Technical Report No. 85

BASIC FIELD DATA COLLECTION PROCEDURES FOR THE GRASSLAND BIOME 1971 SEASON

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GRASSLAND BIOME

U. S. International Biological Program

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ABSTRACT

This report comprises an outline of techniques and methods that will be used in the data collection of the Comprehensive Network during the 1971 collecting season. Participants at the intensive site (Pawnee) are also attempting to incorporate these procedures as a subset of their own activities. Sample field data sheets are also included to indicate the manner of data collection and the basic format of data in the information storage and retrieval system. This report is an outgrowth of Technical Report No. 35, "Field data collection procedures for the Comprehensive Network 1970 season." Participants met to revise the former report in individual groups during the month of December: Producers, Invertebrates, Small Mammals, and Decomposers. A joint meeting in January with representatives from each group further refined the plans and procedures, and included discussion of abiotic measurements, avian studies, and laboratory analytical procedures. Outlined herein are the collection procedures, preliminary analytical procedures, and data collection procedures for evaluating above- and belowground plant biomass, litter, litter accumulation, invertebrate sampling, small mammal sampling, bird investigations, studies of decomposers, micrometeorological data acquisition, and laboratory analysis requirements.

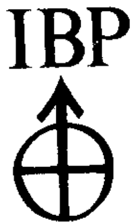
BASIC DESIGN

Whenever possible, each site in the Comprehensive Network will consist of at least one grazed and one ungrazed treatment with two replicates for each treatment. Ideally, these areas should be sufficiently large so that all types of sampling planned in the Comprehensive Network studies can be made within each area (about 30 acres or 13 hectares). Each treatment and replicate should be conspicuously labeled in the field so that all workers may clearly identify their data sheets. Each area should be mapped and marked with stakes to facilitate location of sampling points.

BASIC FIELD DATA

A general information form may be used to record information concerning the site and conditions at the time of sampling. This contains general information and data on plant phenology and is to be completed for each treatment. It will be useful reference material in case there are questions concerning weather conditions at the time of sampling, time required for sampling, etc. A sample form follows.

For convenience and rapidity of processing data, standard field data sheets will be utilized. The sheets are designed for ease of recording raw data in the field and for convenience of transcribing these data to punched cards for machine processing. The data sheets are color coded by data type for convenience of handling and sorting. White and other light colors have been avoided for field use because of reflectance in bright light and resulting eye discomfort. The forms are bound together at the bottom, to prevent their movement by wind when the top is secured by a clipboard.



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GENERAL INFORMATION SHEET

Site____________ Date____________ Time__________ Investigators______

____

Type of study and methods______________________________

____

Weather: Temperature__________ Wind__________ Cloud__________

General comments______________________________

____

Additional measurements taken:______________________________

____

Remarks:____

____

PHENOLOGY	Genus	Species	Subspecies	Phenology	Percent
01 Germinated or sprouted 02 Early vegetation 03 Immature vegetative (non-flowering plants) 04 Prebud 05 Bud stage 06 Early bloom 07 Mid-bloom 08 Full bloom 09 Late bloom 10 Milk stage 11 Dough stage 12 Ripe seed 13 Past ripe 14 Mature vegetative (non-flowering plants) 15 Stem cured 16 Vegetative regrowth 17 Winter dormant	29-30	31-32	33	35-36	38-40

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Each set of data must be accompanied by certain identifying information. These basic data occupy the first 19 columns of an 80-column punched card, and are outlined below (X's indicate number of characters to be punched).

XX	TYPE OF DATA - code for aboveground biomass, litter, small mammal, etc.
XX	SITE - code for Ale, Bison, . . ., Pantex
XXX	INITIALS - for initials of *Field Worker* recording the data
XXXXXX	DATE - two spaces each for day, month, and year, in that order
X	TREATMENT - code for type of treatment
X	REPLICATE - taken from marker sign at field plot
XXXX	PLOT SIZE - quadrat or plot size (area in m^2 for aboveground biomass, area in hectares for birds and small mammals)

HERBAGE DYNAMICS - ABOVEGROUND BIOMASS

Sampling Methods

Aboveground biomass refers to standing live plant material and standing dead plant material. Sampling sites will be located randomly within replicates. The sample size *for the few major species* (those species that collectively account for about 90% of the total biomass) is to be 0.5 m^2, in a circular frame. The number of samples to be clipped per replicate will be that number required to estimate the biomass of the species to within 20% of the true biomass with a probability of 0.8. This number for the initial sampling can be determined from 1970 data. Generally, it will be less than 10. Weight-estimation of these species will be made on the plots before clipping, and on an additional 10X plots which are not clipped, where X is the number of clipped plots. When a quadrat is clipped, the material removed

will be separated by species (for the major species), oven-dried, and weighed. Everything standing will be clipped. Litter will not be included. The standing dead of the current year's growth (recent dead) and of last year's growth (old dead) will be separated by major species where possible.

Estimation of biomass of other important species will require clipping and estimation of larger sample areas. ("Important species" is here defined as those which contribute 5% or more of the total season-long plant biomass.) Circular plots with an area of 2 m^2 or greater should be used. The same numbers of clipped and weight-estimated plots should be used as the smaller plots for major species. Field data will be recorded on the form labeled ABOVEGROUND BIOMASS and sent to the NREL data processing laboratory immediately after field and lab work are completed. That form will contain the information required for determination of the optimum ratio of clipped to weight-estimated plots for the next sampling period.

Aboveground biomass will be sampled *biweekly* during the period of active growth and monthly during the dormant season. Sampling may be less frequent than biweekly if the aboveground biomass is estimated to have changed less than 10% since the previous sampling period. Clipping height for aboveground material is ground level. Crowns will be separated and categorized as to species and living and dead material. Stolens will be combined with the crowns if they are alive; otherwise, they will be combined with the litter.

Twice during the growing season a complete list of species should be generated and reported. This list will include those species which are not of sufficient importance to be included in biomass estimation.

Phenology of the "important species" will be recorded once each sampling period on the white General Information Sheet. Each species should be listed,

the appropriate phenology code designated, and the approximate percentage of the plants in that category estimated.

The aboveground material including crowns will be separated, dried (at less than 60°C) and weighed. A portion will be ground with a 40-mesh screen and will be composited by replicate per group or species per date. At least 5 g of each sample will be sent to NREL for percent ash, calories, nitrogen, phosphorus, and potassium determinations.

A composite sample of each major plant species by replicate will be oven-dried (temperature below 60°C), ground in a Wiley Mill, and one-half of this sample sent to NREL for chemical analysis.

Accuracy

The objective is to obtain an estimate of the mean herbage yield by species with a standard error no greater than 20% of the mean at the 80% level of confidence. When sample data are available, the number of plots required for this degree of precision can be calculated.

Dry weights will be recorded to the nearest 0.01 g.

Data Recording

A sample field sheet for the recording of data follows. A copy of the original form will be immediately forwarded to the Natural Resource Ecology Laboratory where it will be transcribed onto punched cards and analyzed according to the design for preliminary analysis of field data. Results of this analysis must be returned to the field investigator prior to the next sampling period.

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FIELD DATA SHEET - ABOVEGROUND BIOMASS

DATA TYPE	SITE	INITIALS	DATE DAY	DATE MO.	DATE YR.	TREATMENT	REPLICATE	PLOT SIZE	QUADRAT	CLIP-EST.	GROWTH FM.	GENUS	SPECIES	SUBSPECIES	CATEGORY	WEIGHT ESTIMATE	SACK NO.	DRY WEIGHT	CROWN PLOT SIZE	CROWN WEIGHT
1-2	3-4	5-7	8-9	10-11	12-13	14	15	16-19	21-23	25	27	29-30	31-32	34	35	36-40	42-45	47-52	54-57	59-64
01																				

DATA TYPE
01 Aboveground Biomass
02 Litter
03 Belowground Biomass
10 Vertebrate - Live Trapping
11 Vertebrate - Snap Trapping
12 Vertebrate - Collection
20 Avian Flush Census
21 Avian Road Count
22 Avian Road Count Summary
23 Avian Collection - Internal
24 Avian Collection - External
25 Avian Collection - Plumage
30 Invertebrate
40 Microbiology - Decomposition
41 Microbiology - Nitrogen
42 Microbiology - Biomass
43 Microbiology - Root Decomposition
44 Microbiology - Respiration

SITE
01 Ale
02 Bison
03 Bridger
04 Cottonwood
05 Dickinson
06 Hays
07 Hopland
08 Jornada
09 Osage
10 Pantex
11 Pawnee

TREATMENT
1 Ungrazed
2 Lightly grazed
3 Moderately grazed
4 Heavily grazed
5 Grazed 1969, ungrazed 1970
6 Grazed 1970, ungrazed 1971
7
8
9

CATEGORY
1 Live
2 Old dead
3 Recent dead

CLIP-ESTIMATE
1 Harvested
2 Harvest and Est.
3 Estimated
4 Est. for Insect
5 Est. for Reference
6 Est. for Future Clip

GROWTH FORM
1 Perennial grass
2 Annual grass
3 Sedge, rush, etc.
4 Annual forb
5 Biennial forb
6 Perennial forb
7 Half-shrub
8 Shrub
9 Tree
0 Miscellaneous

The following information will be recorded:

XXX QUADRAT NUMBER - number assigned to a particular quadrat by the investigator (This number should be unique for the TREATMENT.) Leave blank if sample for moisture content.

X CLIP-EST - code to indicate whether the quadrat was clipped, clipped and estimated, estimated only, or estimated for other use

X GROWTH FORM - code for stage of growth or type of plant (This is part of the identification and should not be omitted.)

XX GENUS - first two letters of generic name

XX SPECIES - first two letters of species name (if plant is identified only to genus, then put first three letters of generic name in genus-species spaces; if unidentified, use MISC)

X SUBSPECIES - this is a tie-breaker to be used only in case genus and species abbreviations fail to distinguish all plants

X CATEGORY - code for live, standing dead of current year or last year, or (4) perennial live (i.e., *Opuntia*)

XXXXX ESTIMATED WEIGHT IN GRAMS

XXXX SACK NUMBER - (or WET WEIGHT if sample for moisture content)

XXXXXX DRY WEIGHT - include decimal point

XXXX SPECIAL COLLECTION - sack number for special material collected separately (crowns, seeds, flowers)

XXXXXX CROWN WEIGHT - dry weight of special collection (include decimal point)

Preliminary Analysis

After field data are received at the Natural Resource Ecology Laboratory, the following information will be returned to the field investigator:

1. For each replicate (for weight-estimate and separately for clipped plots):
    a. biomass (dry) g/m^2 by quadrat (by category)
    b. biomass (dry) g/m^2 $\bar{x} \pm s$ for replicate (by category)
    c. biomass (dry) g/m^2 $\bar{x} \pm s$ by major species (by category)
    d. percent composition by species (by category)
    e. optimum ratio for weight estimate of live vegetation (separate for 0.5 and 2.0 m^2 samples).
2. For each treatment (for weight-estimate and separately for clipped plots):
    a. biomass (dry) g/m^2 $\bar{x} \pm s$ for treatment (by category)
    b. biomass (dry) g/m^2 $\bar{x} \pm s$ by species (by category)
    c. percent composition by species (by category)

Our objective in the aboveground biomass sampling is to estimate the biomass by species to within 20% of the true biomass with a probability of 0.8. In other words, we are willing to risk that, one time in five, our absolute error may exceed the allowable limits.

When a number (N) of plots are clipped, and the plant material is dried and weighed, the mean weight of herbage per plot, or per unit area, and its associated variance will be calculated. From these preliminary data, a sample size, n, for the area being estimated can be calculated for the next sampling period which will give an estimated total biomass within 20% of the true total biomass with a probability of 0.8. The method of computation is:

$$n = \frac{t^2 s^2}{d^2}$$

where $d^2 = (\overline{x} \cdot 0.2)^2$

and $t = 1.38$ if $N = 10$

$= 1.35$ if $N = 15$

$= 1.33$ if $N = 20$

$= 1.32$ if $N = 25$

$= 1.31$ if $N = 30$

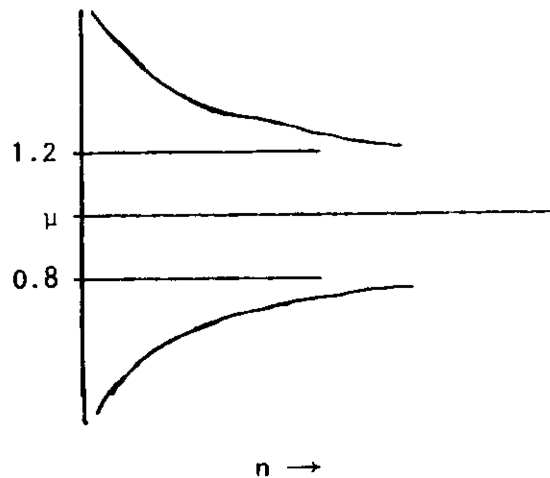
$= 1.30$ if $N = 40$ to 60

$= 1.29$ if $N > 60$

In determining optimum sample size, we must decide on some reasonable confidence limits; i.e., we must be willing to accept a certain probability of error. If we are working at the 95% confidence level, then if an item X is drawn from a normal distribution, the probability is about 0.95 that X lies between $\mu - 1.96\sigma$ and $\mu + 1.96\sigma$. Since we do not know mu and sigma (parameters), we must use our estimate or mean and its standard error. When we do not know the value of 1.96σ, the t distribution enables us to compute confidence limits on the basis of s, our estimate of σ. Note that the t distribution is expressed in units of $s/\sqrt{n}$ (Snedecor and Cochran 1967, p. 61). By using this standard error, we get a confidence interval which is a function of n. Therefore, we can set a value for the confidence interval, and solve for n. In our case:

$$CI = x \pm t_{0.1}\, s/\sqrt{n} = f(n)$$

A plot of the standard error around mu with increasing sample size should show two lines (+ and -) which form an envelope about mu, which converges as the sample size increases.



We want to know at what value of n this envelope is within ±20% of mu. If we look at the standard deviation, which is not a function of n but is an estimate of a parameter of the population, we would find that with increasing sample size the envelope would bounce around and would converge only to the true variance of the population.

Applying what we have just said about using the t distribution and substituting s for sigma, we can convert Cochran's method for estimating sample size (Snedecor and Cochran 1967, p. 516) to the formula given on page 9. From Snedecor and Cochran

$$\text{allowable error} = \frac{2\sigma}{\sqrt{n}}$$

Note again the use of the square root of n. Our allowable error is expressed as a fraction of the mean. Substituting and squaring gives

$$n = \frac{4\sigma^2}{(x \cdot 0.1)^2}$$

He is using 2σ as a convenient approximation of 1.96σ, the 0.95 level of probability. When we substitute s and the t distribution to get our desired confidence interval, we get

$$n = \frac{(t_{0.1})^2 s^2}{d^2}$$

where $d = \bar{x} \cdot 0.2$.

Required Turnaround Time

Sampling is to be done, at most, biweekly during the growing season and monthly at other times of the year, where possible. Because the dry weight-estimate method requires modification of sample size based upon results of the previous sampling period, it is essential that the results of preliminary analysis be in the hands of the field investigator at the time sampling is to be done. In other words, turnaround time must be two weeks maximum during the growing season and monthly at other times of the year. If the investigator finds errors, he should report them so that the data may be reanalyzed.

