Sampling Methods in Behavior Research

PHILIP N. LEHNER

Department of Biology, Colorado State University, Fort Collins, Colorado 80523

(Received for publication August 13, 1991)

ABSTRACT Animals perform a continuous stream of behavior throughout their lives. Because their behavior is not random, appropriate sampling methods can be used to obtain data that accurately reflect the actual behavior and are valid for answering research questions. Answering questions related to several variables assists in narrowing the choices of sampling methods. First, a determination must be made of what behaviors to measure. If the behaviors are few and easily measured, then All Occurrences Sampling is the method of choice because it generates accurate frequency and duration data through continuous recording. Sequence and Sociometric Matrix Sampling are specialized types of All Occurrences Sampling that are restricted to sampling intra- or interindividual sequences and social interactions (e.g., agonistic), respectively. Second, if who (e.g., specific individual, sex, or genotype) performs the behavior is a major component of the research question, then consideration should be given to Focal Animal (Pair, Group) Sampling. Third, if when or where the behavior is performed is of interest (e.g., activity budget), then Instantaneous or Scan Sampling can often be effective. *Ad libitum* Sampling does not produce valid data for analyses, but it is useful when formulating and fine-tuning research questions. One-Zero Sampling is not recommended except when the research question relates to the presence or absence of behaviors only. Other factors to consider in selecting a sampling method are duration of the behavior (event or state), desired scale of measurement (nominal, ordinal, interval, or ratio), and logistics (e.g., time, and equipment and facilities available).

(*Key words*: behavior, sampling methods, techniques, scale of measurement, duration)

1992 Poultry Science 71:643–649

INTRODUCTION

Animals are always behaving. From the point following conception, when movement can first be detected, until death they perform a continuous stream of behavior. It is very difficult and unnecessary to completely record major portions of the stream of behavior in order to answer research questions. Because behavior does not occur randomly, the relative frequency and duration of behaviors can be approximated through sampling. Because samples are only estimates of the true behavior (Crockett, 1991), protocols must be used for sampling that provide valid answers to particular research questions.

In the present paper a discussion of some variables to consider when first selecting a sampling method will be provided. Definitions and a brief discussion of the common behavior sampling methods, including examples of how some of these methods have been used in poultry science studies, will be given. Finally, some additional factors to consider when making a final decision on the appropriate sampling method to use will be discussed.

VARIABLES AFFECTING SAMPLING METHOD SELECTION

The appropriate sampling method will be determined, in part, by how the

research questions (Lehner, 1987) focus the study on particular variables (Table 1), regardless of whether the study is descriptive or experimental. An experiment tests a hypothesis and requires that independent variables be held constant, be allowed to vary naturally, or be manipulated and measurements made on dependent variables (behaviors). In preparing a descriptive ethogram for a species, observations and measurements of the behavior are made under the widest range of conditions for the variables in Table 1.

A question that should be addressed initially is: What behavior is of interest? Is the behavior known or unknown? A determination of the behaviors that occur under specified conditions may be desired. For example, Wood-Gush (1956) released cocks singly into a pen containing a hen and recorded all the males' movements in relation to the hen in order to determine and describe their courtship behavior. In contrast, specific behaviors, such as how ingestion, agonistic, and fearful behaviors of laying hens are affected by cage configuration may be of interest (Anderson and Adams, 1991).

Secondly, a decision must be made whether who performs the behavior is important. The individuals of interest can differ along several parameters (Table 1). Komai *et al.* (1959), for example, measured the effect of genotype on the social aggressiveness of chickens.

The third variable of concern is the temporal aspect of a study. The time when the behaviors occur in response to manipulated stimuli (i.e., treatments) or natural variation in environmental factors may be of interest. For example, Tanaka and Hurnik (1991) measured behavioral responses (e.g., eating) of hens to simulated dawn and dusk. Further, Tanaka and Hurnik (1991) determined where the hens were located spatially (e.g., on the ground, floors, or perches). In this study, they measured the behavioral responses of the hens to changes in the abiotic environment (light), just as Murphy and Wood-Gush (1978) measured the behavioral responses of chickens of two different genotypes to being placed in a strange physical environment (a sound-proof room). The role of the biotic environment was the focus of Kratzer and Craig's (1980) study of the effects of group size and density on mating behavior of cockerels.

