WILDLIFE TOXICOLOGY and POPULATION MODELING

Integrated Studies of Agroecosystems

Edited by

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Statistical Approaches to Data Analysis in Wildlife Ecotoxicology

Michael R. Willig

ABSTRACT

The use of statistics in avian wildlife toxicology is mandated for a variety of reasons associated with identifying sources of variation and isolating the effects related to various treatment factors. Experimental design is essentially the adjustment of data collection methodologies to parallel available analytical techniques that provide resolution to hypotheses of biological interest. Increased focus on the use of *a priori* contrasts and the selection of *a posteriori* comparisons will increase the power of statistical analyses. Bayesian approaches to statistical analysis may become more common in ecotoxicological studies when true replication is not possible or the decision to terminate data collection is predicated upon the data already collected in an ongoing experiment. Multivariate statistical analyses can provide powerful approaches for evaluating hypotheses when they are applied with care, but their use as exploratory analyses has been neglected, especially in relation to construction of subsequent causal hypotheses for evaluation. Future research should examine attributes reflecting changes in the growth rate of populations, or parameters (e.g., age-specific survivorship and age-specific fertility) from which estimated population-level effects can be deduced with facility or be projected by modeling. Given the complexity of ecological systems and their response to stressors, rapid advancement in the field of avian wildlife toxicology will be predicated upon close cooperation between ecologists, biostatisticians, and environmental toxicologists.

KEY WORDS

multivariate statistics, Bayesian statistics, experimental design, population biology, ecological statistics

INTRODUCTION

The domain of topics considered by statistical approaches to data analysis precluded either a broad or comprehensive approach to the subject by the previous four chapters.[1-4] Rather than review or integrate the contents of those four contributions, the primary focus of this chapter is on useful quantitative approaches to wildlife ecotoxicological research which were not considered previously, and on previously considered quantitative approaches from a different perspective.

The use of statistical methodologies in avian toxicological studies is mandated for a variety of reasons associated with inherent sources of natural variation. In addition to responses to different levels of treatment factors, a variety of other sources of variation exist and impinge upon experimental results. In essence, the goal of experimental design and statistics is to identify sources of variation among groups and to distinguish between differences associated with treatment levels per se and those caused by other agents. Extraneous sources of variation should be experimentally controlled or applied to all levels of the treatments in an unbiased fashion. The ultimate goal in designing an experiment should revolve around the resolution of hypotheses. A single field experiment does not always address all hypotheses with equal power. As a result, one should be sure to identify critical questions and assign priority to their resolution before attempting to address peripheral questions within a single experimental design. In essence, one can view experimental design as the adjustment of data collection methodologies to parallel available analytical techniques that provide resolution to hypotheses of biological interest.

PARAMETER ESTIMATION

Measure of the uptake of biocides in target tissues is often required in ecotoxicological studies. Chemical assays frequently result in observations at or below the detection limits of the analytical methodologies. This results in data that are effectively left censored (i.e., data representing observations less than the mean are modified or deleted). Such left censored data often are used in descriptive and inferential statistical analyses after a constant (50% of the detection limit) has been substituted for points at the detection limits; alternatively, the observations below detection limits are excluded from consideration. Both approaches result in statistical biases in parameter estimations (means and variances). Recent work[5,6] suggests promise in the use of maximum likelihood estimation and regression of expected order statistics to circumvent biases in parameter estimation. More accurate parameter estimates are of interest in their own right because increased accuracy will enhance the predictive value of ecological models in which they are incorporated. Although the best statistical design and analysis may be performed on the correlates of fitness for a variety of wildlife taxa exposed to biocides, it is not axiomatic that individual effects will be translated into a demographic response by the population. Increased focus on population-level parameters should constitute a goal of wildlife toxicologists and regulatory agencies.

