Lecture 7

Problems in Running True Expts

- Expt Units too small for biological realism
- Randomisation not possible
- Replication not done or not possible

Pseudoreplication and Hurlbert's paper

Pseudoreplication and ANOVA examples

Introduction

- Studies without randomisation
- Studies without replication
- Studies without both.

Studies Without Randomization of Treatments

Walleye Regulation Change Study- good replication possible but no randomisation possible. Mentioned in first lecture

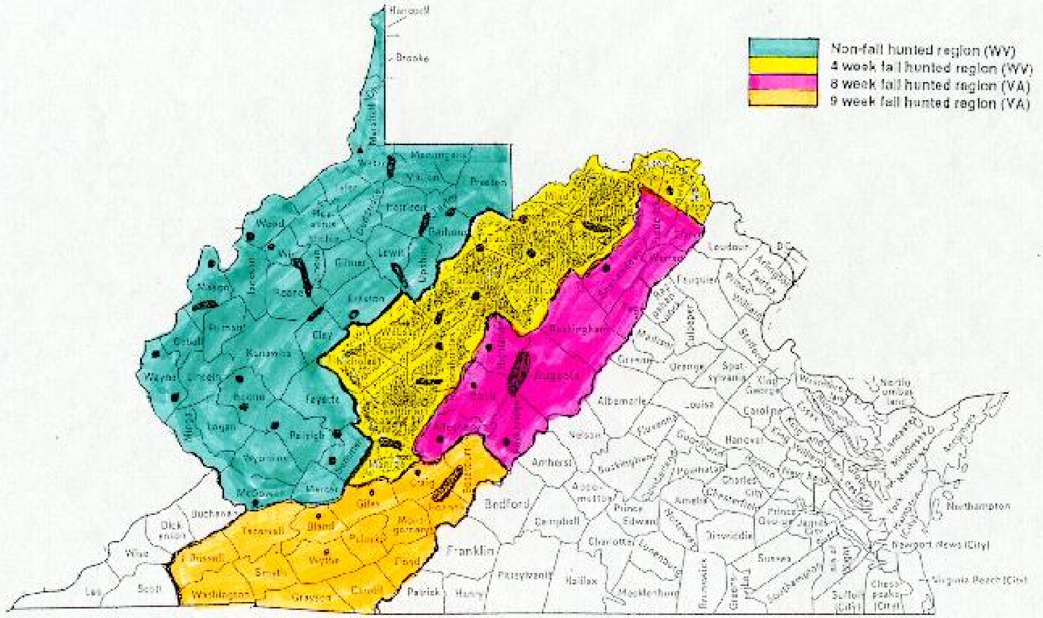
Have you found any studies like this?

We will talk much more about approaches for handling this problem in another lecture or two.

Studies Without Replication of Treatments

- Fish tank study lecture 1-poor design
- Turkey study lecture 1-large scale made it difficult if not impossible. So it was a purely observational study with no replication or randomisation.

ASSESSMENT OF FALL HUNTING IMPACT ON SURVIVAL OF WILD TURKEYS IN THE VIRGINIAS USING RADIO-TELEMETRY SURVIVAL ANALYSIS



Map : Spatial replicates for the four treatments in the states of West Virginia and Virginia

- Have you found any studies like this with no replication?

Background to Hurlbert's Classic Paper and Pseudoreplication

- **"No one would now dream of testing the response to a treatment by comparing two plots, one treated and the other control." Fisher and Wishart (1930).**
- **"field experiments in ecology usually have no replication, or have so few replicates as to have very little sensitivity." Eberhardt (1978).**

Background to Hurlbert's Paper

- Measurative Experiment- the making of measurements at one or more points in space or time.
- Manipulative Experiment -is what I have called an experiment.This is the main focus of the paper.

Manipulative Experiments

Key Features

- Control
- Randomisation
- Replication
- Blocking (Optional but great for increasing precision)

Sources of Confusion in Experiments (Table 1)

With Suggested Solutions!

- Temporal Change

  Control treatments

- Procedural Effects

  Control treatments

- Experimenter Bias

  Randomization

  "Blind Procedures" if Subjective measurement

Sources of Confusion in Experiments (Table 1)

- Experimenter generated variability

    Replication

- Inherent variability among exptl units

  Replication

  Interspersion

  Use of Covariates

- Non Demonic Intrusion (Chance events- expt in progress)

  Replication

  Interspersion

- Demonic Intrusion

  Exorcism (☺) (☺)

Your Critiques/Proposal

Related to this discussion.

Where have you found things most confusing in papers you have read and in your own proposal?

