

# Analysis of Variance

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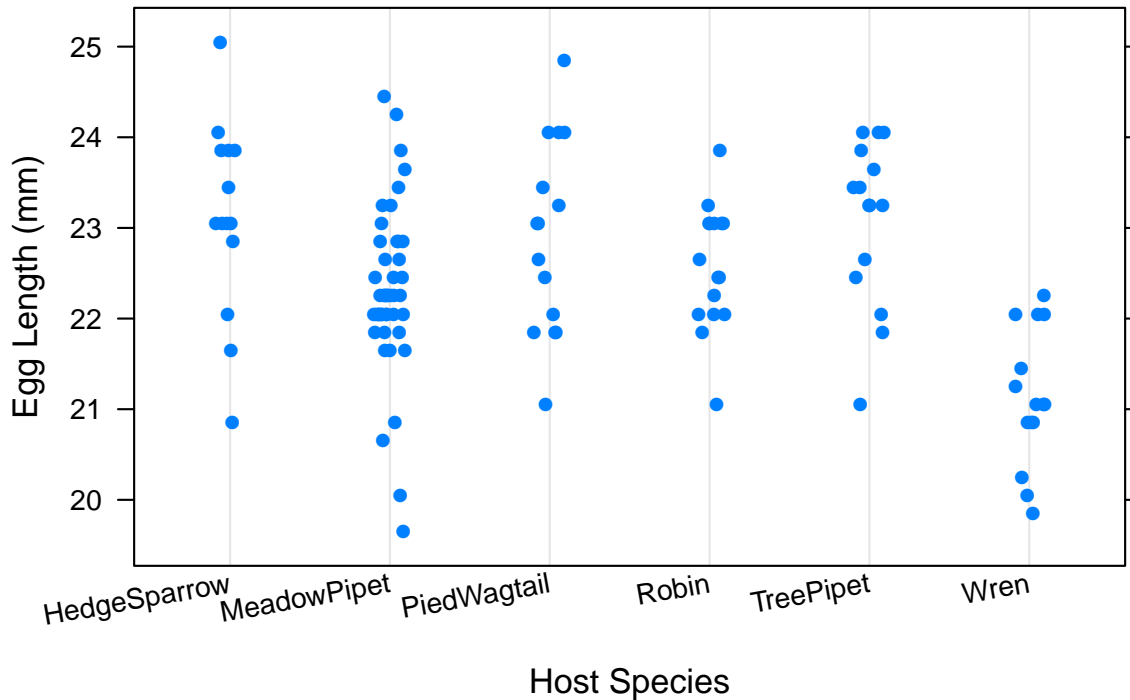
November 11–December 2, 2010

## Cuckoo Birds

### Example

- Cuckoo birds have a behavior in which they lay their eggs in other birds nests.
- The other birds then raise and care for the newly hatched cuckoos.
- Cuckoos return year after year to the same territory and lay their eggs in the nests of a particular host species.
- Furthermore, cuckoos appear to mate only within their territory.
- Therefore, geographical sub-species are developed, each with a dominant foster-parent species.
- A general question is, are the eggs of the different sub-species adapted to a particular foster-parent species?
- Specifically, we can ask, are the mean lengths of the cuckoo eggs the same in the different sub-species?

# Cuckoo Bird Egg Length Distribution



## Comparing More than Two Populations

- We have developed both  $t$  and nonparametric methods for inference for comparing means from two populations.
- What if there are three or more populations?
- It is not valid to simply make all possible pairwise comparisons:
- with three populations, there are three such comparisons, with four there are six, and the number increases rapidly.
- The comparisons are not all independent: the data used to estimate the differences between the pair of populations 1 and 2 and the pair of populations 1 and 3 use the same sample from population 1.
- When estimating differences with confidence, we may be concerned about the confidence we ought to have that all differences are in their respective intervals.
- For testing, there are many simultaneous tests to consider.

