An Overview of Sacrificial Pseudoreplication

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Overview

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Section I

Pseudoreplication

What is Pseudoreplication?

- Pseudoreplication occurs when there is either a single replicate from an experimental unit OR when treatments are segregated
  - Perhaps within a single experimental unit [Figure 1.]
- Results in replicates not being independent
- Cannot test for treatment effects
  - Confounded with spatial effects
- Inferential statistics results become invalid
  - ANOVA assumption violated

What is Pseudoreplication? Cont. (1)

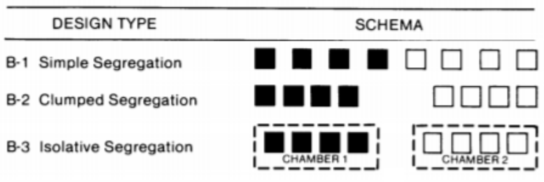

Figure 1: Three design examples where pseudoreplication occurs

- Underlying assumption: experimental units are identical at the time of treatment implementation and remain identical during the duration
- As the number of samples covers the entire unit, the probability that there is a difference between the units approaches 1

What is Pseudoreplication? Cont. (2)

- Null hypothesis assumed: No difference amongst treatment effects
  - Null hypothesis tested: No difference between experimental units
- Probability of a type 1 error increases significantly as the number of replicates increases

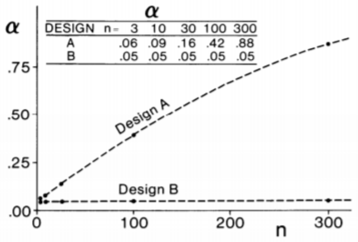

Figure 2: Design A has pseudoreplication whereas Design B does not.

Sacrificial Pseudoreplication

- There are a number of different types of pseudoreplication
  - One of them is known as *Sacrifical Pseudoreplication*
- Occurs when:
  - There IS true replication of treatments but the data of the replicates is pooled together before conducting the statistical analysis
  - Or two (or more) samples from each experimental units are treated as independent

Sacrificial Pseudoreplication Cont. (1)

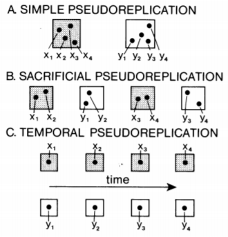

Figure 3: Examples of Various Pseudoreplications

- Sometimes, a significance test is used to compare replicates from different experimental units
  - Often not significant however, this doesn't justify pooling
  - Need to know how different the experimental units are

Sacrificial Pseudoreplication Cont. (2)

- Example:
  - Imagine you had were concerned about malaria in various villages
  - Take 20 villages and randomly split them
  - Give 10 of them a particular pesticide
  - Take a sample of individuals from each village to measure prevalence
  - MISTAKE: Pooling all the individuals in the treated and untreated villages together respectively

Section II

Empiricial Demonstration

Data Set Overview

	plot	beetle_density	treatment
	plot 1	25.31333	trt
	plot 1	13.91215	trt
	plot 2	58.61689	ctrl
	plot 2	67.59083	ctrl
	plot 3	40.20168	trt
	plot 3	46.21766	trt
	plot 4	44.86198	ctrl
	plot 4	62.79626	ctrl

Figure 4: Beetle Density Data Set

- In this data set, we have 4 plots and 2 samples of "beetle" density from each plot. Each of the plots (the experimental unit) is given the control or the treatment.

Analysis

- Here, we see that if we do an ANOVA to compare the effect of treatment to control without considering the dependent nature of the observations, we get the following summary:

```
          Df Sum Sq Mean Sq F value Pr(>F)  
treatment  1   1464  1464.0   9.475 0.0217 *
Residuals  6    927   154.5                 
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Figure 5: One Way ANOVA Results: Control v. Treatment

- Note that there seems to be a significant difference and the degrees of freedom is 6 (due to the incorrect count of replicates). This allows for an easier significance result.

Analysis Cont. (1)

- Instead now, let's run the ANOVA without committing the Sacrificial Pseudoreplication. In other words, we can take the average from each experimental unit to be the single replicate.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
treatment	1	732.0	732.0	4.555	0.166
Residuals	2	321.4	160.7		

Figure 6: One Way ANOVA Results (FIXED df): Control v. Treatment

- Here, we can see that there does not seem to be a significant result.
- Question: Was there an effect of the plots that was confounded with the treatment effect?

Analysis Cont. (2)

- In fact, the beetle densities were not the same on the different plots to begin with
- However, a significant result was still achieved under the assumption violation
- Here is an ANOVA looking at plot effects:

```
            Df Sum Sq Mean Sq F value Pr(>F)
plot         3 2106.8   702.3   9.885 0.0254 *
Residuals    4  284.2    71.0
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Figure 7: One Way ANOVA Results: Plot Comparison

- We see that indeed there was a significant difference of beetle density with respect to plots

Pseudoreplication Error in Analysis

- So then, what exactly occurred?
  - The number of replicates was less which lowered the residual degrees of freedom. This increased the value of the denominator, which resulted in a lower F-test value and thus, the non-significant result.
- ANOVA boils down to a ratio of the between group variance divided by the within group variance.
- The df of the within variance is: N - t
- N went from 8 to 4 and thus df went from 6 to 2

Section III

Solutions and Conclusion

Avoiding Sacrificial Pseudoreplicaion

- The most obvious solution to avoid this kind of pseudoreplication is to use a different design
  - For example, in the context of our beetle data set, you could split a large single plot of land into a 3 x 4 grid, and randomly assign 6 grids to be of the control and the other 6 to be of the treatment, thus taking caring of the confounding of the separate plots
- However, due to various real-world constraints, this may not be possible.
  - Then, the goal becomes to minimize the impact of this constraint

Avoiding Sacrificial Pseudoreplicaion Cont. (1)

- An effort should be made to maximize the independence of your samples if a certain number of replicates is required
  - For example, if you had growth chambers within greenhouses, you could move around the location of the growth chambers (carefully) to other greenhouses receiving the seem nested "outer" treatment
- Also, the study maybe treated as a preliminary one rather than a final conclusion.
- Do the correct analysis - or present both results clearly outlining the assumptions violated for the one involving pseudoreplication.

Summary

- A common mistake made during the analysis phase of an experiment is to treat your replicates as independent when they aren't - this is known as pseudoreplication
  - In fact, Hurlbert found that 48% of studies that he looked at committed this error
- A specific kind is known as Sacrificial Pseudoreplication
  - This is either when you pool results together where a treatment was randomized to the unit rather than the individuals, or you treat two (or more) samples taken from a unit as independent replicates
- A type I error result was presented using example data that demonstrated the dangers of Sacrificial Pseudoreplication with respect to inferential statistics

References

Stuart Hurlbert (1984)
Pseudoreplication and the Design of Ecological Field Experiments
Ecological Monographs Volume 54 - Issue 2

Svetlana Pushkar and Oleg Verbitsky
A Nested ANOVA as Remedy for Sacrificial Pseudoreplication in a Spatial Manipulative Experiment within an Ecnlosed Space

Texas A&M
https://web.ma.utexas.edu/users/mks/statmistakes/pseudorep.html

Influential Points
http://influentialpoints.com/Training/Pseudoreplication_use_and_misuse.htm

University of Alberta, Dr. Gray
https://sites.ualberta.ca/~lkgray/uploads/7/3/6/2/7362679/slides_-_anova_assumptions.pdf

One Way ANOVA Calculation Explanation
https://youtu.be/UrRYITjDOww
Brandon Foltz

Thanks for Listening!