CHAPTER 3

FIELD AND LABORATORY TECHNIQUE

THE FIELD MANUAL

It is quite obvious that someone's field work must precede all laboratory technique. This phase, the field work, has not heretofore been stressed in any published work upon fleas. It is relatively easy to secure without much effort the various fleas that one might call domestic; that is, the human flea, which can be collected in large numbers from almost any hog pen, many lumber camps, or seashore resorts; the western chicken flea, which can readily be shaken in numbers out of almost any hen nest; the cat flea and the dog flea which appear in numbers during the summer months on the household cat and dog, but to successfully gather the other 225 species and subspecies of what one might call the wild native fleas listed herewith from the West, requires much more skill and much more patience. The great majority of these wild native fleas are to be found on the wild rodents of the vicinity. Generally it is much easier to catch the rodents than to find their nests, but in case their nests can be found, many more fleas can be taken from the nests than from the rodent hosts. Only upon rare occasions does a collector actually find wild fleas in any numbers roaming around upon the ground.

For any general collector, the wild rodents, which most of us think of as also embracing the conies, hares and rabbits, will be found to give the most fertile field for collecting. Unless these can be shot and placed in bags from which the fleas cannot escape, it is best in all cases to trap the hosts in traps which keep them alive until the collector can examine them and remove their fleas. While full directions are given for the capture of all animals mentioned with the host indices in following pages, the equipment necessary for the trapping procedure is listed here.

By far the most practical of all trapping devices for small animals is a very small box trap perfected by the writer and used by him exclusively for practically all wild nocturnal rodents. These traps are built around a common snap mouse trap and are large enough only to admit the fist of the collector. A battery of 50 of these is about all one man can care for during a field day.

These small traps can be constructed by the handy collector at the rate of about one per hour. They must be made; they cannot be purchased. Wood apple boxes which can be picked up in almost any gro-

cery store, as empties, can well be used for the stock, or pine boards can be purchased for the purpose. The bases of the traps are made of 1-inch by 4-inch stock (3¼ inches wide) surfaced on 4 sides and cut 7 inches long. The 2 sides and top are cut from quarter inch stock and should measure 4 by 4½ inches, the grain to run the 4-inch way. Now build the box at one end of the base, nailing with inch brads or lath nails. Cut a piece of quarter-inch hardware cloth (screening) to fit the back, and nail in place. The back is made of screen because animals will more readily enter if they can see through. To explain the structure of the door is not so easy. A common tin shingle purchasable at any hardware store will make two doors with enough metal left over for several accessories. Set a common snap mouse trap, the wood base variety, and place it in the opening of the box of the trap. Now slip half the tin shingle over the box trap opening and slightly in front of the spring of the set mouse trap. Hold the mouse trap to the base of the box trap and spring the mouse trap. The snap at half sprung position will hold the tin shingle, which is now the door, tightly in place. Center the mouse trap, and push tightly against the door. With a pencil mark the size of the door, and then carefully mark around the snap of the mouse trap where it contacts the door. Trim the door to size with tin snips, rounding all corners slightly to prevent injury. With a small nail, punch a series of holes around the snap line, these to be used to wire the door securely to the snap. Before wiring the door to the snap cut a three-eighths inch square

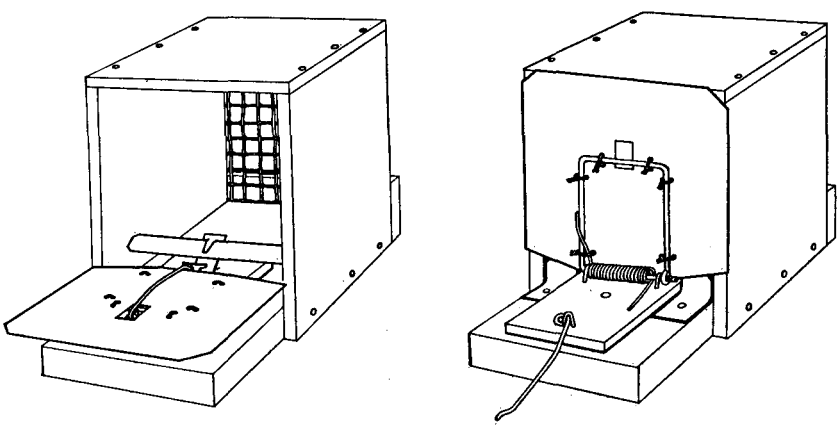

FIG. 2. Box trap designed by author.

