Lecture 6

- Next Lecture
- Brief Review (Factorials, ANCOVA).
- Split Plot Designs
- Repeated Measures Designs

Next Lecture Assignments

Please Read

Hurlbert (1984) Ecological Monographs 187-211.

Heffner et al. (1996). Ecology 2558-2562.

They are both on the webpage. Focus on the first one

Bring Some Examples-

- Expts without replication of treatments,
- Expts without randomization of treatments

Brief Review

Factorial Treatment Structure Randomized Block Design ANOVA

Source	df	SS	MS	F
A	1			
B	1			
AB	1			
Blocks	4			
Residual	12			
Total	19			

5 Blocks, 4 treatments in a 2x2 factorial design.

AB F Test Not Significant-

Focus on Main Effects A and B

AB F Test Significant

Look at graph of all the means to understand the nature of the interaction.

Factorial Designs: Interaction Diagram

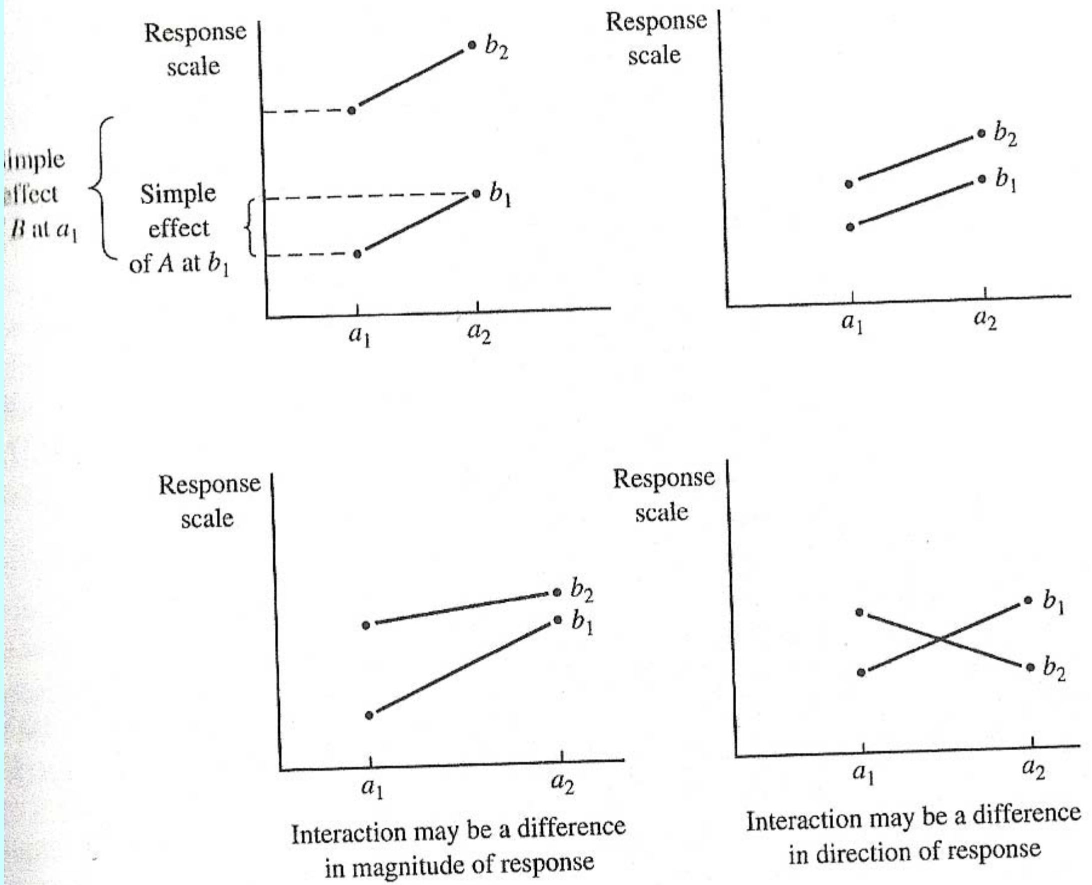


FIGURE 15.1
Illustration of interaction.

Top Panels

No Interaction

Additive Responses

Bottom Panels

Interaction of Different Types

Analysis of Covariance

- An alternative (or addition) to blocking to increase precision for comparison of treatments when there is an important auxiliary continuous variable.
- Can be viewed as a combination of a regression and an ANOVA model.