*What to do?*

# Hypotheses

- The common approach to this problem is based on a single null hypothesis

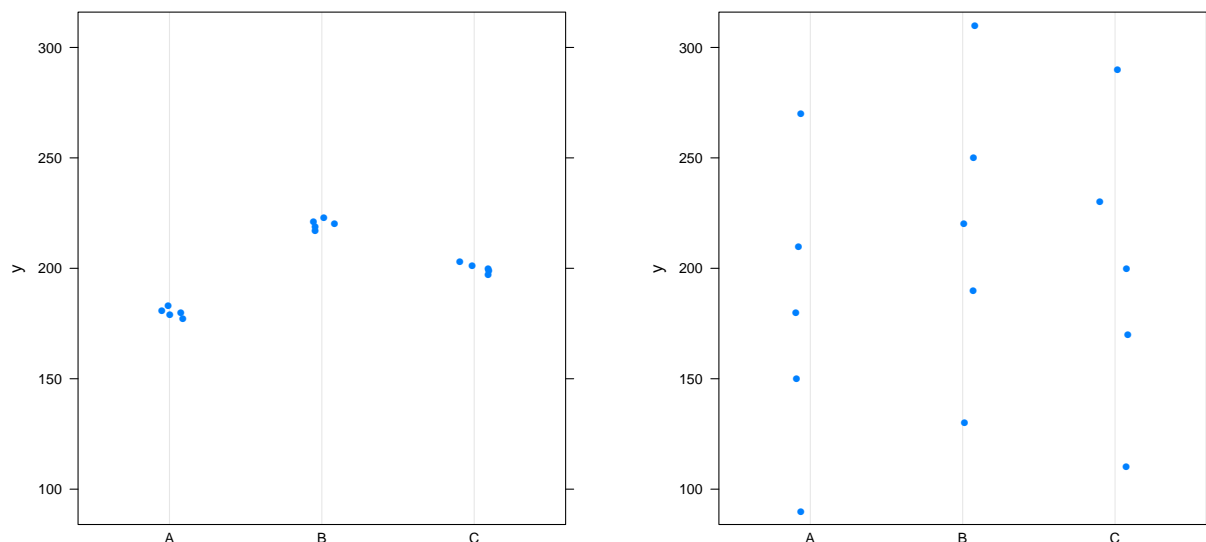
$$H_0: \mu_1 = \mu_2 = \cdots = \mu_k$$

versus the alternative hypothesis that the means are not all the same (so that there are at least two means that differ) where there are  $k$  groups.

- If there is evidence against the null hypothesis, then further inference is carried out to examine specific comparisons of interest.

## Illustrative Example

- The dot plots show two cases of three samples, each of size five.
- The sample means are respectively 180, 220, and 200 in both cases.
- The left plot appears to show differences in the mean; evidence for this in the right plot appears weaker.



# Analysis of Variance

- The previous example suggests an approach that involves comparing variances;
- If *variation among sample means is large relative to variation within samples*, then there is evidence against  $H_0: \mu_1 = \mu_2 = \dots = \mu_k$ .
- If *variation among sample means is small relative to variation within samples*, then the data is consistent with  $H_0: \mu_1 = \mu_2 = \dots = \mu_k$ .
- The approach of testing  $H_0: \mu_1 = \mu_2 = \dots = \mu_k$  on the basis of comparing variation among and within samples is called Analysis of Variance, or ANOVA.

## Notation

- There are  $k$  populations where  $Y_{ij}$  is the  $j$ th observation from the  $i$ th sample.
- There are a total of  $n$  observations with  $n_i$  in sample  $i$ .
- The sample mean and standard deviation of sample  $i$  are  $\bar{Y}_i$  and  $s_i$ .
- The *grand mean*  $\bar{Y}$  is the mean of all observations.
- Note that the grand mean

$$\bar{Y} = \sum_{i=1}^k \left( \frac{n_i}{n} \right) \bar{Y}_i$$

is the weighted average of the sample means, weighted by sample size.

- Note that subscripts for samples and populations range  $i = 1, \dots, k$  and for individual observations range  $j = 1, \dots, n_i$ .

## Modeling Assumptions

We make the following modeling assumptions:

- All observations  $Y_{ij}$  are independent ( $k$  independent random samples from populations of interest).
- $E(Y_{ij}) = \mu_i$  ( $\mu_i$  is the mean of population  $i$ ).
- $\text{Var}(Y_{ij}) = \sigma_i^2$  ( $\sigma_i^2$  is the variance of population  $i$ ).

