Week 8: Differences among more than two samples

Session 1

Spring 2020

iClicker Question 1

I want to compare fish weights in three lakes. I've sampled 32 fishes from each lake.

What statistical analysis should I perform?

A `Multiple linear regression`

B `t-test`

C `one-way ANOVA`

D `Chi-square test`

iClicker Question 2

I want to compare fish weights in two lakes. I've sampled 32 fishes from each lake.

What statistical analysis should I perform?

A `Multiple linear regression`

B `t-test`

C `one-way ANOVA`

D `Chi-square test`

Announcements

This week's material builds on ideas from pairwise group comparisons.

The ANOVA material is more *dense* than what we've covered up until this point.

We're going to have to work on our *statistical intuition* to master these *inferential statistics* concepts.

This week

Tuesday: Differences between more than two samples:

- Analysis of Variance (ANOVA) concepts
  - One-way ANOVA
  - Two-way ANOVA
  - Multipe testing

Thursday

- Continue ANOVA concepts
- Statistical analysis of salamanders

Moving beyond two groups

T-tests are great, but what if we need to analyze more complicated scenarios?

Let's walk through some sampling and experimental scenarios to build intuition.

Scenario context: We're interested in bluegill population densities in Massachusetts lakes.

[1]Image credit: New York Fish and Game Commission

Scenario 1

Having just analyzed some fish counts data in 16 lakes in Massachusetts, Thorsten found a significant ‘lake’ effect using an ANOVA, i.e., the mean number of fish was not the same in all lakes.

1. Thorsten wants to know which *which lakes are different from each other*.
   - Think carefully: what does this actually mean?
   - What is the sampling unit?
   - What did he measure?
   - What would he need to compare?

Scenario 1

Having just analyzed some fish counts data in 16 lakes in Massachusetts, Thorsten found a significant ‘lake’ effect using an ANOVA, i.e., the mean number of fish was not the same in all lakes.

2. What would Thorsten do to find out *which lakes were different from eachother*?

A) A series of t-tests

B) A Tukey Honest Significant Difference test

C) A Kruskal-Wallis test

Scenario 2

I am interested in testing whether there is a significant difference between the population sizes of fish in 30 **low salinity** lakes and 30 **high salinity** lakes:

1. What is different from the last scenario?
2. What is the sampling unit?
3. What specific question(s) should I ask?
4. What, specifically, do I want to compare?

Scenario 2

I am interested in testing whether there is a significant difference between the population sizes of fish in 30 **low salinity** lakes and 30 **high salinity** lakes:

5. Which statistical test could I use?

A) A t-test
B) A One-Way ANOVA
C) A Chi-square test
D) A Two-Way ANOVA

6. Which is the test statistic for the test I chose?

Scenario 3

I am interested in testing whether there is a significant difference between the population density of fish in 30 **low salinity** lakes and 30 **high salinity** lakes.

In fact, I actually sampled 10 **large**, 10 **medium**, and 10 **small** lakes in each of the high and low salinity lakes.

I want to explore whether there are differences in population size based on lake **salinity** and lake **size**.

1. How has our sampling scheme changed?
2. What is the sampling unit?
3. How has our question changed?

Scenario 3

I am interested in testing whether there is a significant difference between the population density of fish in 30 **low salinity** lakes and 30 **high salinity** lakes.

In fact, I actually sampled 10 **large**, 10 **medium**, and 10 **small** lakes in each of the high and low salinity lakes.

I want to explore whether there are differences in population size based on lake **salinity** and lake **size**.

4. Now which statistical test should I use?

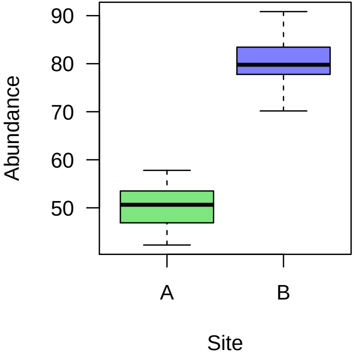
A) A t-test
B) A One-Way ANOVA
C) A Chi-square test
D) A Two-Way ANOVA

5. Now which is the test statistic for the test?

Comparing differences - two samples

Two samples:

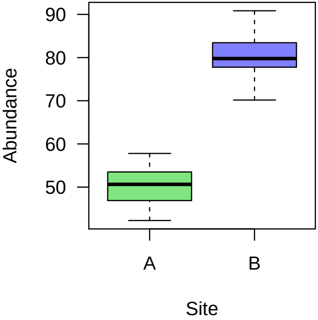
- Which test do we use?



Comparing differences - two samples

Two samples:

- the t-test?
- test whether group means differ significantly
- H_0: there is no significant difference between the means
- H_1: there is a significant difference between the means



Comparing differences - two smaples

Two samples:

- the t-test?
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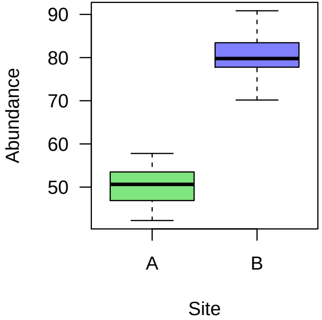
- H_0: there is no significant difference between the means
- H_1: there is a significant difference between the means

Significance based on:

- t-statistic: $t = \frac{|\bar{x}_a - \bar{x}_b|}{\sqrt{\frac{s_a^2}{n_a} + \frac{s_b^2}{n_b}}}$
- degrees of freedom
- p-value



Comparing differences - more than two samples

What about if there are more than 2 samples?