hole just on top of and at the middle of the top line of the snap. The trigger goes through this top hole. With fine iron wire securely wire the door to the snap. Place in position so door is closed, then nail the mouse trap with lath nails securely to the base. To set the trap run the trigger through the small hole in the door as it is forced down to setting position and connect trigger connections as usual.

Three accessories are to be made of the remaining metal of the half shingle. First cut a piece three-eighths of an inch by 3 inches, round off the corners, slip this under the hook which was made for the bait and clamp it in place with a pair of pliers. This is the treadle upon which the animal steps to set off the trap. Cut 2 pieces three-eighths inches by 1¼ inches, round off the corners and bend up to right angle about a quarter inch from one end. Nail these in place, one on either side of the mouse trap so that they fill up the hole below the door. Nail a piece of lath across the back of the trap over the screen and raised about half an inch above the floor—this will prevent the bait from falling out during transit. After a model is once set up, others can be quickly built. The traps should not be built much bigger since the larger the door the more easily it can be pushed open because of the increased leverage. The trap should be just large enough to allow the collector's open hand easy entrance, and just as easy an exit when the fist is pulled out with the trapped animal.

These traps should be made in units of 12, piled in 3 tiers of 4 each and a carrying box made for the 12. Empty dynamite boxes, because of their dovetailed joints, make excellent carrying cases. Each trap of the series of 12 should have a number on it in several places. If numbered the traps can be set out in rotation and picked up in rotation. Lost traps can be more easily located in this way.

These small traps will hold all types of mice, kangaroo rats, house rats, chipmunks, flying squirrels, conies, shrews, large and small weasels, western round-tailed wood-rats and a good percentage of bushy-tailed wood rats, golden-mantled ground squirrels and antelope ground squirrels. They will not hold anything as large as wild pine squirrels and usually the smaller sage rats will not enter them.

It is next to useless for the collector to burden himself with snap traps. On rare occasions they do prove useful. If large numbers of them are set out on a bright moonlight night, a night during which it is known that the moon will set about an hour before dawn, the mice to be caught will run at large during this hour of darkness, get into the traps and will be warm and with fleas when collected at dawn by the collector. On long dark nights snap traps are generally useless because the mice are caught early, the bodies cool and the fleas leave before the collector can pick up the catch in the morning.

Three other types of traps are needed for thorough field preparation. Pocket gophers require a special trap. The small green Macabee gopher trap, which is built in California and which retails for about 25 cents is excellent for small gophers. Larger gophers cannot generally be taken with these, but must be trapped with the Chinch gopher trap, which is built in Oregon and can be purchased for 50 cents. Either trap is set, then forced into an opened burrow and when the gopher tries to close the burrow it is caught and killed by the trap. Where lines of

gopher traps are run, they should be frequently visited to remove the taken animals. Since but one gopher generally inhabits any one system of burrows, traps should be moved to other burrows after the owner is caught.

While the writer has captured moles with Macabee gopher traps, far better results will be secured with the special mole trap recommended by the Bureau of Biological Survey. This is the Out O' Sight Mole trap which is manufactured in the East, but which can be purchased in any hardware store for $1.25. Moles are generally not easy to catch even with the best of traps. Since all mole traps kill, the lines must be frequently visited to remove captured animals.