Analysis of Covariance

Here I illustrate with a Random Complete Block Design with a Covariate (x)

$$y_{ij} = \mu + \tau_i + \gamma_j + \beta(X_{ij} - \overline{X}) + \varepsilon_{ij}$$

Notes:

- Overall mean term
- Treatment effect term
- Block Effect term
- Covariate term (regression)
- Residual term.

Analysis of Covariance: Diagram

We now illustrate the value of the ANCOVA graphically by considering just 2 treatments and one covariate x.

In the plot that follows the Y axis represents the response variable and we consider the two separate regressions (one for each treatment)

Notice the lines are parallel because they are forced to have the same slope (β).

Observed and adjusted differences in the treatment means are shown.

Analysis of Covariance: Diagram

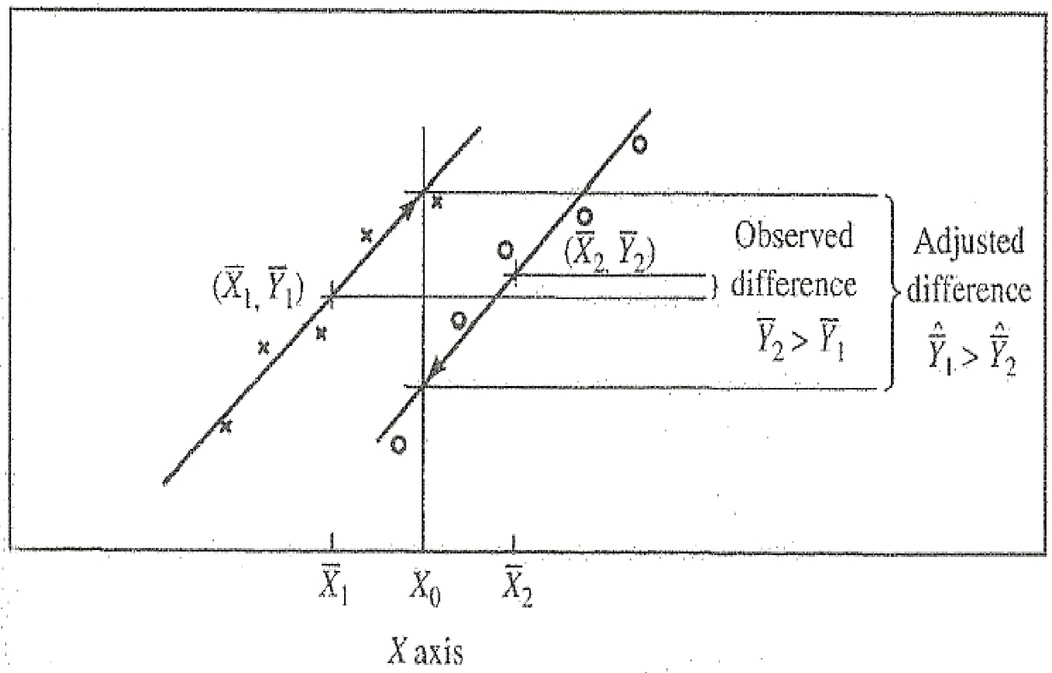


FIGURE 17.1
Error control and adjustment of treatment means by covariance.

Notice how the observed difference in the treat means is confounded by the different values of x. The adjusted treat means are at a common value of x.

Split Plot Design Example
Damschen- Haddad Plants Study

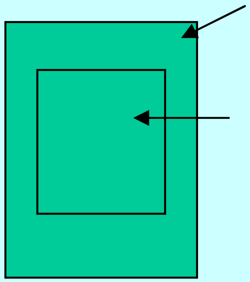
Types of Patches

Randomised at the level of the whole patch

Edge vs Center of Patch (treatments factor B)

Not randomized but applied to a part of the patch

Patch Type
Rectangular



Split Plot Design Another Example Damschen- Haddad Plants Study

Whole Plots

3 Types of Patches (Treatments factor A)

Randomised

Subplots or Split Plots (Within Patches)

Edge vs Center of Patch (treatments factor B)

Not randomized.

Results not presented here but this is what we call a split plot design!! This is a good lead in to this topic!!