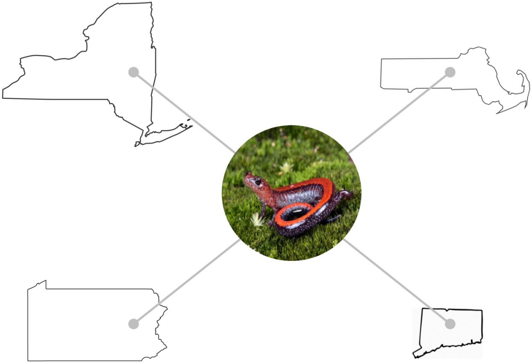
- can you think of any examples?

?

Comparing multiple groups - examples

Regional differences in salamander abundance:

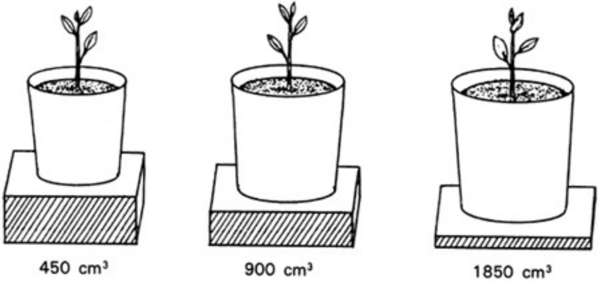
- comparing multiple populations
- quantify the differences between populations



Comparing multiple groups - examples

Plant growth related to available resources (pot size):

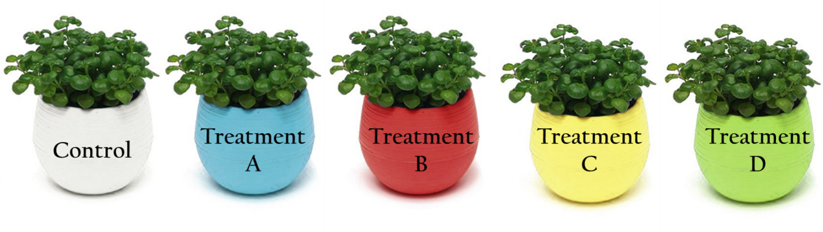
- comparing multiple treatments
- quantify the effects of resource availability



Comparing multiple groups - examples

Plants productivity (dry mass in grams) related to fertilizer treatment

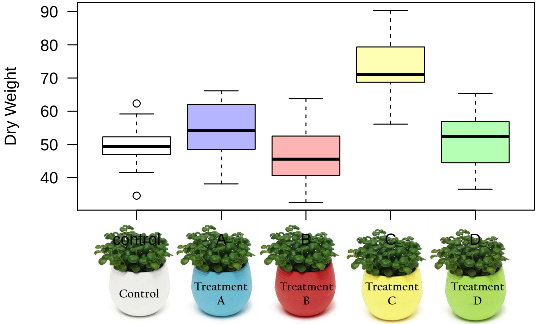
- do our treatments influence biomass production?
- is there a positive effect relative to a control?



Comparing multiple groups - examples

When there are more than 2 groups

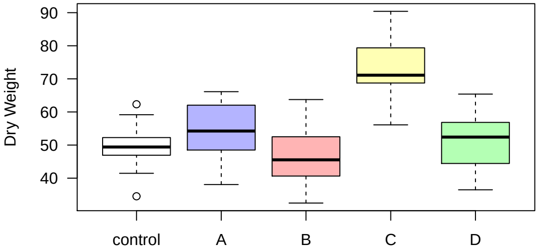
- t-test is probably not optimal:
- We would need to do all possible pairs.
- We might get spurious differences just by chance. Why?



Comparing multiple groups - ANOVA

Analysis of Variance (ANOVA):

- statistical test for testing for differences among >2 groups
- ANOVA and t-test are identical when there are 2 groups
- one factor/group/category (*One-way ANOVA*)



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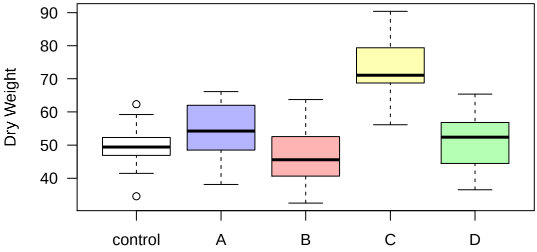
- data are normally distributed



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Comparing multiple groups - ANOVA

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Assumption:

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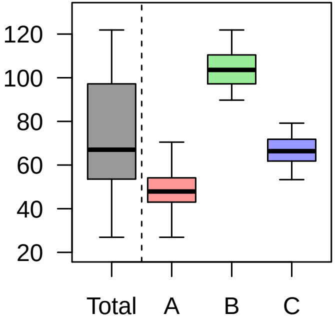
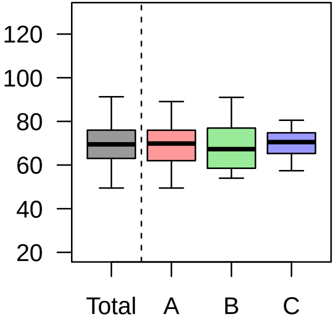
Hypotheses:

- H_0: there are no significant differences between the means
  - all means are equal
- H_1: there are significant differences between the means
  - all means are not equal

ANOVA explained

The ANOVA partitions the *total* variation into *within* sample (group) variation with *between* sample (group) variation to determine whether samples come from a single distribution or not.