TABLE 1. Some variables used to help select behavior sampling methods

Variable	Description and examples
What	Type of behavior (e.g., feeding, mating, and agonistic behavior)
Who	Individuals (e.g., sex, age, social rank, and genotype)
When	Temporal (e.g., season, time-of-day, pre- and posttreatment, and response latency)
Where	Spatial (e.g., geographic, in building, outside, and distance from food, water, or other individuals)
Environment	
Abiotic	(e.g., temperature, humidity, wind speed, and photoperiod)
Biotic	(e.g., animals, vegetation, and observers)

BEHAVIOR SAMPLING METHODS

Developing and implementing sampling methods that result in data that are accurate measures of the true behavior and that provide valid answers to research questions have been a concern of ethologists for decades. Altmann (1974) provided the first comprehensive review of the various behavior sampling methods and provided descriptions and recommended names and uses for seven major types of behavior sampling methods found in the literature. The Altmann (1974) paper became the standard for later discussions of behavior sampling methods (e.g., Sackett, 1978; Lehner, 1979, 1987; Altmann, 1984; Crockett, 1991).

There are eight common behavior sampling methods (Table 2). However, sampling always consists of using either Focal Animal (Pair, Group) or All Animals Sampling paired with another method (e.g., Focal Animal and All Occurrences). Some methods are used consecutively or simultaneously (Altmann, 1984; see below).

Focal Animal Sampling means that behavior is recorded for only one animal during any sample period. It is used when

TABLE 2. Behavior sampling methods

Method	Description
Focal animal (pair, group)	Restricts data recording during a sample period to one animal, pair, or group.
All animals	Data is gathered from all observable animals.
One of the above methods is paired with one of the methods below	
Ad-libitum	Opportunistic sampling with no constraints.
Continuous recording	
All occurrences	Record all occurrences of selected behavior(s).
Sequence	Sampling is restricted to all occurrences of selected intra- or interindividual sequences of behavior.
Sociometric matrix	Record results of interactions between individuals.
Time sampling	
One-zero	Record the occurrence (one) or nonoccurrence (zero) of selected behavior(s) during sequential sample intervals.
Instantaneous or scan	Record behavior of an individual (instantaneous sample) or group of individuals (scan sample) at sequential, predetermined points in time.

the primary interest is in the individual (e.g., sex, age, social rank, or genotype), or when there are too many behaviors or they are occurring too rapidly to accurately record data from several individuals. Focal Animal Sampling is considered synonymous with All Occurrences Sampling or continuous sampling by some researchers (e.g., Altmann, 1984; Crockett, 1991), but all occurrences of a behavior can be recorded from several individuals concurrently. Also, because it can be used in conjunction with All Occurrences, Sequence, One-Zero, or Instantaneous Sampling, the present author concurs with Martin and Bateson (1986) in treating Focal Animal Sampling as a separate sampling method. Focal Animal Sampling was used in Lee and Craig's (1990) study of the effects of beak trimming on the behavior of young pullets by having each of two observers record behavior from one chick at a time. Jones (1980) tested male chicks individually (Focal Animal) in his study of their reaction to eye-like shapes. Most experiments using operant conditioning are restricted to Focal Animal Sampling.

Focal Pair Sampling can be used with a mated pair, but it also refers to data collection from any, but only, two animals at a time, such as during mating, agonistic behavior (Guhl *et al.*, 1945), communication, and parent-young interactions. Focal Group Sampling is often used with social or reproductive units such as flocks or harems. For example, McBride *et al.* (1969), in their research on the behavior of feral domestic fowl, located focal groups (flocks) by hearing cocks crow in the morning.

The recording of behavior from all individuals that are observable is referred to as All Animals Sampling (Table 2). These animals are not members of the same social or reproductive group and may belong to different species. For example, a combined All Animals and Scan Sampling might be used to determine behavioral synchrony between and within captive populations of chickens, turkeys, and Japanese quail kept in separate enclosures.

Ad libitum Sampling means recording any behavior of any individuals that appears relevant to the observer. It is often used during the initial phase of descriptive studies and during reconnaissance observations (Lehner, 1979) when formulating and fine-tuning research questions. Although measures of frequency and duration are sometimes made during *Ad libitum* Sampling (Table 3), they are gathered in a haphazard manner so that they represent only pilot or first approximation data. Wood-Gush and Duncan (1976) used *Ad Libitum* Sampling in the early stages of their study of domestic fowl in the wild. They later used All

TABLE 3. Data provided by sample methods[1]

Sampling method	Frequency	Duration
Ad libitum	?[2]	?
All occurrences	X	X
Sequence	X	?
Sociometric matrix	X	
One-zero	?	X[3]
Instantaneous or scan	X[3]	X[3]

[1]An X indicates data is provided.