Split Plot Designs

One has a basic design like a randomized block for one treatment factor described earlier for a set of large plots (units) but then one also has another treatment factor applied to smaller subplots within the whole plots.

One is interested in main effects of each factor and their interaction.

There are two different error terms used based on the whole plots and the subplots so the analysis is quite complicated.

Split Plot Designs
Linear Additive Model

Randomised blocks (ρ) for one treatment factor (α) for a set of large plots (units)

One also has another treatment factor (β) applied to smaller subplots within the whole plots.

$$y_{ijk} = \mu + \rho_i + \alpha_j + \gamma_{ij} + \beta_k + (\alpha\beta)_{jk} + \varepsilon_{ijk}$$

γ_{ij} - whole plot residual

ε_{ijk} - sub plot residual

Split Plot Design Example ANOVA

6 treatments in a 3x2 factorial design.

5 Blocks

3 Whole Plot Treats Factor A, 15 Whole Plots

2 Split Plot Treatments 30 Sub Plots (15x2)

Split Plot Design Example ANOVA
Whole Plot Only

Source	df	SS	MS	F
Blocks	4			
A	2			
Residual (a)	8			
Total	15			

5 Blocks, 3 treatments on the whole plots

Split Plot Design Example
Full ANOVA Table

Whole Plot Part 15

So 14df

Split Plot Part 30

So 29df

Source	df	SS	MS	F
Blocks	4			
A	2			
Residual (a)	8			
B	1			
AB	2			
Residual (b)	12			
Total	29			

5 Blocks, 6 treatments in a 3x2 factorial design. 3 Whole Plot treats,

2 Split Plot Treatments (15 Whole Plots, 30 Sub Plots)

ANOVA-Incorrect because it ignores the design structure. Consult a statistician!!

Source	df	SS	MS	F
Blocks	4			
A	2			
B	1			
AB	2			
Residual	20			
Total	29			

5 Blocks, 6 treatments in a 3x2 factorial design. 3 Whole Plot treats, 2 Split Plot Treatments (15 Whole Plots, 30 Sub Plots)

A Split Plot Design F Tests

Whole Plots

Factor A Main Effect uses Residual (a) MS as the denominator

Subplots

Factor B and Interaction AB uses Residual (b) MS as the denominator

NOTE: Residual (b) is usually substantially smaller than residual (a) so that the power of the tests for B and AB would be much higher. This should be kept in mind at the design stage.

Split Plot ANOVA Ch 4

- Ch 4 Scheiner and Gurevich Book
- 2 Reps of whole plot treats, 12 treatments in a 2x6 factorial design. 2 Whole Plot treats (CO_2), 6 Split Plot Treatments (Nutrients).(4 Whole Plots, 24 Sub Plots in the whole experiment)
- It is very important to realize that the randomization is a two-stage one because of the nested nature of the design. Figure 4.2 page 72 shows the randomization to the 4 whole plots and then to the 6 subplots within each whole plot.
- Table 4.2 p 73 shows the ANOVA Table. It has some errors which I correct on the next slide.
- Appendix 4.4 shows the SAS code for a split plot ANOVA.

NOTE: Earlier Appendices give SAS code for simpler designs CR Design with factorial treatment structure, RCBlock and Augmented RC Block.

Corrected Split Plot ANOVA

(Table 4.2 in Scheiner and Gurevitch Book)

Source	df	MS	F	p value
CO_2	1	130.67	42.96	0.02
Residual (a)	2	3.04		
Nutrient	5	610.09	386.13	0.00
NutrientxCO_2	5	7.09	4.49	0.02
Residual (b)	10	1.58		
Total	23			

2 Reps of whole plots, 12 treatments in a 2x6 factorial design. 2 Whole Plot treats, 6 Split Plot Treatments (4 Whole Plots, 24 Sub Plots)

Split Plot ANOVA (4.2) Results

- Note that the Residual (a) MS is larger than the Residual (b) MS as expected. Here it is about double the size! In many applications there would be an even larger differential between the two residual MS.
- All F tests are significant here. CO_2 used Residual (a) whereas Nutrient and Nutrient x CO_2 used Residual (b)
- Further analysis would involve examining the 2 x 6 table of treatment means and their standard errors. Here as there is interaction between the factors the marginal means are not very helpful.

Repeated Measures Designs

Introduction

One has a basic design like a randomised block described earlier for one treatment factor but then one measures a variable over time. One is interested in main effects and interactions between the factor and time.

