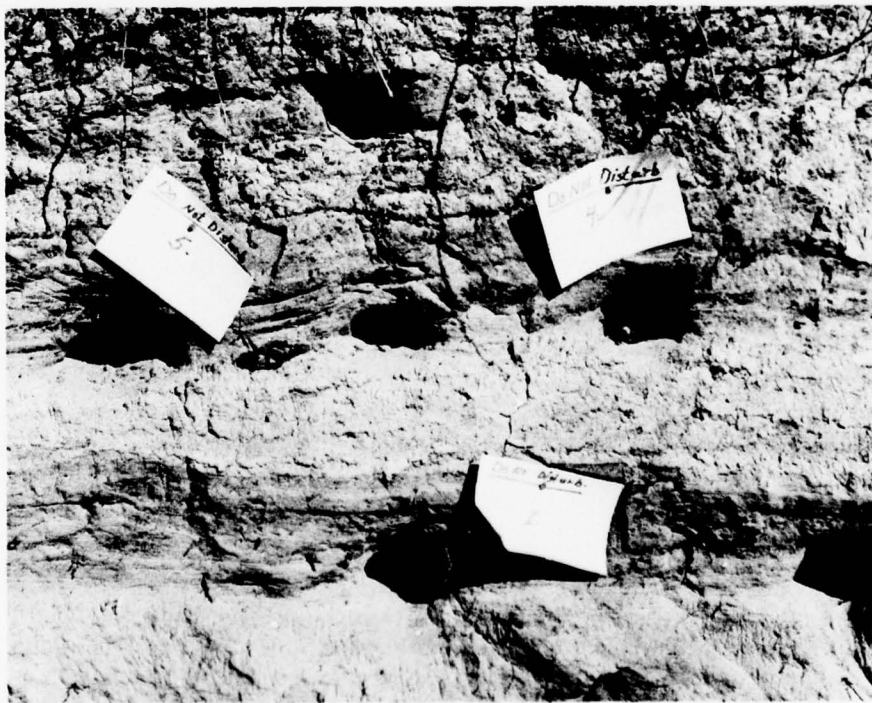


Plate X. Holes of the bank swallow (Riparia riparia). The ectoparasites of this migrant bird overwinter in Alaska, not departing with the bird as popularly assumed

"Ptarmigan tracks were seen in abundance though only two birds were seen. A few squirrel tracks were seen around the site proper although none were sighted or heard. We saw no sign of lynx and I doubt that there are any in the area. There was an absence of wolverine tracks as well as that of marten and ermine.

"The snow (March 31) is 18 to 24 inches deep and thawing rapidly. The river is rapidly overflowing the upper end of the island."

Some study plots were established in the Gerstle River area a short distance in from the highway. Working within the site proper was not deemed feasible by the chemical officers and eventually by us because of the activities going on and the fact that for long periods in early summer, the various access roads are virtually impossible and the use of half-tracked vehicles are required.

#### MAMMALS

The following discussion is limited only to those mammals that were collected during the past year. For a general account of the animals occurring within the Tanana Valley and their overall distribution, the reader is referred to our previous report.

A total of 2,135 mammals have been sampled at this writing, and additional specimens are now enroute. It is likely that our total by June 30 will approximate 3,000. Table 2 and Figure 3 tabulate our collections of all mammals taken. This includes the fur-bearers, such as wolverine, lynx, and wolves, supplied to us by the Regional Office of the Alaska Department of Fish and Game in Fairbanks. Most of these aforementioned animals were from this source; however, Mr. Molchan ran a trap line from Delta

Key to Figure 3:

1. Sorex cinereus
2. Sorex arcticus
3. Mustela rixosa
4. Mustela erminea
5. Mustela vison
6. Martes americana
7. Gulo luscus
8. Lynx canadensis
9. Spermophilus undulatus
10. Marmota caligata
11. Lemmus lemmus
12. Castor canadensis
13. Clethrionomys rutilus
14. Microtus pennsylvanicus
15. Microtus oeconomus
16. Microtus gregalis
17. Zapus hudsonius
18. Erethizon dorsatum
19. Ochotona collaris
20. Lepus americanus
21. Canis latrans
22. Canis lupus
23. Bison bison
24. Rangifer tarandus
25. Bos taurus

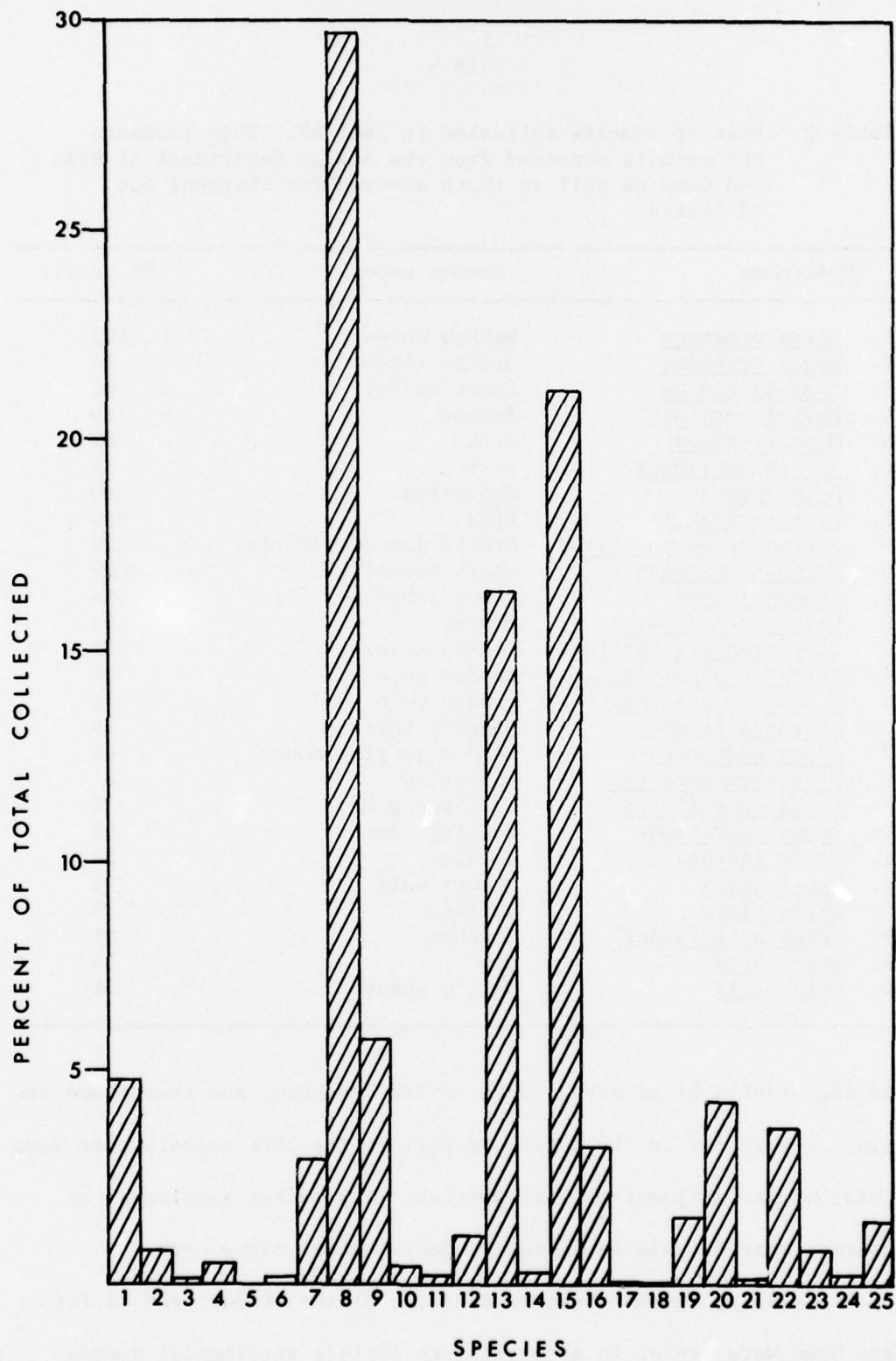


Figure 3. Mammalian species. Percentage by mammalian species of total collected in 1965-66. Key to species number is found on opposite page. A total of 2,135 animals are represented.

Table 2. List of mammals collected in 1965-66. This includes the mammals obtained from the Alaska Department of Fish and Game as well as those secured for shipment out of Alaska.

Host name	Common name	No. coll.
1. <u>Sorex cinereus</u>	Masked shrew	103
2. <u>Sorex arcticus</u>	Arctic shrew	14
3. <u>Mustela rixosa</u>	Least weasel	02
4. <u>Mustela erminea</u>	Ermine	10
5. <u>Mustela vison</u>	Mink	02
6. <u>Martes americana</u>	Marten	02
7. <u>Gulo luscus</u>	Wolverine	63
8. <u>Lynx canadensis</u>	Lynx	635
9. <u>Spermophilus undulatus</u>	Arctic ground squirrel	122
10. <u>Marmota caligata</u>	Hoary marmot	09
11. <u>Lemmus lemmus</u>	Brown lemming	06
12. <u>Castor canadensis</u>	Beaver	23
13. <u>Clethrionomys rutilus</u>	Red-back vole	352
14. <u>Microtus pennsylvanicus</u>	Meadow vole	07
15. <u>Microtus oeconomus</u>	Tundra vole	451
16. <u>Microtus gregalis</u>	Singing vole	69
17. <u>Zapus hudsonius</u>	Meadow jumping mouse	01
18. <u>Erethizon dorsatum</u>	Porcupine	01
19. <u>Ochotona collaris</u>	Collared pika	33
20. <u>Lepus americanus</u>	Varying hare	92
21. <u>Canis latrans</u>	Coyote	04
22. <u>Canis lupus</u>	Timber wolf	78
23. <u>Bison bison</u>	Buffalo	18
24. <u>Rangifer tarandus</u>	Caribou	06
25. <u>Bos taurus</u>	Cow	34
26. <u>Ovis dalli</u>	Dall's sheep	01

to the Gerstle River area, south to Tok Junction, and thus these are also represented in the totals as well as the live animals that were obtained for shipment to Fort Detrick. The latter specimens, of course, were not available for microbiological assay work.