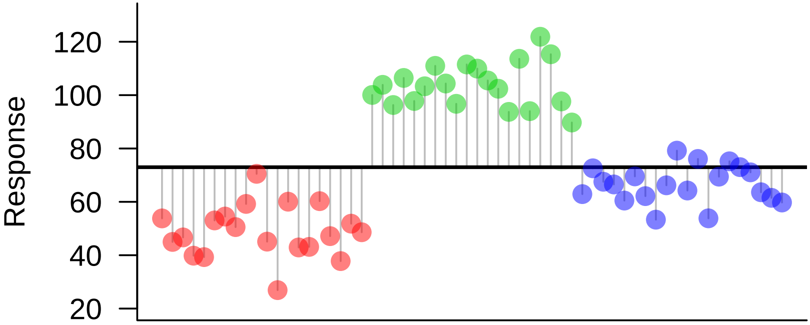




ANOVA and the Sums of Squares

- *Total* sums of squares (SS_T)

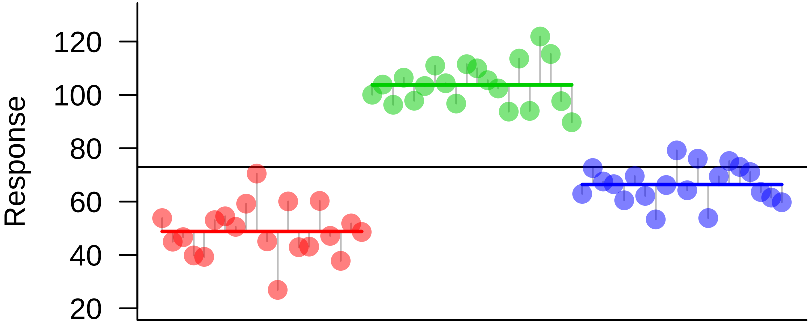
$$SS_T = \sum (x - \bar{x})^2$$



ANOVA and the Sums of Squares

- *Within-sample* sums of squares (SS_W)
- add up the within sample SS

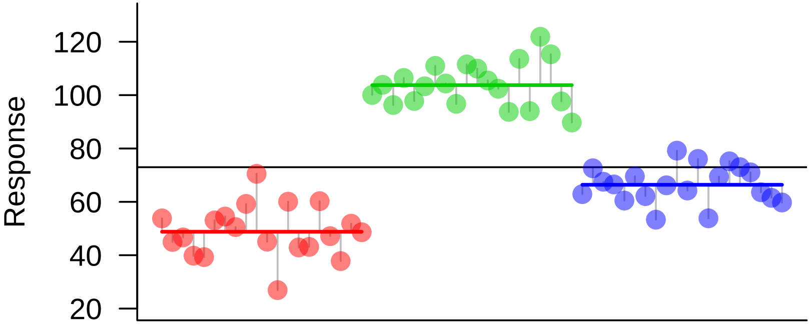
$$SS_W = \sum (x_1 - \bar{x}_1)^2 + \sum (x_2 - \bar{x}_2)^2 + \sum (x_3 - \bar{x}_3)^2$$



ANOVA and the Sums of Squares

- *Within-sample* sums of squares (SS_W)
- more generally (g is the number of groups)

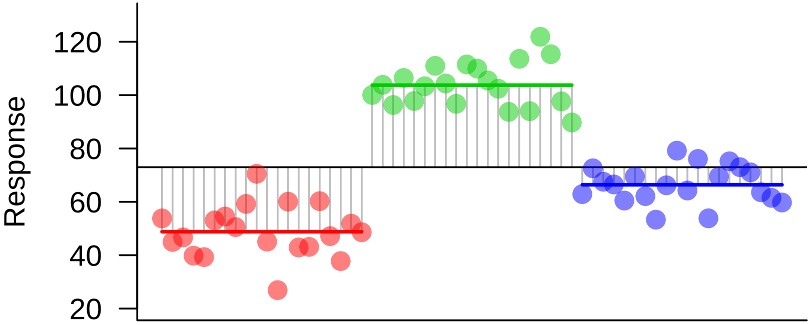
$$SS_W = \sum_g \sum_i (x_{ig} - \bar{x}_g)^2$$



ANOVA and the Sums of Squares

- *Between-sample* sums of squares (SS_B)
- add up the differences in the means

$$SS_B = n_1(\bar{x}_1 - \bar{x})^2 + n_2(\bar{x}_2 - \bar{x})^2 + n_3(\bar{x}_3 - \bar{x})^2$$



ANOVA and the Sums of Squares

- *Between-sample* sum of squares (SS_B)
- more generally (g is the number of groups)

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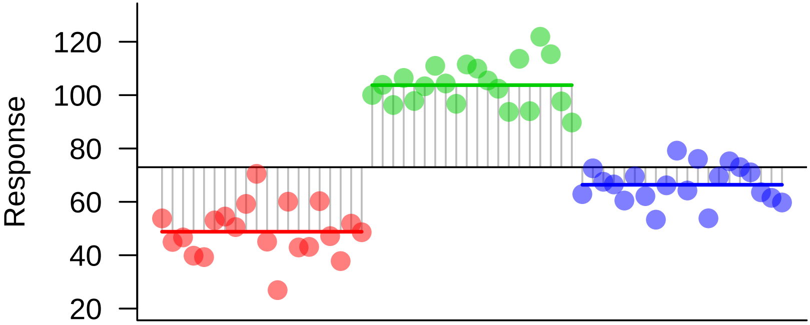
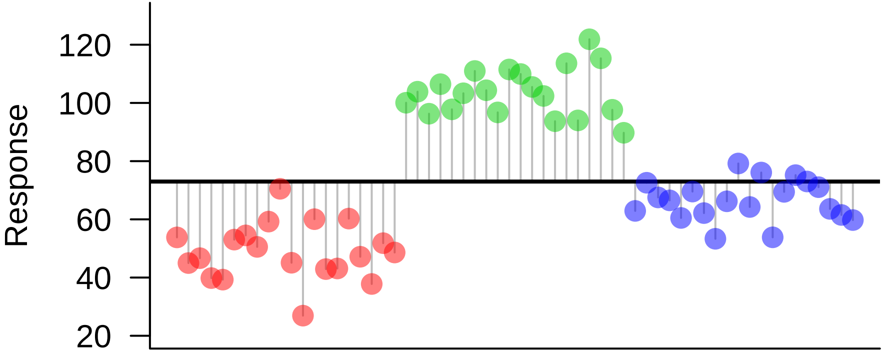