UNIVARIATE APPROACHES

In circumstances in which a particular dependent variable is of inherent interest, univariate analyses are clearly appropriate. In many experimental designs analysis of variance (ANOVA) is the most powerful approach for detecting group differences, but heteroscedasticity (unequal group variances) prevents its application with confidence. Transformations may be employed to produce homoscedasticity, but unless an effective transformation was selected in advance, levels of probability are only approximate. Even in situations in which ANOVA is applied, it may have less statistical power than recently developed alternatives. Rice and Gaines[7] describe a novel approach to analyzing data when variances are unequal; it circumvents these problems by evaluating a modified F statistic, which may be conceptualized as a weighted average of the Behrens-Fisher t statistic.[8] The approach is more powerful than ANOVA because it does not inflate small variance components in creating a pooled within-group mean square.[7]

A second limitation of the ANOVA approach is that it is a two-tailed test of mean differences. In certain ecotoxicological situations, such as those involving biocide dose response, ordered differences clearly constitute the alternative hypothesis (e.g., control <0.50 biocide application <0.75 biocide application). The application of ANOVA in this scenario results in a considerable loss of power, a situation straightforwardly analogous to conducting a two-tailed t test to evaluate a one-tailed hypothesis. Isotonic regression techniques, applicable to both parametric and nonparametric situations, are powerful alternatives to classical ANOVA approaches.[9] Operational difficulties of its application, especially when sample sizes are unequal, can be overcome through simulation to produce p values via microcomputer.[10]

A third limitation of ANOVA is that it does not identify which levels of a treatment factor differ; this facet of the analysis is relegated to the field of *a priori* (planned) and *a posteriori* (unplanned) comparisons.[11] Even though considerable thought should precede the design of experiments, *a priori* comparisons are not frequently conducted in the ecotoxicological literature, perhaps in part because *a posteriori* comparisons seem to address the same questions. This is unfortunate because *a priori* comparisons are more powerful than the ANOVA itself, whereas *a posteriori* comparisons are less powerful than the ANOVA. Two kinds of *a priori* comparisons may be of special interest in ecotoxicological research: those involving trend analysis and those in which a number of treatments are each compared to a single control. Situations in which different doses of a biocide are applied to agricultural fields are amenable to planned comparisons that evaluate if a parallel trend occurs in the dependent variable or else response thresholds exist.[12,13] In addition to the parametric techniques, nonparametric techniques are presented by Hollander and Wolfe [14] for similar questions that do not require interval scale values for the levels of the variables of interest.

In cases similar to those in which the effect of different biocides on acetylcholinesterase activity is being compared to levels in a control, nonorthogonal *a priori* contrasts would be appropriate. In these situations, the level of acetylcholinesterase in each biocide treatment is compared to the level in a control most effectively by the Dunn-Sidak test,[15] although some caution should be employed.[13]

In situations in which all possible comparisons between treatment levels are of interest, researchers provide little (if any) rationale for the selection of a particular *a posteriori* technique, making it difficult to judge the appropriateness of the selection. In an excellent review of unplanned comparisons, Day and Quinn[16] provide a detailed consideration of a large suite of statistical techniques and promote a strategy for their use in evaluating hypotheses. When making all possible pairwise comparisons, they in general conclude that the parametric Ryan's Q test or the nonparametric Joint-Rank Ryan's test should be used to evaluate group differences. They did not recommend the commonly used Duncan's multiple range test or the Student-Newman-Keuls (SNK) procedure, although they did consider Welsch's Step-Up procedure to be only slightly less powerful than Ryan's Q test.

BAYESIAN STATISTICS

Bayesian approaches to statistical analysis may be more appropriate than classical approaches in many ecotoxicological scenarios. This is especially true for situations in which true replication (*sensu* Reference 17) cannot occur for a variety of reasons (cost, agricultural practices, etc.) or for protocols in which the size of the final sample is at least in part determined by the kind of response reflected in data collected during an experiment.[18] In the latter case, probability levels from standard statistical tests may be misleading and biased toward overestimating the "significance" of differences among levels of treatment factors.[19,20] In the former case, parallels exist between studies of large-scale perturbations in ecology and studies in wildlife ecotoxicology. In particular, monitoring the response of nontarget species to the application of biocides in agricultural fields is expensive and often not easily replicated; if replicated, site selection frequently is not random within the inference space of interest to regulatory agencies. Nonetheless, unreplicated experiments such as these can be extremely informative[21,22] when analyses are pursued from a Bayesian perspective.[23,24]

