NRES_798_5_201501

Experimental design
from a statistical perspective

Landscape of statistical methods

Quantile regression models (qr)

General linear models (lm)

Generalized linear models (glm)

Generalized linear mixed models (glmm)

Generalized (non)linear least squares models (gls/gnls)

Generalized additive models (gam)

Mixed effects models (lme)

Nonlinear least squares models (nls)

Generalized additive mixed models (gamm)

Tree models (cart)

Landscape of experimental design

		Independent variable	
		Continuous	Categorical
Dependent variable	Continuous	Regression	ANOVA
	Categorical	Logistic regression	Tabular

Experimental design and analysis

1. What defines the landscape
   – Variable type, treatments, independence (experimental design)
2. Where various statistical tests fall within the landscape
   – Where should statistical trade-offs be made in experimental design, and how will this differ between systems

What defines the landscape?

- Objective and model need to be clearly stated
  - What is the point of the study

  - What is the relevant spatial and temporal scale?
  - What is an “event”?
  - What is the “sample space”?
  - What is the response variable(s)?

Clear statement of the objective of the study

Occurrence
Composition
Density
Rate

Is there spatial or temporal differences in variable Y? — Pattern

What is the effect of factor X on variable Y? — Process

What is the best estimate of parameter θ in Model Z? — Mechanism

1. Ecological hypothesis not established as falsifiable predictions
2. Predictions from a hypothesis not unique
   (same result via different process or mechanism)

3. Traditional ecological experiments (e.g. ANOVA) often not well suited to estimating model parameters

Ecological Field Studies

- Specific objectives
- Study design
- Study execution
- Statistical analysis
- Interpretation of results and conclusions
- Update and retesting the biological model
  - Cycle continues

Ecological Field Studies

- Specific objectives
- Study design
  - Experimental field studies
  - Natural experiments
  - Observational field studies
- Study execution
- Statistical analysis
- Interpretation of results and conclusions
- Update and retesting the biological model
  - Cycle continues

Experimental field studies

- Studies that correspond to the classical experimental framework require:
  - Control, randomization, and replication for experimental unities applied to "treatment" conditions
    - How do ecological experimental studies differ from the "classical" experimental framework?
  - Blocking may be used to increase precision without increasing the number of replicates

What are examples of experimental field studies?

Experimental field studies

- Challenges of manipulative field experiments
  1. Ability to conduct experiments on a sufficiently large scale
     - Large scale experiments often sacrifice replication
     - Scaling from small experiments to large scale inference is often difficult

ELA

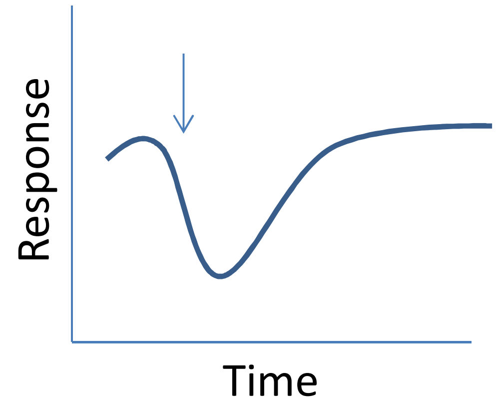
  2. Field experiments often restricted to smaller organisms

  3. Manipulation of only one variable in the field, while keeping other factors constant is challenging

Sevilleta LTER

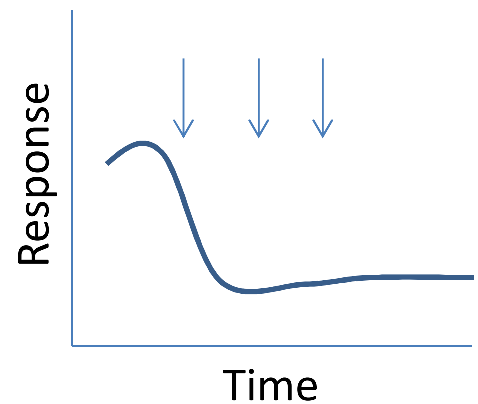
Experimental field studies

- Press experiments
  - Measures resistance of the system to experimental treatments
  - E.g. Nitrogen addition



- Pulse experiments
  - Measures the resilience of the system to experimental treatments
  - E.g. rain shelters, carbon increase

Cedar Creek LTER
CO2 increase



Natural experiments

- Use natural variation instead of manipulation to test X impact on Y
  - Very hard to have only one variable altered in natural experiments
  - Need to include covariates

What are examples of natural experiment?