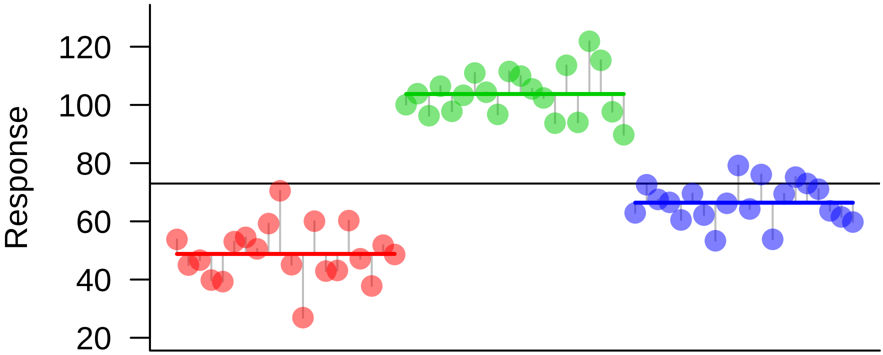
ANOVA and the Sums of Squares

Total:



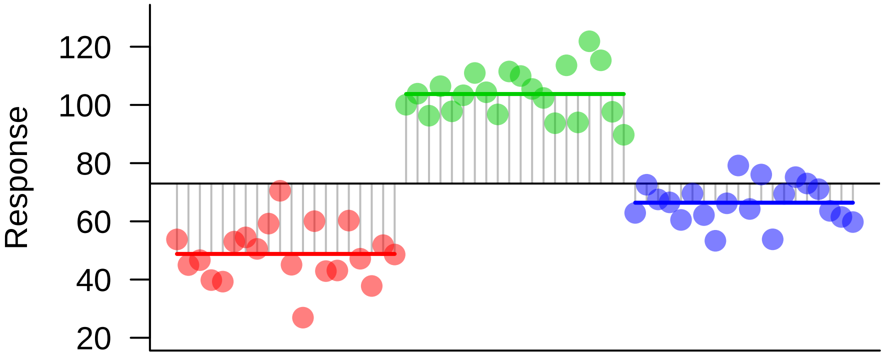
ANOVA and the Sums of Squares

Within group:



ANOVA and the Sums of Squares

Between group:



ANOVA and the Sums of Squares

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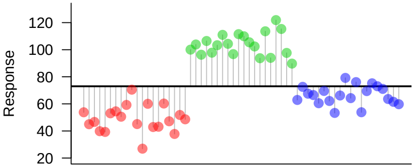

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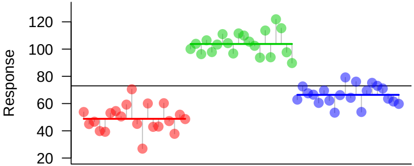

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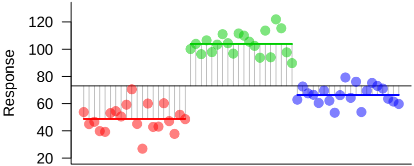

ANOVA degrees of freedom

If we define the following:

- n is the total sample size (number of observations)
- g is the number of groups/samples

ANOVA degrees of freedom

If we define the following:

- n is the total sample size (number of observations)
- g is the number of groups/samples

Then the degrees of freedom (df) are:

- Total: $df_T = n - 1$
- Within: $df_W = g - 1$
- Between: $df_B = n - g$

ANOVA the *mean square*

The mean square (MS) is the sum of squares divided by the degrees of freedom:

$$MS = SS/df$$

So:

- Total: $MS_T = SS_T/df_T$
- Within: $MS_W = SS_W/df_W$
- Between: $MS_B = SS_B/df_B$

ANOVA all the ingredients

	SS	df	MS
Total	$\sum(x-\bar{x})^2$	$n-1$	SS_T/df_T
Within	$\sum_g \sum_i (x_{ig}-\bar{x}_j)^2$	$n-g$	SS_W/df_W
Between	$\sum_g n_g(\bar{x}_g-\bar{x})^2$	$g-1$	SS_B/df_B

ANOVA the statistical test

ANOVA results are usually presented in an ANOVA table

Source of variation	SS	df	MS	F	p
Between	SS_B	df_B	MS_B		
Within	SS_W	df_W	MS_W		
Total	SS_T	df_T	–		

ANOVA the statistical test

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Source of variation	SS	df	MS	F	p
Between	SS_B	df_B	MS_B		
Within	SS_W	df_W	MS_W		
Total	SS_T	df_T	–		