We will also often make the following two additional assumptions:

- all population variances are equal:  $\sigma_i^2 = \sigma^2$  for all  $i$ ;
- all observations are normally distributed:  $Y_{ij} \sim N(\mu_i, \sigma_i^2)$

With the first set of assumptions, note that

- $E(\bar{Y}_i) = \mu_i$  and  $\text{Var}(\bar{Y}_i) = \frac{\sigma_i^2}{n_i}$

and additionally, if the second set of assumptions are made, then

- $\bar{Y}_i \sim N\left(\mu_i, \frac{\sigma^2}{n_i}\right)$

## Variation Among Samples

- We use this formula for the variation among sample means:

$$\sum_{i=1}^k n_i (\bar{Y}_i - \bar{Y})^2$$

which is *a weighted sum of squared deviations of sample means from the grand mean, weighted by sample size.*

- Under the assumptions of independence and equal variances,

$$E\left(\sum_{i=1}^k n_i (\bar{Y}_i - \bar{Y})^2\right) = (k-1)\sigma^2 + \sum_{i=1}^k n_i (\mu_i - \mu)^2$$

where

$$\mu = \frac{\sum_{i=1}^k n_i \mu_i}{n}$$

is the expected value of the grand mean  $\bar{Y}$ .

## Variation Among Samples (cont.)

- The sum

$$\sum_{i=1}^k n_i (\bar{Y}_i - \bar{Y})^2$$

is called the *group sum of squares*.

- If the null hypothesis  $H_0: \mu_1 = \mu_2 = \dots = \mu_k$  is true, then  $\sum_{i=1}^k n_i (\mu_i - \mu)^2 = 0$  and

$$E\left(\sum_{i=1}^k n_i (\bar{Y}_i - \bar{Y})^2\right) = (k-1)\sigma^2$$

- This suggests defining

$$MS_{\text{groups}} = \frac{\sum_{i=1}^k n_i (\bar{Y}_i - \bar{Y})^2}{k-1}$$

to be the *group mean square*.

- If the null hypothesis is true, then  $E(MS_{\text{groups}}) = \sigma^2$ ; otherwise,  $E(MS_{\text{groups}}) = \sigma^2 + \sum_{i=1}^k n_i (\mu_i - \mu)^2 / (k-1) > \sigma^2$ .

## Variation Within Samples

- For each sample, the sample variance

$$s_i^2 = \frac{\sum_{j=1}^{n_i} (Y_{ij} - \bar{Y}_i)^2}{n_i - 1}$$

is an estimate of that population's variance,  $\sigma_i^2$ .

- Under the assumptions of equal variance and independence, each  $s_i^2$  is then an independent estimate of  $\sigma^2$ .
- The formula

$$\sum_{i=1}^k \sum_{j=1}^{n_i} (Y_{ij} - \bar{Y}_i)^2 = \sum_{i=1}^k (n_i - 1) s_i^2$$

is the sum of all squared deviations from individual sample means and has expected value

$$E\left(\sum_{i=1}^k (n_i - 1) s_i^2\right) = (n - k)\sigma^2$$

## Variation Within Samples (cont.)

- The mean square error formula

$$MS_{\text{error}} = \frac{\sum_{i=1}^k (n_i - 1) s_i^2}{n - k}$$

is *a weighted average of the sample variances, weighted by degrees of freedom*.

- Notice that  $E(MS_{\text{error}}) = \sigma^2$  always: it is true when  $H_0: \mu_1 = \mu_2 = \dots = \mu_k$  is true, but also when  $H_0$  is false.

## The F Test Statistic

- We have developed two separate formulas for variation among and within samples, each based on a different *mean square*:
  - ▶  $MS_{\text{groups}}$  measures variation among groups;
  - ▶  $MS_{\text{error}}$  measures variation within groups.
- Define the ratio  $F = MS_{\text{groups}}/MS_{\text{error}}$  to be the *F*-statistic (named in honor of R. A. Fisher who developed ANOVA among many other accomplishments).
- When  $H_0: \mu_1 = \mu_2 = \dots = \mu_k$  is true (and the assumption of equal variances is also true), then both  $E(MS_{\text{groups}}) = \sigma^2$  and  $E(MS_{\text{error}}) = \sigma^2$  and the value of  $F$  should then be close to 1.
- However, if the population mean are not all equal, then  $E(MS_{\text{groups}}) > \sigma^2$  and we expect  $F$  to be greater than one, perhaps by quite a bit.

# The F Distribution

## Definition

If  $W_1$  and  $W_2$  are independent  $\chi^2$  random variables with  $d_1$  and  $d_2$  degrees of freedom, then

$$F = \frac{W_1/d_1}{W_2/d_2}$$

has an  $F$  distribution with  $d_1$  and  $d_2$  degrees of freedom.

- The mean of the  $F(d_1, d_2)$  distribution is  $d_2/(d_2 - 2)$  provided that  $d_2 > 2$ .
- The  $F$  distributions have different shapes, depending on the degrees of freedom, but are typically unimodal and skewed right.
- The R function `pf()` finds areas to the left under  $F$  distribution and the R function `qf()` finds quantiles. These functions work just like `pt()` and `qt()` except that two degrees of freedom need to be specified.