HERBAGE DYNAMICS - LITTER

Sampling Methods

The same sites will be utilized for collection of litter that were used for clipped plots in the aboveground biomass studies. The plot size, therefore, is to be 0.5 m^2. Litter will be collected by quadrats separately in a bag for drying and weighing. All of it should be taken; a vacuum should be used. Material will be oven-dried at 60°C and weighed. To determine the amount of sand and mineral material present after weighing,

the sample should be ashed and the weight of the residue recorded. This weight will be subtracted from the original value in the machine processing of the data. Weights should be determined to the nearest 0.01 g. In addition, a sample of litter, composited by replicate, will be ground in a Wiley Mill with a 40-mesh screen and sent to NREL for analysis of botanical composition. This sample for botanical analysis can be separated from quadrat collections after the dried weights are obtained, and the ashed weights for the quadrats can be calculated from the subsample remaining.

Data Recording

A sample field data sheet for litter collection follows. The information to be recorded is:

XXX QUADRAT - this number should correspond to the number used on the forms for ABOVEGROUND BIOMASS and INVERTEBRATES if the sampling is taken in the same plot

X TYPE OF LITTER COLLECTION - code for distinguishing between total material of quadrat, part of material from quadrat, cleared plot for estimating rate of fall from standing dead, nylon litter bag, etc.

XXXX SACK NUMBER

XXXXXX DRY WEIGHT OF LITTER AND SACK - to nearest 0.01 g (record decimal point)

XXXX DRY WEIGHT OF SUBSAMPLE

XXXXXX ASH WEIGHT - weight of material after ashing of subsample

XXXXXX PREVIOUS DATE - for computation of time interval between weighings if litter bags or other types of samples used

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FIELD DATA-SHEET - LITTER

DATA TYPE	SITE	INITIALS	DATE			TREATMENT	REPLICATE	PLOT SIZE	QUADRAT	TYPE	SACK NO.	DRY WT.	SACK WT.	ASH WT.	PREVIOUS DATE			
			Day	Mo	Yr										Day	Mo	Yr	
1-2	3-4	5-7	8-9	10-11	12-13	14	15	16-19	21-23	25	27-30	32-37	39-42	44-49	51-52	53-54	55-56	

DATA TYPE
01 Aboveground Biomass
02 Litter
03 Belowground Biomass
10 Vertebrate - Live Trapping
11 Vertebrate - Snap Trapping
12 Vertebrate - Collection
20 Avian Flush Census
21 Avian Road Count
22 Avian Road Count Summary
23 Avian Collection - Internal
24 Avian Collection - External
25 Avian Collection - Plumage
30 Invertebrate
40 Microbiology - Decomposition
41 Microbiology - Nitrogen
42 Microbiology - Biomass
43 Microbiology - Root Decomposition
44 Microbiology - Respiration

SITE
01 Ale
02 Bison
03 Bridger
04 Cottonwood
05 Dickinson
06 Hays
07 Hopland
08 Jornada
09 Osage
10 Pantex
11 Pawnee

TREATMENT
1 Ungrazed
2 Lightly grazed
3 Moderately grazed
4 Heavily grazed
5 Grazed 1969, ungrazed 1970
6
7
8
9

TYPE
1 Quadrat, total
2 Quadrat, part
3 Cleared plot
4 Litter bag

HERBAGE DYNAMICS - BELOWGROUND BIOMASS

Sampling Methods

Belowground biomass will be sampled by collection of soil cores. Cores should be collected at least four times a year. One core should be taken in each clipped 0.5 m^2 quadrat. The core will be divided into 10 cm intervals. The total length of the core should be sufficient to account for at least 90% of the root material in the soil profile. In order to remove roots from soil core segments, each sample should be washed, and the root material that can be floated off retained as the sample. The sample should be oven-dried at 60°C, weighed to the nearest 0.01 g, ashed, and the residue weighed. Rhizomes should not be separated. In processing of soil cores, consideration should be given to the requirements of the INVERTEBRATE studies and the MICROBIOLOGY investigations. Take separate cores for lab analysis.

Composite samples of root ash by depth increment from each replicate should be sent to the NREL. In addition, two *very clean* root samples should be collected during the growing season (one early, after about 10% of the plant growth, and one after maximum growth) for determination of ash content of roots.

Data Recording

A sample field data sheet for recording results of belowground biomass follows. The information required is

XXX QUADRAT - the same number as used to identify quadrat on other data forms

XXX CORE DIAMETER - to nearest 0.1 cm

X HORIZON - code for soil horizon

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FIELD DATA SHEET - BELOWGROUND BIOMASS

DATA TYPE	SITE	INITIALS	DATE			TREATMENT	REPLICATE	PLOT SIZE	QUADRAT	CORE DIAM.	HORIZON	TOP DEPTH	BOTTOM DEP.	LENGTH	WASH WT.	DRY WT.	ASH WT.	CROWN DRY WT.
			Day	Mo	Yr													
1-2	3-4	5-7	8-9	10-11	12-13	14	15	16-19	21-23	25-27	29	31-33	35-37	39-41	43-47	49-54	56-61	63-68

DATA TYPE
01 Aboveground Biomass
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SITE
01 Ale
02 Bison
03 Bridger
04 Cottonwood
05 Dickinson
06 Hays
07 Hopland
08 Jornada
09 Osage
10 Pantex
11 Pawnee

TREATMENT
1 Ungrazed
2 Lightly grazed
3 Moderately grazed
4 Heavily grazed
5 Grazed 1969, ungrazed 1970
6
7
8
9

HORIZON
1 AO
2 A
3 B
4 C

XXX TOP DEPTH - depth to top of segment, cm

XXX BOTTOM DEPTH - depth to bottom of segment, cm

Note: In the WASH WEIGHT column, use codes (1 crowns, 2 rhizomes, 3 roots).

HERBAGE DYNAMICS - LITTER ACCUMULATION

Either de-littered plots or accumulation screens will be used to evaluate rates of transfer from live or standing dead to litter. Size of these plots will be a minimum of 15 x 15 cm. A minimum of 30 plots per treatment should give acceptable results, based on last year's experience at Osage. Accumulated material should be harvested at monthly invervals. Harvesting will be accomplished by clipping around the periphery of each plot to remove the material that has fallen there. A slightly raised frame of screen wire was used at Osage to retard decomposition of the material between collecting periods.

VERTEBRATES - SMALL MAMMALS

Sampling Methods - Live Trapping

Small mammal populations will be sampled by marking, releasing, and recapturing animals in a grid of live traps. The grid will consist of 12 x 12 stations, with two traps per station. Stations will be 15 m apart. The grid will cover an area of 6.8 acres (2.8 hectares). There will be five consecutive days of trap-mark-release. This will provide basic data for estimation of population density. Density of major species will be estimated four times during the year at the southern sites (Jornada, Pantex, Osage) and three times at the other sites (with the exception of Pawnee, where there will be six sampling periods coordinated with other studies of consumer populations).

Rodents will be marked by a system of toe amputation and/or with numbered metal ear tags. A toe can be removed by clipping with a pair of fine pointed scissors or with fingernail clippers. The incision should be made cleanly and at the base so as to remove the entire toe. If only a portion of the toe is removed, it is sometimes difficult to recognize on recapture. In addition, natural amputations sometimes occur and these can be confused with toe-clips that are not cleanly made. Fig. 1 shows the method to be used. Looking at the underside of the animal, the feet are read from left to right and top to bottom to give the four digits of the identification number, one digit per foot. The toes on each foot are read from left to right. If there are four toes on the foot, then there are four possible numbers that can be assigned to that particular foot, 1, 2, 3, or 4. If there are five toes on the foot, there are five possible numbers. Clipping of one toe from each foot gives a four-digit identification number. Animals with toes like the illustration will provide a possible 899 different combinations of four-digit numbers to be assigned to that species. This requires clipping of no more than one toe per foot. In the case of a natural amputation, which occurs after marking, there will be two toes missing from the foot. In such cases, that digit of the identification number is indicated as a 9. This system often permits the original identity of the animal to be determined without confusion.

Sampling Methods - Snap Trapping

Kill trapping will be conducted to obtain information on diets. In the process, some demographic data will be derived from these specimens. Animals trapped will be removed and saved for autopsy. At autopsy the animals will be weighed and measured, the testes of the males will be measured to the

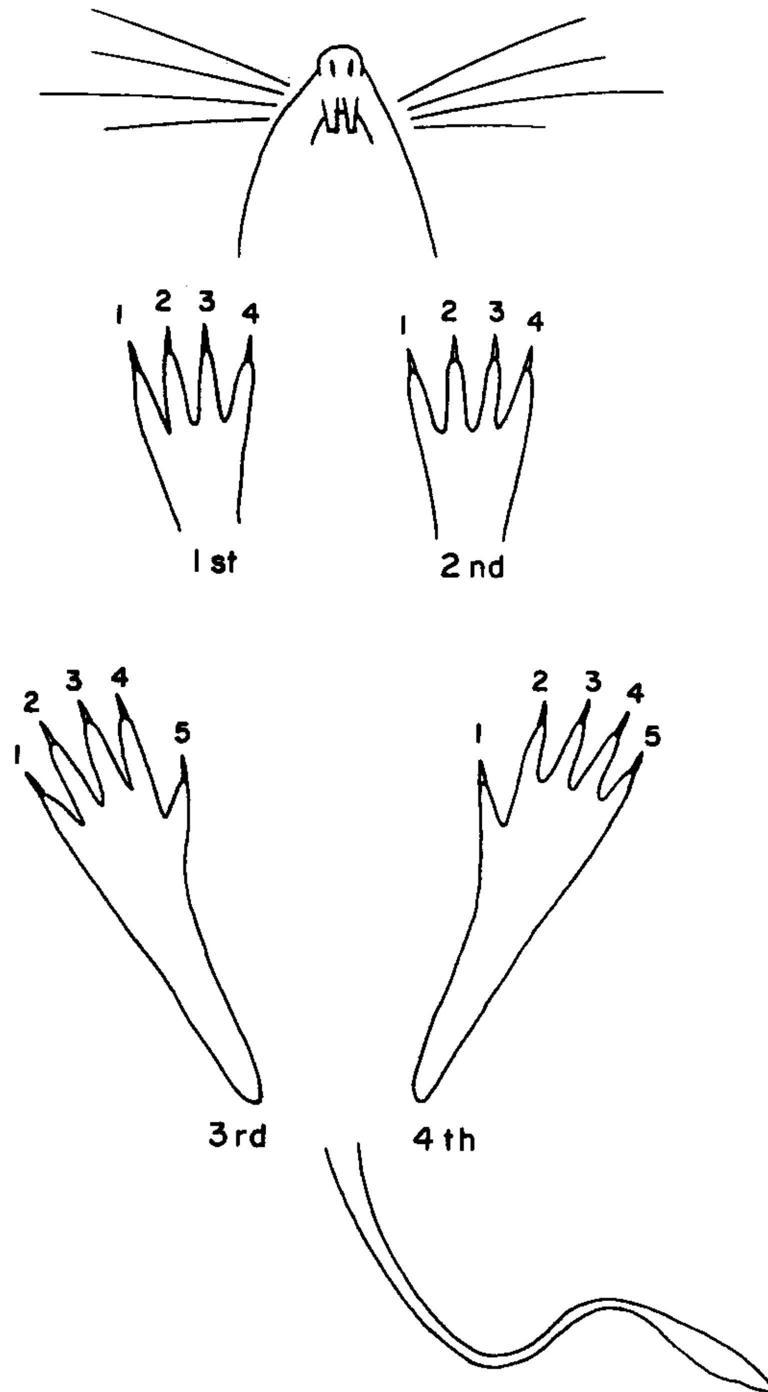

Fig. 1. Four-digit number for rodent marking.

nearest millimeter, both length and width. For females, the condition of the mammary glands and the reproductive tracts will be recorded. *Nulliparous* females give no evidence of ever having been pregnant (no embryos, placental scars, corpora lutea, or lactation). *Primiparous* females are, or have been, pregnant at least one time (pregnant, but no additional placental scars; one set only of placental scars and/or corpora lutea). *Multiparous* females are, or have been, pregnant more than one time (pregnant, with additional set(s) of placental scars and/or lactation; two or more sets of placental scars and/or corpora lutea). The condition of the mammae will be noted. *Nipples* may be small (non-lactating, nulliparous females); enlarged but not prominent (non-lactating, parous females); or enlarged and prominent (lactating, parous females). The *total number of embryos* will be recorded, including those that appear to be abnormal or resorbing. The number being *resorbed* will be recorded separately, and will include only those embryos which are distinctly smaller, and undergoing tissue lysis and disorganization. The *embryo length* will be recorded as the crown-rump length in millimeters of the embryo as it lies in its normal position. The total weight of the embryos and reproductive tract combined, will be recorded to the nearest 0.01 g. Numbers of new and old *placental scars* will be recorded. New scars are distinguished by plentiful blood supply to the implantation site and/or a large amount of dark pigment deposited at the site. Old scars are distinguished by lack of blood supply, and fainter pigmentation. The number of *corpora lutea* can be determined from the intact ovaries by examining the surface of the ovaries for firm, spheroidal, pinkish to yellowish structures. Both eyes of each specimen will be preserved in 10% neutral Formalin and labeled. Eyes *should not* be frozen. The eyeballs should be punctured to

allow penetration of the preservative. Later determination of the dry weight of the paired lenses will be used in estimation of the relative ages of the animals. Total stomach contents of all individuals of each major species will be preserved in Formalin and labeled. The label should contain the following information:

i. Site
ii. Date collected
iii. Collector's number
iv. Code for genus and species
v. Sex
vi. Ages (ad. or juv.)

Sampling Methods - Pocket Gophers

Pocket gophers will be censused by a mark-release-recapture method. Live-traps will be set in exposed burrows where animal activity is evident.

Sample Methods - Lagomorphs

A minimum 10-mile sample transect will be taken from a moving vehicle at night with a strip 25 yards on either side of the path of the vehicle. This will require a driver and an observer. A different distance may be used depending on habitat and observer preference. Odometer readings to the nearest tenth will be recorded at the start of the run and each time a jackrabbit is flushed at the point of flush on the route. An odometer reading will be recorded at the termination of the run. At each mile of the route, the distance in yards to an estimated 25 yards from the line of travel will be measured and recorded.

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FIELD DATA SHEET -- JACKRABBIT CENSUS

Data Type	Site	Initials	Date			Route	Strip Width	Hour	Genus	Species	Subspecies	Odometer	Measured
			Day	Month	Year								
1-2	3-4	5-7	8-9	10-11	12-13	14-15	17-19	21-24	26-27	28-29	30	32-35	36-40
15													

Route

Investigator's identifier

Hour

24 hour clock

Strip Width

Width in feet of census strip

Measured

Distance in feet to estimated point

Accuracy

The accuracy of the live trapping technique for small mammals varies with different species. There are procedures for evaluating the variance of the estimates obtained. The magnitude of the variance will depend on the sample size, and therefore on the density of the population under investigation. At least two separate estimates of the small mammal population density will be derived from the data obtained by live trapping. Data on pocket gophers will provide an index of the population density for these species. Further effort will be required to relate density indices to actual population densities.