Common steel traps have a wide variety of uses in the field. Most burrowing rodents can be taken in them. For some animals one has but to throw the set into the burrow and stake it down, other animals are very particular into what they step. Mountain beavers, gray diggers, wood-rats, and conies will readily step into bare traps, but sets for sage rats, Oregon ground squirrels, and some other rodents require skillfully hidden sets. Sets can be hidden by burying them shallowly after placing a ball of horse hair or soft moss under the pan.

Box traps, steel traps, mole traps, and gopher traps in fair numbers will give the necessary trapping devices to catch the host material of almost any vicinity.

The novelty of long lines of small box traps is the variety of mice and other small rodents in each night's catch. Box traps are generally set for anything that will enter them. The plan is not exactly to set the traps at random, but each location should be carefully considered as to how much shelter the small animal will have to get to the trap. The small mice depend primarily on natural shelter for their safety. Naturally then, the most profitable places to set traps are under logs, in sheltered cavities of one type or another, along fence rows, in old deserted buildings, in rim rocks. At times the collector can find dusting spots and small springs to which the rodents come to dust and drink. These afford excellent places for sets. In desert regions where there is little shelter between bushes it becomes necessary to set along the tiny trails and highways of the animals which can generally be seen running over the sands.

The matter of bait is one of question. Almost everything in the line of food has been tried to coax small animals into box traps, but a bait which practically climaxes all others as far as the tastes of the majority of small rodents is concerned is quick cooking rolled oats. A small handful of this thrown into a trap will attract a mouse for a long ways. Common rolled oats give only about 50 per cent of the results secured by quick cooking. Apple has always been an excellent bait but the moisture content runs rapidly through the intestine to badly soil the inside of the trap and muss up the feet and fur of the animal. Small

carnivores, such as weasels and grasshopper mice, do not come into these small traps more quickly if they are baited with meat, but if they happen along they enter probably only to investigate the odors left in the traps by mice.

By far the most useful gun for the field is a .410-gauge shotgun. The small shot hardly tear the pelts at all, and therefore bleeding is brought to a minimum. Two and one-half inch shells will stop chipmunks, small squirrels, 3 inch shells will stop animals up to the size of marmots. For long distance work and large rodents a .22 caliber rifle is the most effective. A pneumatic air rifle is indispensable. It can be used to shoot pack-rats in small traps and in locations where firearms are prohibited it is a most handy weapon even for large squirrels.

The trapping day and its schedule plays an important part in the field work. Box trap lines should be set as close to dusk as possible without hurrying. Such a timing allows all available nocturnal rodents to enter the traps before morning comes with its host of daybreak loving chipmunks and ground squirrels. In the morning the chipmunks will find these traps remaining open and enter them. If the chipmunks enter in the evening they simply dirty up the traps and take the possible place of a mouse that cannot be shot during the day, as all chipmunks can.

Collections from box trap lines should be made as early after sunrise as possible. The sun irritates the small animals and soon they worry themselves to death. In the case of desert trap lines, the heat of the sun also causes quick death to the nocturnal forms.

It is the custom of this writer to kill all small mice at the trap, thus doing away with the necessity of again looking through them. Insert the hand, close it carefully and quietly over the mouse, withdraw, then force the thumb behind the ear until the neck is broken. No bleeding results if the job is done properly. Drop the mice into the clear glass jars of a biology collecting vest. Handle all mice carefully, any rough treatment will cause their fleas to hop off. In so far as there seems to be little or no correlation between the mouse and the number of fleas it will carry, it is generally not necessary to keep them in individual jars until their fleas are removed. Under no conditions mix different species or subspecies of hosts in these collecting jars. Practically all mice, chipmunks and kangaroo rats can be handled in this way. Squirrels, domestic rats, wood-rats or other animals of this size and nature are best taken back to the field laboratory and then shot with an air gun while still in the trap, or removed with a gloved hand. Such treatment prevents infection to the hands of the collector through scratches and bites of the infuriated animals.