Other variations include having different treatments applied at different time points. This is sometimes called a cross over design.

There are a variety of possible approaches to analysis.

Repeated Measures Designs

Analysis Approaches

- Summary Measures Approach
- Split Plot-Compound Symmetry
- Split Plot-adjustments to F tests
- General Multivariate Approaches (MANOVA)

Repeated Measures Designs

Summary Measures Approach

Here we simply reduce all the data over time to one value and do a simple analysis of variance on the summary data. (Now there are no time points)

Possibilities:

-Average- possibly analyse the averages of all the time values within an expt unit.

-Slope- fit a linear regression to each time series (ie for each exptl unit) and use the slope as your variable. This is a measure of the time trend for that experimental unit.

-NOTE: This approach is used and I have sometimes recommended it when consulting with ecologists but it is often limiting which leads to the others which follow.

Repeated Measures Designs

Split Plot

Compound Symmetry Approach

•Similarities to a split plot in terms of analysis at least for the simplest form.

•Based on what is called compound symmetry of the correlation structure. This is where all time points have equal correlation irrespective of how far apart they are.

•This assumption may be valid in some cases but not in others.

Incorrect ANOVA (often done) which ignores the repeated measures structure.

Source	df	SS	MS	F
Treatments	2			
Blocks	4			
Time	2			
Treat x Time	4			
Residual	32			
Total	44			

5 Blocks, 3 treatments where there are 3 time points in a repeated measures design but it is pretended that they are independent.

Repeated Measures Design ANOVA

Simplest Possible Reasonable Analysis (like a Split Plot)

Source	df	SS	MS	F
Treatments	2			
Blocks	4			
Residual (a)	8			
Time	2			
Treat x Time	4			
Residual (b)	24			
Total	44			

5 Blocks, 3 treatments where there are 3 time points in a repeated measures design

Repeated Measures Designs
Adjustments to the "Split Plot Analysis"

- Some statisticians I talked to suggested the split plot type analysis is likely robust to other correlation structures than compound symmetry.
- Alternately a suggestion to adjust (downwards) the df of the F tests based on results of a test of the compound symmetry assumption. (Details not given here).

Repeated Measures Designs
General Multivariate Approaches

- Complex but allow much more realistic flexible correlation structures than compound symmetry.
- Based on Multivariate Analysis of Variance MANOVA).
- I recommend that you consult a statistician before you use something like this. (We need the work!! Although I am not an expert on this topic at all (☺)).