Interspersion of Treatments

- Statisticians emphasize importance of randomization
- Hurlbert concern is with randomization methods not always giving good interspersion of treatments
- Examples given now from paper

Some Design Types Illustrated

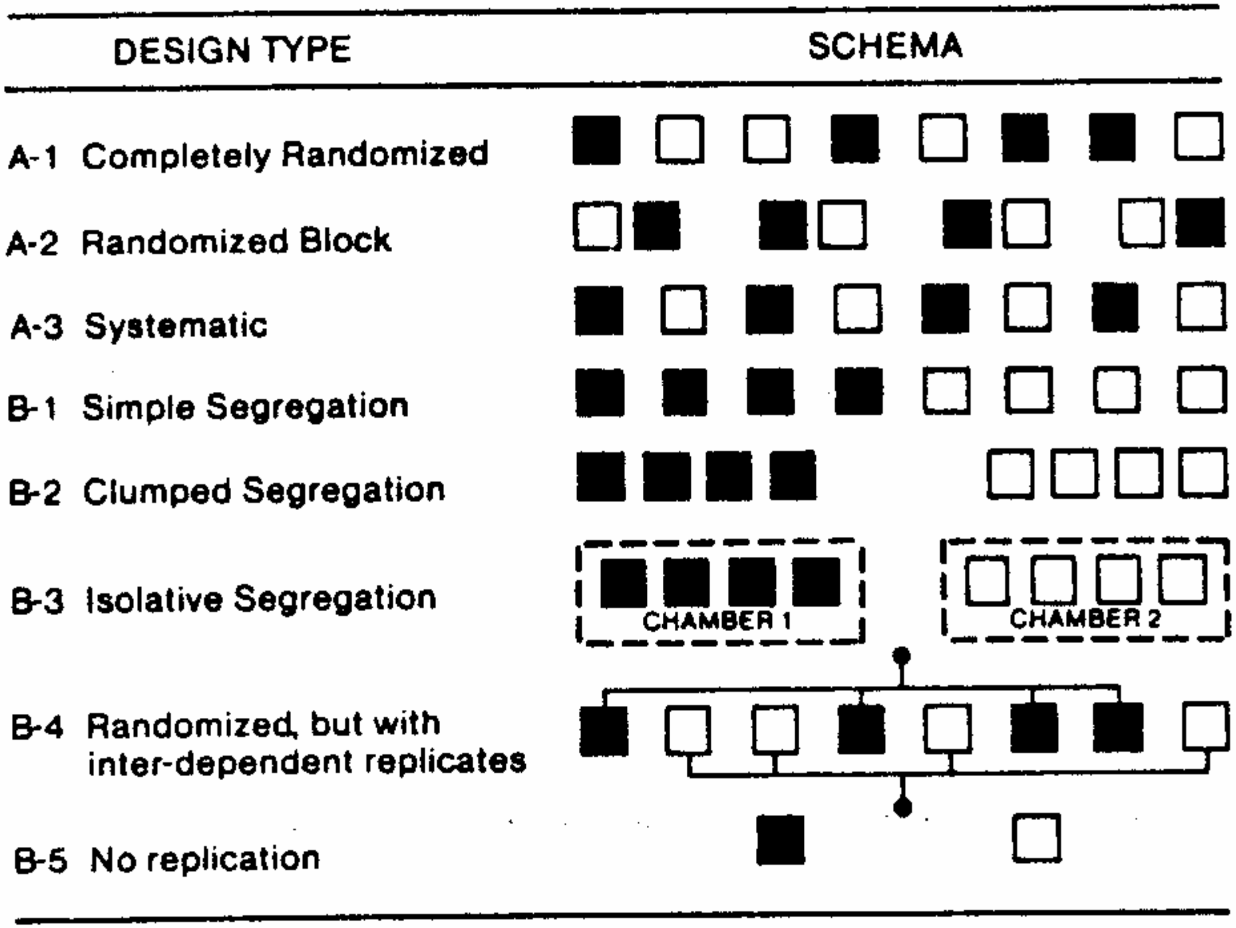


FIG. 1. Schematic representation of various acceptable modes (A) of interspersing the replicates (boxes) of two treatments (shaded, unshaded) and various ways (B) in which the principle of interspersion can be violated.