Those specimens obtained from the Alaskan Department of Fish and Game were useful in attempting to isolate etiological agents from the tissues but unfortunately could not be used in getting

serum samples. This is a highly regrettable situation, because information of much value likely could be secured were it feasible to collect the serum. I have thought of this problem a great deal and have not been able to resolve a satisfactory method whereby we could induce the trappers to take blood samples. For the present our data from these important animals will have to continue to come in small lots when it is possible to capture one and draw blood either at death or very shortly thereafter.

It is felt that the following comments on commonly collected animals will be of interest: Sorex cinereus (masked shrew) is found in virtually all of our study regions, with the possible exception of the higher elevations which approach the limit of vegetation. At times, the shrew can become very common and, like many of the mammals of the Far North, is subject to rather violent fluctuations. Shrews are considered primarily to be invertebrate eaters, but in the subarctic they will feed on plant materials, such as birch seeds, which they encounter in their snow tunnels.

Another shrew which we encounter from time to time is Sorex arcticus, commonly known as the arctic shrew. It is found on a smaller scale within the taiga but supposedly is more abundant in the true boreal situations. Within the taiga, the arctic shrew should be most abundant on north-facing slopes, where the vegetation would be black spruce and Labrador tea, and the permafrost very close to the surface.

Mustela erminea (ermine or short-tail weasel) is widely dis-

tributed throughout much of Alaska and even occurs high in the Alaska Range. Its presence, and particularly, numbers, depends, directly on available biomass of prey. Consequently, when voles and hares are at a high peak, the weasels may become very common.

Mustela rixosa (least weasel) is the smallest representative of the order Carnivora. It is widely distributed but not often collected. This species is closely dependent upon the state of the vole populations, much more so than the larger preceding species. It is a surprise to find one in a Sherman live trap, for their weight is not considerably greater than that of a huge Microtus.

Gulo luscus (wolverine) is sparsely distributed throughout the taiga but is supposedly more common in the upland tundra of the Alaska and Brooks Range. I strongly suspect that this animal is more common within the taiga than is usually supposed, but since it moves with a fair amount of secrecy, is not often seen. This summer, two subadults were observed in the upland tundra region, which constitutes the second and third living specimen that I have seen in the interior of Alaska over a ten-year period. It is a wide-ranging animal and preys on a variety of herbivorous mammals.

Vulpes fulva (red fox) is one of the common carnivores in our study region and is known by several other names, such as cross fox and silver fox. The foxes are true scavengers and will feed on anything that is edible. Voles and hares constitute a large part of their diet. Many of the old trapper thought that when the mouse sign was good, they would have good fox trapping the following winter.

There is some question as to whether the red fox is an endemic species in North America or whether it was brought with the white man to the eastern United States. The latter may be true in recent historical times, but I believe that those found in the boreal regions of North America are truly endemic mammals.

Canis lupus (gray or timber wolf) was once a widely distributed animal in North America but is now rare south of Canada. Due to bounty systems imposed by various agencies and the keen activity of aerial bounty hunters, the wolf population in Alaska is considered relatively low. In fact, some biologists have stated that the wolf is "now a rare animal" as recent as a year ago. However, with the bounty system removed, the wolf has made a surprising come-back within the interior of Alaska. As the small bands of caribou grow larger on the northern slopes of the Alaska Range, I would anticipate the wolves becoming more abundant. In addition to feeding upon the caribou and moose, they are opportunists and will eat whatever they can catch. Their diet varies with season, place, and the presence of such other animals as voles, hares, ground squirrels, and moose.

Lynx canadensis (lynx) is one of the important carnivores in the taiga food web, but it is highly specialized for taiga conditions. It is light of weight and has huge feet to give it good floatation for traversing winter snow cover. However, studies to date indicate that it does not migrate any considerable distance. It has an extremely efficient fur insulation, thereby making it virtually immune

to low winter temperatures. It is highly adapted to feeding on the snowshoe hare, and in the absence of the snowshoe hare, will, in effect, disappear. The peak in its population generally follows that of the snowshoe hare by approximately two years.

Spermophilus undulatus (= Citellus parryi; = Citellus undulatus) (arctic ground squirrel) is one of the common animals in the upland tundra. Certain subspecies are found in cleared areas below the timberline, and one of them is known to invade mixed aspen and spruce forests. This mammal is not abundant throughout the Tanana Valley, invading as far as Fort Greely from the south, and getting barely over the White Mountains into the Tanana Valley near Eagle Summit. If not molested, this animal can become extremely tame and seems to have a predilection for inhabiting the edificarian habitat with man.

Tamiasciurus hudsonicus (red squirrel) is a characteristic mammal of the boreal forests. It is closely associated with white spruce and is seldom common in areas where spruce are lacking. While the squirrels make arboreal nests and occupy them a great deal during the warmer months of the year, they also make subterranean nests. When the temperature is below  $-25^{\circ}$  F or  $-30^{\circ}$  F, the squirrels spend the bulk of their time in the subterranean burrows and in tunnels that they have made just under the snow. When the breakup occurs, it looks as though a giant Microtus has been tunneling in the area.

Castor canadensis (beaver) is, or at least was, a very

common animal in the taiga. In our study region, the beavers may be found on virtually any stream or slough. I have actually seen them build dams across rivers such as the Tolovana, only to have them washed out by heavy rains. They have a predilection for the sloughs, which have a very sluggish current. While much has been reported regarding this animal and the muskrat concerning tularemia, virtually nothing is known of their role in the epizootology of this disease in Alaska. Epidemiological studies conducted by Hopla in previous investigations point to the fact that this animal, as well as the muskrat, is probably important in the spread of the disease to man within the native village.

Lemmus lemmus (brown lemming) is a fringe animal in our study region and is found almost entirely in the upland tundra. In some areas, it interdigitates with the lowland taiga. While the populations of the brown lemming are said to peak at great densities, I have never seen this happen in the study areas adjacent to the Denali Highway. In our area, they are closely associated with the red-backed vole, the singing vole, and the tundra vole. While I have found their fleas occasionally upon Ochotona, I have as yet to capture animals from this type of habitat.

Clethrionomys rutilus (red-backed vole) is one of the most common mammals in the undisturbed areas of Alaska. However, once a region has been burned over and secondary growth is established, Microtus oeconomus will usually replace C. rutilus. Its populations fluctuate greatly, but not with any type of a general cycle or

trend. For example, the voles may be extremely abundant in a small area at one study site, yet two to three miles away they may actually be very rare. Without question it is one of the most widely distributed animals in Alaska and from this standpoint alone bares considerable investigation. It adapts to the edificarian habitat much in the manner that Peromyscus will further to the south. I have always found this type of behavior somewhat incongrous for this vole, largely because all studies to date indicate that is is virtually replaced in the secondary growth of timber and grass once land has been cleared. From this, it would seem logical that the tundra vole would be the most logical vole to find around man's dwellings.

Microtus oeconomus (tundra vole) is also one of the most common mammals in Alaska. In the disturbed areas, there is little or no question in my mind but that it is the most common one. However, it is rare in mature spruce, whereas the red-backed vole will appear abundantly. It seems particularly to like weedy and bushy road burns and other similar habitat. Also, its populations can be very abundant in the upland tundra. This species is widely distributed in the Old World.

Microtus gregalis (= Microtus miurus) (singing vole) is found in the low Arctic tundra and the shrubby upland tundra. It appears to be a semi-colonial form, and its distribution appears to be governed to a large extent by the snow cover.

Ochotona collaris (pika) is a true upland tundra mammal, living

in the rocky or talus slopes. Very little is known about its ecology in the subarctic, but I have selected it as a mammal of study simply because it has several disjunct populations within our study area. Since it is strictly localized, yet co-mingles with other animals, such as the hoary marmot, the red-backed vole, the arctic ground squirrel, and various species of shrew, it is interesting to see what happens with the ectoparasite populations. Also, since there is little interchange between colonies of pika, it is thought that this may offer some valuable information with regards to certain epizootics that supposedly occur within this group of mammals.

Lepus americanus (snowshoe or varying hare) is one of the most common mammals within the taiga. There are years when, without doubt, it is the most obvious, and other years when it would appear to be one of the rarest mammals. Its population fluctuations are well known but poorly understood. It is also the principal host of the one tick, Haemaphysalis leporis-palustris, that occurs within our study area with any frequency. The varying hare is also one of the mammals definitely incriminated in the epizology of tularemia in Alaska. Infected ticks have been removed from it on several occasions, and I have had at least one positive identification.

It is unfortunate at this time that the varying hare population is at such a low ebb. Game biologists had offered to "eat their hats" if we could secure more than 20 snowshoe hares throughout the winter. The number that we did acquire is attributed to the

tenacity of Messrs. Stoker, Molchan, and Schuck. It is an animal that is difficult to maintain in captivity unless proper climatic conditions are maintained. In obtaining specimens to ship outside, we had to give up modern laboratory facilities because of the excessive temperatures involved in maintaining other animals and switch to an abandoned barn at one of the older homesteads in the Delta Region. Once this was done, we were able to maintain the animals alive for a reasonable period of time and thus accumulate sufficient stock to ship. Young of the year animals can be held together in relatively large groups without particular difficulty during the summer months. This cannot be done with adults, because mating behavior is in process throughout the summer and they become very competitive for space in an enclosed area.