Natural experiments

- Snapshot experiments
  - Replicated in space
  - E.g. 20 plots sampled during a day/year
  - Benefit
    - Rapid, independence of spatial replicates can be more robust

- Trajectory experiments
  - Replicated in time
  - E.g. 2 plots samples yearly over 20 years
  - Independence of samples needs to be considered (whale vs. daphnia population)
  - Benefit
    - Covariate often assumed to remain constant
    - Structure corresponds to how many ecological models are formulated (e.g. population growth)

Observational field studies

- Many studies don't fall into the true experimental framework:
  - Inventory
  - Monitoring
  - Habitat Studies
  - Other

No perturbation (experimental or natural)

Rely on natural variation in dependent and independent variables

- "Quasi" experiments may meet some experimental requirements, but not all

Examples of field studies, experiment or not

- E.g. Breach of tailing pond
- Knapp et al. 2001:
  - impact of trout introduction to lakes in the Sierra Nevada
  - Compared invertebrate communities in naturally fishless lakes, stocked lakes , and lakes that were formally stocked with fish

Experimental field studies

- Classical experimental framework allows stronger inferences than most observational studies (cause and effect)
- However, in ecology there are often severe practical limitation to carrying out true experiments at the appropriate spatial and temporal scales

Experimental design (classic)

- Treatment (sample space)
  - Set of conditions that are of interest to the scientist
- Experimental Unit (event)
  - What the treatments are applied to
  - Area of land in region under study
  - Picking the right size for this unit can be very important
- Measurement (response variable)
  - Plant/animal response monitored over time

Experimental design principles

- Treatment, experimental unit, measure

- Control
- Randomization (validity)
- Replication (precision
- Blocking

- R.A. Fisher, 1935, “Experimental Design”

Experimental design principles

- Control
  - Ideally, except for random variation, the only difference between experimental units is the treatment effect.

  - In many experiments a “control” treatment will be used (include only mechanics of treatments)

E.g. Animal movement/treatment prior to experiment

Experimental design principles

- Randomization (validity)
  - We need randomisation in the assignment of treatments to experimental units so that no unintended bias is incorporated into how assignments are made

Experimental design principles

- Randomization
  - Confounding factors
    - Systematic spatial or temporal variation that is extraneous to the focal treatments
  - Randomization minimizes the confounding of treatments with **unknown** or **unmeasured** variables in the study area
  - Measurement of covariables is not a substitution for randomization and replication

Experimental design principles

- Replication (precision)
  - Replicate experimental units are needed of each treatment to allow us to estimate the inherent stochasticity (variation) in our data
  - The more replicate the better precision (but possibly not accuracy if systematic uncertainty induces bias)