[2]Frequency and duration are sometimes measured during *Ad libitum* Sampling, but because these data are recorded neither randomly, nor systematically, they are not valid for experimental analyses and are of limited descriptive value. Durations are recorded sometimes during Sequence Sampling. Frequencies obtained from One-Zero samples are not true frequencies (see text).

[3]Durations measured by One-Zero Sampling and frequencies and durations measured by Instantaneous or Scan Sampling can be reasonably accurate if certain criteria are met (see text).

Occurrences Sampling to measure nest site selection, nesting, laying, and incubating in the same population (Duncan *et al.*, 1978).

All Occurrences Sampling requires the observer to make a complete record of every occurrence of one or more predetermined behaviors for every individual in the sample. This method is generally used to measure both frequencies and durations (Table 3) and is sometimes referred to as continuous sampling (Crockett, 1991) or continuous recording (Martin and Bateson, 1986). This sampling method provides the most complete and accurate data if the observer restricts the number of behaviors and animals recorded per sample period. However, if several behaviors are recorded it can be very labor-intensive (Altmann, 1984), especially if several animals are included in the sample. Guhl (1953) measured sexual behavior when five cocks were introduced daily, singly, and consecutively into a pen of hens. He recorded all occurrences of courting, treading, crouching, and avoiding.

Sequence Sampling is a type of All Occurrences Sampling that is used, primarily, to study rather predictable chains of behavior, such as courtship behavior (Guhl *et al.*, 1945) or when the order of behaviors (intra- or interindividual) is central to the research question. Because the sequence of behaviors is of primary interest, measures of duration are generally not taken(Table 3).

Sociometric Matrix Sampling is used to study interactions between individuals, which results in a matrix summarizing the interactions. It can be argued that this is a method of recording data during Focal Pair (Group) and All Occurrences Sampling. For example, winner or loser charts of agonistic interactions are often cast into an ordered matrix to reflect a dominance hierarchy (e.g., Guhl, 1956). Frequencies are generally the only data of interest with this sampling method (Table 3).

One-Zero Sampling is used to record the occurrence or nonoccurrence of behavior(s) during consecutive sample intervals. A 1 is scored for the sample interval if the behavior occurred, and a 0 is scored if it did not occur. The number of sample intervals in which the behavior occurred is sometimes referred to as a "modified frequency" (Sackett, 1978) or "Hansen frequency" (named after Hansen, 1966). However, the resultant data are not true frequencies (Table 3) but rather the numbers, or proportions, of all sample intervals in which the behavior occurred. It is generally recommended that One-Zero Sampling be avoided when possible (Altmann, 1974, 1984; Kraemer, 1979; Lehner, 1979), as it usually provides poor measures of both frequency and duration (Simpson and Simpson, 1977; Tyler, 1979; Martin and Bateson, 1986). However, if the sample intervals are short enough so that several occur during a long-duration behavior, then this method can produce reasonable estimates of duration if the sample size is large. One-Zero Sampling does allow a large number of behaviors and animals to be measured, and it generally results in high interobserver reliability (Crockett, 1991), but these positive characteristics must be weighed against the relative inaccuracy of the data. It can be a useful method if only the presence or absence of a behavior (e.g., hatchability) is important (Altmann, 1984).

Instantaneous or Scan Sampling is used to record behavior at predetermined points in time (e.g., 12-s intervals; Lee and Craig, 1990). Instantaneous Samples are taken from Focal Animals, and Scan Sampling is used for groups. Instantaneous Sampling at 8-min intervals was used by Webster and Hurnik (1991) in their

study of the behavior of laying hens. Duncan and Wood-Gush (1972) filmed displacement preening at 32 frames per second, which allowed them to analyze the film frame by frame as instantaneous samples every 1/32 s. Film analysis results in instantaneous samples at such short intervals that it is essentially continuous recording in All Occurrences Sampling. When sampling intervals are short relative to durations of behavior and the behavior occurs at high rates, then Instantaneous Sampling can provide a reasonably accurate measurement of both frequency and duration (Rhine and Ender, 1983; Martin and Bateson, 1986) (Table 3). Scan Sampling is often used to determine activity budgets and behavioral synchrony (Altmann, 1974, 1984). For example, Tanaka and Hurnik (1991) used scan sampling to determine the number of hens on the ground, three floors, the top perches, and in front of nests under simulated dawn and dusk conditions.