Alces alces (moose) is the largest herbivore within the taiga. Indeed, it invades the upland and northern tundra. It is a relatively common animal within the Tanana Valley, and in certain valleys to the south (on the other side of the Alaska Range) is even more common. Because it is widely sought as a game and meat animal, considerable effort needs to be expended to ascertain the abundance of certain organisms such as those found in brucellosis and Q fever. Its co-mingling with domestic animals and the buffalo herd introduced into Alaska could be a very important consideration here.

Rangifer tarandus (caribou) is not an important animal within the taiga proper. However, this past year we have learned of

several discrete small bands on the north slopes of the Alaska Range. Little is known about these small bands of caribou, and apparently they are not molested by hunters to any significant degree, largely because of the difficulty in procuring specimens. Mr. Frank Jones of the Alaska Department of Fish and Game considers them to be non-migratory in the sense that they do not apparently join up with the larger herds. Nothing is known of their behavior, and I think this is one of the most intriguing problems remaining unsolved insofar as the large game mammals are concerned. Our observations to date indicate that these caribou abandon the higher slopes of the Alaska Range and come down into the "lowlands" of the upland tundra adjacent to the spruce forests, crossing through much of the spruce forests to other open areas.

Since these small bands have apparently been isolated from the main herds of the caribou for some years, serological studies upon them would be most germane. Within our study area at Delta Creek, their range overlaps with that of the buffalo. In considering these caribou, I have wondered, when the populations build to a certain density, if they do not spill over and join with some of the larger herds, such as that of the Nelchina, thus helping to explain the larger herds which then migrate considerable distances. In this respect, then, their behavior would not be unlike that which has been reported for some of the smaller rodents.

Bison bison (American buffalo) is not native to Alaska but was introduced in the 1920's from the National Bison Range in

Montana. Insofar as I know, these animals were not tested for such diseases as brucellosis or Q fever prior to this shipment. Its range in our study region is limited to the vicinity of Delta Junction, Gerstle River, Donnelly, and Delta Creek, where it frequents open, grassy meadows, fresh burns in the herbaceous stage of recovery, and also occurs along the gravel outwashes of the larger creeks and rivers. Frequently in the winter, the latter type of habitat is blown free of snow, and relatively large concentrations of these animals will be found there grazing on the leguminous forage that grows so luxuriently in this type of habitat during the summer months.

#### AVES

##### Resident Birds:

Alaska has few resident birds, and among these the most important are discussed briefly below.

Canachites canadensis (spruce grouse) is a relatively sedentary, permanent resident of the mature taiga. As its name implies, it is the most abundant in the spruce forests. It is a herbivore, feeding on buds, twigs, and seeds, and to a certain degree, upon insects. Occasionally it is found infested with the hare tick, Haemaphysalis leporis-palustris.

Lagopus lagopus (willow ptarmigan) is a semi-permanent resident of the taiga, only because it performs an altitudinal migration to the upland tundra for nesting during the summer and returns to the taiga in the winter months. It is primarily a

herbivore, but insects and other invertebrates are also eaten.

Lagopus mutus (rocky ptarmigan) is a resident of the upland tundra, so that we encounter it only rarely within our study region. Taxonomically, it is difficult to separate from the willow ptarmigan, and I suspect many records have been confused with regards to these two species in the past.

Surnia ulula (hawk owl) is a resident of the interior of Alaska and differs from many in being extremely active during the daylight hours. The major part of its diet consists of voles and other small rodents, although insects and small birds have been taken. When the vole populations are abundant, this owl appears to be relatively common. During the summer of 1961, it was the most abundant that I have known it in the interior of Alaska. A method of trapping this bird needs to be devised, for it would be extremely interesting to do serological studies on it.

Perisoreus canadensis (gray or Canadian jay) is a permanent resident of the taiga in all of its successional stages. It is a true scavenger but at times can be a carnivore. Frequently it becomes tame around the edificarian habitat, so much so that it will alight on a person's shoulder. In such circumstances it is most difficult to set a trap line because it attempts to rob the bait from traps. Despite the fact that it is a relatively commonplace bird within the taiga, little or nothing is known about its breeding and nesting habits.

Corvus corax (raven) is one of the most intriguing resident

birds in the boreal region. While it is primarily a scavenger, there are times when it becomes an active carnivore, particularly when the vole populations are high or when they are especially available, as during spring breakup. Despite numerous attempts to capture it, we have had little success. It is one of the birds that we wish to capture in relatively large numbers in order that serological studies can be undertaken. It is hypothesized such results could prove highly interesting with regards to tularemia.

Acanthis flammea (redpoll) is a very common bird in the taiga regions, usually occurring in small flocks during the winter months. The individuals nesting in a particular region may not be the same individuals which winter there. It is an omnivore, but insofar as is known, its feeding habits are mainly herbivorous. During the winter months, it can be particularly numerous along the exposed gravel outcrops of river beds.

Migratory Birds:

While it was mentioned that the resident birds were relatively few in number, the interior of Alaska has a dense population of the migratory ones. Inasmuch as our sample of the migratory birds is so small, the reader is referred to Table 3 for the ones collected by us. Until more collections have been made, it does not seem pertinent to discuss each one in detail. Principally, we are interested in the migratory birds that are primarily ground nesters. It is my thinking that such forms would stand a better chance of being

Table 3. Tabulation of the migratory and resident birds secured in 1965-66.

Host name	Common name	No. coll.
Migratory:		
<u>Bombycilla garrula</u>	Bohemian waxwing	12
<u>Empidonax sp.</u>	Flycatcher	13
<u>Junco hyemalis</u>	Slate-colored junco	21
<u>Hylocichla ustulata</u>	Olive-backed thrush	15
<u>Parus hudsonicus</u>	Boreal chickadee	01
<u>Totanus flavipes</u>	Lesser yellowlegs	01
<u>Vermivora cletata</u>	Orange-crowned warbler	04
<u>Passerella iliaca</u>	Fox sparrow	10
<u>Dendrocia petechia</u>	Yellow warbler	11
<u>Turdus migratorius</u>	Robin	21
<u>Melospiza lincolni</u>	Lincoln's sparrow	12
<u>Passerculus sandwichensis</u>	Savannah sparrow	94
<u>Mareca americana</u>	Baldpate	06
<u>Bucephala albeola</u>	Bufflehead	01
Resident:		
<u>Lagopus mutus</u>	Rock ptarmigan	13
<u>Lagopus lagopus</u>	Willow ptarmigan	24
<u>Pica pica</u>	Magpie	12
<u>Acanthus flammea</u>	Redpoll	14
<u>Corvus corax</u>	Raven	10

infected with either of the two organisms in which we are mainly interested. Certain of these migratory birds, such as the white-crowned sparrow, are very common, as are the Savannah and fox sparrows. The robin is relatively abundant and, contrary to its nesting habits further in the south, frequently nests upon the ground. Another aspect that is of interest with some of these ground-inhabiting birds is the fact that they can be hosts for the immature stages of the rabbit tick. This was found to be particularly true during the summer of 1964, but for reasons unknown to us at the present

time, we had no positive records from 1965. Perhaps further investigation will help to clarify this question.

#### ARTHROPODS

Siphonaptera: Of the 52 species of fleas known to occur in Alaska, 24 were encountered this past year. Certain species were found to be in greater abundance than the previous summer, and we had the highest host index (infestation per host) that I have experienced in Alaska. In part, this may have resulted from better techniques on the part of the field crews over the past summer but cannot account for the discrepancy for the years 1961 and 1962, when meticulous care was taken.

The most interesting of the records from the biological standpoint were those pertaining to Epitedia wenmanni. In addition to this, we collected specimens of an apparently undescribed species of flea belonging to the genus Rhadinopsylla. Specimens of this new flea were secured in 1962 but were obtained in extremely small numbers from ermine. It was thought at that time that this was not the true host, and the collections this summer at the Delta Creek study site in the black spruce forests from Clethrionomys rutilus have verified this concept. Figure 4 summarizes the collection data on the fleas. The percentage expressed for each species is that out of the total number of 3,167. The percentage of positive collections is also represented for each species, from a total of 1,115 positive collections.

Tables 4 through 9 show the relationship of certain fleas with

the more common rodent species. Most of the data in these tables are relatively straightforward, but as one studies Tables 7 and 8, some very interesting contrasts in infestations of these two common voles occur. It is of particular interest to note that while the red-backed vole was not taken in as large numbers as the tundra vole (for example, 252 specimens of the former, as compared to 408 of the latter), yet Catallagia dacenkoi fulleri was taken 41 times for a total of 132 fleas from the red-backed vole, as compared to 43 times and only 59 fleas on the tundra vole. Equally interesting

Table 4. Fleas associated with the masked shrew, Sorex cinereus.

Siphonaptera	Tot. no. fleas	No. pos. coll.	Ave. host
<u>Catallagia dacenkoi fulleri</u>	03	02	1.1
<u>Corrodopsylla curvata curvata</u>	41	23	1.2
<u>Malaraeus penicilliger dissimilis</u>	01	01	
<u>Megabothris calcarifer gregsoni</u>	01	01	
<u>Megabothris quirini</u>	03	02	1.1
<u>Peromyscopsylla ostsibirica longiloba</u>	01	01	

observations are made in regards to Malaraeus penicilliger dissimilis, Megabothris calcarifer gregsoni, Megabothris quirini, and Peromyscopsylla ostsibirica longiloba. No attempt has been made to analyze this data or to weigh it statistically other than for the rough presentation in the tables. These data show which of the two voles these fleas

Table 5. Fleas associated with the arctic ground squirrel, Spermophilus undulatus.