Data Recording - Live Trapping

A sample data sheet for the recording of live trapping results follows. Separate sheets will be used by different observers, or on different days, or as continuation sheets if more than one is required on any one day.

XX GENUS - first two letters of the generic name

XX SPECIES - first two letters of the species name

X SUBSPECIES - single letter to be used only as a tie-breaker in case previous columns fail to separate two species

X CONDITION - used to indicate whether an animal escaped or is in poor condition; leave blank if normal

X MARK - this is the condition of the mark on the animal; blank if normal

XXXX NUMBER - four-digit identification of the individual (see toe-clip diagram)

X MALE - code for reproductive condition of male animals

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FIELD DATA SHEET - VERTEBRATE - LIVE TRAPPING

DATA TYPE	SITE	INITIALS	DATE			TREATMENT	REPLICATE	PLOT SIZE	GENUS	SPECIES	SUBSPECIES	CONDITION	MARK	NUMBER	MALE	FEMALE	WEIGHT	MOLT	LOCATION		PREVIOUS NO.
			Day	Mo	Yr														Row	Col	
1-2	3-4	5-7	8-9	10-11	12-13	14	15	16-19	21-22	23-24	25	27	29	31-34	36	38	40-44	46	48-49	51-52	54 57

DATA TYPE
- 01 Aboveground Biomass
- 02 Litter
- 03 Belowground Biomass
- 10 Vertebrate - Live Trapping
- 11 Vertebrate - Snap Trapping
- 12 Vertebrate - Collection
- 20 Avian Flush Census
- 21 Avian Road Count
- 22 Avian Road Count Summary
- 23 Avian Collection - Internal
- 24 Avian Collection - External
- 25 Avian Collection - Plumage
- 30 Invertebrate
- 40 Microbiology - Decomposition
- 41 Microbiology - Nitrogen
- 42 Microbiology - Biomass
- 43 Microbiology - Root Decomposition
- 44 Microbiology - Respiration

SITE
- 01 Ale
- 02 Bison
- 03 Bridger
- 04 Cottonwood
- 05 Dickinson
- 06 Hays
- 07 Hopland
- 08 Jornada
- 09 Osage
- 10 Pantex
- 11 Pawnee

FEMALE
- 0 Adult, vulva inactive
- 1 Subadult, vuvla inactive
- 2 Juvenile, vulva inactive
- 3 Adult, vulva turgid
- 4 Subadult, vulva turgid
- 5 Juvenile, vulva turgid
- 6 Adult, vulva cornified
- 7 Subadult, vulva cornified
- 8 Juvenile, vulva cornified
- 9 Pregnant

CONDITION
- 0 Normal
- 1 Escaped
- 2 Torpid
- 3 Dead

TREATMENT
- 1 Ungrazed
- 2 Lightly grazed
- 3 Moderately grazed
- 4 Heavily grazed
- 5 Grazed 1969, ungrazed 1970
- 6
- 7
- 8
- 9

MOLT
- 0 No evidence
- 1 Post-juvenile
- 2 Post-subadult
- 3 Adult (vernal)
- 4 Adult (autumnal)
- 5 Molt of unknown stage
- 6 Undetermined

MALE
- 0 Adult, non-breeding
- 1 Subadult, non-breeding
- 2 Juvenile, non-breeding
- 3 Adult breeding ?
- 4 Subadult breeding ?
- 5 Juvenile breeding ?
- 6 Adult breeding
- 7 Subadult breeding
- 8 Juvenile breeding
- 9 Undetermined

MARK
- 0 Normal
- 1 Unmarked
- 2 Ear tag
- 3 Toe Clip
- 4 Ear tag and toe clip
- 5 Natural amputation

X FEMALE - code for reproductive condition of female animals

XXXXX WEIGHT - weight in grams if animal is weighed in field (to nearest 0.1 g)

X MOLT - condition of molt, blank if not evident

XX ROW - location of capture identified by number of row in grid

XX COLUMN - number of column in grid

XXXX PREVIOUS NUMBER - in case of a natural amputation, the old number of the animal

Data Recording - Snap Trapping

Two field sheets are required for recording of data from sacrificed animals taken in snap traps. Data on animals captured in the snap traps are recorded on the sheet for MAMMAL COLLECTION. Information included is:

XX GENUS - first two letters of genus name

XX SPECIES - first two letters of species name

X SUBSPECIES - tie-breaker, if required

XXXXXXX SPECIMEN NUMBER - number assigned by collector (letters and/or numbers)

X MARK - source of specimen and type of mark, according to code

XXXX LENGTH - total length, mm

XXX TAIL - tail length, mm

XXX FOOT - hind foot, mm

XXX EAR - ear from notch, mm

XXXXX WEIGHT - weight in grams, to nearest 0.1 g (before stomach is removed)

X MOLT - pelage condition, according to code

FIELD DATA SHEET - MAMMAL COLLECTION

DATA TYPE	SITE	INITIALS	DATE			TREATMENT	REPLICATE	PLOT SIZE	TRAP DAY	HOUR	GRID TRAP		GENUS	SPECIES	SUBSPECIES	SPECIMEN NUMBER	MARK	LENGTH	TAIL	FOOT	EAR	WEIGHT	MOLT	PARASITES	STOMACH WEIGHT	FOOD	EYE LENS	SPECIMEN	MAP REFERENCE		
			Day	Mo.	Yr.						Row	Col																	TWN	RNG	S

MARK
0 None
1 Snap-trap grid, unmarked
2 Snap-trap grid, marked
3 Live-trap grid, unmarked
4 Live-trap grid, marked
5 Other trapping

MOLT
0 No evidence
1 Post-juvenile
2 Post-subadult
3 Adult (vernal)
4 Adult (autumnal)
5 Molt of unknown stage
6 Undetermined

PARASITES - EYE LENS
0 Not saved
1 Preserved

SPECIMEN
0 Not saved
1 Skin
2 Skull
3 Skin and skull
4 Skeleton
5 Liquid preservative

FOOD
0 None
1 Stomach only
2 Cheek pouch only
3 Both

DATA TYPE
01 Aboveground Biomass
02 Litter
03 Belowground Biomass
10 Vertebrate - Live Trapping
11 Vertebrate - Snap Trapping
12 Mammal - Collection
13 Snap Trap Effort
14 Mammal Reproductive
20 Avian Flush Census
21 Avian Road Count
22 Avian Road Count Summary
23 Avian Collection - Internal
24 Avian Collection - External
25 Avian Collection - Plumage
30 Invertebrate
40 Microbiology-Decomposition
41 Microbiology-Nitrogen
42 Microbiology-Biomass
43 Microbiology-Root Decomposition
44 Microbiology-Respiration

SITE
01 Ale
02 Bison
03 Bridger
04 Cottonwood
05 Dickinson
06 Hays
07 Hopland
08 Jornada
09 Osage
10 Pantex
11 Pawnee

TREATMENT
1 Ungrazed
2 Lightly grazed
3 Moderately grazed
4 Heavily grazed
5 Grazed 1969, ungrazed 1970
6
7
8
9

X	PARASITES - 1 if collected
XXX	STOMACH WEIGHT - wet weight in grams, to nearest 0.1 g
X	FOOD - indicate collection, according to code
X	EYE LENS - 1 if collected
X	SPECIMEN - type of specimen preserved, according to code
XXXXXXXX	MAP REFERENCE - Township, Range, and Section

Additional data on internal anatomy of specimens collected is recorded on the field sheet entitled MAMMAL REPRODUCTIVE. This sheet is a continuation of the last, and therefore 42 columns are identical to the previous information on the specimen. Additional data required, beginning in column 44, is:

X	EXTERNAL - reproductive condition--male
XXXXX	TESTES - length and width, mm
X	EPIDIDYMUS - condition, according to code
X	SEMINAL VESICLES - condition, according to code
X	EXTERNAL - reproductive condition--female
X	MAMMARY - condition, according to code
X	PUBIC SYMPHASIS - condition, according to code
XX	NORMAL EMBRYOS - numbers, in left and right horns
XXX	EMBRYO LENGTH - crown-rump length, mm
XX	RESORBING - number disintegrating, left and right
XX	NEW SCARS - recent placental scars, left and right
XX	OLD SCARS - old placental scars, left and right
XX	CORPORA LUTEA - number visible on surface of each ovary, left and right

FIELD DATA SHEET - MAMMAL REPRODUCTIVE

DATA TYPE	SITE	INITIALS	DATE			TREATMENT	REPLICATE	PLOT SIZE	TRAP DAY	HOUR	GRID TRAP		GENUS	SPECIES	SUBSPECIES	SPECIMEN NUMBER	MALE					FEMALE																	
			Day	Mo.	Yr.						Row	Col					EXTERNAL	TESTES LN	TESTES WD	EPIDID	SEM VES	EXTERNAL	MAMMARY	PUBIC SEM	NORMAL EMBRYOS L	NORMAL EMBRYOS R	EMBRYO LENGTH	RESORB L	RESORB R	SCARS NEW L	SCARS NEW R	SCARS OLD L	SCARS OLD R	CORPORA LUTEA L	CORPORA LUTEA R	TRACT WEIGHT	CORP ALB	SPEC SOURCE
1-2	3-4	5-7	8-9	10-11	12-13	14	15	16-19	21-22	23-26	27-28	29-30	31-32	33-34	35	36-42	44	45-47	48-49	50	51	52	53	54	55	56	57-59	60	61	62	63	64	65	66	67	68-72	71	72

MALE
- 0 Adult, non-breeding
- 1 Subadult, non-breeding
- 2 Juvenile, non-breeding
- 3 Adult breeding ?
- 4 Subadult breeding ?
- 5 Juvenile breeding ?
- 6 Adult breeding
- 7 Subadult breeding
- 8 Juvenile breeding
- 9 Undetermined

FEMALE
- 0 Adult, vulva inactive
- 1 Subadult, vulva inactive
- 2 Juvenile, vulva inactive
- 3 Adult, vulva turgid
- 4 Subadult, vulva turgid
- 5 Juvenile, vulva turgid
- 6 Adult, vulva cornified
- 7 Subadult, vulva cornified
- 8 Juvenile, vulva cornified
- 9 Undetermined

SEMINAL VESICLES
- 0 No observation
- 1 Minute
- 2 Small
- 3 Well developed

EPIDIDYMUS
- 0 No observation
- 1 Not convoluted
- 2 Slightly convoluted
- 3 Convoluted

MAMMARY
- 0 No observation
- 1 Small
- 2 Large
- 3 Lactating

SOURCE
- 1 Snap trap grid
- 2 Live trap grid
- 3 Other trap line
- 4 Misc. collection

PUBLIC SYMPHASIS
- 0 No observation
- 1 Closed
- 2 Slightly open
- 3 Open

DATA TYPE
- 01 Aboveground Biomass
- 02 Litter
- 03 Belowground Biomass
- 10 Vertebrate - Live Trapping
- 11 Vertebrate - Snap Trapping
- 12 Mammal - Collection
- 13 Snap Trap Effort
- 14 Mammal Reproductive
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- 21 Avian Road Count
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- 23 Avian Collection - Internal
- 24 Avian Collection - External
- 25 Avian Collection - Plumage
- 30 Invertebrate
- 40 Microbiology-Decomposition
- 41 Microbiology-Nitrogen
- 42 Microbiology-Biomass
- 43 Microbiology-Root Decomposition
- 44 Microbiology-Respirption

SITE
- 01 Ale
- 02 Bison
- 03 Bridger
- 04 Cottonwood
- 05 Dickinson
- 06 Hays
- 07 Hopland
- 08 Jornada
- 09 Osage
- 10 Pantex
- 11 Pawnee

TREATMENT
- 1 Ungrazed
- 2 Lightly grazed
- 3 Moderately grazed
- 4 Heavily grazed
- 5 Grazed 1969, ungrazed 1970
- 6
- 7
- 8
- 9

XXX TRACT WEIGHT - grams of total intact reproductive tract, including embryos

X CORPORA ALBICANS - total number visible

X SPECIMEN SOURCE - origin of specimen, according to code

Preliminary Analysis

Data from the live trapping grid will be analyzed by a computer program based upon the Jolly stochastic model (Jolly 1965), and by a regression method based upon appearance of unmarked animals (Zippin 1956). This provides estimates of numbers with standard errors. Lagomorph results will be analyzed as a census of a sample area the length of the route traveled (Flinders and Hansen, manuscript), and separately by the plot-removal method (Hanson 1968).

Required Turnaround Time

Data will be forwarded for analyses upon completion of collection. These data should be analyzed and returned to the investigator prior to the next collection of data sets.

Functional Role of Mammals

In addition to population samples, the functional role of major species will be evaluated. The major species by site and their presumed important functions are listed in the following table.

Table 1. Functional aspects of small mammal activity to be investigated at IBP Grassland Biome Sites.

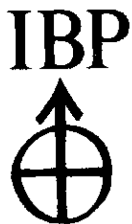
Site	Species	Function
Bridger	*Thomomys talpoides*	Root consumption and harvest (soil) movement
	Microtus montanus	Diet Harvest rates Runway area as proportion of ground surface Burrow counts
Cottonwood	*Thomomys talpoides*	Root utilization Soil movement
	Peromyscus or other	Diet Seed utilization Insect utilization
Osage	*Microtus orchrogaster*	Diet Harvest rate Runway area Burrow counts
	Sigmodon hispidus	Diet Harvest rate Runway area Burrow counts
Pantex	*Peromyscus maniculatus*	Diet Seed utilization Insect utilization
	Sigmodon hispidus	Diet Harvest rate Runway area Burrow counts
	Lepus californicus	Diet Seed dispersal
	Perognathus flavescens	Seed utilization Seed dispersal

Table 1. (Continued)

Site	Species	Function
Jornada	*Dipodomys ordii*	Seed utilization Seed dispersal Excavation
	Dipodomys spectabilis	Seed utilizaton Seed dispersal Excavation
	Lepus californicus	Diet Seed dispersal
	Spermophilus spilosoma	Diet Excavation
Ale	*Perognathus parvus*	Diet Seed dispersal
	Peromyscus maniculatus	Diet Seed utilizaton Insect utilization
Pawnee	*Lepus* sp.	Diet Movement
	Spermophilus tridecemlineatus	Diet
	Onychomys leucogaster	Diet

Runways, Mounds, and Excavations

The modifications that the major species of small mammals make in the form of runways, mound building, and burrowing or excavation activities will be mapped on graph paper. These data will be analyzed by the use of a planimeter to ascertain the total area modified. The results will be summarized on a separate data sheet for submission to the Grassland Biome central office.



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FIELD DATA SHEET - SMALL MAMMAL EXCAVATION SUMMARY

Data Type	Site	Initials	Date			Treatment	Plot Size	Plot Number	Structure	Genus	Species	Subspecies	Area
			Day	Month	Year								
1-2	3-4	5-7	8-9	10-11	12-13	14	15-20	21-25	27	29-30	31-32	33	35-45
16													

Structure

1 Mound

2 Runway

3 Burrows

4

5

6

VERTEBRATES - AVIAN POPULATIONS

Sampling Methods

Avian populations will be censused during the early nesting period at each site except Jornada in two replicate 26.1 acre (10.6 hectare) areas. Each area will be grided with marker stakes at 61 m intervals for reference points. The breeding birds of each area will be flushed several times over a two-day period. The locations of individual birds will be noted on grid maps of the areas. From the points where individuals are flushed, the outlines of breeding territories will be drawn on the maps. On the basis of this, the breeding population of each sample area will be established, and the breeding bird density will be estimated. Information will also be obtained from these data on species composition and, along with weights of birds collected elsewhere, biomass per unit area.