- F is the test statistic for the ANOVA

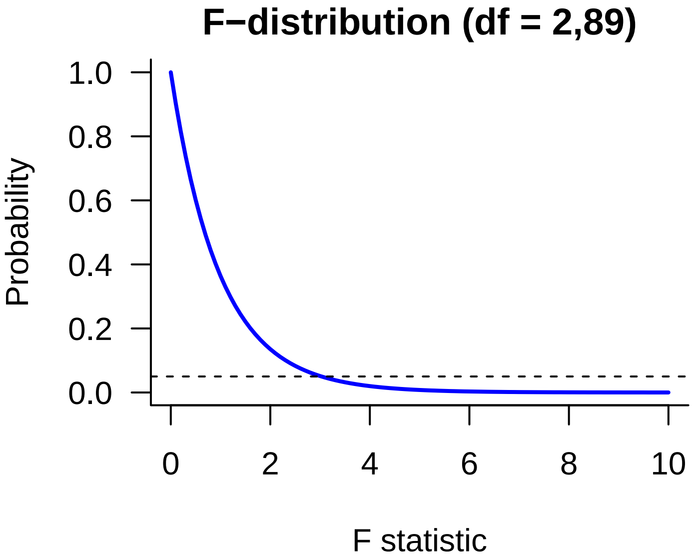
$$F = \frac{MS_B}{MS_W}$$

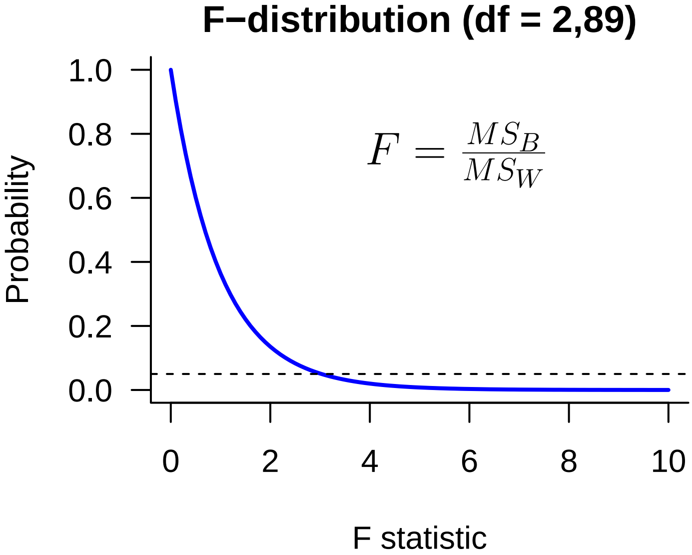
ANOVA the statistical test

ANOVA results are usually presented in an ANOVA table

Source of variation	SS	df	MS	F	p
Between	SS_B	df_B	MS_B		
Within	SS_W	df_W	MS_W		
Total	SS_T	df_T	–		

- p is the probability of observing the F statistic with a given degrees of freedom if the null hypothesis is true:
    - null hypothesis is ‘no difference between the means’
    - based on the F-distribution


F−distribution (df = 2,89)
Probability
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
F statistic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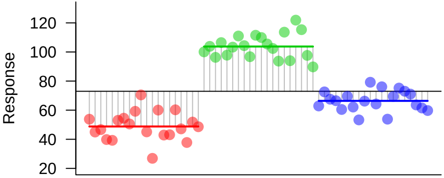
ANOVA and the Sums of Squares



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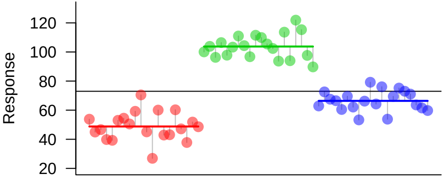
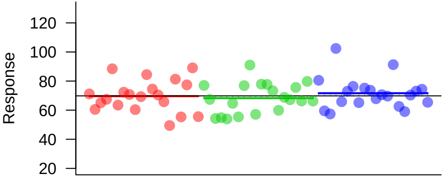


ANOVA and the Sums of Squares



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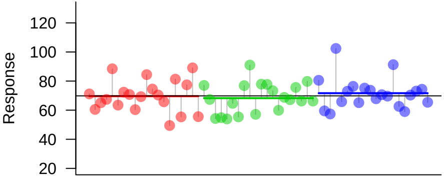

ANOVA the p value

Hypotheses:

- H_0: there are no significant differences between the means
  - all means are equal
- H_1: there are significant differences between the means
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When do we reject or fail to reject the null hypothesis?

ANOVA the p value

Hypotheses:

- H_0: there are no significant differences between the means
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When do we reject or fail to reject the null hypothesis?

- if F is large, then p is small
- if $p < 0.05$ we reject the null hypothesis
- if $p > 0.05$ we *fail to* reject the null hypothesis

Pairwise comparisons with ANOVA

The F statistic tells us whether there are differences, but *not* what the differences are:

- *Cannot* use t-tests to make pairwise comparisons
  - multiple t-tests will lead to significant results by chance

Pairwise comparisons with ANOVA

The F statistic tells us whether there are differences, but *not* what the differences are:

- Instead we conduct *Post-hoc* testing
  - Tukey Honest Significant Difference test (Tukey HSD)
  - accounts for multiple tests being conducted
  - calculation of a t-statistic
  - a pair, so degrees of freedom is 1
  - 5% critical value for $df = 1$ is 4.303
    - if $t > 4.303$ then $p < 0.05$

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$$t_{a,b} = \frac{|\bar{x}_a - \bar{x}_b|}{\sqrt{\frac{MS_W\left(\frac{1}{n_a} + \frac{1}{n_b}\right)}{2}}}$$

Pairwise comparisons with ANOVA

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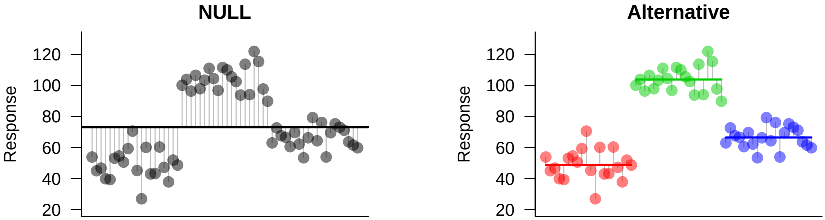
	A	B	C
A	-	$t_{A,B}$	$t_{A,C}$
B	-	-	$t_{B,C}$
C	-	-	-

ANOVA Recap

Comparing differences between >2 samples (groups) using ANOVA

- null hypothesis:
  - no difference between the samples
  - data are from the same population
- alternative hypothesis:
  - sample means are different
  - data from the different populations