Damschen Corridor Study on Plant Species Richness

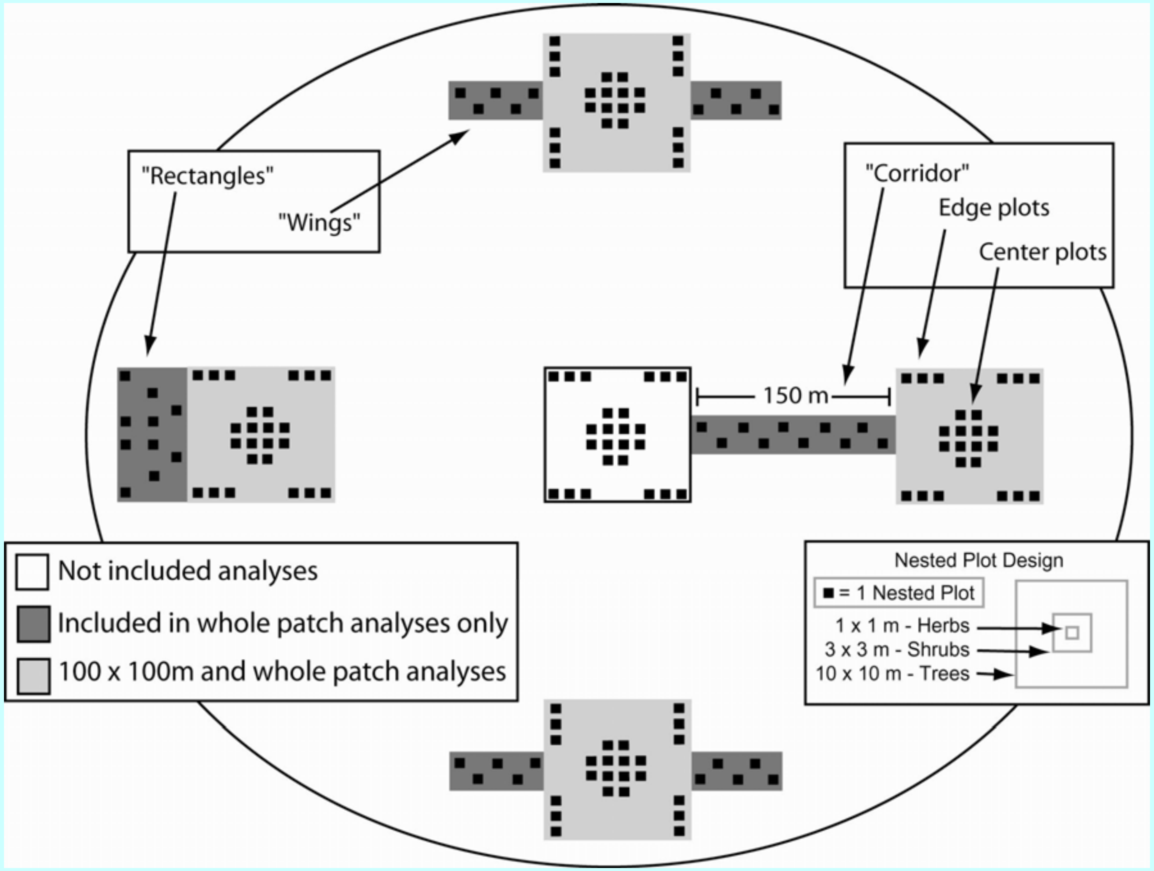
- To study the plant community and how dispersal may be aided by corridors. We focus on species richness
- Recall treatment factor applied to the patches has these types

  Connected

  Isolated Winged

  Isolated Rectangular

Figure 1.3. Patch and plot design. The center patch (shown in white) was never included in analyses, while light gray were included only in analyses of the central 100 x 100 m area of each patch, and both light and dark gray areas were included for whole patch analyses. The black squares represent one nested plot with a 1 x 1 m, 3 x 3 m, and 10 x 10 m plot sampling herbs, shrubs, and trees, respectively.



Features of Study

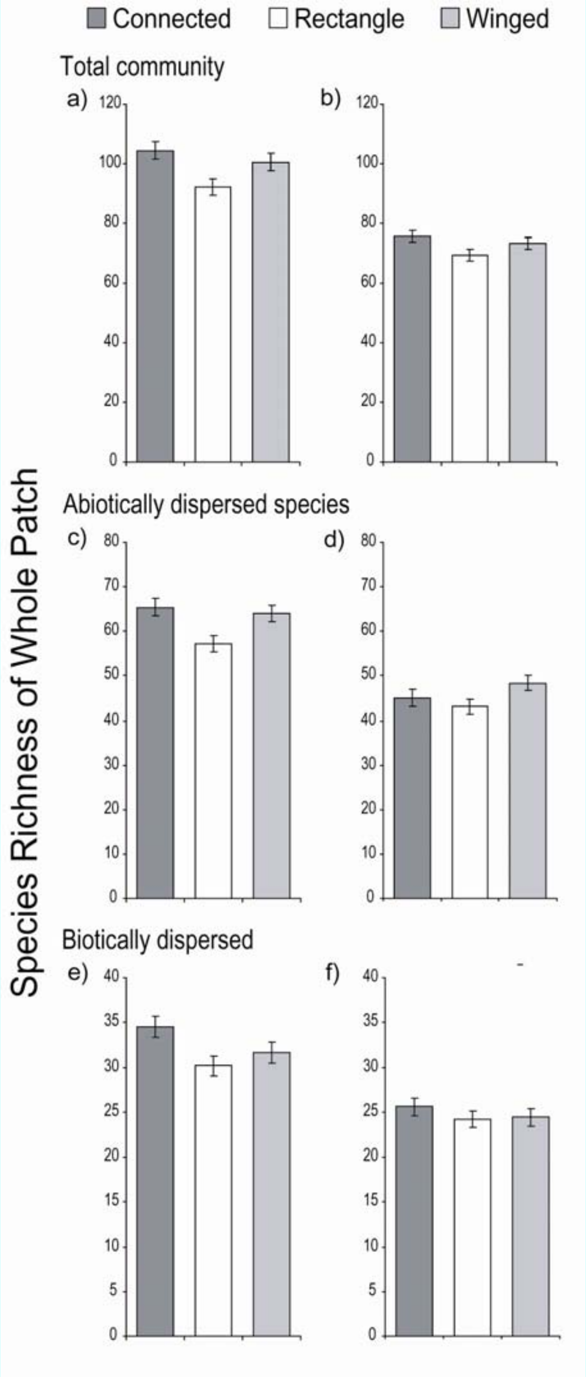
- Complex Subsampling Issues not discussed here
- True Split Plot could be used (Edge vs Center subplot factor). This analysis is not shown here.
- Repeated Measures (Patch types over Years) is shown in some detail. She used the “split plot” approach to analysis.