Siphonaptera	Tot. no. fleas	No. pos. coll.	Ave. host
<u>Oropsylla idahoensis</u>	313	63	4.6
<u>Thrassis sp.</u>	02	01	
<u>Megabothris abantis</u>	01	01	

Key to Figure 4:

1. Epitedia wenmanni
2. Orchopeas caedens durus
3. Hoplopsyllus glacialis lynx
4. Malaraeus penicilliger dissimilis
5. Megabothris calcarifer gregsoni
6. Tarsopsylla octodecimentata coloradensis
7. Amphalius runatus necopinus
8. Corrodopsylla curvata curvata
9. Ceratophyllus riparius
10. Ctenophyllus armatus terribilis
11. Megabothris groenlandicus
12. Chaetopsylla floridensis
13. Catallagia dacenkoi fulleri
14. Monopsyllus vison
15. Megabothris quirini
16. Rhadinopsylla
17. Ceratophyllus scopulorum
18. Ceratophyllus lunatus tundrensis
19. Oropsylla alaskensis
20. Ceratophyllus idius
21. Oropsylla idahoensis
22. Amphipsylla marikovskii ewingi
23. Thrassis pristinus
24. Peromyscopsylla ostsibirica longiloba

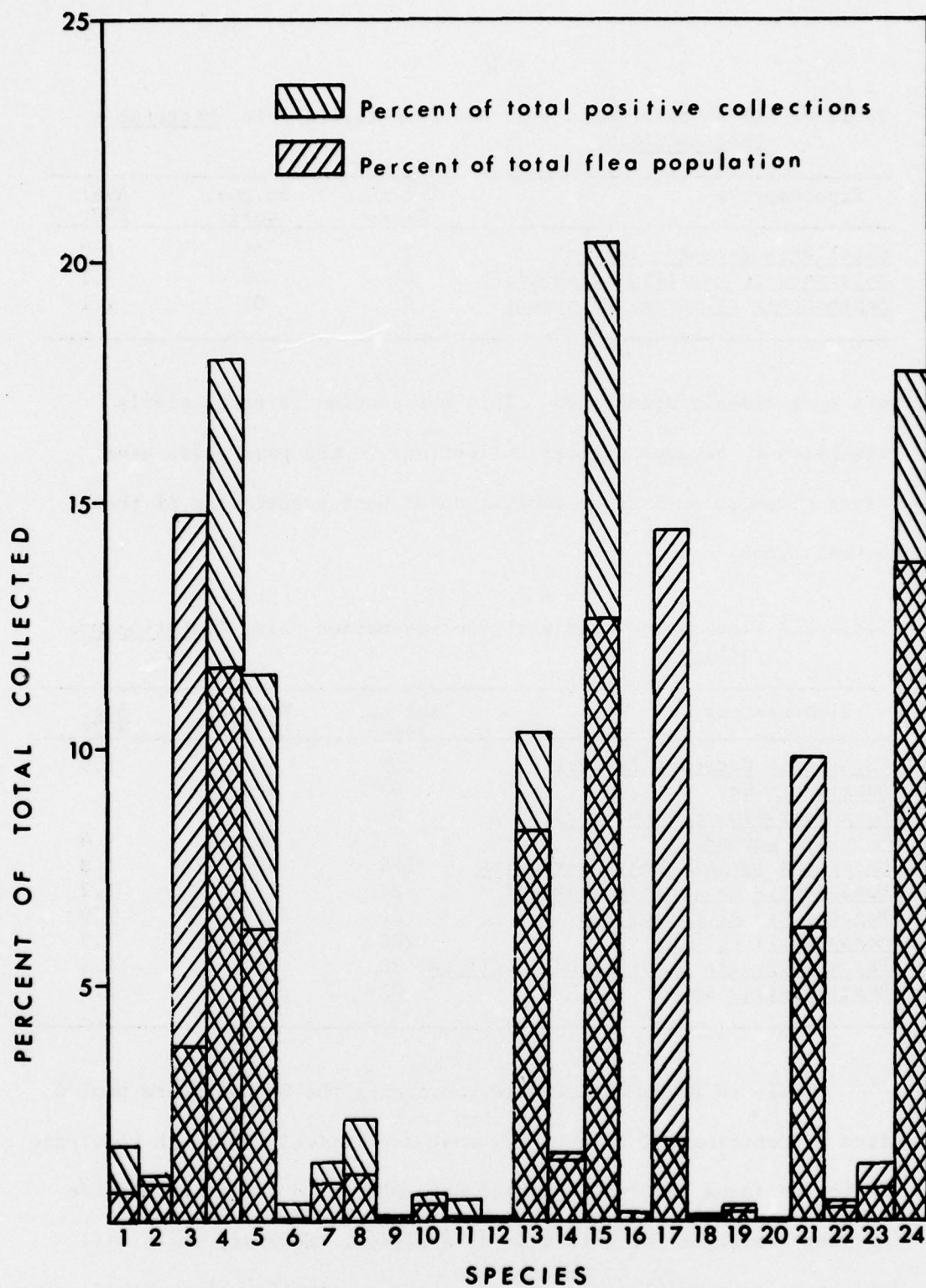


Figure 4. Species of Siphonaptera and number of positive collections for each flea are represented by percent of totals for each. A total of 3,167 fleas were collected by 1,115 positive collections.

Table 6. Fleas associated with the long-tailed vole, Microtus longicaudus.

Siphonaptera	Tot.no. fleas	No. pos. coll.	Ave. Host
<u>Catallagia dacenkoi fulleri</u>	04	04	1.0
<u>Malaraeus penicilliger dissimilis</u>	06	05	1.1
<u>Megabothris calcarifer gregsoni</u>	01	01	1.1

are most closely associated. This association is particularly significant, because smaller collections in the past years have never given this clear an indication of host association of these boreal fleas.

Table 7. Fleas associated with the red-backed vole, Clethrionomys rutilus.

Siphonaptera	Tot.no. fleas	No. pos. coll.	Ave. Host
<u>Catallagia dacenkoi fulleri</u>	132	41	3.9
<u>Catallagia sp.</u>	02	01	
<u>Corrodopsylla curvata curvata</u>	01	01	
<u>Epitedia wenmanni</u>	12	08	1.4
<u>Malaraeus penicilliger dissimilis</u>	175	88	1.8
<u>Megabothris calcarifer gregsoni</u>	22	20	1.2
<u>Megabothris groenlandicus</u>	01	01	1.0
<u>Megabothris quirini</u>	200	112	1.9
<u>Peromyscopsylla ostsibirica longiloba</u>	30	18	1.1
<u>Rhadinopsylla sp.</u>	07	03	2.1

Table 10 presents the data concerning the Siphonaptera pooled from microbiological assay. Now that a relatively thorough knowledge as to the fauna involved has been accumulated, a larger proportion of them available for this type of analysis. However, there will always be some problem when we are working with the voles, for females of certain fleas cannot be separated from each other in the absence

Table 8. Fleas associated with the tundra vole, Microtus oeconomus.

Siphonaptera	Tot. no. fleas	No. pos. coll.	Ave. host
<u>Amphipsylla marikovskii ewingi</u>	02	02	
<u>Catallagia dacenkoi fulleri</u>	59	43	1.2
<u>Ceratophyllus idius</u>	01	01	
<u>Corrodopsylla curvata curvata</u>	03	03	
<u>Eptedia wenmanni</u>	13	10	1.3
<u>Malaraeus penicilliger dissimilis</u>	69	53	1.2
<u>Megabothris calcarifer gregsoni</u>	159	96	1.4
<u>Megabothris sp.</u>	02	02	
<u>Monopsyllus vison</u>	06	01	
<u>Oropsylla idahoensis</u>	01	01	
<u>Peromyscopsylla ostsibirica longiloba</u>	306	140	2.3

of males. Hopefully, other characteristics than those applied in museum studies can be obtained to make these differentiations possible. There is no problem of identification, even with these difficult females, to genus.

Table 9. Fleas associated with the pika, Ochotona collaris.

Siphonaptera	Tot. no. fleas	No. pos. coll.	Ave. host
<u>Amphalius runatus necopinus</u>	29	13	2.3
<u>Ctenophyllus armatus terribilis</u>	10	06	1.4
<u>Megabothris groenlandicus</u>	01	01	
<u>Oropsylla idahoensis</u>	01	01	

Culicidae: This year, 389 pools of mosquitoes approximating 19,450 specimens, were collected for microbiological assay purposes. This is in contrast with 254 pools and 9,788 specimens collected the previous year. Table 11 shows the tabulations for the break-down by genus and species. Inasmuch as crews were in the field at an

Table 10. Specimens of Siphonaptera pooled for microbiological assay. In general, animals and/or nests were selected which had one predominant flea. Such was not the case with the voles.

Species	Host	No. coll.
<u>Hoplopyllus glacialis lynx</u>	<u>Lynx canadensis</u> - lynx	4
<u>Ceratophyllus idius</u>	<u>Tachycineta thalassina</u> - violet green swallow	6
<u>Ceratophyllus riparius</u>	<u>Riparius riparius</u> - bank swallow	7
<u>Ceratophyllus scopulorum</u>	<u>Petrochelidon pyrrhonota</u> - cliff swallow	5
<u>Oropsylla idahoensis</u>	<u>Spermophilus undulatus</u> - arctic ground squirrel	12
<u>Malaraeus penicilliger dissimilis</u>	<u>Clethrionomys rutilus</u> - red-backed vole	4
<u>Megabothris</u> sp.	<u>Microtus oeconomus</u> - tundra vole	
	<u>Clethrionomys rutilus</u> - red-backed vole	3
<u>Megabothris quirini</u>	<u>Microtus oeconomus</u> - tundra vole	
	<u>Clethrionomys rutilus</u> - red-backed vole	2
<u>Megabothris calcarifer gregsoni</u>	<u>Microtus oeconomus</u> - tundra vole	
	<u>Clethrionomys rutilus</u> - red-backed vole	1
	<u>Microtus oeconomus</u> - tundra vole	
		44

earlier date this year, we had an opportunity to sample the genus Culiseta. I think this is an important point, because the adult mosquitoes belonging to this genus overwinter as adults. Data collected to date (Hopla - manuscript) have indicated that this mosquito does not take a blood meal prior to entering hibernation. However, I have observed more specimens of Culiseta alaskaensis attempting to secure a second blood meal under natural conditions more often than all other species of Aedes combined.