ANOVA Recap

Comparing differences between >2 groups using ANOVA

Source of variation	SS	df	MS	F	p
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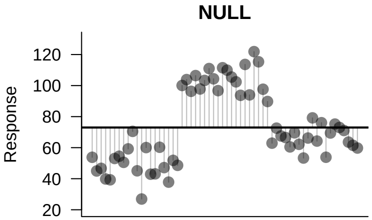
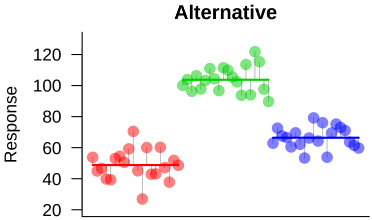
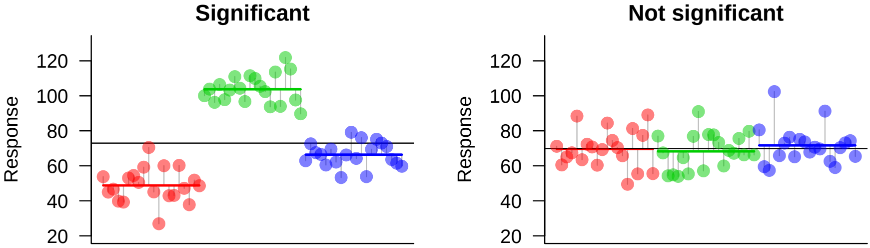




ANOVA Recap

Comparing differences between >2 groups using ANOVA

- Essentially comes down to:
  - a model with one mean *or* a model with a mean per group
  - which model best explains the data
  - which model significantly reduces the sums of squares



More than one factor with ANOVA

So far we have looked at multiple levels within a single factor

- factor: a single categorical predictor variable
- level: the categories within a factor

In some cases, we may be interested in >1 factor

- 2 factors: *two-way* ANOVA
- 3 factors: *three-way* ANOVA
- $\cdots$ multi-way ANOVA

Two-way ANOVA

Let's use a grazing example:

	Site	
Grazing Treatment	Top	Lower
Lo	9	7
Lo	11	6
Lo	6	5
Mid	14	14
Mid	17	17
Mid	19	15
Hi	28	44
Hi	31	38
Hi	32	37

Two-way ANOVA

Lets use the example from the book (in `R` looks like this):

```
graze
```

```
##     graze  Site Abundance
## 1      Lo   Top         9
## 2      Lo   Top        11
## 3      Lo   Top         6
## 4     Mid   Top        14
## 5     Mid   Top        17
## 6     Mid   Top        19
## 7      Hi   Top        28
## 8      Hi   Top        31
## 9      Hi   Top        32
## 10     Lo Lower         7
## 11     Lo Lower         6
## 12     Lo Lower         5
## 13    Mid Lower        14
```

Two-way ANOVA

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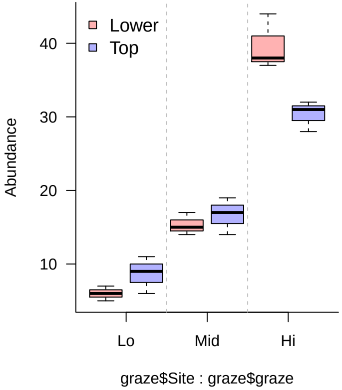
## 9      Hi   Top        32
## 10     Lo Lower         7
## 11     Lo Lower         6
## 12     Lo Lower         5
## 13    Mid Lower        14
```

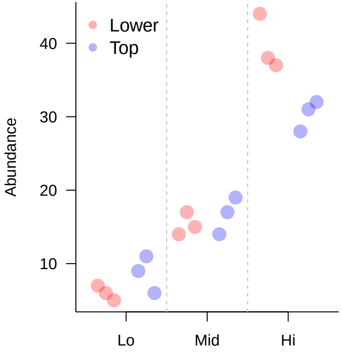


Two-way ANOVA

Lets use the example from the book (in R looks like this):