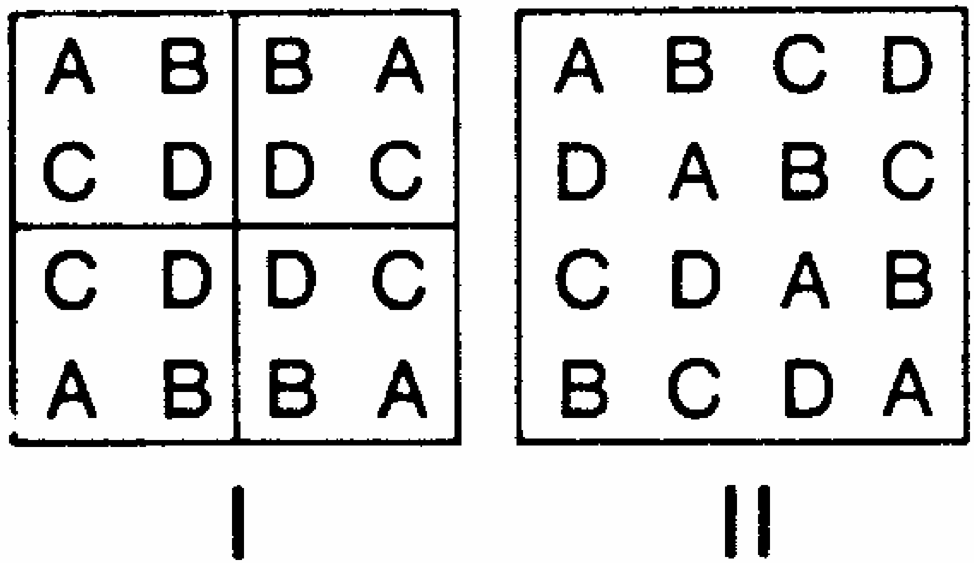


FIG. 3. Examples of segregated arrangements of four treatments, each replicated four times, that can result from use of restricted randomization procedures: (I) randomized block design, (II) Latin square design.

Interspersion of Treatments

My Comment

He seems rather pedantic here

Just use some common sense and re randomize. I will explain as we go further.

What is Pseudoreplication?

Any kind of inappropriate use of multiple measurements which purport to come from independent exptl units randomly applied to a treatment but in fact do not.

- **Simple**-Sub Samples taken from one experimental unit
- **Sacrificial** –True replicates are pooled prior to analysis
- **Temporal**-Repeated measurements taken on an experimental unit over time.

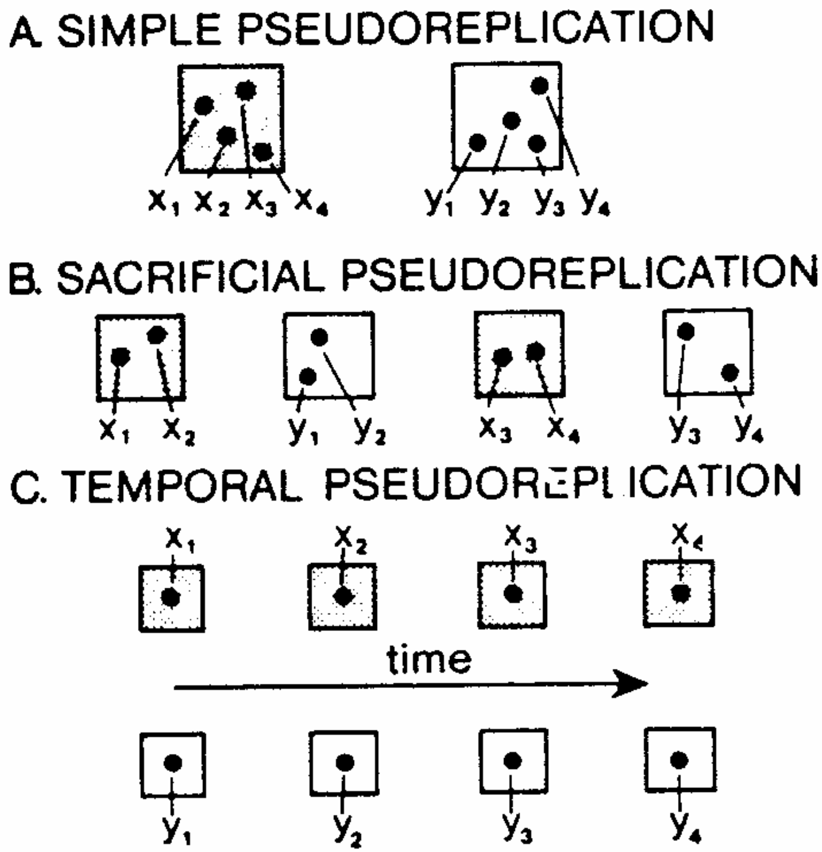


FIG. 5. Schematic representation of the three most common types of pseudoreplication. Shaded and unshaded boxes represent experimental units receiving different treatments. Each dot represents a sample or measurement. Pseudoreplication is a consequence, in each example, of statistically testing for a treatment effect by means of procedures (e.g., *t* test, *U* test) whicn assume, implicitly, that the four data for each treatment have come from four independent experimental units (=treatment replicates).