Since the submission of the report in 1965, I have had an

Table 11. Tabulation of mosquito pools collected at Fairbanks and in the Delta area during June and July of 1965. The pools averaged 50 specimens but ranged from 20 to 150.

Species	Mosquito Pools
<u>Aedes</u> : sp. (Black-legged group)	198
<u>hexodontus</u>	59
<u>intrudens</u>	63
<u>punctor</u>	44
<u>excrucians</u>	15
<u>stimulans</u>	02
<u>Culiseta</u> :	
<u>alaskaensis</u>	60
<u>impatiens</u>	25
<u>Culex</u> <u>territans</u>	02
	—
Total	389

opportunity to analyze the biting records collected in the study areas adjacent to Fairbanks during the summer of 1961-62. Figure 5 shows the feeding activity according to a 24-hour break-down. Ultimately, it is hoped time will be found to separate these down by species. Once this is done, the species feeding pattern can be fairly well established.

Observations on the feeding habits of Alaskan mosquitoes have been summarized in a manuscript submitted to the Bulletin of the Brooklyn Entomological Society, which should appear in print some time this summer. Most of the data for this paper was presented at the American Mosquito Control Association Meetings in Atlanta, Georgia, this past March. It is anticipated that I will cooperate with a request submitted by Dr. Archie Hess to send specimens to his laboratory in order that an attempt can be made to determine the

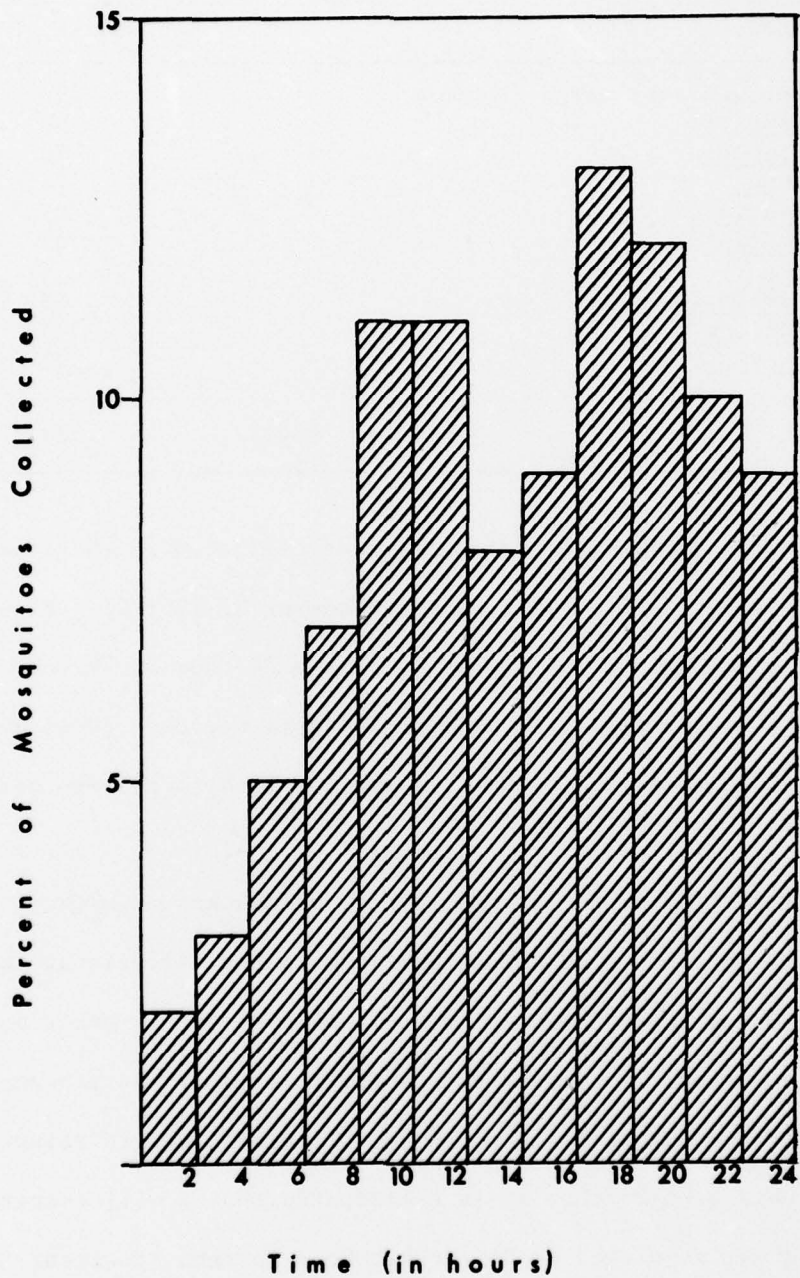


Figure 5. Summary of mosquito biting records at Nordale Woods near Fairbanks, summer of 1961-62. Time is expressed at 2-hour intervals on the military clock. This study was based on a total of 9,466 biting records.

source of blood meals in the subarctic mosquitoes according to the techniques published by Templis.

Acarina: No pools of Acarina were obtained from the snowshoe hares collected this year. Those hares taken during the summer months were apparently in tick-free areas, and the migratory and resident birds taken this summer failed to produce any specimens. We will continue to watch for animals which might serve as hosts for the rabbit tick, inasmuch as this would be a significant contribution to its biology. When snowshoe hare populations have crashed in a particular area, it does not seem feasible that sufficient numbers of the ticks can find adequate hosts if they must rely primarily upon the varying hare. Our studies from the previous summer indicated that nymphs seem to engorge reasonably well upon a wide variety of passerine birds. However, I am at a complete loss to explain their absence from these birds this year.

#### MICROBIOLOGICAL ASSAY

Table 12 tabulates the mammals from which tissues and sera were pooled this past year. It will be noted that this list complies closely to that listed in Table 2 which summarizes the mammals collected during the same period. The only discrepancy will be with Clethrionomys rutilus, Microtus oeconomus, and Lepus americanus. The difference is caused in the fact that these three animals were live trapped for purposes other than for tissue and serology studies. The list is presented in this table and also reflects the animals from which the ectoparasites were recovered

Table 12. Number of tissue and sera pools collected from Alaskan mammals.

Host name	Common name	Tissue	Sera
<u>Sorex cinereus</u>	Masked shrew	103	00
<u>Sorex arcticus</u>	Arctic shrew	14	00
<u>Mustela rixosa</u>	Least weasel	02	04
<u>Mustela erminea</u>	Ermine	10	00
<u>Mustela vison</u>	Mink	00	02
<u>Martes americana</u>	Marten	10	21
<u>Gulo luscus</u>	Wolverine	63	01
<u>Lynx canadensis</u>	Lynx	635	22
<u>Spermophilus undulatus</u>	Arctic ground squirrel	122	74
<u>Marmota caligata</u>	Hoary marmot	09	00
<u>Tamiasciurus hudsonicus</u>	Red squirrel	18	05
<u>Lemmus lemmus</u>	Brown lemming	06	00
<u>Castor canadensis</u>	Beaver	23	13
<u>Clethrionomys rutilus</u>	Red-backed vole	252	19
<u>Microtus pennsylvanicus</u>	Meadow vole	07	05
<u>Microtus oeconomus</u>	Tundra vole	408	123
<u>Microtus gregalis</u>	Singing vole	69	00
<u>Zapus hudsonius</u>	Meadow jumping mouse	01	01
<u>Erethizon dorsatum</u>	Porcupine	01	01
<u>Ochotona collaris</u>	Collared pika	33	00
<u>Lepus americanus</u>	Varying hare	22	14
<u>Canis latrans</u>	Coyote	04	00
<u>Canis lupus</u>	Timber or gray wolf	78	00
<u>Vulpes fulva</u>	Red fox	05	00
<u>Bison bison</u>	Buffalo	18	12
<u>Rangifer tarandus</u>	Caribou	06	01
<u>Bos taurus</u>	Cow	00	32
<u>Ovis dalli</u>	Dall's sheep	00	01

with the exception of the wolverine and the lynx, the bulk of which were available to us only as carcasses from the Alaska Department of Fish and Game.

Serology: Q fever, the complement fixation test (CFT) as carried out by us is similar to the method of Welsh (1959). It consists of an overnight incubation of 2 - 4° C of the combined serum, antigen,

and complement, followed by 30 minutes at 37° C in a water bath after the addition of sensitized sheep cells. Two whole units of complement, two units of antigen, and two units of hemoglobin are then added. A 50 percent visual hemolytic end point is used; proper controls are run with each test. The antigen, QII 795 is one of the broadest-spectrum Phase II antigens known; Ohio 314 Phase I antigen is on hand for use when needed.