Experimental design principles

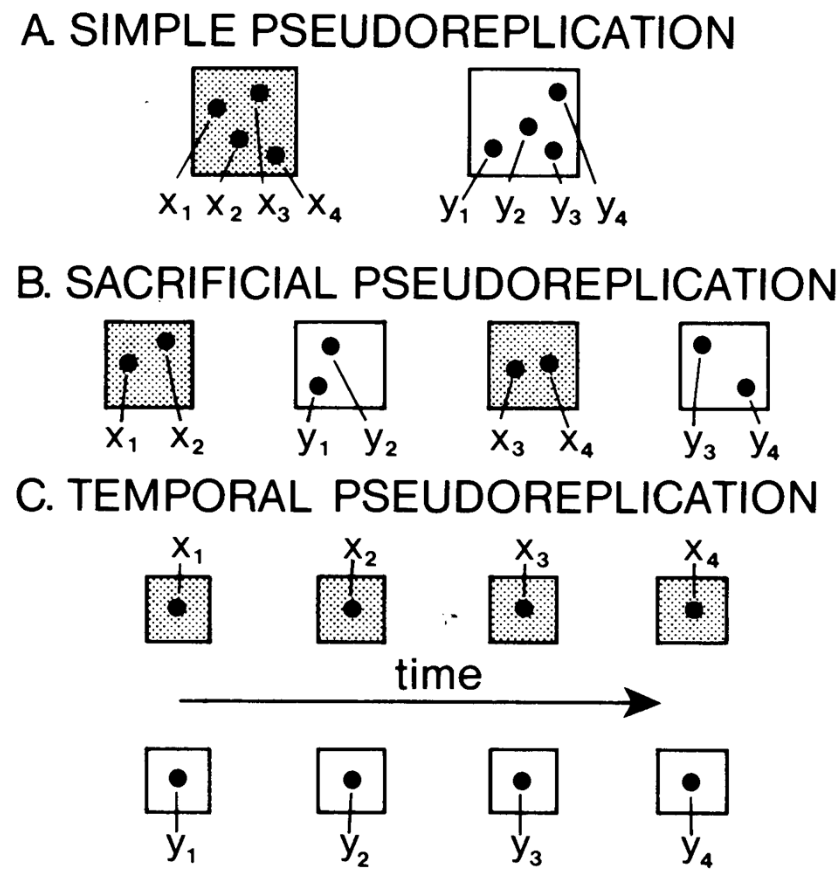
- Replication
  - Dependent on ecological and statistical effect size
  - Contingent on time and money to replicate treatments
  - Rule of 10
    - 10 replicate observations for each treatment level
    - E.g. 250 replicates feasible
      - 5 species, 5 treatments/species, 10 replicate
    - Generally applicable to small scale manipulation studies

Pseudoreplication and the Design of Ecological Field Experiments

Stuart H. Hurlbert

Ecological Monographs, Volume 54, Issue 2 (Jun., 1984), 187-211.

Pseudoreplication



Experimental design principles

- Replication in large experiments
  - Replication not possible (cost, time, scale)

BACI (Before, After, Control, Impact)

	Before	After
Control	1	1
Treatment	1	1

Experimental design principles

- Replication
  - Ensure sufficient space, time between samples
  - Sufficiently separate to ensure independence
    - Process dependent
  - Sufficiently close to occur in comparable environments
    - Landscape heterogeneity

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Confounding elements and how they can be dealt with

TABLE 1. Potential sources of confusion in an experiment and means for minimizing their effect.

Source of confusion	Features of an experimental design that reduce or eliminate confusion
1. Temporal change	Control treatments
2. Procedure effects	Control treatments
3. Experimenter bias	Randomized assignment of experimental units to treatments Randomization in conduct of other procedures "Blind" procedures*
4. Experimenter-generated variability (random error)	Replication of treatments
5. Initial or inherent variability among experimental units	Replication of treatments Interspersion of treatments Concomitant observations
6. Nondemonic intrusion†	Replication of treatments Interspersion of treatments
7. Demonic intrusion	Eternal vigilance, exorcism, human sacrifices, etc.

* Usually employed only where measurement involves a large subjective element.

† Nondemonic intrusion is defined as the impingement of chance events on an experiment in progress.

Experimental design principles

- Blocking
  - Not essential but blocking can improve precision by reducing unexplained variation
    - partitioning variance through experimental design
    - Equivalent methods can be used to estimate observer bias, instrument bias, etc.
  - Usually each treatment occurs only once in each block (e.g. randomized complete block)
  - Blocks are constructed to be homogeneous within, but may be very different from each other

Experimental design principles

- Complete random design
  - Treatments allocated randomly to experimental units (with aim to have equal number of replicates per treatment)
- Randomized complete block design
  - Treatments randomly allocated to experimental units within homogeneous blocks (usually one replicate per block)