Combinations of sampling methods are the rule in behavior studies. As mentioned above, Focal Animal (Pair, Group) and All Animals sampling methods are always paired with another method (Table 2). For example, Focal Pair and All Occurrences Sampling was used by Webster and Hurnik (1990) in their study of the effects of movable roosts, cage mates, and genetic stock on feather pecking and climbing the cage. Jones and Mench (1991) used Focal Animal and All Occurrences Sampling in their study of the behavioral correlates of male mating success. Other combinations of sampling methods are frequently used. For example, *Ad libitum* records of unusual behaviors are generally made while using another sampling method such as Focal Animal and All Occurrences. Altmann (1984) suggested that behavioral synchrony in a group can be determined by taking scan samples at the beginning and end of Focal Animal and All Occurrences sample periods.

OTHER FACTORS TO CONSIDER WHEN SELECTING A SAMPLING METHOD

Duration of Behavior

A *state* is an ongoing, duration meaningful behavior. An *event* is a momentary behavior that happens so rapidly that it is normally recorded only as an occurrence and not a duration. Events are often changes in states, such as a hen flying (state), landing on the ground (event), and then walking (state). All the sampling methods discussed above will be effective in recording the occurrence of states (note concerns about One-Zero Sampling discussed above), but only continuous recording sampling methods (All Occurrences, Sequence, and Sociometric Matrix) will effectively sample events. If durations of the state are important, then All Occurrences Sampling is the method of choice (Table 3), but Instantaneous or Scan Sampling can be used effectively if certain criteria are met (see above).

Scale of Measurement

The four scales of measurement are: nominal, ordinal, interval, and ratio. Nominal data are counts (frequencies) of occurrences of behaviors that differ qualitatively. For example, seven waltzes, ten feather-rufflings, and twelve wing-flaps might be recorded during a Focal Animal and All Occurrences sample of a cock's agonistic behavior. Ordinal data are ranked by a common qualitative property. The relative order of the ranks is known, but the distance between them is neither continuous nor known. For example, the waltzes (above) could be ranked as of high, medium, or low intensity, which would have provided higher resolution data than merely recording its occurrence (nominal data), but the ranks still would not contain a continuous measure with known intervals. Interval data is the same as ordinal data except that the differences between the categories are of known and uniform distances, which allow additivity. However, the zero point is unknown or has been arbitrarily assigned (e.g., 0 C was arbitrarily assigned and is not the absence of temperature). Latencies to respond to a stimulus are, for example, interval data because the uniform, continuous measurement of time is used, but the arbitrary zero point of stimulus onset is assigned because the point in time when the animal first perceives the stimulus is unknown. Ratio data are the same as interval data except that there is a

known, true zero point (absence of the factor being measured). Recording the duration of a feeding bout and the spatial distance between members of a flock are both examples of the use of the ratio scale of measurement.

The scale of measurement is important because it imposes some restrictions on the use of statistical tests. Parametric tests, which are more powerful than nonparametric tests (when both are applicable; Zar, 1984), should be restricted to interval and ratio data (e.g., Drew and Hardman, 1985) because these data meet the criteria of continuous measures with equal intervals. However, these criteria are sometimes disregarded and nominal or ordinal data are transformed (e.g., arc-sine) to meet the other two criteria of normal distribution and homogeneity of variance. The present author prefers the conservative approach and recommends that only the less powerful, nonparametric statistical tests be used with nominal and ordinal data (Lehner, 1979). The capacity to accurately use the various scales of measurement and generate accurate nominal (e.g., frequencies) or ratio (e.g., durations) data differs between the sampling methods (Table 3).

Logistics

Unfortunately, the selection of a sampling method is also determined, in part, by logistics. This includes time constraints on the availability of facilities, subjects, treatments, observers, and equipment. The type and quality of recording equipment, as well as the ability and experience of observers, will help dictate the resolution and amount of data that can be obtained. Within these constraints, and those mentioned above, the rule to follow is: select the sampling method that will provide only enough accurate and valid data of sufficient resolution to answer your research question. The proper sampling method is both effective and efficient.

REFERENCES

Altmann, J., 1974. Observational study of behaviour. Behaviour 49:227–267.

Altmann, J., 1984. Observational sampling methods for insect behavioral ecology. Florida Entomol. 67: 50–56.

Crockett, C., 1991. Data collection in the zoo setting. *In*: Wild Mammals in Captivity. S. Lumpkin and Kleiman, ed. University of Chicago Press, Chicago, IL (in press).