Pseudo Replication Discussion

- Your Reactions/ Where is it confusing?/ What is a true replicate?

Examples of Pseudoreplication

What are some you have found?

Pseudoreplication Example

Previous Fire Expt: Control vs 2 Fire Regimes (ie no burn vs. two burn treatments. True expt we have say 5 plots each trt in CRD.)

Use of Subsamples (simple pseudoreplication)

- Five subsamples from the one control experimental unit and five from each treatment exptl unit.(15 numbers but only 3 units)

Use of temporal “pseudoreplication”

- Data taken from one control plot 5 years and each treated plot over five years. (15 numbers but only 3 units)

Sacrificial Pseudoreplication

- 2 Treatments (Fox and No Fox) on microtus sex ratio and there 2 reps of each
- Correct analysis –analyse with two sample t test with 2 reps of each trt.

TABLE 6. A hypothetical example of sacrificial pseudoreplication resulting from misuse of chi-square.

Question: Does fox predation affect the sex ratio of *Microtus* populations?

Experimental design: Establish four 1-ha experimental plots in a large field where foxes hunt; put fox-proof fences around two plots selected at random (A_1, A_2), keep the other two plots as controls (B_1, B_2); 1 mo later sample *Microtus* population in each plot.

	Results of sampling				
	Plot	% males	No. males	No. females	Statistical analysis
Foxes	A_1	63	22	13	Test for homogeneity with χ^2
	A_2	56	9	7	Result: $\chi^2 = .019$, $P > .50$ So: pool the data (see below)
No foxes	B_1	60	15	10	Test for homogeneity with χ^2
	B_2	43	97	130	Result: $\chi^2 = 2.06$, $P > .15$ So: pool the data (see below)
	Pooled data				
Foxes	$A_1 + A_2$	61	31	20	Test for homogeneity with χ^2
No foxes	$B_1 + B_2$	44	112	140	Result: $\chi^2 = 3.91$, $P < .05$ *Conclusion:* foxes affect sex ratio

Chi-Square Tests and Sacrificial Pseudoreplication

- Inappropriate analysis-use chi square tests (last slide) after pooling the two replicates. This is called sacrificial replication
- Key Issue-What is the experimental unit here?
- The correct experimental unit is the plot and not the individual microtus in a plot.

Results of Hurlbert's Classic Paper

- Hurlbert(1984) Ecological Monographs 54:,187-211 checked 176 studies from 1960 to 1984.
- Found 27% overall or 48% of those making statistical inferences used "pseudoreplication".
- Different disciplines broken down in next table.
- Advice to Statisticians, Biologists, Editors very impt!!

TABLE 4. Occurrence of pseudoreplication in various segments of the biological journal literature, as determined by several student reviewers.

Subject matter	Journal	Number of reports: Examined	... which adequately described design and used inferential statistics	... and which committed pseudoreplication	Reviewer
Marine field experiments	*Journal of Experimental Marine Biology and Ecology*	50	18	7	.. Johnson
Marine organisms	*Marine Behaviour and Physiology; Biological Bulletin*	44	25	15	M. Chiarappa
Heavy metal effects on marine plankton	Articles in bibliography of Davies (1978)	50	5	1	A. Jones
Temperature effects on fish	Various	50	29	7	T. Foreman
Salt-marsh plants	Various	50	31	4	P. Beare
Temperature-plant relationships	Various	50	11	7	J. Gilardi
Life-history traits of animals	Various	44	38	8	M. Russell
Animal physiology	*Physiological Zoology*	50	?*	7	C. Gasior
Effects of ionizing radiation	*Radiation Research; Health Physics*	50	34	1	J. DeWald
Animal ecology	*Journal of Animal Ecology*	50	?*	2	M. Rehse
Plant-herbivore interactions	Various	49	?*	3	M. Blua
	Totals	537	191+	62	

* The number of studies falling under this heading was not reported.

Results of Hefner et al.(1996) Ecology Paper

- This was a follow up study by Hefner et al.(1996) Ecology.
- They found about a rate of 14% pseudoreplication.
- I find this rate is still high.Papers like the fish tank example from an earlier lecture are still too common.

ANOVA and PseudoReplication Sometimes there are subtle effects

Two examples of This

Completely Random Design Example 1.