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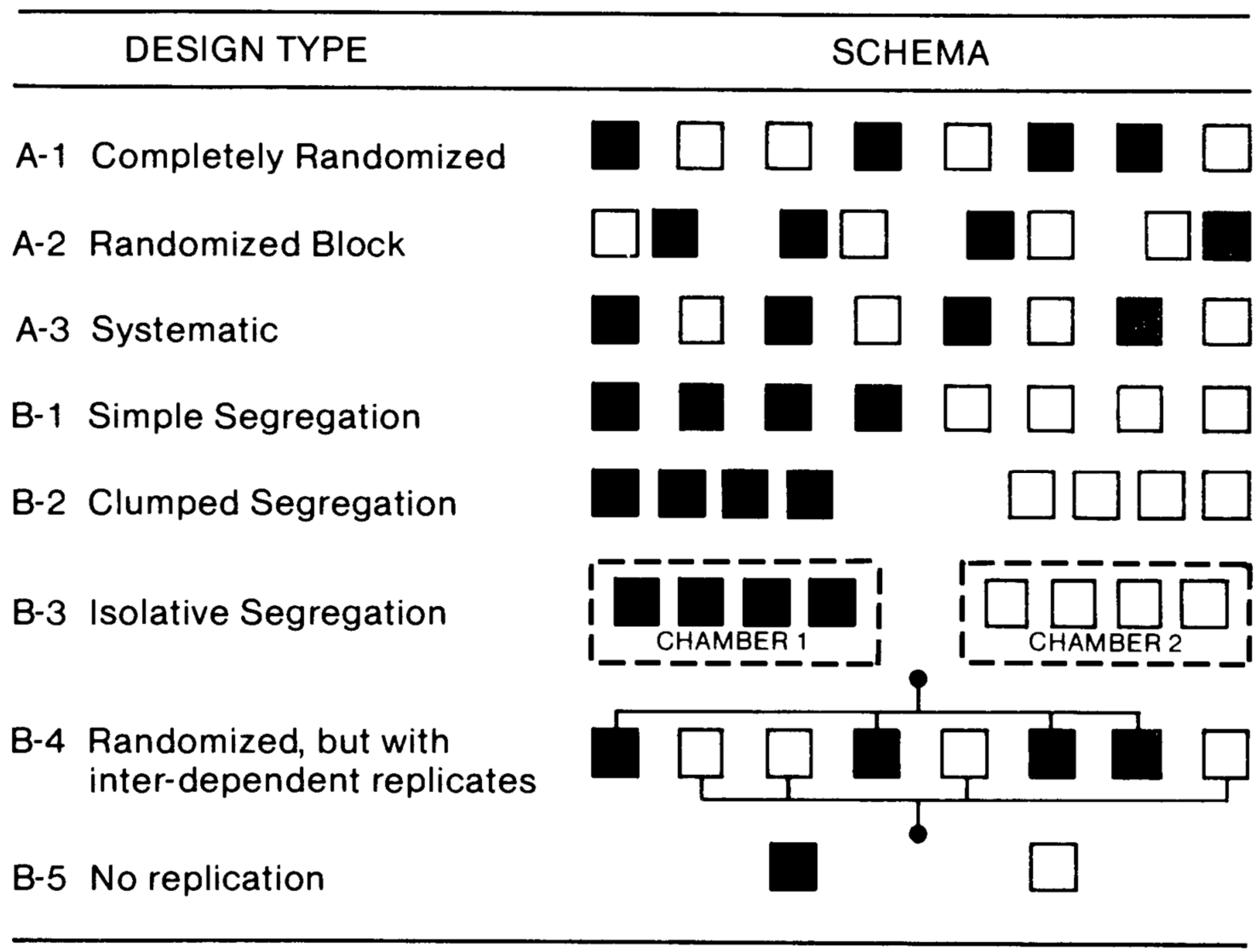

Essential questions to ask when designing experiments

- Designing effective field studies
  - Are plots large enough to obtain realistic results?
  - Is the grain and extent of the study appropriate for the scientific inference we want to make?
  - Does the range of treatments span the range of possible environmental conditions?
  - Have appropriate controls been established (impact of treatment can be identified)?
  - Is replication and randomization used appropriately
  - Have all replicates been manipulated in the same way (controls aren't confounded)?
  - Have appropriate covariates been measured in each replicate?

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