```r
head(graze, 9)
```

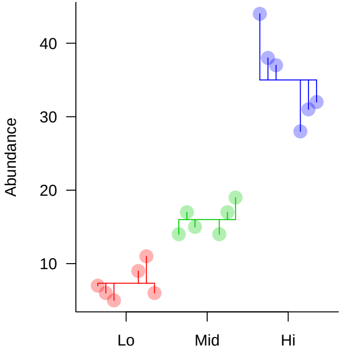
```
##   graze Site Abundance
## 1    Lo  Top         9
## 2    Lo  Top        11
## 3    Lo  Top         6
## 4   Mid  Top        14
## 5   Mid  Top        17
## 6   Mid  Top        19
## 7    Hi  Top        28
## 8    Hi  Top        31
## 9    Hi  Top        32
```



Conducting the ANOVA

Step one:

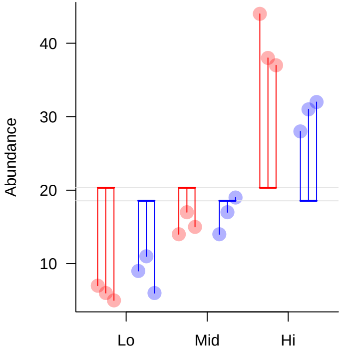
- SS for each factor
  - graze
  - site
- $SS_{graze} = \sum(x_{i,graze} - \bar{x}_{graze})^2$
- Ignore site grouping



Conducting the ANOVA

Step one:

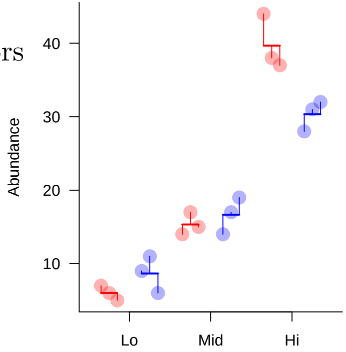
- SS for each factor
  - graze
  - site
- $SS_{site} = \sum(x_{i,site} - \bar{x}_{site})^2$
- Ignore graze grouping



Conducting the ANOVA

Step two:

- SS for each combinations of factors
- Treat all groupings as unique
- $SS_{within} = (x_{i,g} - \bar{x}_g)^2$



Conducting the ANOVA

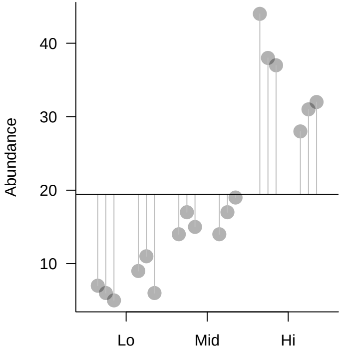
Step three:

- Sums of squares of both factors
- $SS_{both} = SS_{total} - SS_{graze} - SSsite - SS_{within}$

Conducting the ANOVA

Step four:

- Total sums of squares
- $SS_{total} = \sum(x_i - \bar{x})^2$
- the *null* model
- Ignore all group structure



Conducting the ANOVA - sums of squares

	SS	df	MS	F	p
Graze	SS_{graze}				
Site	SS_{site}				
Both factors(interaction)	SS_{both}				
Within group	SS_{within}				
Total	SS_{total}				

Degrees of freedom

In general:

- Factor 1 (F1): number of levels - 1
- Factor 2 (F2): number of levels - 1
- Within: n - (levels in F1 $\times$ levels in F2)
- Total: n - 1

Degrees of freedom

In general:

- Factor 1 (F1): number of levels - 1
- Factor 2 (F2): number of levels - 1
- Within: n - (levels in F1 $\times$ levels in F2)
- Total: n - 1

Grazing example:

- Graze: $3 - 1 = 2$
- Site: $2 - 1 = 1$
- Within: $18 - (3 \times 2) = 12$
- Total: $18 - 1 = 17$

Degrees of freedom

In general:

- Factor 1 (F1): number of levels - 1
- Factor 2 (F2): number of levels - 1
- Within: n - (levels in F1 $\times$ levels in F2)
- Total: n - 1

	SS	df	MS	F	p
Graze	SS_{graze}	df_{graze}			
Site	SS_{site}	df_{site}			
Both factors(interaction)	SS_{both}	df_{both}			
Within group	SS_{within}	df_{within}			
Total	SS_{total}	df_{total}			

Mean squares

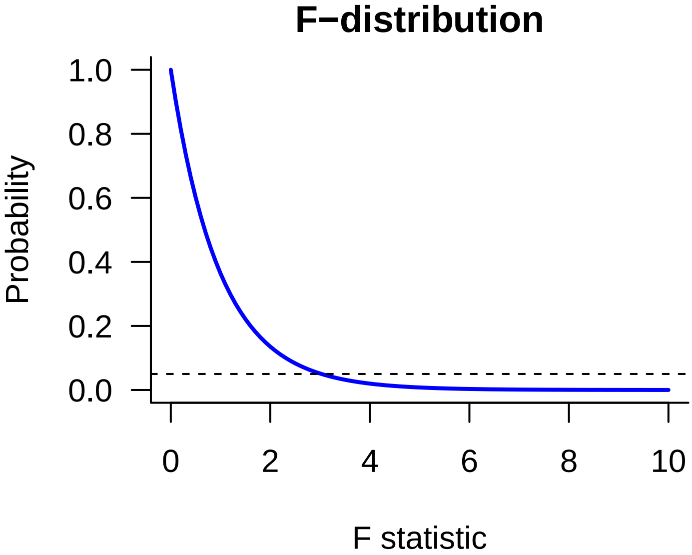
- the mean squares are calculated by dividing the sums of squares by the degrees of freedom for each element

	SS	df	MS	F	p
Graze	SS_{graze}	df_{graze}	$MS_{graze} = \frac{SS_{graze}}{df_{graze}}$		
Site	SS_{site}	df_{site}	$MS_{site} = \frac{SS_{site}}{df_{site}}$		
Both factors	SS_{both}	df_{both}	$MS_{both} = \frac{SS_{both}}{df_{both}}$		
Within group	SS_{within}	df_{within}	$MS_{within} = \frac{SS_{within}}{df_{within}}$		
Total	SS_{total}	df_{total}			

F statistic

- the F-statistic is calculated by taking the element of interest divided by the within group MS (the *error* term)

	SS	df	MS	F	p
Graze	SS_{graze}	df_{graze}	$MS_{graze} = \frac{SS_{graze}}{df_{graze}}$	$\frac{MS_{graze}}{MS_{within}}$	
Site	SS_{site}	df_{site}	$MS_{site} = \frac{SS_{site}}{df_{site}}$	$\frac{MS_{site}}{MS_{within}}$	
Both factors	SS_{both}	df_{both}	$MS_{both} = \frac{SS_{both}}{df_{both}}$	$\frac{MS_{both}}{MS_{within}}$	
Within group	SS_{within}	df_{within}	$MS_{within} = \frac{SS_{within}}{df_{within}}$		
Total	SS_{total}	df_{total}			


F−distribution
1.0
0.8
0.6
0.4
0.2
0.0
Probability
0
2
4
6
8
10
F statistic

ANOVA in practice - R

- Read in the data as a data frame

```
graze
```

```
##     graze  Site Abundance
## 1      Lo   Top         9
## 2      Lo   Top        11
## 3      Lo   Top         6
## 4     Mid   Top        14
## 5     Mid   Top        17
## 6     Mid   Top        19
## 7      Hi   Top        28
## 8      Hi   Top        31
## 9      Hi   Top        32
## 10     Lo Lower         7
## 11     Lo Lower         6
## 12     Lo Lower         5
## 13    Mid Lower        14
```

Any ANOVA in practice - R

- Conduct *any* test using formula syntax