At Jornada, the procedure will be identical to that given above, except that census plots will be 91.8 acres (37.3 hectares) in size. The grid dimensions will remain unchanged. A 10.6 ha plot will be nested within each 37.3 hectare plot to serve as a basis for standardized comparisons with censuses at other plots.

In addition, bird populations will be censused at each site in mid-winter. The strip-census method (Emlen, in press) will be used. After establishment of the appropriate strip widths for each species, two replicate censuses, each one-half mile in length, will be conducted on successive days (weather permitting). Comparisons between flush-census estimates and Emlen strip-census estimates made during the breeding period will permit seasonal comparisons. At Jornada, additional censuses will be made, using the strip-census technique on a bi-monthly basis.

A general index of bird density in the region and seasonal changes in abundance will be obtained by roadside censuses conducted during spring migration, fall migration, and mid-winter migration by local personnel. In addition, the same observer will conduct one roadside census during the breeding period plot sampling. IBP investigators will conduct a count on the same route the following day to provide a basis for site comparisons. The census will cover a specific route at each of the sites. The route will be censused in early morning. Observations will be made at a total of 30 stops along a 14.5-mile route. The stops will be one-half mile apart, except that stops occurring in non-grassland habitats will be skipped. At each stop, all birds observed during a three-minute observation period will be recorded. During the three-minute period, a circle one-quarter mile in radius will be observed. Any birds noted in this location during this time period will be recorded. At the end of the roadside census on a particular date, the records will be compiled on a summary sheet.

Specimens will be collected, in both summer and mid-winter, in similar habitat at some distance from the study area for determinations of weight and dietary composition. Crop, stomach, and lower digestive tracts will be removed and preserved in 5% Formalin for laboratory analysis. Sampling of the food items brought to nestlings by adults will be made using the pipe-cleaner collar technique. A pipe cleaner is twisted around the neck of the nestling bird tight enough to prevent its swallowing bulky insects but loose enough to prevent injury to the bird. The bolus of food which collects in a nestling's gullet during a 30-minute period will be removed with forceps and preserved in 5% Formalin for later analysis. At any nest, each nestling will be subjected to this procedure no more often than once per day.

Data Recording - Flushing Census

A sample field data sheet, captioned AVIAN FLUSH CENSUS, for recording the exact movements of a few birds in a limited area follows. From this information the movements of particular individuals can be plotted, or the relative positions of territories can be outlined.

Data Recording - Road Census

Observations by species will be recorded for each of the up to 30 stops on the road census on the attached sheets. Stops are selected for uniformity of habitat. Avoid special microhabitats, such as streams. Each sheet provides positions for recording the results from twenty stops. Two sheets will be required for tabulating results from one census. Each of the two sheets will be totaled separately, and the results tabulated in three columns of a summary sheet. *Only the summary sheet will be submitted to the Natural Resource Ecology Laboratory*. A sample summary sheet follows. Information to be recorded is:

X	GROUP - general group of birds
XX	GENUS - first two letters of generic name
XX	SPECIES - first two letters of species name
X	SEX - leave blank if undetermined
XX	PAGE TOTALS, 1 - summary of records in first 10 stops
XX	2 - summary of records in second 10 stops
XX	3 - summary of records in third 10 stops
XX	NUMBER OF STOPS - total number of stations at which species occurred

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FIELD DATA SHEET – AVIAN FLUSH CENSUS

DATA TYPE	SITE	INITIALS	DATE			TREATMENT	REPLICATE	GROUP	GENUS	SPECIES	SUBSPECIES	BASELINE SIZE	Y-AXIS SIZE	CONVERSION	HOUR	AREA (ha)
			Day	Mo	Yr											
1-2	3-4	5-7	8-9	10-11	12-13	14	15	17	18-19	20-21	22	23	24	25 27	28-31	33-36
20																

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FIELD DATA SHEET - AVIAN ROAD COUNT

GROUP
0 Waterfowl
1 Falconiform
2 Galli- and Gruiform
3 Charadriiform
4 Doves, owls, nighthawks, woodpeckers
5 Flycatchers, swallows, jays, titmice, wrens
6 Thrashers, bluebirds, gnatcatchers, pipits
7 Shrikes, starlings, vireos
8 Icterids
9 Fringillids

SEX
0 Unknown
1 Male
2 Female

GROUP	GENUS	SPECIES	SEX	STATIONS																			
				_1	2	3	4	5	6	7	8	9	0	_1	2	3	4	5	6	7	8	9	0

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FIELD DATA SHEET - AVIAN ROAD COUNT SUMMARY

DATA TYPE	SITE	INITIALS	DATE			TREATMENT	REPLICATE	PLOT SIZE	GROUP	GENUS	SPECIES	SEX	PAGE TOTALS					NO. STOPS	
			Day	Mo	Yr								1	2	3	4	5		
1-2	3-4	5-7	8-9	10-11	12-13	14	15	16-19	21	22-24	26-27	29	31-32	34-35	37-38	40-41	43-44	46-48	

DATA TYPE
01 Aboveground Biomass
02 Litter
03 Belowground Biomass
10 Vertebrate - Live Trapping
11 Vertebrate - Snap Trapping
12 Vertebrate - Collection
20 Avian Flush Census
21 Avian Road Count
22 Avian Road Count Summary
23 Avian Collection - Internal
24 Avian Collection - External
25 Avian Collection - Plumage
30 Invertebrate
40 Microbiology - Decomposition
41 Microbiology - Nitrogen
42 Microbiology - Biomass
43 Microbiology - Root Decomposition
44 Microbiology - Respiration

SITE
01 Ale
02 Bison
03 Bridger
04 Cottonwood
05 Dickinson
06 Hays
07 Hopland
08 Jornada
09 Osage
10 Pantex
11 Pawnee

TREATMENT
1 Ungrazed
2 Lightly grazed
3 Moderately grazed
4 Heavily grazed
5 Grazed 1969, ungrazed 1970
6
7
8
9

Data Recording - Avian Collections

Information from birds collected in the field will be recorded on three data forms. Any one or all may be used, according to the information required by the investigator. The first data form provides for recording data on the INTERNAL parts of the bird.

X	GROUP - general group of birds
XX	GENUS - first two letters of generic name
XX	SPECIES - first two letters of species name
X	SUBSPECIES - code for tie-breaker, if required
XXXX	SPECIMEN NUMBER - number assigned by collector, when associated with initials (columns 5-7) provides unique identification for specimen
XXXX	TIME - 0001 to 2400, time of collection
XX	STATE - first two letters of state name, except as otherwise indicated in key; if state name is two words, then first letter of each
XXXX	COUNTY - first four letters of county name
XXX	TOWNSHIP - two numbers plus letter (04N)
XXX	RANGE - two numbers plus letter (03W)
XX	SECTION
XX	HABITAT
X	CAPTURE - method of collection
X	SEX
X	BROOD PATCH
XXXX	GONAD LENGTH - mm, (total mass for female)
XXXX	GONAD WIDTH - mm

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FIELD DATA SHEET - AVIAN COLLECTION - INTERNAL

DATA TYPE	SITE	INITIALS	DATE			TREATMENT	REPLICATE	PLOT SIZE	GROUP	GENUS	SPECIES	SUBSPECIES	SPEC. NO.	TIME	STATE	COUNTY	TWNSHP	RANGE	SECTION	HABITAT	CAPTURE	SEX	BROOD PATCH	GONAD		FOLLICLE	OVIDUCT	SKULL OSS.	CROP	STOMACH	BURSA	FAT			SPECIMEN
			Day	Mo	Yr																			LENGTH	WIDTH							Subcut-Ant	SubcutPost	Visceral	
1-2	3-4	5-7	8-9	10-11	12-13	14	15	16-19	21	22-23	24-25	26	27-30	32-35	36-37	38-41	42-44	45-47	48-49	51-52	53	55	56	57-60	61-64	65-68	69-70	71	72	73	74-75	76	77	78	79

DATA TYPE
- 01 Aboveground Biomass
- 02 Litter
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- 10 Vertebrate - Live Trapping
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- 42 Microbiology - Biomass
- 43 Microbiology - Root Decomposition
- 44 Microbiology - Respiration

SITE
- 01 Ale
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- 10 Pantex
- 11 Pawnee

TREATMENT
- 1 Ungrazed
- 2 Lightly grazed
- 3 Moderately grazed
- 4 Heavily grazed
- 5 Grazed 1969, ungrazed 1970
- 6
- 7
- 8
- 9

CAPTURE
- 0 Not noted
- 1 Gun
- 2 Mist net
- 3 Trap
- 4 Found dead

BROOD PATCH
- 0 Not noted
- 1 Feathers dropped
- 2 Vascular
- 3 Edematous
- 4 Regressing
- 5 No evidence

STATE
- AK Alaska
- AZ Arizona
- CT Connecticut
- MD Maryland
- ME Maine
- MN Minnesota
- MS Mississippi
- MR Missouri
- NV Nevada
- TN Tennessee

FAT
- 0 Not noted
- 1 None
- 2 Little
- 3 Moderate
- 4 Much
- 5 Extreme

SKULL
- 0 Not noted
- 1 Quarter or less
- 2 Half or less
- 3 Three-quarters or less
- 4 Up to fully oss.

SPECIMEN
- 0 Not saved
- 1 Skin
- 2 Skeleton
- 3 Skin & skeleton

HABITAT
- 0 Not noted
- 1 Grassland
- 2 Grass-forb
- 3 Savannah
- 4 Shrub-steppe
- 5 Deciduous
- 6 Coniferous
- 7 Riparian

CROP/STOMACH
- 0 Not noted
- 1 Empty
- 2 Quarter
- 3 Half
- 4 Three-quarters
- 5 Full

GROUP
- 0 Waterfowl
- 1 Falconiform
- 2 Galli- and Gruiform
- 3 Charadriiform
- 4 Doves, owls, nighthawks, woodpeckers
- 5 Flycatchers, swallows, jays, titmice, wrens
- 6 Thrashers, bluebirds, gnatcatchers, pipits
- 7 Shrikes, starlings, vireos
- 8 Icterids
- 9 Fringillids

SEX
- 0 Unknown
- 1 Male
- 2 Female

XXXX	FOLLICLE - largest, mm
XX	OVIDUCT DIAMETER - mm
X	SKULL OSSIFICATION
X	CROP - 1 if collected
X	STOMACH - 1 if collected
XX	BURSA - external length
X	FAT, SUBCUTANEOUS ANTERIOR
X	FAT, SUBCUTANEOUS POSTERIOR
X	FAT, VISCERAL
X	SPECIMEN

A separate AVIAN COLLECTION form will be used for recording of EXTERNAL characteristics and measurements of the bird. The information to be recorded is:

X	GROUP
XX	GENUS
XX	SPECIES
X	SUBSPECIES
XXXX	SPECIMEN NUMBER
X	SEX
X	DEVELOPMENT
XX	AGE - a two-digit number
X	AGE UNITS - units of age estimate, see key
XXXXX	WEIGHT - one space is for decimal
XXXXX	WING LENGTH - mm, wrist to tip of longest primary
XXXXX	TAIL LENGTH - base of central rectrices to tip of longest

GRASSLAND BIOME

U.S. INTERNATIONAL BIOLOGICAL PROGRAM

FIELD DATA SHEET - AVIAN COLLECTION - EXTERNAL

DATA TYPE	SITE	INITIALS	DATE			TREATMENT	REPLICATE	PLOT SIZE	GROUP	GENUS	SPECIES	SUBSPECIES	SPECIMEN NUMBER	SEX	DEVELOPMENT	AGE	AGE UNITS	WEIGHT	WING LENGTH	TAIL LENGTH	BILL			TARSUS	MIDDLE TOE	
			Day	Mo	Yr																Length	Height	Width			
1-2	3-4	5-7	8-9	10-11	12-13	14	15	16-19	21	22-23	24-25	26	28-31	32	34	35-36	37	39-43	45-49	51-55	57-60	61-64	65-68	70-74	76-79	

DATA TYPE
01 Aboveground Biomass
02 Litter
03 Belowground Biomass
10 Vertebrate - Live Trapping
11 Vertebrate - Snap Trapping
12 Vertebrate - Collection
20 Avian Flush Census
21 Avian Road Count
22 Avian Road Count Summary
23 Avian Collection - Internal
24 Avian Collection - External
25 Avian Collection - Plumage
30 Invertebrate
40 Microbiology - Decomposition
41 Microbiology - Nitrogen
42 Microbiology - Biomass
43 Microbiology - Root Decomposition
44 Microbiology - Respiration

SEX
0 Unknown
1 Male
2 Female

DEVELOPMENT
0 Not noted
1 Nestling
2 Juv. or imm.
3 Subadult
4 Adult

AGE UNITS
0 No observation
1 Hour
2 Days
3 Weeks
4 Months
5 3 months
6 Years

SITE
01 Ale
02 Bison
03 Bridger
04 Cottonwood
05 Dickinson
06 Hays
07 Hopland
08 Jornada
09 Osage
10 Pantex
11 Pawnee

GROUP
0 Waterfowl
1 Falconiform
2 Galli- and Gruiform
3 Charadriiform
4 Doves, owls, nighthawks, woodpeckers
5 Flycatchers, swallows, jays, titmice, wrens
6 Thrashers, bluebirds, gnatcatchers, pipits
7 Shrikes, starlings, vireos
8 Icterids
9 Fringillids

TREATMENT
1 Ungrazed
2 Lightly grazed
3 Moderately grazed
4 Heavily grazed
5 Grazed 1969, ungrazed 1970
6
7
8
9

XXXX BILL LENGTH - outer rim of nostril to tip

XXXX BILL HEIGHT - at nostril

XXXX BILL WIDTH - at nostril

XXXXX TARSUS

XXXX MIDDLE TOE

An additional AVIAN COLLECTION form is provided to record data on the condition of the *plumage*. The information will be recorded only for selected feathers and feather tracts. The condition of the molt will be noted for the 1st, 5th, and 9th right primaries, for the 1st, 5th, 7th, and 9th right secondaries, and for the 1st, 3rd, and 6th right rectrices. The generation or plumage of the same flight feathers will then be noted. The condition of the molt for specific feather tracts of the body will follow.

Preliminary Analysis

Results of the flush census technique will be plotted on a map of the area showing the territory occupied by individual birds. On the basis of the average area of the territories, an estimate of population density will be computed. Biomass will be estimated utilizing weights of specimens collected.