Bayesian statistics analyze data to produce final probabilities (posterior probabilities) that are independent of the probabilities of data not obtained in the experiment (i.e., the probabilities of outcomes more extreme than those obtained in the experiment are not considered and do not exaggerate the strength of evidence against a null hypothesis). However, adherents to the Bayesian school of statistics, or subjectivists, do require the specification of the probability of truth of the hypothesis before, or at least separate from, the data generated to evaluate the question of interest. These prior probabilities must be subjectively chosen by the investigator and ought to reflect the biological insight of the investigator. The elegance of the approach is that the interpretation of data is formally related to the consumer's *a priori* view of the truth of the hypothesis, and this prior view must be specified for all to see. For the Bayesian statistician, defining the subjectivity of any test is an important element in understanding data analysis.

MULTIVARIATE APPROACHES

Multivariate analyses of data have become increasingly common in the ecological literature since their early application to questions in phytoecology[25] and numerical taxonomy[26] over three decades ago. Many comprehensive summaries of their application have appeared in the intervening years, with James and McCulloch[27] most recently classifying the plethora of techniques available, summarizing their objectives and limitations, and providing useful guidelines for their incorporation in ecological studies. All too often, multivariate inferences are made without due consideration to sampling design and methodological assumptions. Moreover, the utility of multivariate data analysis as an exploratory method that can suggest descriptive models or future hypotheses has been neglected in the ecological literature, in general, and ecotoxicological studies, in particular. Observations and previous experience with a system can directly lead to insights about cause and effect relationships and are the bases of the hypothetico-deductive method (Figure 1). When straightforward patterns are not easily discerned because multiple factors affect relationships among variables, multivariate approaches can be used as exploratory or heuristic devices capable of suggesting descriptive models that can, in turn, provide a framework for a causal model. In either case, controlled experiments in the field or laboratory or quasiexperimental designs involving time-series analysis or repeated measures can subsequently be evaluated by multivariable techniques.[27,28]

It is difficult, if not impossible, to provide effective directions for avoiding the "Scylla of oversimplification" that occurs when complex relationships among intercorrelated characters are evaluated via univariate techniques without risking exposure to the "Charybdis of assuming that patterns in data necessarily reflect factors in nature, that they have a common cause, or worse, that statistical methods alone have sorted