Repeated measures analysis for total species richness in the whole patch.

- Factors include:
- Patch type (connected, unconnected winged, unconnected rectangular) significant $p = 0.03$
- year (2001, 2002, 2003) repeated measure significant $p < 0.0001$.
- interaction between year and patch type ns $p=0.86$
- the log soil moisture by weight for each patch is included as a covariate (ANCOVA) significant $p= 0.03$. This is not surprising is it?- as soil moisture would vary by year and individual patch and is so crucial to plant reproduction.
- These results are for species richness when including all species based on presence absence surveys on the whole plot. The figure which follows shows some other analyses as well (abiotically and biotically dispersed species treated separately and different types of sampling).

Repeated measures analysis for total species richness in the whole patch.

- Because there is no interaction between the two factors the important results are for the patch types averaged over years and are presented in the next figure.
- She used contrast statements in SAS but I will just summarize the key results here.
- Connected and Isolated Winged similar and greater species richness than Isolated Rectangular. True for all species and then those dispersed abiotically. (panel a and b)
- Biotically dispersed species results (panel c) are less clear but connected patches appear to have greater species richness than the two isolated patch types which are similar.



Connected
Rectangle
Winged
Species Richness of Whole Patch
Total community
a)
b)
Abiotically dispersed species
c)
d)
Biotically dispersed
e)
f)

Experimental Design Summary

-What is the exptl unit? How big are units to be? Animal movement/plant dispersal and their influence on scale?

-What are the treatments? How many treatments? What structure on the treatments? Factorial?

-Is Randomization feasible?

-How many replicates? Power? Pilot Study?

-Blocking factors available?Use if possible. If not perhaps use ANCOVA to increase precision

Experimental Design Summary

-- Is scale the right one biologically? Did you have to make plot sizes smaller than ideal because of costs?

--Usually there are Multiple Objectives with many variables to measure which will often necessitate design compromises. (Which variable to look at for power analysis?)

--Quite a lot of studies will have nested design structures which lead to split plot designs. Remember the subplot factor and the interaction tests will have a lot more power than the whole plot factor. If this is not what you want try and come up with a better design that gets away from this approach –if possible.

--Many studies long term with measurements over time will mean that repeated measure analyses will be very important.

-- Do not ignore split plot or repeated measure structures in analysis. Those analyses are wrong!!!

Experimental Design Summary

- What variables have to be measured and how will it be done?
- Will subsampling have to be used to measure different variables?

  -Count trees on the whole plot?

  -Count small plants on much smaller subplots?
- There are important sampling design issues on how the sampling and sub-sampling should be done. We will spend a whole section of the class on sampling designs.

Spatial (and Temporal Scales) of Interest May Create Practical Difficulties with True Expts

Exptl Units may be too Small For Biological Realism

Role of Microcosms (Pro and Con)

Ecology 77 (3) Special Issue in 1996

Lawton, Drake et , Carpenter articles

How should one do Expts in Ecology?

Whole Ecosystem "Experiments"

Carpenter et al.(1995) Science 269 324-327

Ecological Experiments with Model Systems

Lawton (1995) Science 269 328-331.