INVERTEBRATES

Sampling Methods

Invertebrate sampling will be conducted to obtain quantitative estimates of numbers and biomass of major groups. The area of the sample is to be 0.5 m^2. A trap will be used that can be set down rapidly over the sample area to contain the flying insects present (the Quick Trap). Sampling will

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U.S. INTERNATIONAL BIOLOGICAL PROGRAM

FIELD DATA SHEET - AVIAN COLLECTION - PLUMAGE

DATA TYPE	SITE	INITIALS	DATE			TREATMENT	REPLICATE	GROUP	GENUS	SPECIES	SUBSPECIES	MOLT										GENERATION OF REMIGES & RECTRICES										BODY PLUMAGE														
												Prim,rt			Sec,rt				Rect,rt			Prim,rt			Sec,rt				Rect,rt			Crown	Nape	Auricular	Cervical	Int scap	Dorsal	Pelvic	Humeral	Sternal	Abdominal	Femoral	Crural	Marg cov	Sec cov	Prim cov
			Day	Mo	Yr							1	5	9	1	5	7	9	1	3	6	1	5	9	1	5	7	9	1	3	6															
1-2	3-4	5-7	8-9	10-11	12-13	14	15	17	18-19	20-21	22	24	25	26	28	29	30	31	33	34	35	37	38	39	41	42	43	44	46	47	48	50	52	54	56	58	60	62	64	66	68	70	72	74	76	78

DATA TYPE
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- 42 Microbiology - Biomass
- 43 Microbiology - Root Decomposition
- 44 Microbiology - Respiration

SITE
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- 09 Osage
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- 11 Pawnee

GROUP
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- 7 Shrikes, starlings, vireos
- 8 Icterids
- 9 Fringillids

TREATMENT
- 1 Ungrazed
- 2 Lightly grazed
- 3 Moderately grazed
- 4 Heavily grazed
- 5 Grazed 1969, ungrazed 1970
- 6
- 7
- 8
- 9

GENERATION
- 0 No observation
- 1 Juvenal
- 2 1st basic (=1st winter)
- 3 2nd basic (=2nd winter)
- 4 Adult (generation unknown)

MOLT
- 0 No observation
- 1 Old
- 2 Dropped
- 3 Pin
- 4 Quarter grown
- 5 Half grown
- 6 Three-quarter grown
- 7 Full and unsheathing
- 8 New

BODY PLUMAGE
- 0 No observation
- 1 No molt
- 2 Some follicles molting
- 3 Incomplete replacement

be done between the hours of 10 AM and 4 PM, with consideration for weather conditions. Material will be removed from the trap by means of a D-vac vacuum insect net, with a modification for use with this trap. Collections of invertebrates will be made simultaneously on the same plots as herbage samples so that the insect data can be statistically correlated with the weight in grams of aboveground plant material of various species. The general procedure will be as follows:

i. Drop traps will be dropped on 0.5 m^2 plots on which weight-estimates of plants have been made by the vegetation samples on the same or the preceding day.

ii. Two types of pastures will be sampled, i.e., grazed and ungrazed.

iii. There will be two replicates in each pasture or treatment area, and a minimum of 5 to 10 samples per replicate.

iv. Plant material will be clipped at ground level or one inch above ground level and placed in a container. This material will subsequently be subjected to Berlese funnel extraction.

v. As in the previous year, the plots will then be subjected to D-vac, using the nozzle in such a fashion that all the material down to the bare earth, including litter and mulch, will be collected. This bag of material will also be subjected to Berlese funnel extraction.

vi. Invertebrate investigators on all sites will sample every two weeks throughout the season or at the same times as the producer investigators, but never less than the producers.

In the case of large plants which occur infrequently, the botanists will be determining their distribution using large plots, the size as yet to be determined. Invertebrate specialists will devise a method for sampling host-specific insects on these plants in such a way that the collected data can be correlated with the plant distribution, presumably on a m^2 or hectare basis. One suggested approach is to cover individual plants with plastic bags and clip at base of the plant. Insects can then be washed off with benzene, alcohol, or some other solvent.

The information that invertebrate specialists obtain will be comparable between sites and will be given in both numbers and biomass. In this regard, the suggested size of Berlese funnels is as follows: a 14-inch diameter lip with a 12-inch diameter funnel and a 10 inch depth. Other size Berlese funnels are certainly permissible, but experience has shown that the larger ones are more efficient. Whatever the size used, it is necessary that the efficiency of the funnels be included with the data reported, i.e., the percent extraction. This can be determined by hand sorting at least one sample from each replicate. Insects and associated invertebrates can then be removed from the alcohol and counted.

The previous year's data has suggested that different insect groups may vary in importance at the different sites. Therefore, it is presumed that data collected at one site would not be equally applicable at another. It is expected, therefore, that studies on the function of important insect groups will be initiated on the various sites in conjunction with the data being obtained on all groups. These studies should be aimed at evaluating effects on energy flow as well as describing function.

Where feasible, it is suggested that answers be found for the following questions posed by other consumer groups, producers, decomposers, and abiotic. These data are needed to develop their submodels.

i. In considering insects as decomposers, i.e., feeding on dead organic matter, what and how much is consumed per unit area?

ii. The same information has been requested by the producers, i.e., how much is consumed by herbivores and how much by sap feeders?

iii. Data derived from our bimonthly sampling needs to be analyzed to provide the small mammal researchers with information on the best times to take collections of insectivores.

iv. Estimates of insect abundance and biomass are needed by the bird researchers to ascertain whether insectivorous birds are selectively or randomly harvesting.

v. Information such as dry weights, individual biomass, caloric values per gram of dry weight, and respiration values are needed by people working with insects or with insectivores. While some of this information can be gleaned from the literature, some will have to be done by the individual researcher, and the insects which are important on an individual site will determine the choice.

vi. The data sheet has been changed to conform with the changes suggested at the June 1970 meeting. Of particular interest is the use of four columns to designate order and family rather than three. Also in the columns concerned with the adult stage of an insect there will be room to indicate both biomass and numbers

for the two sexes, if needed. Additional information on the proper completion of this form follows.

Data Recording

A sample field data form follows:

XX QUADRAT - same number used in vegetation studies if same quadrat used (A quadrat numbers should be recorded even if no insects are collected from it. This enters into determination of sample size.)

X TROPHIC LEVEL - this refers to the group of insects according to the key provided

XXXXX HOST - name code for host plant (see ABOVEGROUND BIOMASS for code)

XXXX ORDER - first four letters of the order name (columns 31-34)

XXXX FAMILY - first four letters of the family name, if known (columns 35-38)

XX GENUS - first two letters of the genus name, if known

XX SPECIES - first two letters of the species name, if known

XX LIFE STAGE - refers to developmental stage of the insect in the sample - includes sex and biomass (11 = Ad. ♂, 12 = Ad. ♀)

XXXXXX TOTAL NUMBER - total count of insects of this group in the sample

XXXXXX DRY WEIGHT - ovendry weight in grams (with decimal point) of subsample from total (subsample refers to a large known number of this particular insect)

XXX SUBSAMPLE NUMBER - the number of insects used in the dry-weight determination

GRASSLAND BIOME

U.S. INTERNATIONAL BIOLOGICAL PROGRAM

FIELD DATA SHEET - INVERTEBRATE

DATA TYPE	SITE	INITIALS	DATE			TREATMENT	REPLICATE	PLOT SIZE	QUADRAT	TROPHIC	HOST	ORDER	FAMILY	GENUS	SPECIES	SUBSPECIES	LIFE STAGE	TOTAL NO.	DRY WT.	NO. WEIGH
			Day	Mo	Yr															
1-2	3-4	5-7	8-9	10-11	12-13	14	15	16-18	20-21	23	25-29	31-33	35-37	39-40	42-43	45	47-48	50-55	57-62	64-66

DATA TYPE
- 01 Aboveground Biomass
- 02 Litter
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- 20 Avian Flush Census
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- 22 Avian Road Count Summary
- 23 Avian Collection - Internal
- 24 Avian Collection - External
- 25 Avian Collection - Plumage
- 30 Invertebrate
- 40 Microbiology - Decomposition
- 41 Microbiology - Nitrogen
- 42 Microbiology - Biomass
- 43 Microbiology - Root Decomposition
- 44 Microbiology - Respiration

SITE
- 01 Ale
- 02 Bison
- 03 Bridger
- 04 Cottonwood
- 05 Dickinson
- 06 Hays
- 07 Hopland
- 08 Jornada
- 09 Osage
- 10 Pantex
- 11 Pawnee

TROPHIC
- 0 Unknown
- 1 Plant feeding (tissue)
- 2 Plant feeding (sap)
- 3 Plant feeding (pollen and nectar)
- 4 Plant feeding (seed)
- 5 Predator
- 6 Parasitoid
- 7 Parasite
- 8 Scavenger
- 9 Non-feeding stage

TREATMENT
- 1 Ungrazed
- 2 Lightly grazed
- 3 Moderately grazed
- 4 Heavily grazed
- 5 Grazed 1969, ungrazed 1970
- 6
- 7
- 8
- 9

LIFE STAGE
- 00 Undetermined
- 10 Adult
- 20 Pupae
- 30 Egg
- 40 Nymph or Larva
- 41 Nymph or Larva, early
- 42 Nymph or Larva, middle
- 43 Nymph or Larva, late
- 50 Instar
- 51 Instar, 1st
- 52 Instar, 2nd
- 53 Instar, 3rd

Non-feeding [illegible]
[illegible]
Adult [illegible]

Note: The last two categories (DRY WEIGHT and SUBSAMPLE NUMBER) of information must be recorded on the data sheets separately for each replicate, even if the information is obtained from only one subsample. The program for summarizing data does not search beyond the replicate for weight information.

Preliminary Analysis

The data will be analyzed to provide mean and standard deviation of the numbers and the biomass of invertebrates by trophic level for each treatment area sampled.

MICROBIOLOGY - BIOMASS

Sampling Methods

Biomass of microorganisms in the soil will be determined either by direct counts or standard plate counts of samples. Generally, samples will be obtained from duplicate cores taken simultaneously with belowground plant biomass studies. Composite samples may be formed from 10 to 20 g of soil from each of the core samples of a given depth throughout the replicate. Subsamples with depth should correspond to those used for root biomass distribution.

Direct Count Procedures

The composite sample will be thoroughly mixed, and portions will be removed for microscopic examination. Samples should be taken at selected intervals during the growing season, at least monthly. Bacterial counts will be made according to the method outlined by D. Parkinson in the IBP Manual on Microbiological Methods (in preparation). Fungal hyphal lengths

will be determined according to the agar-film method of Thomas, Nicholas, and Parkinson (1965), which may be modified for phase microscopy.

Plate Count Procedures

Plate counts for bacteria and actinomycetes will follow standard procedures. These will each involve 10 g soil for the initial dilution, three-minute homogenization, at least four replicate plates at each dilution, plate count or half-strength nutrient agar, incubation at 30°C, and counting only after maximum colony development. All biomass measurements will necessitate moisture determinations, so counts or measurements can be placed on a dry soil basis.

Data will be reported on the following data sheet. Sample weights reported will be on an ovendry basis. If decimals are used, allow one space for this.

Data Recording

A sample data form for recording information on biomass of microorganisms follows:

X	HORIZON - code for soil horizon
XXX	DEPTH TO TOP OF CORE - cm
XXX	DEPTH TO BOTTOM OF CORE - cm
XXXXX	WEIGHT OF SOIL FROM CORE - to nearest 0.1 g
XXXX	WEIGHT OF MICROSAMPLE - to nearest 0.1 g
X	TYPE - code for type of microbiological material
XXX	COUNT or LENGTH - the number (of spores) or length (of hypha) in the microscope field, number of colonies on plate

GRASSLAND BIOME

U.S. INTERNATIONAL BIOLOGICAL PROGRAM

FIELD DATA SHEET - MICROBIOLOGY - BIOMASS

DATA TYPE	SITE	INITIALS	DATE			TREATMENT	REPLICATE	PLOT SIZE	HORIZON	DEPTH. TOP	DEPTH, BOTTOM	CORE WT.	SAMPLE WT.	TYPE	COUNT-LENGTH	TYPE	COUNT-LENGTH	
			Day	Mo	Yr													
1-2	3-4	5-7	8-9	10-11	12-13	14	15	16-19	21	23-25	27-29	31-35	37-40	42	44-46	48	50-52	

DATA TYPE
- 01 Aboveground Biomass
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- 42 Microbiology - Biomass
- 43 Microbiology - Root Decomposition
- 44 Microbiology - Respiration

SITE
- 01 Ale
- 02 Bison
- 03 Bridger
- 04 Cottonwood
- 05 Dickinson
- 06 Hays
- 07 Hopland
- 08 Jornada
- 09 Osage
- 10 Pantex
- 11 Pawnee

TREATMENT
- 1 Ungrazed
- 2 Lightly grazed
- 3 Moderately grazed
- 4 Heavily grazed
- 5 Grazed 1969, ungrazed 1970
- 6
- 7
- 8
- 9

TYPE
- 1 Bacteria
- 2 Bacterial spore
- 3
- 4
- 5 Fungus

HORIZON
- 1 AO
- 2 A
- 3 B
- 4 C

X TYPE

XXX COUNT or LENGTH

Etc.

MICROBIOLOGY - DECOMPOSITION

Sampling Methods

Rates of decomposition will be evaluated by putting sample material in nylon net bags with 1 to 2 mm mesh as containers and placing these in the soil for later recovery and drying, weighing, and ashing to determine the rate of loss of sample material. Sample material may be cellulose filter papers, native litter from standing live plant material, or standard litter distributed to all sites. Plant root material from washed soil cores should also be used. The sample will be placed at a depth of 5 to 7 cm in the soil. Samples should be placed in the soil at monthly intervals during the growing season and retrieved at monthly intervals. A sufficient number should be placed in each replicate so that at least three may be recovered at each time interval. Upon collection, the sample material will be removed from the nylon bag and oven-dried at 60°C (plant material) or 90°C (cellulose filter paper) for 24 hours. The sample material will be weighed, then ashed, and the ash weighed for determination of the amount of sand and mineral material weighed with the original sample. At the same time, a sample of soil should be similarly dried, ashed, and weighed to determine the loss of organic material and carbonates from soil upon ignition. Ashing will be at 600°C for four hours.

Data Recording

A sample data sheet for recording information on decomposition of organic material follows:

X	SAMPLE MATERIAL - code for the type of material in the bag
XX	DEPTH - cm
XXXXXX	DATE OF BURIAL - two spaces each for day, month, and year, in that order
XXX	NUMBER OF DAYS
XXXXX	ORIGINAL DRY WEIGHT - to nearest 0.1 g
XXXXX	WEIGHT AT RETRIEVAL - (dry)
XXXXX	WEIGHT AFTER IGNITION
XXXXX	SOIL SAMPLE WEIGHT
XXXXX	SOIL WEIGHT AFTER IGNITION

MICROBIOLOGY - CO_2 EVOLUTION

Sampling Methods

Carbon dioxide evolution will be estimated in the field by use of a canopy covering a known area of soil from which live plant cover has been removed, and by placing an alkaline CO_2 absorbing solution under the canopy for 24 hours. Replicate samples should be taken with and without litter on soil surface. Trapped CO_2 may be estimated by titrametric, gasometric, or spectral methods. The method will determine the absorption period. The closed system may be as simple as a metal cylinder (at least 10 cm diameter) driven to at least a 20 cm depth. Care should be taken to avoid heat build-up within the chamber. At least four replicates of each treatment should be done. Data should be expressed as mg CO_2 released per m^2 per day.