The capillary agglutination test (CAT) has been used as a supplementary test to complement fixation and is thought to be more reliable than testing sera from Clethrionomys and Microtus. Microtine rodents are generally regarded as poor reproducers of complement-fixing antibodies. In addition, the CAT is particularly useful when working with small amounts of sera as frequently is the case when dealing with small rodents such as the voles. We produce our own antigen for the CAT from the Ohio 314 strain and in addition have obtained antigens from the Zoonoses Investigation Unit, Communicable Disease Center. The seed stock of the various strains of Q fever organisms mentioned above were furnished by Dr. David Lackman of the Rocky Mountain Laboratory.

Tularemia employs the standard tube agglutination test utilizing an avirulent strain (JAP) of F. tularensis as a source of antigen. However, in an attempt to save time this past year we have been using the Lederle tube antigen which is highly reliable and is stated to give a positive reading at a 1:20 dilution. In the past years I have used the hemagglutination test but feel

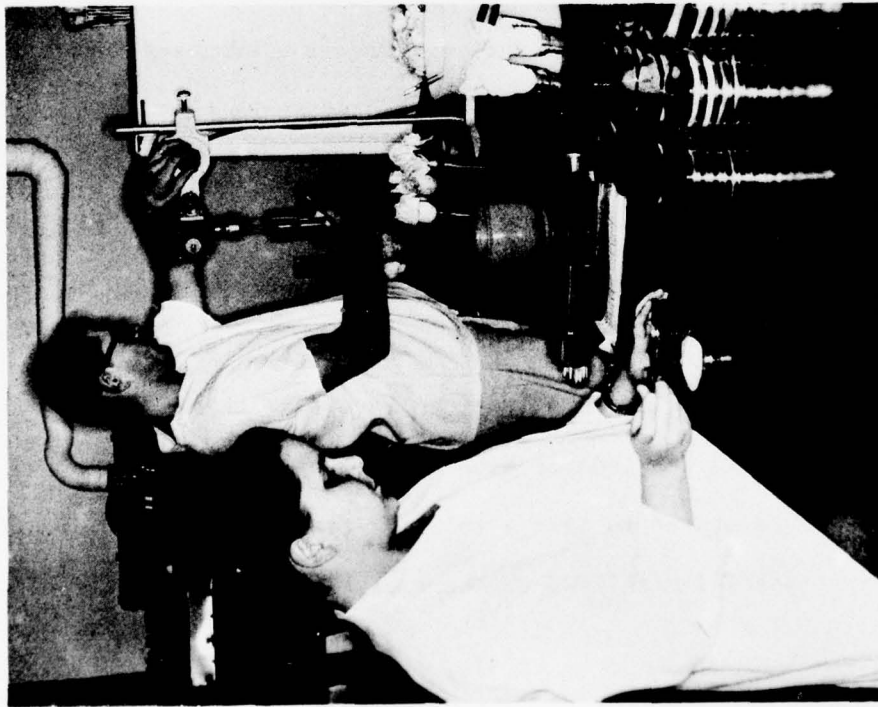


Plate XI. Tissue room. Mr. Atkin is grinding tissues with the aid of a teflon grinder. Mrs. Keezer is plating a suspension of the ground tissue on cystine-glucose-blood agar.

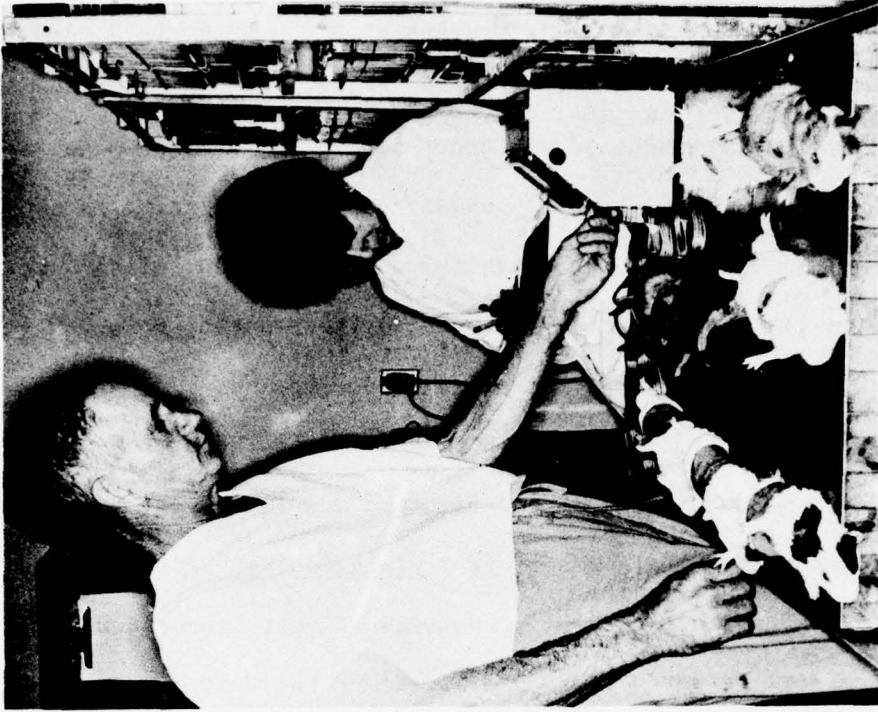


Plate XII. Mr. Davis and Mrs. Cleveland taking and recording temperatures of guinea pigs.

that the simplicity of the test outweighs the slight advantage in sensitivity that the former test may have. In performing the test we incubate for two hours at 37° C in a water bath followed by overnight refrigeration between 2° and 4° C. Positive tests are always rechecked. The seed stock for the strain of tularemia organisms used in making up our own antigen was supplied by Dr. Cora R. Owens of the Rocky Mountain Laboratory. The advantages of using an avirulent strain are considerable, particularly in a building that is occupied by more than one type of microbiological assay research. It has been my experience and that of other investigators that this strain is fully as antigenic as the more virulent ones. All sera for both tularemia and Q fever tentatively identified as positive are rechecked to insure the results can be repeated. To my thinking, a titre of 1:80 is significant when testing for tularemia antibodies and 1:32 when testing for Q fever by the CFT.

Results: Table 13 summarizes the results insofar as the serological survey is concerned. Succinctly I have only considered sera positive for the antibodies of Q fever when they were verified by a recheck and there was agreement between the CFT and the CAT. While many workers consider 1:8 as positive when working with this particular antibody, I feel safer at 1:32, particularly since we are working with a variety of rodents for which little or no background information is available. With regards to the titres reported for tularemia antibodies I have listed all those from 1:20 and up,

Table 13. Antibody survey of mammalian and avian sera pools,  
June 1, 1965 - March 30, 1966.

Host	Area	Lab. No.	Q Fever	CAT	Tul.
<u>Citellus undulatus</u>	Thompson Pass	377	1:32	+	-
" "	" "	382	1:64	+	-
" "	Richardson Hwy.	430	1:64	+	-
" "	" "	449	1:128	+	-
" "	" "	486	1:32	+	-
" "	" "	620	1:16	+	-
" "	Delta Area	483	1:32	+	-
" "	" "	485	1:256	+	-
" "	Brock Road	513	-	-	1:20
<u>Lepus americanus</u>	Richardson Hwy.	486	1:32	+	-
" "	" "	620	1:16	+	-
<u>Microtus oeconomus</u>	Manley Hot Springs	440	1:128	+	-
" "	" "	505	1:256	+	-
" "	" "	466	-	-	1:40
" "	" "	508	1:128	+	-
" "	" "	592	1:64	+	-
" "	" "	598	1:16	+	-
" "	" "	616	1:64	+	-
" "	" "	639	1:16	+	-
" "	" "	641	1:16	+	1:40
" "	" "	665	-	-	1:20
" "	" "	685	-	-	1:40
" "	" "	689	-	-	1:40
" "	Delta Area	421	-	-	-
" "	" "	609	-	-	1:20
" "	Bolio Road	493	1:256	+	-
" "	" "	495	1:128	+	-
" "	" "	643	1:64	+	-
" "	Livengood	456	1:128	+	-
" "	" "	610	1:512	+	-
" "	" "	652	-	-	1:40
" "	Miller House	647	1:32	+	-
" "	Paxson Lake	488	1:128	+	-
" "	Brock Road	635	1:64	+	-
<u>Clethrionomys rutilus</u>	Manley Hot Springs	601	1:32	+	-
" "	" "	603	1:16	+	-
" "	" "	619	1:16	+	-
" "	" "	626	1:32	+	-
" "	" "	633	1:32	+	-
" "	Delta Area	604	-	-	1:20
" "	" "	608	-	-	1:20
" "	" "	625	1:16	+	-
" "	" "	680	-	-	1:20
" "	Farmer Loop Rd.	645	-	-	1:20
" "	Paxson Lake	494	1:32	+	-

Table 13 (continued)

Host	Area	Lab. No.	Q Fever	CAT	Tul.
<u>Clethrionomys rutilus</u>	Brock Road	596	1:32	+	-
" "	"	669	-	-	1:40
<u>Castor canadensis</u>	"	496	1:16	+	-
<u>Empidonax</u>	"	667	-	-	1:20
<u>Zapus hudsonius</u>	"	672	-	-	1:320
<u>Tamiasciurus hudsonicus</u>	Gerstle Road	605	-	-	1:20
<u>Ursus middendorffi</u>	Lake Hgoshik(?)	587	1:32	+	-
<u>Gulo luscus</u>	"	589	1:32	+	1:80
<u>Lynx canadensis</u>	Tok	541	-	-	1:20
<u>Rangifer tarandus</u>	Paxson Lake	457	1:16	+	-
" "	"	502	-	-	1:20
<u>Ursus horribilis</u>	Talkeetna	550	-	-	1:40
<u>Bison bison</u>	Delta Area	384	-	-	1:20
" "	"	396	-	-	1:20
" "	"	417	-	-	1:20
" "	"	418	-	-	1:40
" "	"	422	-	-	1:40
" "	"	450	-	-	1:40
" "	"	455	-	-	1:40
" "	"	570	1:16	+	-
" "	"	636	-	-	1:20
" "	"	671	-	-	1:20
<u>Canis latrans</u>	"	545	-	-	1:40
" "	"	554	-	-	1:20
" "	"	569	1:16	+	-
<u>Bos taurus</u>	Clearwater	501	-	-	1:40
" "	"	512	-	-	1:40
" "	"	520	1:16	+	1:40
" "	"	526	-	-	1:20

but I feel that significance can only be attached at 1:80 or higher dilution. All the sera identified as positive for tularemia antibodies were cross-checked against brucellosis. Frequently we found that sera positive for tularemia antibodies would also cross-agglutinate with Brucella antigen. When this occurred, only those sera showing a higher titre for tularemia antibodies were considered as positive. In reviewing the antibody titres for tularemia, only

two animals had titres of 1:80 or higher. They are the wolverine (lab. no. 589) and the jumping mouse (lab. no. 672) with 1:80 and 1:320 respectively.