Looking Ahead to Next Lecture

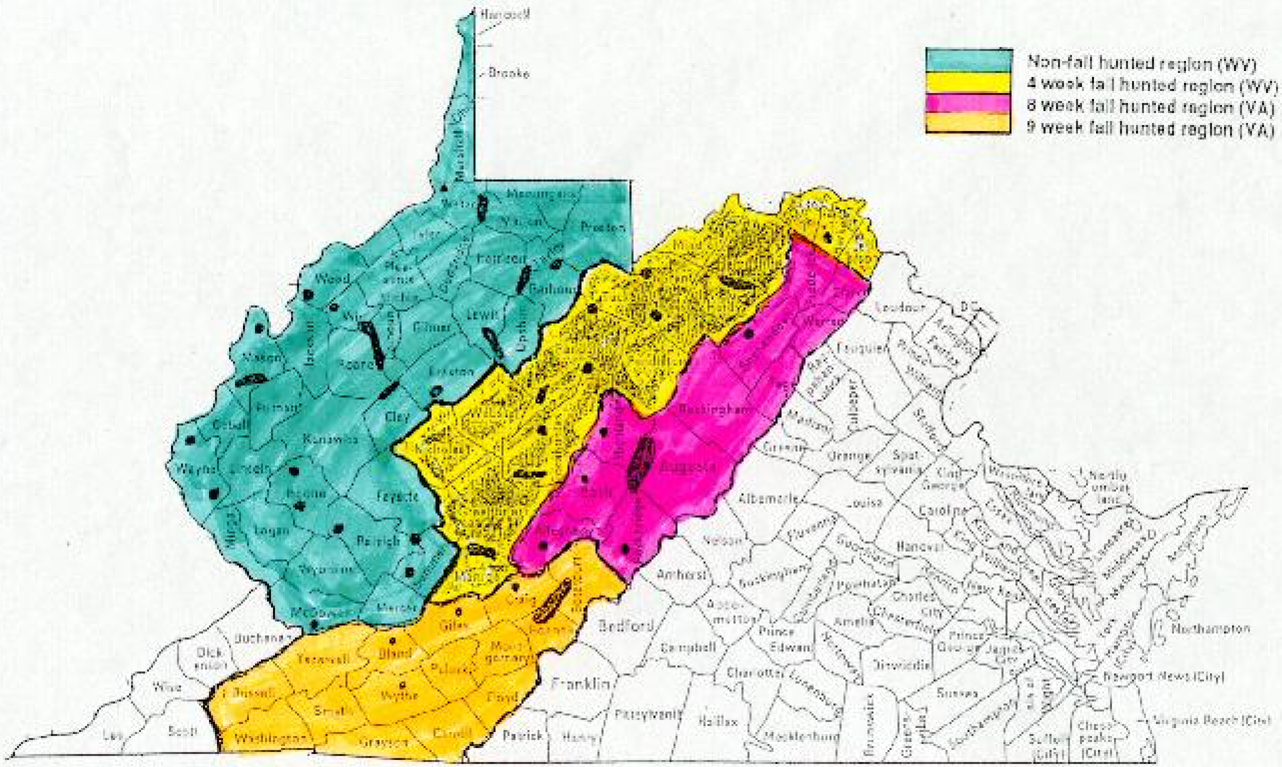
Hurlbert (1984) Ecological Monographs 187-211. This has become a classic paper! He invented a new word-Pseudoreplication. You may have already found examples or *"committed the sin"* yourself!

We will talk also more generally about problems with using true expts in Ecology.

- Expts without replication of treatments,
- Expts without randomization of treatments
- Bring examples to discuss.

Remember These from Lecture 1.

Turkey Study: No Replication or Randomization



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

Randomisation, No Replication

3. Fish Growth Chamber Example

Lankford and Targett (2001). Low-Temperature Tolerance of Age-0 Atlantic Croakers: Recruitment Implications for US Mid-Atlantic Fisheries. Transactions of American Fisheries Society 130: 236-249.

Cold induced winter mortality of age 0 croaker is thought to be very important in estuarine nursery areas on the east coast of the US.

No Randomisation, Replication

4. Liming Streams Example

- Rundle et al. 1995.Freshwater Biology 34,165-175
- Reported in Downs et al.2002.Monitoring Ecological Impacts : Concepts and practice in flowing waters. Cambridge University Press.p 234-248.
- River Tywi in Wales.Remediation study on streams affected by acid rain.1984-1994.
- 3 Control and 3 Limed sites (Matched but not Randomised) with measurement for 3 yrs before and 3 years after the liming. (BACI)
- Measures are numbers of macro invertebrates of acid sensitive taxa.
- Results show that there is a significant positive impact but it only has a short term effect.