## Sampling Distribution

- If we have  $k$  independent random samples and:
  - ▶ the null hypothesis  $H_0: \mu_1 = \mu_2 = \dots = \mu_k$  is true;
  - ▶ all population variances are equal  $\sigma_i^2 = \sigma^2$ ;
  - ▶ individual observations are normal,  $Y_{ij} \sim N(\mu, \sigma^2)$ ;

then,

- ▶  $(k - 1)MS_{\text{groups}}/\sigma^2 \sim \chi^2(k - 1)$ ;
- ▶  $(n - k)MS_{\text{error}}/\sigma^2 \sim \chi^2(n - k)$ ;
- ▶  $MS_{\text{groups}}$  and  $MS_{\text{error}}$  are independent;

- It follows that

$$F = \frac{MS_{\text{groups}}}{MS_{\text{error}}} \sim F(k - 1, n - k)$$



## ANOVA Table

- The  $F$  statistic is the test statistic for the hypothesis test  $H_0: \mu_1 = \mu_2 = \dots = \mu_k$  versus  $H_A$ : not all means are equal.
- The steps for computing  $F$  are often written in an ANOVA table with this form.

Source	df	Sum of Squares	Mean Square	F	P value
Groups	$k - 1$	$SS_{\text{groups}}$	$MS_{\text{groups}}$	$F$	$P$
Error	$n - k$	$SS_{\text{error}}$	$MS_{\text{error}}$		
Total	$n - 1$	$SS_{\text{total}}$			

## Total Sum of Squares

- The total sum of squares is the sum of squared deviations around the grand mean.

$$SS_{\text{total}} = \sum_{i=1}^k \sum_{j=1}^{n_i} (Y_{ij} - \bar{Y})^2$$

- It can be shown algebraically that

$$\sum_{i=1}^k \sum_{j=1}^{n_i} (Y_{ij} - \bar{Y})^2 = \sum_{i=1}^k n_i (\bar{Y}_i - \bar{Y})^2 + \sum_{i=1}^k (n_i - 1) s_i^2$$

or

$$SS_{\text{total}} = SS_{\text{groups}} + SS_{\text{error}}$$

## Return to the Cuckoo Example

- The function `lm()` fits linear models in R.
- The function `anova()` displays the ANOVA table for the fitted model.

```
> cuckoo.lm = lm(eggLength ~ hostSpecies, data = cuckoo)
> anova(cuckoo.lm)
```

### Analysis of Variance Table

Response: eggLength

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
hostSpecies	5	42.940	8.5879	10.388	3.152e-08 ***
Residuals	114	94.248	0.8267		

---

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## Interpretation

*There is very strong evidence that the mean sizes of cuckoo bird eggs within populations that use different host species are different (one-way ANOVA,  $F = 10.4$ ,  $df = 5$  and 114,  $P < 10^{-7}$ ). This is consistent with a biological explanation of adaptation in response to natural selection; host birds may be more likely to identify an egg as not their own and remove it from the nest if its size differs from the size of its own eggs.*

## Summary Statistics

- The table can also be constructed from summary statistics.
- Note for example that the mean square error in the ANOVA table is a weighted average of the sample variances.

Host Species	$n$	mean	sd	variance
HedgeSparrow	14	23.12	1.07	1.14
MeadowPipet	45	22.30	0.92	0.85
PiedWagtail	15	22.90	1.07	1.14
Robin	16	22.57	0.68	0.47
TreePipet	15	23.09	0.90	0.81
Wren	15	21.13	0.74	0.55

## Example Calculations

- *Degrees of freedom depends only on sample sizes.*
- $14 + 45 + 15 + 16 + 15 + 15 = 120$  so there are 119 total degrees of freedom.
- There are  $k = 6$  groups, so there are 5 degrees of freedom for group.
- The difference is 114 degrees of freedom for error (or residuals).
- *$MS_{error}$  is the weighted average of sample variances*

$$\begin{aligned} MS_{error} &= \frac{(13)(1.07)^2 + (44)(0.92)^2 + (14)(1.07)^2 + (15)(0.68)^2 + (14)(0.90)^2 + (14)(0.74)^2}{114} \\ &\doteq 0.827 \end{aligned}$$

## More Calculations

- The grand mean:

$$\frac{(14)(23.12) + (45)(22.30) + (15)(22.90) + (16)(22.57) + (15)(23.09) + (15)(21.13)}{120} \\ \doteq 22.46$$

- Group sum of squares:

$$(14)(23.12 - 22.46)^2 + (45)(22.30 - 22.46)^2 + (15)(22.90 - 22.46)^2 + (16)(22.57 - 22.46)^2 + (15)(23.09 - 22.46)^2 + (15)(21.13 - 22.46)^2 \\ \doteq 42.94$$

- You should know how to complete a partially filled ANOVA table and how to find entries from summary statistics.