From the serological evidence it would seem that Q fever must be a relatively wide-spread phenomena in the endemic animals of Alaska. As to the origin of this condition, our studies have not progressed nearly far enough to made a valid interpretation. For example, Tabert and Lackman (1965) tested a group of Eskimos in Hooper's Bay thinking they had an area where Q fever was not known to occur. However, on the basis of RIPT data and skin tests, apparently two natives of 176 individuals had positive titres. Our findings agree pretty much with those reported last year and in certain samples sent to Dr. Lackman for conformitory results, we have had relatively close agreement.

The Radioisotope Precipitation Test (RIPT): This test was developed by Gerloff (1962) for detecting antibodies for Type 2 polio virus. Later it was adapted by Hoyer et al. (for measuring reactions of Coxiella burneti with antibodies). Lackman has continued to develop the RIPT as a technique that can be applied to human, guinea pig, mouse, cattle, and other mammalian serologic studies. He is of the opinion that it is simple to perform and permits a more nearly complete epidemiological study of Q fever than was possible with other serological tools currently available.

Our equipment for this test duplicates that used at the Rocky Mountain Laboratory and we had anticipated being able to use it in

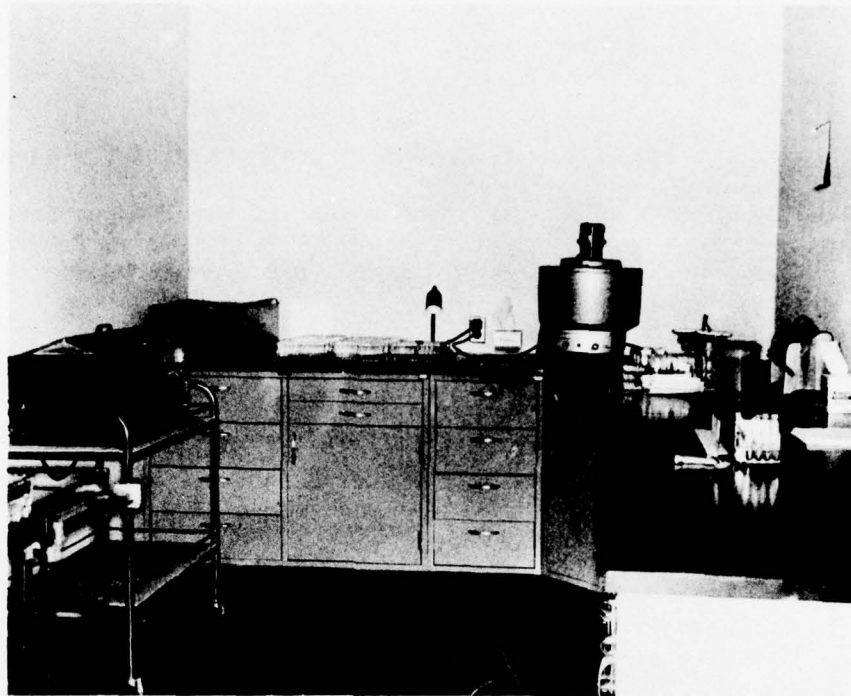


Plate XIII. Serology laboratory for complement agglutination tests and CAT tests.

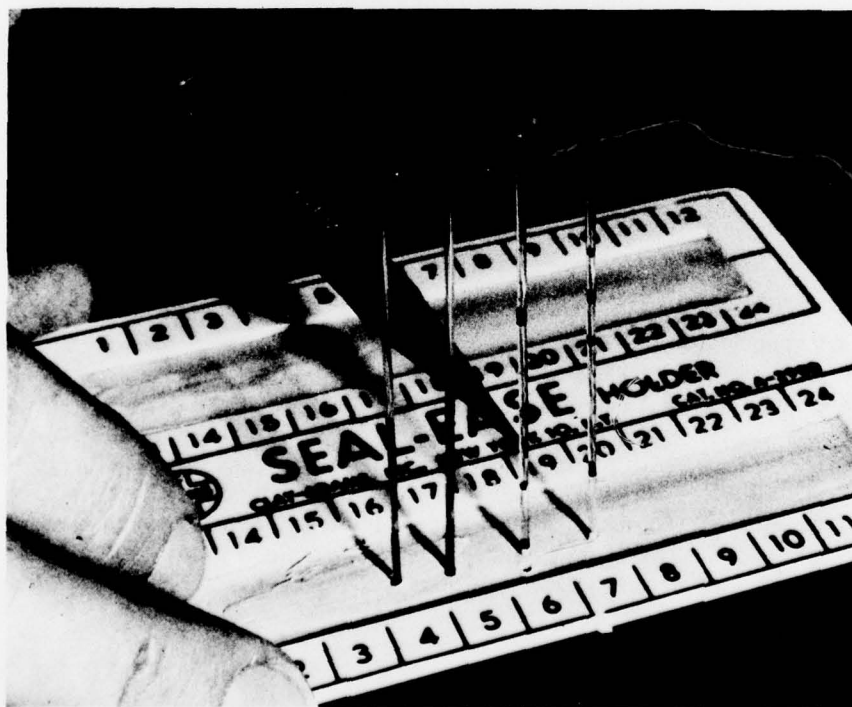


Plate XIV. Positive CAT test for Q fever organisms. This particular titre was obtained from a bison.

all sera this fall. However, technical problems with the equipment supplied by Nuclear of Chicago have taken far more of our time than anticipated. We have this under control and are now beginning preliminary testing. It is anticipated that within the next few months we will have valid data to present utilizing this technique. We follow the technique of Tabert and Lackman (1965) which is as follows:

"RIP test procedure. The RIP test employed was essentially the one described by Hoyer et al. The purified phase I Q fever antigen was labeled with  $I^{131}$  by persulfate oxidation.  $I^{131}$ , oral (Squibb), in less than 1 ml distilled water, no preservative added, was used. The antigen was washed four times to remove unbound iodine and resuspended in 5 ml of 0.02 M phosphate buffered saline, pH 7.1. The labeled antigen was diluted 1:300 to 1:1000 for use, depending on the radioactivity present. The diluted antigen, 0.04 ml, and diluted serum, 0.04 ml, were added carefully to the bottom of a 13- by 100-mm test tube with a Lang-Levy constriction pipet. Sera were diluted 1:32 for screening and in twofold dilutions to 1:32,768 for titration. The diluent for both antigen and sera was 0.02 M phosphate buffered saline, pH 7.1 to which had been added 0.05 M ethylenediaminetetraacetic acid (Versene) to a final concentration of 1:25. After incubation and rotation for 1 hr at 37°C, 0.2 ml of diluted AGG (anti-gammaglobulin) were added to each tube with a Cornwall syringe. The dilution of AGG was previously determined by titration with a known positive Q fever serum. Incubation and rotation were continued for 1 hr at 37°C and the tubes were stored overnight at 4°C.