Drew, C. J., and M. L. Hardman, 1985. Designing and Conducting Behavioral Research. Pergamon Press, New York, NY.

Duncan, I.J.H., C. J. Savory, and D.G.M. Wood-Gush, 1978. Observations on the reproductive behaviour of domestic fowl in the wild. Appl. Anim. Ethol. 4:29–42.

Duncan, I.J.H., and D.G.M. Wood-Gush, 1972. Analysis of displacement preening in the domestic fowl. Anim. Behav. 20:68–71.

Guhl, A. M., 1953. Social behavior of the domestic fowl. Technical Bulletin Kansas Agricultural Experiment Station 73:3–48.

Guhl, A. M., 1956. The social order of chickens. Sci. Am. 194:42–46.

Guhl, A. M., N. E. Collias, and W. C. Allee, 1945. Mating behavior and the social hierarchy in small flocks of white leghorns. Physiol. Zool. 18: 365–390.

Hansen, E. W., 1966. The development of maternal and infant behavior in the rhesus monkey. Behaviour 27:107–149.

Jones, M.E.J., and J. A. Mench, 1991. Behavioral correlates of male mating success in a multisire flock as determined by DNA fingerprinting. Poultry Sci. 70:1493–1498.

Jones, R. B., 1980. Reactions of male domestic chicks to two-dimensional eye-like shapes. Anim. Behav. 28:212–218.

Komai, T., J. V. Craig, and J. Wearden, 1959. Heritability and repeatability of social aggressiveness in the domestic chicken. Poultry Sci. 38:356–359.

Kraemer, H. C., 1979. One-zero sampling in the study of primate behavior. Primates 20:2237–2244.

Kratzer, D. D., and J. V. Craig, 1980. Mating behavior of cockerels: effects of social status, group size and group density. Appl. Anim. Ethol. 6:49–62.

Lee, H. Y., and J. V. Craig, 1990. Beak-trimming effects on the behavior and weight gain of floor-reared, egg-strain pullets from three genetic stocks during the rearing period. Poultry Sci. 69: 568–575.

Lehner, P. N., 1979. Handbook of Ethological Methods. Garland STPM Press, New York, NY.

Lehner, P. N., 1987. Design and execution of animal behavior research: An overview. J. Anim. Sci. 65: 1213–1219.

Martin, P., and P. Bateson, 1986. Measuring Behaviour: An Introductory Guide. Cambridge University Press, Cambridge, England.

McBride, G., I. P. Parer, and F. Foenander, 1969. The social organization and behaviour of the feral domestic fowl. Anim. Behav. Monogr. 2:127–181.

Murphy, L. B., and D.G.M. Wood-Gush, 1978. The interpretation of the behavior of domestic fowl in strange environments. Biol. Behav. 3:39–61.

Rhine, R. J., and P. B. Ender, 1983. Comparability of methods used in the sampling of primate behavior. Am. J. Primatol. 5:1–15.

Sackett, G. P., 1978. Observing Behavior, Vol. II: Data Collection and Analysis Methods. University Park Press, Baltimore, MD.

Simpson, M.J.A., and A. E. Simpson, 1977. One-zero and scan methods for sampling behavior. Anim.

Behav. 25:726–731.
Tanaka, T., and J. F. Hurnik, 1991. Behavioral responses of hens to simulated dawn and dusk periods. Poultry Sci. 70:483–488.
Tyler, S., 1979. Time-sampling: A matter of convention. Anim. Behav. 27:801–810.
Webster, A. B., and J. F. Hurnik, 1990. Behavior, production, and well-being of the laying hen. 1. Effects of movable roosts, relationship of cage mates, and genetic stock. Poultry Sci. 69: 2118–2127.
Webster, A. B., and J. F. Hurnik, 1991. Behavior, production, and well-being of the laying hen. 2. Individual variation and relationships of behavior to production and physical condition. Poultry Sci. 70:421–428.
Wood-Gush, D.G.M., 1956. The agonistic and courtship behaviour of the brown leghorn cock. Br. J. Anim. Behav. 4:133–142.
Wood-Gush, D.G.M., and I.J.H. Duncan, 1976. Some behavioural observations on domestic fowl in the wild. Appl. Anim. Ethol. 2:255–260.
Zar, J. H., 1984. Biostatistical Analysis. 2nd ed. Prentice-Hall, Inc., Englewood Cliffs, NJ.