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FIELD DATA SHEET - MICROBIOLOGY - DECOMPOSITION

DATA TYPE	SITE	INITIALS	DATE			TREATMENT	REPLICATE	PLOT SIZE	MATERIAL	DEPTH	DATE BURIED			NO. DAYS	WT. ORIGINAL	WT. RETRIEV.	WT. IGNITION	SOIL WT.	SOIL IGNIT.
			Day	Mo	Yr						Day	Mo	Yr						
1-2	3-4	5-7	8-9	10-11	12-13	14	15	16-19	21	23-24	26-27	28-29	30-31	33-35	37-41	43-47	49-53	55-59	61-65

DATA TYPE
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TREATMENT
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- 4 Heavily grazed
- 5 Grazed 1969, ungrazed 1970
- 6
- 7
- 8
- 9

SAMPLE MATERIAL
- 1 Cellulose
- 2 Litter
- 3 Standing dead
- 4
- 5

Samples for evaluation of soil respiration in the laboratory may be taken from the soil cores in the same way that samples were collected for evaluation of microbial biomass. The samples will be used to evaluate CO_2 evolution in closed chambers and with varying conditions of temperature and moisture to extrapolate to field conditions. Samples should not be allowed to dry to avoid the partial sterilization stimulation of gaseous evolution.

SAMPLING FOR N FIXATION STUDIES

It is the plan to make a few N fixation measurements on cores from the Comprehensive Network sites this season. Six cores are needed for the measurements planned, so eight from each site should be taken to provide a couple of spares. These will be approximately 9 cm in diameter and 10 to 12 cm deep. If the sample are taken in the cans which are sent to the sites, they will be the right size.

Eight cans will be sent to each site. These will have the bottoms removed and the bottom edges sharpened. These should simply be pressed into the moist soil and lifted out, can and core and all. If a can does not lift out easily, digging down alongside with a spade and forcing the spade in under the can helps to detach it from the soil and roots below. If the soil is not moist enough for the can to enter easily it can be wet artificially.

The time of sampling is not critical, but sampling at the time of maximum plant growth rate, just prior to the time of peak standing crop biomass, is preferred. A couple of weeks of advance notice as to when cores can be expected from the various sites should be given. Samples should be

taken from ungrazed or lightly grazed areas, and on soil that is typical of most sites. It should be made certain that the cores are moist but *not saturated*. They should be bundled up and sent by the fastest available transportation. They may be wrapped in plastic, but it should not be airtight. They should not go anaerobic in transit. Bus express may be the best.

Samples should be sent to:

Dr. John O. Reuss
Department of Agronomy
Colorado State University
Fort Collins, Colorado 80521

ABIOTIC FACTORS

Measurement System

Measurements will be made at the Comprehensive Network sites of the climatic variables which are considered driving forces of the ecosystem. These are factors that are known to be related to organism physiological processes, though they may not necessarily include the means by which the organisms are coupled to the environment. The objective is not to make a detailed study of microclimate, nor to evaluate detailed models of biotic processes, but to evaluate those factors related to biological productivity and trophic level transfer rates.

Commercially available sensors will be used. A multichannel digital recorder, with attendant integration and analog/digital conversion circuitry, will be operated on a continuous basis. In addition, a strip chart hygrothermograph will be located in a standard Weather Service Shelter.

Automatic Data Recording System

A magnetic tape recording system capable of logging 18 channels of meteorological information at hourly intervals will be placed in a representative location at each Comprehensive Network site in a partially buried fiber-glass bunker. The following measurements will be taken at hourly intervals.

Ungrazed Treatment Area

Channel No.	Measurement
1	Time (2 digits days and 1 digit hours)
2	Time (1 digit hours and 2 digits minutes)
3	Precipitation (recording rain gauge)
4	Air moisture (at average canopy height)
5	Air temperature (+5 cm)
6	Air temperature (+50 cm)
7	Air temperature (+2 m)
8	Soil temperature (-2.5 cm)
9	Soil temperature (-10 cm)
10	Soil temperature (-25 cm)
11	Soil heat flux (-8 cm)
12	Wind speed (2 m above soil surface)
13	Total radiation (2 m above soil surface)
14	Net radiation (2 m above soil surface)
15	Soil moisture (-5 cm)
16	Soil moisture (-50 cm)

Grazed Treatment Area

Channel No.	Measurement
17	Soil moisture (-5 cm)
18	Soil moisture (-50 cm)
19	Net radiation (2 m above soil surface)
20	Soil heat flux (-8 cm)

Manuals for the operation of the recording system will be furnished to each site. A manual showing nominal values for sensor output and calibration procedures will also be furnished. While the data acquisition system is designed to be automatic and should function without attendance, past experience indicates quality data can only be obtained by frequently checking sensors and comparing recorded values to nominal values of output whenever possible. It is therefore recommended that the system be checked at every possible opportunity.

Data tapes should be changed at weekly intervals during the first two months of system operation. If results are satisfactory, the interval between tape changes may be extended to one month.

The data from tapes will be processed and converted to measurement units by the NREL, and a copy of resulting printout will be returned to the individual responsible for abiotic measurements at each site with notes as to the appearance of data and recommendations for operational changes if needed. Site personnel will also review the data and look for obvious measurement errors. Computer processing of tapes will be completed and returned to site coordinators within 10 working days after receipt of the data tape.

Strip Chart Data

A hygrothermograph will be operated inside a standard Weather Service Instrument Shelter for the determination of daily maximum-minimum air temperatures and to determine an index and backup for atmospheric water content. A sling psychrometer will be used to periodically calibrate the hair hygrometer in the hygrothermograph. Hygrothermograph charts will be changed weekly if possible, and data from the charts will be sent to the NREL monthly (*only* the form will be sent first). Only after the computer output is returned to the site, will the strip charts be sent to NREL.

SITE FACTORS

A description of the site at which the environmental measurements will be taken should include:

1. Physical Description:
   a. Elevation (m).
   b. Longitude and lattitude (° and ').
   c. Land slope (%) measured downhill (representing the local slope around the sensors).
   d. Direction of slope (°) measured downhill, corrected for declination.
2. Soil Description:
   a. Soil type or series name(s) on which production studies and environmental measurements are being made (must be same).
   b. Soil map (if available).
   c. Soil profile description (Soil Conservation Service).

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FIELD DATA SHEET--HYGROTHERMOGRAPH

Data Type	Site	Initials	Date			Hour	Temp.	Humid.	Therm.	Psy.	Hour	Temp.	Humid.	Therm.	Psy.	Hour	Temp.	Humid.	Therm.	Psy.
			Day	Month	Year															
1-2	3-4	5-7	8-9	10-11	12-13	15-18	20-22	24-25	27-29	31-32	34-37	39-41	43-44	46-48	50-51	53-56	58-60	62-63	65-67	69-70
50																				

Temp.

Selected temperature reading from hygrothermograph

Humid.

Selected humidity reading from hygrothermograph

Therm.

Reading from standard thermometer at same hour as hygrothermograph reading

Psy.

Reading from sling psychrometer at same hour as hygrothermograph reading

3. Descriptive Soil Analysis:

   a. Texture will be determined by the hydrometer method[1/] with readings to provide separations at 2 μ, 20 μ, 50 μ, 200 μ, and 2 mm. Samples will be treated to remove organic matter[1/], dissolve carbonates[1/], and disperse colloids[1/] (to be done at NREL).

      Samples for texture analysis will be collected (i) from the top 4 cm, (ii) from a sample representative of the A horizon, and (iii) from a sample representative of the B horizon. Fifty-gram samples will be obtained. Soil depth from which samples (ii) and (iii) were taken will be reported.

   b. Organic matter will be determined by the wet combustion technique[1/]. Determinations will be made for the same depth increments corresponding to root samples.

   c. Bulk density will be determined by the core method[1/]. Determinations will be made at the same depths at which the thermocouple psychrometers are located, plus the 0 to 4 cm surface layer. Ten samples are required for representative values for each depth.

   d. Soil pH will be determined for composite samples of the A horizon, and likewise for the B horizon. pH measurements will be made on a soil paste.

[1/] See Black, C. A. [ed.]. 1965. Methods of soil analysis. Parts 1 and 2, No. 9. Amer. Soc. Agron., Inc., Madison, Wisconsin. 1572 p.

e. Exchangeable Ca, Mg, Na, K, Total N, P (Contact John O. Reuss).
   Re: Comparable determination.

f. Cation exchange capacity (Contact John O. Reuss).
   Re: Comparable determination.

g. Soil water characteristic curves will be determined for tensions of -0.1, -0.25, -0.50, -1.0, -3.0, and -15.0 bars. These characteristic curves will be determined for soil depths corresponding to thermocouple psychrometer placement (to be done at NREL).

4. Dynamic Soil Analysis.

   a. Dynamic soil anslyses will be made of ammonia, nitrate, and bicarbonate phosphorus by NREL.

   b. A single 400 g sample from each 10 cm interval of depth representing a composite sample for the replicate will be sent to NREL.

HANDLING OF LABORATORY SAMPLES

Chemical Analyses of Plant and Animal Samples

Investigators submitting samples to the Grasslands Ecology Research Laboratory for chemical analyses should use: (i) the standardized processing and storage scheme (Table 2), and (ii) REQUEST FORM FOR LABORATORY ANALYSIS (Form NREL-60). Table 2 is used by the investigators requesting analysis to determine processing required prior to sending samples to the laboratory. Form NREL-60 is used both for indicating the required analyses and for sample identification. A MINIMUM OF 5 g OF DRY MATTER MUST BE SUBMITTED FOR EACH ANALYSIS. Samples will be pooled within replications; no individual plot or animal samples will be analyzed except by special agreement.

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REQUEST FORM FOR LABORATORY ANALYSIS

Date of Request ______________ Name of Investigator ______________

FORM NO	SITE	INITIALS	DATE OF COLLECTION	TREATMENT	REPLICATE	SOURCE	ORDER-FAM OR NON-TAXONOMIC IDENT.	TAXONOMIC IDENT.			INVESTIGATORS SPECIMEN OR DATA NO.	GERL LAB NO.	ANALYSES REQUESTED											
													DIETS			CHEMICAL ANALYSES								
								GENUS	SPECIES	SUB-SP			HAND SORT	MIC LO	MIC HI	DRY MATTER	ASH	GROSS ENERGY	KJEDAHL N	P	CWC	ADP	LIGNIN	CARBON
1-2	3-4	5-7	8-9 10-11 12-13	14	15	16-17	18 - 23	24 26	27 29	30 32	33 - 36	39 - 44	47	48	49	50	51	52	53	54	55	56	57	58
60																								

SITE
- 01 Ale
- 02 Bison
- 03 Bridger
- 04 Cottonwood
- 05 Dickinson
- 06 Hays
- 07 Hopland
- 08 Jornada
- 09 Osage
- 10 Pantex
- 11 Pawnee

TREATMENT
- 1 ungrazed
- 2 lightly grazed
- 3 moderately grazed
- 4 heavily grazed
- 5 gr-70 un-71
- 6
- 7
- 8
- 9
- A Diet Pasture Lt.
- B Diet Pasture Mod
- C Diet Pasture Heavy
- D Stress - O
- E Stress - W
- F Stress - N
- G Stress - WN
- H
- I
- J
- K
- L
- M

SOURCE
- 01 mouth & cheeks
- 02 esophageal
- 03 rumen
- 04
- 05 stomach
- 06 crop
- 07
- 08 caecum
- 09 colon
- 10
- 11 fecal
- 12
- 13 caches & stores
- 14
- 15 hand-clippings
- 16 hand-plucks
- 17 animal clips & wastes
- 18 mechanical harvested
- 19 litter & detritus
- 20 aboveground plants
- 21 belowground plants
- 22 reproductive
- 23 standing dead
- 24
- 25
- 26
- 27
- 28
- 29
- 30
- 31
- 32
- 33
- 34
- 35
- 36

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REQUEST FORM FOR LABORATORY ANALYSIS FOR SOILS

Date of Request ______________ Name of Investigator ______________

FORM NO.	SITE	INITIALS	DATE OF COLLECTION			TREATMENT	REPLICATE	SERIES NAME	CM TO TOP	CM TO BOTTOM	INVESTIGATORS SPECIMEN OR DATA NO.	GERL LAB NO.	RESULTS OF ANALYSIS										
													TOTAL N	NO_3	NO_2	NH_4	C/N RATIO	BICARB. P	TEXTURE	ORGANIC MATTER			
1-2	3-4	5-7	8-9	10-11	12-13	14	15	16-22	23-24	25-26	27-30	31-36	37-40	41-44	45-48	49-52	53-56	57-60	61-62	63-66	67-70	71-74	
61																							

SITE
- 01 Ale
- 02 Bison
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- D Stress - O
- E Stress - W
- F Stress - N
- G Stress - WN
- H
- I
- J
- K
- L
- M

Col. 31-74 Filled in by Lab

Note that in Table 2 for the various analyses (i.e., energy, nitrogen, phosphorus, etc.) for various types of samples (i.e., soil, herbage, etc.), the processing instructions are indicated as a numeral, and the grinding instructions are indicated with a letter. Processing and grinding instructions for particular analyses are indicated by the numeral-letter combination for a particular analysis and type of sample.

Analyses to be conducted are indicated on REQUEST FORM FOR LABORATORY ANALYSIS simply by a check in the appropriate column following the sample description and investigator's data number.

Example 1. A diet sample is to be analyzed for dry matter, nitrogen, and gross energy. The reference code is 5-a (see Table 2) for these three analyses. Processing instruction 5 is "dry in a forced air oven at 45°C to constant weight," and grinding instruction "a" is "through 20 mesh screen in a Wiley mill." The investigator would, before sending samples to the GERL, dry the material in a forced air oven at 45°C to constant weight then grind through a 20 mesh screen in a Wiley mill.

Example 2. A sample of gastro-intestinal tract contents and a sample of urine from the same animal requires analysis for phosphorus on both samples. The coding from the gastro-intestinal sample would be 6-a. Processing instruction 6 is "preserve with thymol in the field and dry as in 5." Grinding instruction "a" is "through 20 mesh screen in a Wiley mill"; therefore, the investigator would preserve with thymol in the field, then dry with a forced air oven at 45°C to constant weight, then grind through 20 mesh screen in a Wiley mill.

Table 2. Processing and storage schemes[a/] for samples collected for Grasslands Ecology Research Laboratory.

Analyses[b/],[c/]	Type of Sample					
	Soil	Herbage	Diet	GI Contents and Feces	Urine	Body Carcasses or Tissues
Energy	7-x	5-a	5-a	5-a	3-x	4-b
Nitrogen	7-x	5-a	5-a	5-x	3-x	4-b
Phosphorus	7-x	5-a	5-a	6-a	3-x	4-b
Ash	7-x	5-a	5-a	6-a	3-x	4-b
Fiber	7-x	5-a	5-a	6-a	---	---
Lignin	7-x	5-a	5-a	6-a	---	---
Dry matter	7-x	1-x	---	2-x	---	4-b
Minerals	7-x	5-a	5-a	6-a	3-x	4-b
Soluble carbohydrates	7-x	1-b	1-b	5-a	3-x	4-b
Pigments or lipids	7-x	1-b	1-b	2-x	---	4-b

a/ Processing:
1. Place in dry ice then store in freezer at 0°C.
2. Preserve with thymol then store in freezer at 0°C.
3. Reduce pH below 3 with HCl then store in freezer at 0°C.
4. Store in freezer at 0°C.
5. Dry in forced air oven at 45°C to constant weight.
6. Preserve with thymol in field then dry as in 5.
7. Place in air tight container.