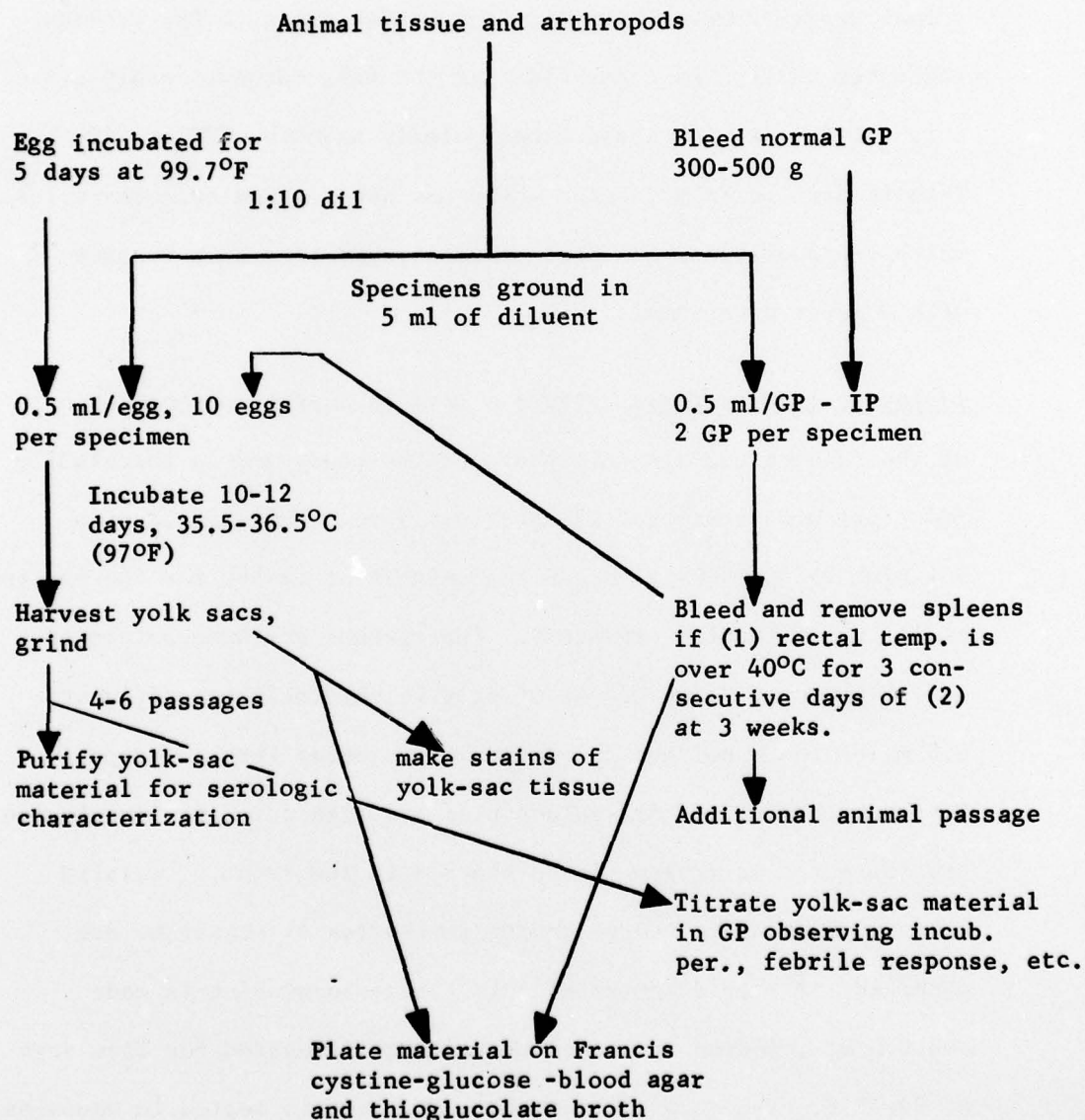
"Five tenths ml diluent were added to each tube and the mixture was shaken vigorously with a cyclo-mixer just before centrifugation at 175 X G for 10 min in a centrifuge with a horizontal head. The tubes were not allowed to stand after centrifugation, as the uncombined antigen would settle if allowed to remain for a very long period. From each tube, 0.5 ml of supernatant fluid was removed with constriction pipets and placed in a planchet. The planchets were dried and the radioactivity was determined by Nuclear-Chicago gas flow counting equipment."

From Tabert and Lackman's (1965) findings it is apparent that the RIPT detects a higher frequency of antibody response than either

the CFT or the CAT particularly in regards to humans. There is still some question mark as to what interpretation can be implied to animals other than man. The increased sensitivity observed by these workers with human sera was not present with the other animal sera. However, personal communications with Dr. Lackman indicates that he is convinced that the RIPT unquestionably gives more valid data with these other animals than the CFT or CAT. This is particularly germane where our studies indicate the voles, which are poor producers of complement, may have some contact with Q fever organisms.

Isolation of Organisms: Figure 6 is a schematic representation of the figures used in this phase of the study and is practically identical with that used the previous year. Male guinea pigs weighing 250 to 500 grams are the animals of choice for the routine isolation of the two organisms. The tissues are homogenized in teflon grinders using 0.5 ml of sterile skim milk as a diluent; 0.5 ml of the resultant suspension is injected into the animal intra-peritoneally. All guinea pigs are bled prior to inoculation and the serum is frozen to be checked in the event a valid rise in temperature occurs and/or antibodies or organisms are observed. A 1:10 dilution of this tissue homogenent is made and 0.5 ml injected into each of ten eggs incubated for five days at 99.7° F. The remainder of the suspension is sealed in ampoules and stored at -60° C. Mosquitoes and other arthropods are handled in essentially the same way as the tissue samples except the

Figure 6. Schematic representation of the procedure used for isolation of C. burneti and F. tularensis.



mosquitoes are rinsed in two rinses of sterile skim milk in an attempt to free them from external contaminants. The ticks and fleas are rinsed in 5% phenol followed by sterile distilled water for the same reason. Penicillin (1000 units) is added to the skimmed milk diluent at the time of grinding.

Rectal temperatures of the guinea pigs are measured daily for three weeks. If a reading of 40° C or above is encountered for three days the animals are bled and transfers of the blood made into embryonated hens eggs. Such animals are then sacrificed and the spleen removed. These spleens are homogenized in the grinders as previously described and injected into the embryonated eggs. Additional samples of the suspension are cultivated on cystine-glucose-blood agar and thioglycolate broth. Each of two mice receive 5 ml of this same suspension intra-peritoneally as a more delicate test than culture media for F. tularensis.

After three weeks all animals not showing an elevation in temperature are bled and the sera tested by the complement fixation, capillary agglutination and the standard agglutination test. The animals are then necropsied, spleen removed and are processed as described above. Tissues from animals showing an antibody titre for either of the two organisms is watched with special care after the reinoculation.

After inoculation the eggs are incubated for 97° F for 11 days; embryos which died before the third day of incubation are discarded. Embryos which survive beyond the third day are harvested as soon



Plate XV. Planchet and counter system used for the RIP test. We have had innumerable delays in developing this system due to the erratic performance of the counter and recorder. The system was replaced and now appears to be in good working condition.



Plate XVI. Entomological technician (Miss Megan Young) preparing arthropods for study.

Table 14. Serological results of first animal passaged tissues. These findings are only tentative and the tissue of the first "passage animals" are now inoculated into the second animal. No organisms for Q fever or tularemia have been identified from this material.

Host	Lab. No.	Temp. after			
		4 days	CFT	CAT	Tul.
<u>Empidonax</u> sp.	750	38°	1:16	-	0
<u>Aedes</u> sp.	758	37.5°	1:16	+	0
<u>Microtus pennsylvanicus</u>	844	38°	1:16	-	0
<u>Microtus pennsylvanicus</u>	845	37°	1:16	-	0
<u>Tamiasciurus hudsonicus</u>	852	37°	1:16	-	0
<u>Microtus oeconomus</u>	854	38°	1:16	-	0
<u>Microtus oeconomus</u>	1137	38°	1:16	-	0
<u>Microtus miurus</u>	1181	37°	1:32	-	0
<u>Clethrionomys rutilus</u>	1289	38°	1:32	+	0
<u>Bucephala albeola</u>	1315	38°	1:80	-	0
<u>Lynx canadensis</u>	1326	38°	1:16	+	0
<u>Lynx canadensis</u>	324	37°	1:64	+	0
<u>Lynx canadensis</u>	342	37°	1:16	-	0
<u>Lynx canadensis</u>	461	37.5°	1:128	+	0
<u>Lynx canadensis</u>	496	37.5°	1:16	+	0
<u>Aedes pionips</u>	593	37.5°	1:16	+	0
<u>Aedes excrucians</u>	597	37.5°	1:32	+	0
<u>Culiseta alaskaensis</u>	610	38°	1:32	+	0
<u>Lepus americanus</u>	613	37°	1:16	+	0
<u>Turdus migratorius</u>	620	38°	1:32	+	0
<u>Microtus oeconomus</u>	623	37.5°	1:16	-	0
<u>Tamiasciurus hudsonicus</u>	682	37.5°	1:512	+	0
<u>Tamiasciurus hudsonicus</u>	689	39°	1:32	-	0
<u>Aedes</u> sp.	697	38°	1:16	-	0
<u>Aedes excrucians</u>	715	37.5°	1:16	-	0
<u>Aedes intrudens</u>	727	37.5°	1:16	-	0
<u>Tamiasciurus hudsonicus</u>	749	37.5°	1:128	+	0

after death as possible and those alive on the 11th are sacrificed and the yolk sacs removed.

Smears of yolk sac material are stained with Gimenez and examined for the presence of rickettsiae. If the rickettsiae are found, the yolk sacs are pooled and the egg passage is continued in an attempt to obtain a rich growth of organisms. If a satisfactory yield has been obtained the yolk sacs from the last passage are harvested and ether extracted to purify the rickettsiae so the organisms can be used as an antigen for serological characterization and complement fixation.

The biological properties of the isolates of Q fever and tularemia are determined by the same techniques as described previously. As of this writing, we have not actually isolated either of the two organisms.

Table 14 tabulates the possible positive results of the first animal passage. At this point, the serology studies can only be considered suggestive, particularly in view of the fact that several are from voles, and that no marked elevation of temperature occurred. It is perhaps significant to note that all antibodies are for Q fever organisms and not for tularemia.

#### REFERENCES

- Bell, J.F., Luoto, L., Casey, M.L. and Lackman, D.B., *J. Immun.*, 93:403, 1964.
- Bell, J.F., Lackman, D.B., Meis, A. and Hadlow, W.J., *Milt. Med.*, 129:591, 1964.
- Coombs, R.R.A. and Stoker, M.G.P., *Lancet*, 261:15, 1951.

Gerloff, R.K., Hoyer, B.H. and McLaren, L.C., J. Immun., 89:  
559, 1962.

Hoyer, B.H., Gerloff, R.K., Luoto, L. and Ritter, D., To be published.

Lackman, D.B., Frommhagen, L.H., Jensen, F.W. and Lennette, E.H.,  
Amer. J. Hyg., 75:158, 1962.

Lackman, D.B., Gilda, G. and Philip, R.N., Health Lab Sci.,  
1:21, 1964.

Luoto, L., J. Immun., 77:294, 1956.

Ribi, E. and Hoyer, B.H., J. Immun., 85:314, 1960.

Tabert, G. and Lackman, D.B., J. Immun., 94(6):959, 1965.