## Variance Explained

### Definition

The *proportion of variability explained by the groups*, or  $R^2$  value, is defined as

$$R^2 = \frac{SS_{\text{groups}}}{SS_{\text{total}}} = 1 - \frac{SS_{\text{error}}}{SS_{\text{total}}}$$

and takes on values between 0 and 1.

- In the cuckoo example, the proportion of the variance explained is  $42.94/137.19 \doteq 0.31$ .

## Estimation

- The ANOVA analysis provides strong evidence that the populations of cuckoo birds that lay eggs in different species of host nests have, on average, eggs of different size.
- It is more challenging to say in what ways the mean egg lengths are different.
- Estimating the standard error for each difference is straightforward.
- Finding appropriate multipliers for those differences may depend on whether or not the researcher is examining a small number of predetermined differences, or if the researcher is exploring all possible pairwise differences.
- In the former case, a  $t$ -distribution multiplier is appropriate, except that the standard error is estimated from all samples, not just two.
- In the latter case, there are many approaches, none perfect.

## Standard Error

- When estimating the difference between two population means, recall the standard error formula (assuming a common standard deviation  $\sigma$  for all populations)

$$SE(\bar{Y}_i - \bar{Y}_j) = \sigma \sqrt{\frac{1}{n_i} + \frac{1}{n_j}}$$

- In the two-sample method, we pooled the two sample variances to estimate  $\sigma$  with  $s_{\text{pooled}}$ .
- In ANOVA, the square root of the mean square error,  $\sqrt{MS_{\text{error}}}$  pools the data from all samples to estimate the common  $\sigma$ .
- This is only sensible if the assumption of equal variances is sensible.

## Example

- For the cuckoo data, we have this estimate for  $\sigma$ .

$$\sqrt{MS_{\text{error}}} \doteq \sqrt{0.827} \doteq 0.91$$

- With six groups, there are 15 different two-way comparisons between sample means.
- The standard errors are different and depend on the specific sample sizes.
- It can be useful to order the groups according to the size of the sample means.

## Example

Population	Mean	$n$	Population	Mean	$n$	Difference	SE
MeadowPipet	22.30	45	Wren	21.13	15	1.17	0.27
Robin	22.57	16	Wren	21.13	15	1.45	0.33
PiedWagtail	22.90	15	Wren	21.13	15	1.77	0.33
TreePipet	23.09	15	Wren	21.13	15	1.96	0.33
HedgeSparrow	23.12	14	Wren	21.13	15	1.99	0.34
Robin	22.57	16	MeadowPipet	22.30	45	0.28	0.27
PiedWagtail	22.90	15	MeadowPipet	22.30	45	0.60	0.27
TreePipet	23.09	15	MeadowPipet	22.30	45	0.79	0.27
HedgeSparrow	23.12	14	MeadowPipet	22.30	45	0.82	0.28
PiedWagtail	22.90	15	Robin	22.57	16	0.33	0.33
TreePipet	23.09	15	Robin	22.57	16	0.52	0.33
HedgeSparrow	23.12	15	Robin	22.57	16	0.55	0.33
TreePipet	23.09	15	PiedWagtail	22.90	15	0.19	0.33
HedgeSparrow	23.12	14	PiedWagtail	22.90	15	0.22	0.34
HedgeSparrow	23.12	14	TreePipet	23.09	15	0.03	0.34

## Confidence Intervals

- Each of the fifteen differences can be estimated with confidence by using a  $t$ -multiplier times the SE for the margin of error.
- The  $t$ -multiplier is based on the confidence level and the error degrees of freedom.
- In the example, for a 95% confidence interval, the multiplier would be  $t^* = 1.98$ .
- Each of the fifteen confidence intervals would be valid, but it would be incorrect to interpret with 95% confidence that *each of the fifteen confidence intervals contains the corresponding difference in means*.