While it is important to know where to trap, it is just as important to know where not to trap. Generally it is useless to trap in situations where farm cats are at large. Their patience generally rewards them

with a meal off all of the more daring mice, the trapper having little or no chance where they run. It is generally useless to run a trap line at the same location a second night. All small mice seem to honor the other mouse's territory and apparently will encroach upon another's domain only after all smell of the owner has disappeared. Catches on a second night in one place fall off so sharply that it does not pay to try again the following night. While many mice can be taken from under new haycocks, most of these have abandoned old nests eleswhere to re-establish under the new haycocks and have thus left most of their fleas behind. Mouse takes from under new haycocks may be large, but the number of flea takes from the mice is generally small. Old haycocks, on the other hand, are almost always well worth-while turning over. An old well established mouse nest may often net the collector 100 fleas. Nests so collected should be placed in paper bags until they can be sorted over for fleas.

After all animals of a night's catch have been carried back to the field laboratory, preparations must be made for securing the fleas. The only equipment necessary is 70 per cent alcohol, chloroform, vials, labels, dissecting needles and a good sized white enameled dishpan. Some collectors use a comb to comb fleas off their host. Dr. Jordan suggests putting the fleas in 70 per cent alcohol to which has been added a drop or two of glycerine. This prevents hardening. The main point in removing the fleas from the hosts is that the operator get comfortable and out of the wind. Fleas will leave the hosts more readily if the hosts are cold. The collector should sit with the dishpan held between the knees. With some slight force drop the hosts out of their containers into the dishpan. The force with which they hit the bottom will jar most of the live fleas off. These can be picked out of the pan with a dissecting needle dampened in the vial of 70 per cent alcohol waiting for the reception of the fleas. After pummeling the host for a short time to force its fleas off, take the host by the tail and hind feet with one hand, then with the other rapidly run the fingers downward against the fur. This operation will force more fleas from the host. Holdenried of the Hooper Foundation said to the writer on their first trip into the field together, "I have seen fleas removed from hosts by collectors in a lot of different ways, but you are the first one I ever saw milk them off." The next operation is directed against stubborn fleas. Pour a small amount of chloroform into a clear pint jar. Dangle the host in the jar while forcing the hand over the opening for pressure. Most remaining fleas will hop off the host and die in the jar.

One school of collectors in the West holds that chloroform applied to fleas ruins them for plague work. This group of collectors uses hydrocyanic acid gas for the removal of fleas from hosts. The average collector is, however, not in a position to use this technique. A second school of collectors, just as interested in plague as the first, claims that chloroform in no way effects fleas as far as plague is concerned. This

group of collectors uses chloroform altogether in removing fleas from their hosts.

After application of one or the other of these gases, dead fleas should be brushed out of the fur. In case chloroform is used, the hosts should be laid aside for a day and worked over again.

Fleas collected for plague inoculation purposes are bottled in a special salt solution and delivered to headquarters by air mail, special delivery.

All hosts which cannot be positively identified by the collector should be built into study skins with skull attached and turned over to a mammologist for identification.

It is a tedious task to remove fleas from most rodent nests. It can only be accomplished by the hunt and pick method. Bit by bit pull the nest to pieces and remove the fleas. Wherever possible the host should be collected with the nest for positive identification.

A good collector places his label inside of the vial, most of us lick them and stick them on the outside so that they can fall off and cause confusion.

Field notes—every collector has his own ideas on field notes. Perhaps the writing of these gives the collector a few moments of relaxation before setting out to collect the traps set for diurnal animals or to shoot a few chipmunks and squirrels of one type or another.

THE LABORATORY MANUAL

It is not very often that one finds time in the field to mount or determine fleas. The customary procedure is to bring all specimens collected into a laboratory in the vials in which they were originally placed. When one is ready to proceed with the laboratory work it is the generally recognized custom to return all specimens to water.