Grinding:
a. Through 1 mm or 20 mesh screen in Wiley mill.
b. Through 1 mm or 20 mesh screen in Wiley mill with dry ice.
x. No grinding.

b/ A minimum of 5 g of dry matter should be collected for each analysis.

c/ Sample containers, labels, and marking pens will be provided to provide uniformity and facilitate handling and storage.

The urine sample to be analyzed for phosphorus is instruction 3-x. Processing instruction is "reduce pH below 3 with HCl and store in a freezer at 0°C." Then, of course, no grinding is required.

Form NREL-60 is the chemical and botanical analysis request sheet. Extra copies of this form are available on request. The investigator enters a sample description (see example), his number (the specimen number), and indicates by checks analyses to be conducted. The laboratory number is entered by the GERL technicians.

Botanical and Diet Analyses

Botanical and diet analyses are requested on Form NREL-60. These samples can include plant materials such as litter or diet samples. Samples for which only plant identification is needed should have slides prepared before sending to GERL; samples which contain seeds or insect parts should be sent intact.

Preparation of slides for botanical analyses. The method discussed here should be followed. In the field, samples intended only for taxonomic determination studies can be frozen, air dried, or preserved in alcohol. Samples intended for energy determinations or chemical analyses, as well as in addition to taxonomic determinations, should be air dried or oven-dried (45°C) or frozen as soon as possible, and should not be placed in a preservative. Dietary samples from large herbivores (all stomach, fistula, rumen, or fecal material) should be air dried and then ground in a Wiley laboratory mill over a 1 mm (20 mesh) screen before microscope slides are made. Mammals that weigh less than 200 g live weight usually chew their food fine enough so grinding is not necessary. Samples removed from insect crops cannot be

ground because of the small quantities involved. Dietary material from these small animals is transferred directly to slides without drying or washing. Dietary samples from omnivores, where identification of insect fragments is needed, should not be ground at all until the insect fragments are removed, as insects are best identified from a low power hand-sorting procedure.

Materials needed for this method include:

Microscope slides - glass, plain, standard size, laboratory grade

Cover slips - glass, 22 x 40 mm for most animals

glass, 22 x 22 mm for insects

Slide labels

Plastic squeeze bottles with spout (2)

Teasing needles (2)

Spatula with narrow, flexible blade

Sponge

200 mesh screen

Drying oven and racks

Waring blender (1 quart)

Hertwig's solution - clearing agent

270 g chloral hydrate crystals

19 cc 1N HCl

60 cc glycerin

Combine glycerin and HCl, add chloral hydrate crystals, stir until crystals dissolve. The crystals will dissolve faster if the mixture is warmed and agitated in a blender.

Hoyer's solution - mounting medium

200 g chloral hydrate crystals

50 cc water

20 cc glycerin

30 g photo purified gum arabic

Combine glycerin and water, add chloral hydrate crystals and stir until crystals dissolve. Warm the solution and stir in gum arabic. The mixture may be agitated with a blender after the gum arabic is added. Let stand until the solution clears.

Two slides should be prepared from each sample, if possible. Plant fragments should be spread evenly over the slide and should not overlap. At 100 power magnification, there should be about three large fragments per field.

Place approximately 10 cc of ground or blended sample in a 0.1 mm (200 mesh) screen and wash under running water for one minute (Fig. 2). Remove a small amount of the washed material from the screen, with a spatula, and spread near one end of a microscope slide (Fig. 3). Add three or four drops of Hertwig's solution to the wet material on the slide, then carefully boil off most of the Hertwig's by holding the slide above a small alcohol burner (Fig. 4). It is important, at this point, not to char the sample by overheating.

When most of the Hertwig's has boiled off, add enough Hoyer's solution to cover an area about two-thirds as large as a cover slip. With a teasing needle, mix the plant material with the Hoyer's and spread evenly over an

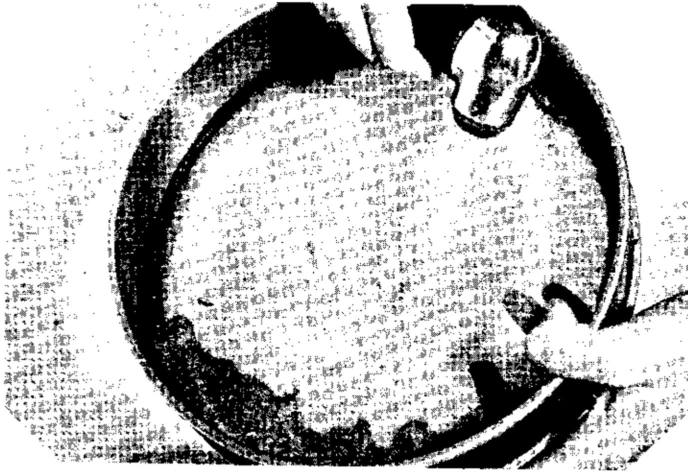

Fig. 2. The ground sample is washed with water to remove dirt and small plant fragments over a 0.1 mm (200 mesh) screen.

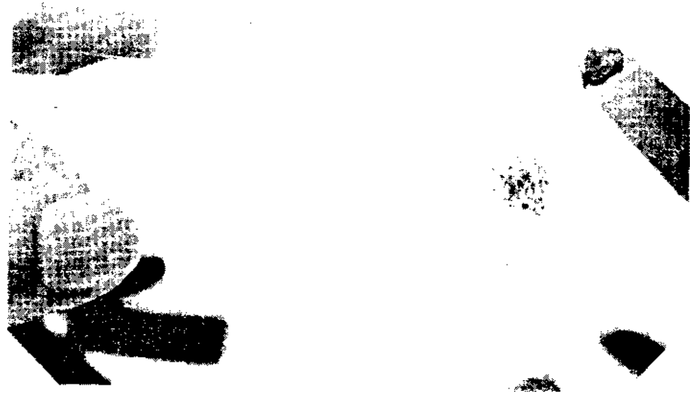

Fig. 3. A small amount of the sample is placed on a slide. About three or four identifiable particles per field is the desired density.

Fig. 4. Hertwig's solution is boiled off over an alcohol burner.

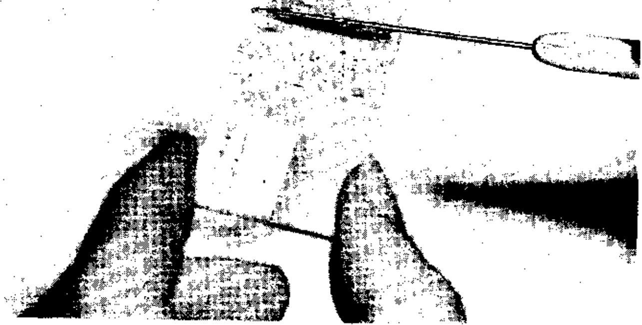

Fig. 5. Hoyer's solution is mixed with the sample and spread evenly over the slide.

area as large as a cover slip (Fig. 5). Place a cover slip on the preparation and heat the slide over the burner until the Hoyer's starts to boil. Immediately wipe the bottom surface of the slide with a cold, damp cloth or sponge to draw air bubbles out of the Hoyer's solution. Press a teasing needle gently on top of the cover slip to squeeze out excess mounting medium and remove any remaining air bubbles. (Very tiny bubbles usually disappear during the drying process and are not detrimental.) Apply a thin ring of Hoyer's solution around the edge of the cover slip, if needed, to form a seal as the slide dries.

Slides are placed flat on racks, in a drying oven at 55°C, for two or three days, or until the Hoyer's solution has hardened, then stored in a dry place. Hoyer's solution forms a permanent mounting medium when hardened, but is soluble in water allowing easy cleaning or reuse of slides.

Reference collection. A collection of all species of plants present at a site should be available for making reference slides. The appropriate slides of leaf, stem, root, flower, and seed should be prepared for each species. The separate parts of each plant are placed in a Waring blender with enough water to at least cover the blades. Less than a teaspoon of plant material is needed, and plants may be green or dried.

After one to two minutes at high speed, the contents of the blender are poured into a 0.1 mm mesh screen and washed. Reference slides are made directly from this material, following the same procedure as for dietary samples, but applying more material to the slides.

Woody material may be ground in a Wiley mill before being added to the blender. Very hot water also helps soften tissue and removes plant pigments.

Reference collections of insects are also needed for identification of insect fragments.

Methods of insect identification and quantitation. The procedure is to recover insects or parts of insects from the gut of the animal, then to identify each insect represented by comparing the insect or its parts with an identified reference collection. The reference collection is assembled from insects collected at each site and identified in advance with the aid of entomologists. The reference collection should be started during the first season of field work at each site.

The length of each insect eaten is estimated on the basis of direct measurement with a micrometer or ruler, or else by inference from size of fragments. Insects in the reference collection provide a basis for comparisons in estimating lengths from fragments. The number of each kind of insect eaten is determined by counting the parts repeated in a stomach sample, such as the number of head capsules, thoraces, elytra, etc., and using whichever part gives the highest count. Adult and immature stages of insects are identified and each handled on the same basis. The length of insect, as recorded at the time of examining the stomach sample, serves as the index to the dry weight of the insect eaten. A chart or graph for calibration of length with dry weight for each type of insect, i.e., family, is usually made up by collecting specimens of different sizes, oven-dyring them, and then weighing them. Life history stages of different forms are similarly calibrated. Note that it is *not* necessary to measure the volume or weight of insect remains present in the gut.

Methods for seed identification and quantitation. The seeds, seed coats, or kernels are recovered from the general mass of food remains

previously removed from the gut under a dissecting microscope. Identifications are then made by comparison with a reference collection of seeds from locally occurring seed plants. In addition, quantitative data are desired, as with insects. Measurements of seed size are made to obtain mean length of each seed type as it occurs in the sample being examined. The length serves as the index to dry weight for seeds of each species and size. Calibration of length with dry weight is done with information assembled separately on seed weights from the literature or by collecting fresh seeds, then oven-drying, and weighing them. Some discretion is necessary, due to variations in feeding mechanisms employed which results in the presence or absence of seed coatings. The numbers of seeds eaten are determined by counting the seeds or parts of seeds.

Field handling. The animal should be collected at the time it is feeding. A tie-on label should be attached to the leg with the identifying number to be listed on Form NREL-60, and the other information requested on the form should be completed. Digestion must be stopped rapidly either with alcohol or by freezing. Alcohol is acceptable if no chemical analyses are to be made; but if chemical analyses are to be done, alcohol is unacceptable. Field freezing is preferred. If using alcohol, 70% ethanol should be forced deeply into the esophagus with a medicine dropper (calibrated). The amount of alcohol accepted should be recorded. The animal should be wrapped tightly in Saran Wrap, retaining any alcohol spilled from the animal. THE ANIMAL MUST BE KEPT COOL IN THE FIELD. At the laboratory, the package should be weighed without unwrapping; the weight of the label, alcohol, and Saran Wrap subtracted; and the weight of the animal recorded. Freeze until further handling is possible.

Laboratory handling. Materials needed include:

Dissecting scope with zoom device (10X - 80X)

Illuminator and spotlight

Balance with one-tenth gram readings

Ethanol

200 mesh screen

Petri dishes (10 mm to 15 mm deep) (3)

Plastic sacks (approx. 17 cm x 8 cm)

Spatula (8 cm to 12 cm wide)

Dissecting scissors

Tweezers (fine pointed) (2)

Teasing needles (2)

Envelopes (approx. 15 cm x 8 cm)

Cards for recording insects and densities

Data should be recorded from the animal specimen. The animal should be thawed, then skinned, and abdominal cavity opened, and stomach (proeventriculus and gizzard) removed. The esophagus and pharynx should be examined for possible food items. If any are found, they should be placed in a sample vial. The stomach should be cut open and the contents added to the vial. A label should be slipped in with the identifying number and other identifying information. Add 70% alcohol to the vial or freeze the sample. Sex, gonad condition and size, and amount of fat deposit should be recorded on AVIAN COLLECTION, INTERNAL (Form NREL-23) or MAMMAL COLLECTION (Form NREL-12A).

The identification of foods should be carried out as follows:

i. Get diet specimen and data form (Form NREL-62).

ii. Empty specimen into small glass dish. If specimen is preserved in alcohol, pour off murky fluid and replace with fresh 70% alcohol. Retrieve any food fragments inadvertently poured off with old fluid.

iii. Use dissecting microscope at 10 or higher power magnification. Sort stomach contents enough to allow visual estimate of percent plant remains and percent animal remains on basis of bulk.

iv. Sort grit and count number of pieces greater than 0.5 mm.

v. Sort all plant items.

vi. For each seed type, record identification, the number present, and size. If identification is not apparent, record color, dimensions, surface characters, and make a sketch. Estimate and record percent of plant matter each type represents. Seeds needing further work should be placed in mini-vials with sample number on cork.

vii. For plant fibers and leafy tissues, indicate the percent of plant matter in sample they represent. Record characteristics and place a generous sample in a mini-vial for microscopic identification at GERL; record sample number on cork.

viii. Sort all animal items. Several dishes may be used.

ix. Identify animals to lowest feasible taxonomic category. Record identification and also parts present, color, developmental stage of each specimen, and length. If wide range in size is

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LAB DATA SHEET--MICROSCOPIC ANALYSIS

Data form number (1-2)	Site (3-4)	Source (5-6)	Date (7-12)	Treatment (13)	Replicate number (14)
Project (15-20)	Data type (21-22)	Code list (23-27)	Lab use (28-41)	Number of slides (42-43)	Fields per slide (44-46)
Animal species code (47-50)	Sex (51)	Age class (52)	Unused (53-63)	Data cards (64)	Year (66-67)
Lab number (68-72)					

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	
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DATA DESCRIPTION FOR FORM NREL-62

Microscopic Analysis

Columns	Information for Each Slide
Header Card	
1 - 2	Data form number--62
3 - 4	Site 01 Ale 02 Bison 03 Bridger 04 Cottonwood 05 Dickinson 06 Hays 07 Hopland 08 Jornada 09 Osage 10 Pantex 11 Pawnee
5 - 6	Source 01 Mouth and cheeks 02 Esophageal 03 Rumen 04 05 Stomach 06 Crop 07 08 Caecum 09 Colon 10 11 Fecal 12 13 Caches and stores 14 15 Hand clippings 16 Hand plucks 17 Animal chips and wastes 18 Mechanical harvested 19 Litter and detritus 20
7 - 12	Date taken by investigator--day, month, year (in that order)

Microscopic Analysis (Continued)

Columns	Information for Each Slide
13	Treatment 1 Ungrazed 2 Lightly grazed 3 Moderately grazed 4 Heavily grazed 5 Grazed 1969, Ungrazed 1970 6 7 8 9
14	Replicate number
15 - 20	Project--investigator's last name
21 - 22	Data type 06 Hand sorted 07 Macroscopic 08 16 Microscopic - Lo 17 Microscopic - Hi 18
23 - 27	Number of master list of codes used for composition determination
28 - 41	Investigator's number and other lab identification
42 - 43	Number of slides read per specimen or number of grids per sample
44 - 46	Number of fields per slide or number of points per grid
47 - 50	Animal species code
51	M or F--sex
52	A or J--age class
53 - 63	Unused
64	Number of data cards to follow for that slide
66 - 67	Decade and year lab number was assigned
68 - 72	Lab number--sequential within that year (columns 66 - 67) across all data sets

Microscopic Analysis (Continued)

Columns	Information for Each Slide
Composition Analysis Card(s)--as needed	
1 - 63	Up to nine groups (as needed) of seven columns each, with the first four for alphameric code (relative to master list as specified on header card), and the last three for number of times the coded category was observed.
64	Number of cards to follow
66 - 72	As on header card

found, record numbers of each size. Sketch enigmatic specimens for future reference.

x. Store specimens in main vial, separate vials or mini-vials, all labeled. Need for maintaining segregation will vary according to complexity of dietary sample and completeness of immediate identifications. If all data are obtained, lump; if further work is needed, use several vials. Unusual specimens may be added to reference collection when identified.

xi. Count and record total number of animal prey items.

xii. Count and record total number of seeds.