```r
oneway.site <- aov(Abundance ~ Site, data = graze)
summary(oneway.site)
```

```
##             Df Sum Sq Mean Sq F value Pr(>F)
## Site         1   14.2   14.22   0.087  0.771
## Residuals   16 2602.2  162.64
```

Any ANOVA in practice - R

- Conduct *any* test using formula syntax

```r
oneway.site <- aov(Abundance ~ Site, data = graze)
summary(oneway.site)
```

```
##             Df Sum Sq Mean Sq F value Pr(>F)
## Site         1   14.2   14.22   0.087  0.771
## Residuals   16 2602.2  162.64
```

Any ANOVA in practice - R

- Conduct *any* test using formula syntax

```r
oneway.graze <- aov(Abundance ~ graze, data = graze)
summary(oneway.graze)
```

```
##             Df Sum Sq Mean Sq F value   Pr(>F)
## graze        2 2403.1  1201.6   84.48 6.84e-09 ***
## Residuals   15  213.3    14.2
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1
```

Any ANOVA in practice - R

- Conduct *any* test using formula syntax

```r
oneway.graze <- aov(Abundance ~ graze, data = graze)
summary(oneway.graze)
```

```
##             Df Sum Sq Mean Sq F value   Pr(>F)
## graze        2 2403.1  1201.6   84.48 6.84e-09 ***
## Residuals   15  213.3    14.2
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1
```

Any ANOVA in practice - R

- Conduct *any* test using formula syntax

```
twoway.additive <- aov(Abundance ~ Site + graze, data = gra
summary(twoway.additive)
```

```
##             Df Sum Sq Mean Sq F value   Pr(>F)
## Site         1   14.2    14.2    1.00    0.334
## graze        2 2403.1  1201.6   84.48 1.54e-08 ***
## Residuals   14  199.1    14.2
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1
```

Any ANOVA in practice - R

- Conduct *any* test using formula syntax

```
twoway.additive <- aov(Abundance ~ Site + graze, data = gra
summary(twoway.additive)
```

```
##             Df Sum Sq Mean Sq F value   Pr(>F)
## Site         1   14.2    14.2    1.00    0.334
## graze        2 2403.1  1201.6   84.48 1.54e-08 ***
## Residuals   14  199.1    14.2
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1
```

Any ANOVA in practice - R

- Conduct *any* test using formula syntax

```
twoway.interaction <- aov(Abundance ~ Site * graze, data =
summary(twoway.interaction)
```

```
##             Df Sum Sq Mean Sq F value   Pr(>F)
## Site         1   14.2    14.2   2.462  0.14264
## graze        2 2403.1  1201.6 207.962 4.86e-10 ***
## Site:graze   2  129.8    64.9  11.231  0.00178 **
## Residuals   12   69.3     5.8
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.
```

Any ANOVA in practice - R

- Conduct *any* test using formula syntax

```
twoway.interaction <- aov(Abundance ~ Site * graze, data =
summary(twoway.interaction)
```

```
##              Df Sum Sq Mean Sq F value   Pr(>F)
## Site          1   14.2    14.2   2.462  0.14264
## graze         2 2403.1  1201.6 207.962 4.86e-10 ***
## Site:graze    2  129.8    64.9  11.231  0.00178 **
## Residuals    12   69.3     5.8
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.
```

Group Exercise - *salamANOVA*

We will conduct three analyses using the *salamANOVA*. We are interested in whether salamander snout-to-vent length (SVL) varies by sex and/or site. The data look like this:

```
str(sals)
```

```
## Error in str(sals): object 'sals' not found
```

- `Site`: there are four sites (`P1A`, `P1B`, `P2A`, `P2B`)
- `Sex`: `M` (male) and `F` (female)
- `SVL`: the snout-to-vent length in mm

Group Exercise - *salamANOVA*

Analysis 1: Does SVL vary by sex?

- What is the null hypothesis?
- Make a plot to visualize the hypothesis.
- What statistical test will you use to test H_0?
- What is the:
  - test statistic for this particulat test (e.g., t, F, etc)
  - degrees of freedom (calculate this)
  - significance level
- Conduct the analysis:
  - what is the value of the test statistic
  - what the p-value
- Write a short paragraph reporting the conclusion, use values from the statistical test to suppo, supported by the results from the test.

Group Exercise - *salamANOVA*

Analysis 2: Does SVL vary by site?

- What is the null hypothesis?
- Make a plot to visualize the hypothesis.
- What statistical test will you use to test H_0?
- What is the:
  - test statistic for this particulat test (e.g., t, F, etc)
  - degrees of freedom (calculate this)
  - significance level
- Conduct the analysis:
  - what is the value of the test statistic
  - what the p-value
- Write a short paragraph reporting the conclusion, use values from the statistical test to suppo, supported by the results from the test.

Group Exercise - *salamANOVA*

Analysis 3: Does SVL vary by sex and/or site?

- What is the null hypothesis?
- Make a plot to visualize the hypothesis.
- What statistical test will you use to test H_0?
- What is the:
  - test statistic for this particulat test (e.g., t, F, etc)
  - degrees of freedom (calculate this)
  - significance level
- Conduct the analysis:
  - what is the value of the test statistic
  - what the p-value
- Write a short paragraph reporting the conclusion, use values from the statistical test to suppo, supported by the results from the test.

Group Exercise - *salamANOVA*

Assignment: Statistical analysis of variation in salamnder SVL.

- Write a report with four sections:
  1. Analysis 1
  2. Analysis 2
  3. Analysis 3
  4. Reflection: how does analysis 3 compare to analyses 1 and 2?
- Sections 1 to 3 sould report on each of the prompts in the previous slides.
- Section 4 is an opportunity to demonstrate your undertanding of the material covered over the previous weeks.