A great deal of equipment is not necessary to work these insects in the laboratory. Bleach is necessary. This should be a 10 per cent solution of sodium hydroxide. Also at hand should be a 1 per cent solution of acetic acid, 25, 50, 75, and 96 per cent alcohol, carbol-xylol, cedar oil, xylene and mounting media. Carbol-xylol can be made by placing a pound bottle of carbolic acid crystals in warm water until it dissolves, then pouring half of it out of the bottle and replacing the half with warmed xylene. The solution should be strong enough so that when cold there are carbolic acid crystals precipitated on the bottom. The best of microscope slides and cover glasses should always be used. For the sake of beauty the author has always preferred to use round cover glasses.

Two things are of utmost importance in mounting fleas; first, the bleaching; second, the consistency of the mounting media.

Preparations are best carried through in watch glasses, those with ground glass edge. These stack nicely and are evaporation proof, and data can be written on the ground glass edge.

The student should always remember that before bleaching there are many things of interest to be found on the inside of the flea which bleach will completely ruin for study. Some of these things of interest are the internal anatomy, about which little has been recorded for fleas; eggs in the abdomen of the female, these frequently differing with the species in size, shape and number; the Cysticercoid stage of the tapeworm, which at times can be seen in large numbers in the abdomens of fleas; nematodes, which sometimes completely pack the abdominal cavity; threadworm larvae which wind in seemingly endless length in the flea's body. All these should be studied before bleaching begins. To bring these things to light, remove the fleas from 70 per cent alcohol and place them in 96 per cent alcohol. After a few hours pipet off the alcohol and flood the specimens with carbol-xylol. They will begin to clear immediately. Check with microscope for interesting points and any found worth preserving can be prepared for mounting by being placed in xylene for a short time, then mounted in balsam. Very satisfactory identifications can often be made from such mounts but they are never mounts that one can look at with pride because of the cloudiness of the specimens. Carbol-xylol treatment at this time in no way affects the specimens for bleach treatment. To return the specimens to bleach, thoroughly wash balsam off them with xylene and then place in carbol-xylol. From carbol-xylol run specimens through 96, 50, 25 per cent alcohol and then to water. From water place specimens in bleach.

Remove fleas to be bleached from their vial of 70 per cent alcohol. Place each batch in a watch glass and cover with water. Place data upon the watch glass edge. Allow to stand in water for 24 hours. Drain off and then submerge the specimens in bleach. It is difficult to say how long a flea should remain in the bleach. Experience in this line is generally the best teacher. Pale fleas such as Corypsylla and Corypsylloides require very little bleaching, dark fleas such as the wild rabbit fleas seem to take endless time. If specimens are watched carefully, bleaching can be hastened by heating the bleach. The process should never be one of boiling and never should one heat the bleach to such an extent that the delicate edges of the sclerites become disfigured. Bleaching should continue until the visceral mass has completely disappeared. The author has always preferred a slow room temperature bleaching. This gives good definition to the sclerite edges and there is generally no distortion. Do not over bleach. Under bleached specimens can always be returned to the bleach, but over bleached specimens are often a total loss.

After the bleaching has been completed the specimens should be thoroughly rinsed in water, then for 24 hours suspended in a 1 per cent solution of acetic acid. This is, of course, to neutralize any bleach remaining in the specimens. After washing the acetic acid off with water, the fleas are to be run through the alcohol solutions, 25, 50, 75 and 96 per cent, several hours each. From the alcohols run the speci-

mens into carbol-xylol. After a few hours check for clearness with microscope. If there is any trace of cloudiness, place specimens in cedar oil. If this does not clear up the cloudiness run fleas backwards through the process and rebleach. Before mounting the specimens should be as clear as glass, and in females the spermatheca and the marginal outline of the VII sternite should be very clear. In males the modified abdominal segments should show up well.