In stomachs of larger volume, the contents can be divided between two or more petri dishes until their density is adequate for sufficient light penetration to clearly distinguish individual insect fragments. If the mixture in the petri dish is not clear, the contents can be strained on a 200 mesh screen under tap water and deposited again in the petri dish.

The petri dish is then placed on a white background under a dissecting scope, preferably one having a zoom device for higher magnification of the more minute insect fragments. A dissecting scope with a zoom device of 10X - 80X ocular power has been found satisfactory. The insects are then identified and recorded on the appropriate forms. Tweezers and/or teasing needles are used to manipulate the insect fragments for positive identification.

This procedure deals with three sorts of foods: animal items, seeds, and plant fibers or soft tissues of non-fruit origin. For animal items and for seeds, the above analysis should give identifications, numbers of items,

and biomass (dry weight) eaten of each item. For plant fibers and soft parts of non-fruit origin, identification will be as described in the previous section. However, the amounts of these substances present in the dietary sample should be estimated from the original dietary analysis outlined above.

Soil Samples

Identification of soil samples will be recorded on REQUEST FORM FOR LABORATORY ANALYSIS FOR SOILS (Form NREL-61). One time analyses of major soils on all sites will include:

i. Soil texture

ii. Soil organic matter

iii. Soil bulk density

iv. Soil pH

v. Exchangeable cations (Ca, MG, Na, K)

vi. Nitrogen and phosphorus analysis

vii. Moisture release curves (at five tensions from .33 through 15 atmospheres and possibly additional measures at 40 and 60 atmospheres).

Data Processing

Description of forms. REQUEST FORM FOR LABORATORY ANALYSIS (Form NREL-60) is the form for botanical and chemical analyses, and REQUEST FORM FOR LABORATORY ANALYSIS FOR SOILS (Form NREL-61) is for soil analyses. These forms include the following information:

Form NREL-60

Column	Item
1- 2	Data form number
3- 4	Site
5- 7	Initials
8-13	Date
14	Treatment
15	Replicate
16-17	Source (stomach, aboveground, belowground, etc.
18-23	Order, family, or non-taxonomic identification
24-32	Taxonomic identification
33-36	Investigator's number
39-44	GERL lab number
47-72	Analyses requested

Form NREL-61

Column	Item
1- 2	Data form number
3- 4	Site
5- 7	Initials
8-13	Date
14	Treatment
15	Replicate
16-22	Series name
23-25	Texture
26-27	cm to top
28-29	cm to bottom
30-32	Horizon
33-36	Investigator's number
39-44	GERL lab number
47-72	Analyses requested

Data processing requirements. Each sample will be recorded in the data bank by GERL lab number. The file on each number will include:

i. The sample descriptions from Form NREL-60 or NREL-61.

ii. The status and results of analyses.

Data will be retrievable by specifying any of the identifying information on types of analyses or combinations of identification and types of analyses. Printout will include both sample identification and results of analyses.

Results of botanical and microscopic diet analysis will be computed in the following way. If 20 locations were examined on each of 50 slides made from the contents of stomachs of 50 herbivores taken in the same study area, the conversion of frequency to density is as follows: 50 slides x 20 locations = 1,000 total locations. If plant species A occurred in 700 of these locations, the percent of frequency would be:

$$F = \frac{\text{number of locations in which species A occurs}}{\text{total number of locations examined}} \times 100$$

$$F = \frac{700}{1000} \times 100$$

$$F = 70\%$$

Converting F to mean density/1000 locations, we have:

$$70 = 100(1 - e^{-D})$$

$$\frac{70}{100} = (1 - e^{-D})$$

$$0.7 = 1 - e^{-D}$$

$$0.7 - 1.0 = -e^{-D}$$

$$-(-0.3) = -(-e^{-D})$$

$$0.3 = e^{-D}$$

Now, to find the value of D, we look up the X value of $e^{-X} = 0.3$ in a standard mathematical table of "Values of Exponential Functions," and we see that D (or X) equals 1.20.

With a frequency of 70%, we have a mean density of 1.20 particles of species A per location, D = 1.20.

There are two requirements that must be met before percent of frequency can be converted to density (Curtis and McIntosh 1950). The fragments of plants must be distributed randomly over the slide, and the density of particles must be such that the most common species does not occur in more than 86% of the fields of the microscope. Thorough mixing of material and adjustments of the quantity placed on the slides will take care of both these requirements.

Recent technological advances (Sparks and Malechek 1968) have added another dimension to the microscopic technique. Density of particles per location is converted to relative density:

$$\frac{\text{Density of particles of species A}}{\text{Total density of particles of all species}} \times 100 \ .$$

The relative density of a species is then used to estimate percent of dry weight of that species in the mixture. Equations of regression that express the relationship between estimated percent of dry weight (X) and actual percent of dry weight (Y) were developed for three categories of plants: grasses, forbs, and grass-forb combinations. The ratio between estimated percent of dry weight (relative density) and actual percent of dry weight was approximately 1:1 for all three categories. Student's (t) test showed that there was no significant difference between the equations of regression for grasses and forbs, and that the calculated equations of regression for grasses, forbs, and grass-forb combinations were not statistically different from the equation Y = X. Therefore, the percent of dry weight of a mixture can be predicted directly from the relative density.

Storr (1961) and Heady and Van Dyne (1965) reported that specific gravity (weight per unit area) of plant material is not consistent at different stages of maturity nor is it consistent from species to species. The 1:1 relationship between estimated percent of dry weight may be consistent with all species or at all stages of maturity. However, unless the parts of plants in the diet being analyzed are grossly different from those reported by Sparks and Malechek (1968), the added accuracy gained by using an equation of prediction more complicated than $Y = X$ would probably not be worthwhile.

Results of macroscopic separation will be computed in the following way. Let us say we wish to calculate the mean daily consumption of each of these foods by a lark bunting in May. Studies in bioenergetics tell us that the daily gross intake of food for a small bird living in a cage can be calculated. The account below explains and illustrates the computation.

S. C. Kendeigh[2/] has worked out equations which can provide an estimate of the energy required to sustain for a day a small bird in caged existence (existence energy). Important variables are weight of bird and temperature of the environment.

2/ S. C. Kendeigh, 1963, Relation of existence energy requirements to size of bird. American Zoologist 3(4):497 (Abstract No. 77).

Existence energy is the food metabolized by a bird maintaining constant weight in a small cage at a constant temperature and photoperiod. The regression line for eight species, varying from 10 to 4000 grams, 9-12-hour photoperiod and 30°C is represented by the equation: $M = 1.5860\ W^{0.601}$ and at 0°C: $3.8578\ W^{0.550}$, where M is kcal/bird-day, and W is weight in grams. Regression lines for a 15-hour photoperiod are not significantly different. The lower weight exponent at 0°C than at 30°C indicates that small birds are affected by low temperatures to a relatively greater extent than are large birds. The weight exponent at 30°C, lower than that given for standard metabolism ($M = 0.8446 + W^{0.659}$) by King and Farner (Marshall's "Biology and comparative physiology of birds," Vol. II: 215-288, 1961), also indicates that small birds expend proportionally more energy for existence than do large birds. The difference of values for the Y intercept, from 0.845 for standard metabolism to 1.5860 at 30°C to 3.8578 at 0°C, reflect the influence of feeding and activity compared with fasting and rest and of low temperature stress. These equations are considered only preliminary formulations. Because of the small number of species included, only the equations for standard metabolism and existence metabolism at 0°C are statistically significant.

After publishing the above in 1964, Kendeigh revised his equations (January 12, 1966):

Existence energy at 30°C: $M = 1.368\ W^{0.674}$

$(\log M = 0.136 + 0.562 \log W)$

Existence energy at 0°C: $M = 3.974\ W^{0.562}$

$(\log M = 0.599 + 0.562 \log W)$

M = kcal/bird-day

W = weight in grams

In order to determine the amount of food needed per day by a small bird living in the wild, the value for existence energy must be raised by two factors. One factor is for efficiency of digestion, and the other is for additional energy used in activity. Kendeigh is currently employing a digestive efficiency factor of 80%, in work reported at the September 1971 IBP meeting in Holland. Schartz and Zimmerman (1970) found that dickcissels in the wild needed 1.4 times existence energy. In the following calculation, therefore, the value of 80% is used for efficiency of digestion and 1.4 as the factor for normal activity in the wild, since the latter is the best estimate now available for a small, grassland bird.

A sample calculation will now be made, using the two equations, for 0°C and 30°C. Take a bird with body weight of 38.7 g (lark bunting male). *First*, the calculations for 30°C:

1. Take log of both sides of equation: $M = 1.368\ W^{0.674}$

   $$\log M = \log 1.368 + 0.674 \log W$$

2. Substitute 38.7 g for W

   $$\log M = 0.136 + (0.674)(1.588) = 1.206$$

3. Find antilog of log M

   antilog of 1.206 = 16.1

   Hence, M = 16.1 kcal/bird-day.

4. Calculate existence energy as kcal/kg-day:

   $$\frac{16.1 \text{ kcal/bird-day}}{38.7 \text{ g}} = \frac{x \text{ kcal/kg-day}}{1000 \text{ g}}$$

   Also, insert the efficiency factor (for 80%):

   $$\frac{16.1 \times 1000 \times 1.25}{38.7} = 520 \text{ kcal/kg-day} = \text{gross energy intake at } 30^\circ\text{C}$$

Second, for 0°C, same steps:

$$M = 1.368\ W^{0.674}$$

$$\log M = 0.599 + (0.562)(1.588) = 1\ 491$$

$$\text{antilog } 1.491 = 31.0$$

$$M = 31.0 \text{ kcal/bird-day}$$

$$\frac{31.0 \times 1000 \times 1.25}{38.7} = 1001 \text{ kcal/kg-day} = \text{gross energy intake at } 0°\text{C}$$

A line is plotted (Fig. 6). Lines for birds of other sizes can be added to the graph.

Third, calculate the mean daily energy intake for male lark bunting in May:

In May at the Pawnee Site, the mean monthly temperature is 12.7°C.

Reading from the graph, gross energy at this temperature is 810 kcal/kg-day.

Derive gross energy intake needed per bird-day in the wild in May:

$$\frac{810 \times 38.7 \times 1.4}{1000} = 43.9 \text{ kcal/bird-day}$$

Since we know the proportions of the different foods eaten and the caloric equivalents for those foods, we can calculate the mean amounts of all foods eaten per day. Caloric equivalents are obtained from the literature or from the IBP central laboratory.

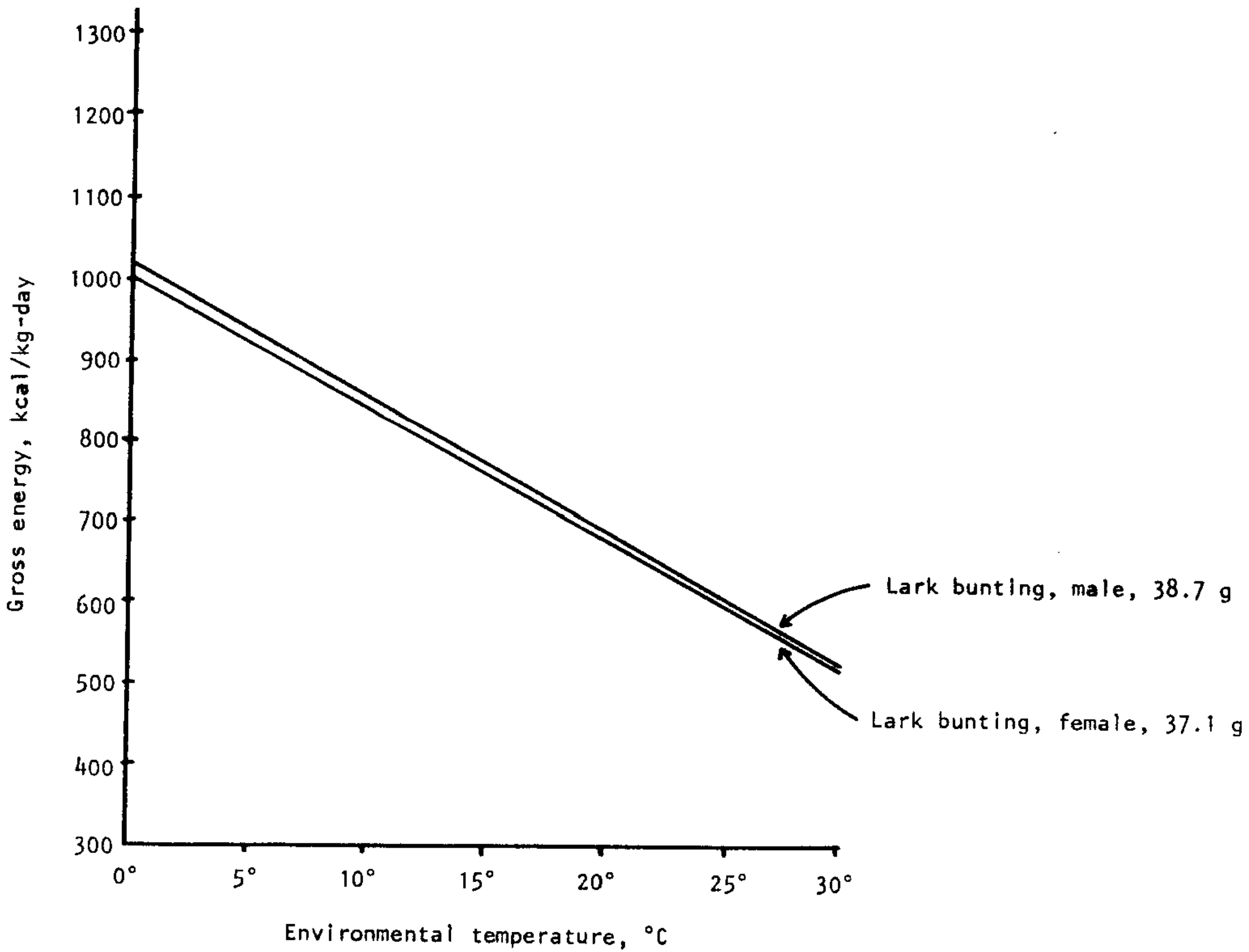

Fig. 6. Lines for gross energy requirements at constant temperatures for small birds based on Kendeigh's equations for existence energy in relation to size of bird and an assumed digestive efficiency of 80%.

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