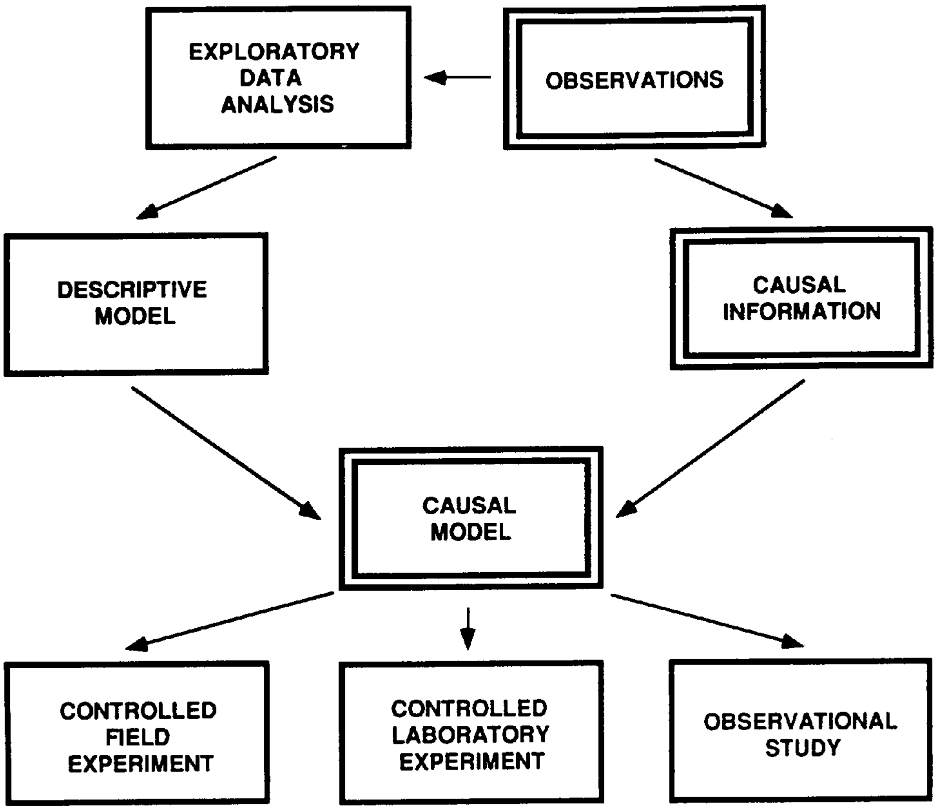

FIGURE 1. Research protocol emphasizing the role of multivariate statistics (applicable to issues surrounded by single boxes) in the formulation of models and in the evaluation of hypotheses.[27,28] Observation can directly yield insight into the cause and effect relationship between factors which then forms the basis for formulation of hypotheses in the hypothetico-deductive method (indicated by the linked double boxes). At other times, observations may be complex or confounded by a variety of variables, reducing the likelihood that inference of causal relationships will be directly obtained. In such circumstances, multivariate data analysis can act as a heuristic device to explore relationships among factors. These multivariate exploratory analyses produce a descriptive model which then gives rise to a causal model (indicated by the single boxes linking observations and causal model). Regardless of the nature of the study (field or laboratory), multivariate statistics may be used to evaluate specific hypotheses (controlled experiments) or detect patterns in the analysis of trends (observational studies).

out multiple causes" for the observed patterns.[27] Perhaps the best that can be expected is that the knowledge that both pitfalls exist provides a navigational tool for avoiding their consequences.

DEMOGRAPHIC INTERFACES

Effects at the level of the individual may be immediate or delayed, and may or may not have an effect upon population-level processes. Although the best statistical design and analysis may be performed on the correlates of fitness for a variety of wildlife taxa exposed to biocides, it is not axiomatic that individual effects will be translated into a demographic response by the population. Indeed, if the major concern surrounding the use of biocides is their effects on population parameters, then the research focus should examine attributes that can reflect changes in the growth rate of populations, or the parameters (e.g., age-specific survivorship and age-specific fertility) from which estimated population-level effects can be deduced with facility or be projected by modeling. The impact of biocides on population-level processes should receive consideration as an important component in regulatory decisions by governmental agencies.

It is well known, for example, that limited removal of individuals via hunting can have little or no effect on subsequent population levels. Moreover, the local effect of removing individuals from a population can be mediated by the immigration of individuals from surrounding habitats, resulting in the absence of a net change in the density of individuals over the long term. These same lessons from wildlife management can be applied to ecotoxicological research examining the effects of biocides on nontarget species rather than the impact of hunting on game animals. The extent to which ecological filters such as these operate will confound extrapolations from individual-level effects to those at the population level. Even the size and proximity of habitats surrounding agricultural fields can have an effect on population-level effects within the area targeted for biocide treatment.

Answers concerning the effects of biocides on wildlife populations cannot be obtained from a universally applicable general design. Only a clear statement of the biological question and a delineation of the level at which particular effects are to be manifested can yield a successful research design. The fields of ecology and wildlife management provide the general principles upon which effects can be predicted and the fields of statistics and modeling can provide a variety of technical approaches to analyzing data. It seems clear, given the complexity of ecological systems and their responses to stressors, that rapid advancement in the field of avian toxicology will depend upon close cooperation between ecologists, biostatisticians, and environmental toxicologists.

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