After specimens have been bleached and cleared to the satisfaction of the worker they should be changed from the clearing solution to xylene in which they should remain from a few hours to a day, depending on how much of a hurry the worker is to finish the job. When one is ready to mount, transfer the specimens to new xylene. Dry the cleansing alcohol from a slide and cover glass, place the slide in position on the mounting mat. Put a drop or two of balsam at center of slide, and gently spread over area to be covered by the cover glass. Dip the point of a dissecting needle into the balsam, then apply sticky tip to the flea to be mounted and lift it from the xylene into the balsam, center, and with a pair of forceps drop cover glass into place. The specimen or specimens should not drift. In multiple mounts care should be taken that all specimens are completely submerged in the balsam or air will seep into the fleas and cause them to turn black. In case specimens drift, raise cover glass, reset specimens and replace cover. Specimens which turn black must be unmounted, run back to the alcohol solutions and left there until the air escapes, then run back through the process again. In case balsam does not completely spread under cover glass, add a bit more with a quill or dissecting needle. Mounts look very much better if not too much mounting media is used and the cover glass rests snugly upon the specimens.

Before mounting fleas, one should prepare a mounting mat. On a white ruled index card place a microscope slide and mark around it with a pencil. Remove slide and divide outline of slide into 3 equal parts, 1 inch square. Divide the middle square with 2 lines running from opposite corners. They will cross at the center of the square. Center at the cross point a ¼-inch round cover glass, and outline with pencil. Repeat process with various other sizes of square and round cover glasses. With such a mat the specimens can be centered on the slide, as can the cover glasses and labels which go on the ends.

Various mounting media should be given trial before the student decides upon one. The author has always worked with balsam. The skill with which one mounts will depend entirely on how well he understands the working of the media. The author has always preferred to use a thin balsam in a comfortably warm laboratory. Balsam does not set rapidly but drying can be hastened in an oven. Balsam should be thinned to the consistency desired by adding xylene.

It is generally felt that all microscope slides and cover glasses should

be washed in alcohol and dried with a linen handkerchief just prior to using.

Few persons agree as to the arrangement of fleas upon slides. However, fleas should never be thrown into the media to drift where they will as the cover glass settles down. There should always be some definite arrangement. For exchange purposes the writer prefers to place on a slide a male and a female of the same species, the male above, the female below, both with legs up, bodies parallel with top and bottom of slide, heads to the left, and equidistant between the center of the slide and the edge of a ¾-inch round cover glass. Rare specimens should be mounted singly in the center of a ½-inch round cover glass. To show variation, as many as 50 medium-sized fleas can be nicely arranged in rows under a ¾-inch cover glass.

It is generally not a good practice to mix species of fleas under a cover glass.

After viewing hundreds of specimens of fleas of other collectors which were placed upon slides in every imaginable position, the writer decided to mount all his specimens with head to the left. After using this method for some years, the writer was informed by Gus Augustson that Alfonso Dampf of Mexico City had written him that his specimens as well as Hubbard's were all placed on the slides backwards—that the International Congress had decided that fleas should be mounted with their heads to the right. Augustson changed over, but the writer still prefers to mount his specimens with heads to the left.

Although students of fleas have labeled slides with india ink on the glass slide ends, with special write-on-glass inks, and write-on ground ends, all meticulously prepared slides have a neat paper label pasted on each end. Labels should be affixed at the time the mount is made. In this way the label will dry out thoroughly and make a better inking surface as the balsam dries in the oven. Some key number must be placed upon the label in pencil so that, when labels are hard enough to ink in, the specimen can be referred back to the field log for collection data. If the hand is not too heavy, labeling should be done with a crow quill pen and black india ink. Type specimens should be further marked with some colored ink. If the hand is heavy any good fine writing steel pen will do. What goes on each label on the slide is a matter of choice. This collector places common name of host, location of collection, date, personal log number and name on right hand label; technical name of flea and technical name of host on left hand label. All data on labels should be carefully and neatly printed.

Before affixing labels or printing upon them, make sure legs of specimens are up.

When the slide is finished and ready to be stored away it should represent the collector and his skill, and should be something of which he can be justly proud.