Start with 3 treats, 3 replicates

Source	df	SS	MS	F
Treats	2			
Residual	6			
Total	8			

ANOVA and Pseudoreplication

Correct Analysis

Completely Random Design-3 reps and now add 5 time points

Source	df	SS	MS	F
Treats	2			
Residual (a)	6			
Total	8			
Time	4			
Time x treats	8			
Residual (b)	54			
Total	74			

Blue – Whole Plot

Black – Sub Plot(Time)

ANOVA and Pseudoreplication

Completely Random Design-3 reps and also 5 time points

Incorrect Analysis

Source	df	SS	MS	F
Treats	2			
Time	4			
Time x treats	8			
Residual	60			.
Total	74			.

ANOVA and Pseudoreplication

Completely Random Design- Example 2 has 1 rep and 5 time points. **Correct Analysis**.

Source	df	SS	MS	F
Treats	2			
Residual(a)	0			
Total	2			
Time	4			
Time x treats	8			
Residual(b)	0			.
Total	14			.

ANOVA and Pseudoreplication

Completely Random Design-1 rep and 5 time points

Incorrect 1

Source	df	SS	MS	F
Treats	2			
Time	4			
Time x treats	8			
Residual	0			.
Total	14			.

ANOVA and Pseudoreplication

Completely Random Design-1 rep and 5 time points

Incorrect 2

Source	df	SS	MS	F
Treats	2			
Time	4			
Residual	8			.
Total	14			.

Finally Residual df- Too bad they are wrong!!

Classical Experiments- Summary

- Specify Objectives- Comparing "treatments"
- Study Design – Includes

  Control, Randomisation, Replication,

- Statistical Analysis- Varied

  Centrality of ANOVA and Linear Models

- Clearcut Interpretation of Results and Conclusions

  "Design based" interpretation allows establishment of "causation". Observational studies are usually "model based".

Experiments Check List

- Specify Objectives and Scope of Inference

  What are your treatments and what are the experimental units they will be applied to?

  What are the spatial and temporal scales?

  Is it practical? If not then iterate to arrive at a reasonable compromise with respect to costs and satisfying objectives.

Experiments Check List

- Decide how to measure a response

Usually many variables of interest each with their own measurement issues

You may just get one measurement of a variable on the whole plot. However, often you may need spatial and temporal subsamples and these might require a complex sampling design just to get one or a series of repeated measures on each experimental unit.

(Animal popn density could require a capture-recapture study on each of your experimental units)

Sampling is a major topic we cover a bit later in the semester

Experiments Check List

- List Factors that can effect the Response.

Design Factors

Factors to Vary-Treatments and Control Treatments

Factors to Fix- Control Settings

Confounding Factors

Factors to control by design (blocking)

Factors to control by analysis (covariates)

Factors to control by randomization

Experiments Check List

- Plan the Time Line of the Experiment.
- Outline Statistical Analysis

Linear Models and ANOVA usually central to this. Make sure that you are aware of any complications.

factorial treatment structure, split plot nested treatment structures or repeated measures.

Experiments Check List

Replication

- Be very clear about what the experimental unit is so that you can try to avoid pseudo replication (simple, time, sacrificial).

- Determine No. of Replicates

  Need pilot study or Prior Info on Variation

  Use power analysis or effect size of a CI to do calculation.

Determine No. Of Replicates

- Simplest approach is to use half width of a CI to do calculation.

$$(\overline{X}_1 - \overline{X}_2) \pm z_{\alpha/2}\sqrt{\frac{2s^2}{r}}$$

$$d = z_{\alpha/2}\sqrt{\frac{2s^2}{r}}$$

$$r = (z_{\alpha/2})^2 2s^2 / d^2$$

- Need to specify confidence level and half width (d).
- Need to carry out pilot study for estimate of variance or use value from a past study.

Looking Ahead to Next Lecture

- “Quasi” Experiments vs. “True” Experiments!
- What do we gain and what do we lose?

“Quasi Experiments” in Ecology

- Many Ecological Studies look somewhat like experiments
- Yet they are not true experiments with all the characteristics we defined earlier. Could call them

“Quasi Experiments”.

- One important class of examples is assessment of some impact, perturbation, manipulation (hunting, power plant, fire, oil spill etc) on an ecological system.
- Perturbation may be planned or unplanned. Could be part of a research effort or could be part of an environmental impact assessment.

Difficulties with traditional experimental designs

- No reasonable control areas often

  (Expense, Practicality, Uniqueness)

- Lack of randomization

- Lack of replication

  (Expense, Practicality)