## 95% Confidence Intervals

Population	Population	$a$	$b$
MeadowPipet	Wren	0.63	1.71
Robin	Wren	0.80	2.09
PiedWagtail	Wren	1.12	2.43
TreePipet	Wren	1.30	2.62
HedgeSparrow	Wren	1.32	2.66
Robin	MeadowPipet	-0.25	0.80
PiedWagtail	MeadowPipet	0.07	1.14
TreePipet	MeadowPipet	0.25	1.33
HedgeSparrow	MeadowPipet	0.27	1.37
PiedWagtail	Robin	-0.32	0.98
TreePipet	Robin	-0.13	1.16
HedgeSparrow	Robin	-0.11	1.21
TreePipet	PiedWagtail	-0.47	0.84
HedgeSparrow	PiedWagtail	-0.45	0.89
HedgeSparrow	TreePipet	-0.64	0.70

## Simultaneous confidence intervals

- If we want to be 95% confident that *all population mean differences* are contained in their intervals, we need to increase the size of the multiplier.
- This issue is known as *multiple comparisons* in the statistics literature.
- The method described in the text, *Tukey's honestly significant difference (HSD)* is based on the sampling distribution of the difference between the largest and smallest sample means when the null distribution is true, but assumes equal sample sizes.
- Other methods use slightly smaller multipliers for other differences; for example, the multiplier for the difference between the first and second largest sample means would be smaller than that for the largest and smallest sample means.
- It suffices to know that if you care about adjusting for multiple comparisons, that the multipliers need to be larger than the  $t$ -multipliers and that there are many possible ways to accomplish this.

## Tukey's HSD in R

- R contains the function `TukeyHSD()` which can be used on the output from `aov()` to apply Tukey's HSD method for simultaneous confidence intervals.
- The method adjusts for imbalance in sample size, but may not be accurate with large imbalances.



## Cuckoo Data

```
----- file cuckoo.txt -----  
eggLength hostSpecies  
19.65 MeadowPipet  
20.05 MeadowPipet  
20.65 MeadowPipet  
20.85 MeadowPipet  
21.65 MeadowPipet  
...  
21.45 Wren  
22.05 Wren  
22.05 Wren  
22.05 Wren  
22.25 Wren  
----- end of file -----
```

## Reading in the Data

- Here is code to read in the data.
- We also use the lattice function `reorder()` to order the populations from smallest to largest egg length instead of alphabetically.
- This reordering is not essential, but is useful.
- The command `with()` allows R to recognize the names `hostSpecies` and `eggLength` without the dollar sign.
- The `require()` function loads in lattice if not already loaded.

```
> cuckoo = read.table("cuckoo.txt", header = T)  
> require(lattice)  
> cuckoo$hostSpecies = with(cuckoo, reorder(hostSpecies,  
+   eggLength))
```

# Fitting the ANOVA model

- We greatly prefer using `lm()` instead of `aov()`, but `TukeyHSD()` requires the latter.

```
> fit = aov(eggLength ~ hostSpecies, data = cuckoo)
```

## Tukey's HSD

```
> TukeyHSD(fit)
```

```
Tukey multiple comparisons of means  
95% family-wise confidence level
```

```
Fit: aov(formula = eggLength ~ hostSpecies, data = cuckoo)
```

```
$hostSpecies
```

	diff	lwr	upr	p adj
MeadowPipet-Wren	1.16888889	0.383069115	1.954709	0.0004861
Robin-Wren	1.44500000	0.497728567	2.392271	0.0003183
PiedWagtail-Wren	1.77333333	0.810904595	2.735762	0.0000070
TreePipet-Wren	1.96000000	0.997571262	2.922429	0.0000006
HedgeSparrow-Wren	1.99142857	1.011964373	2.970893	0.0000006
Robin-MeadowPipet	0.27611111	-0.491069969	1.043292	0.9021876
PiedWagtail-MeadowPipet	0.60444444	-0.181375330	1.390264	0.2324603
TreePipet-MeadowPipet	0.79111111	0.005291337	1.576931	0.0474619
HedgeSparrow-MeadowPipet	0.82253968	0.015945760	1.629134	0.0428621
PiedWagtail-Robin	0.32833333	-0.618938100	1.275605	0.9155004
TreePipet-Robin	0.51500000	-0.432271433	1.462271	0.6159630
HedgeSparrow-Robin	0.54642857	-0.418146053	1.511003	0.5726153
TreePipet-PiedWagtail	0.18666667	-0.775762072	1.149095	0.9932186
HedgeSparrow-PiedWagtail	0.21809524	-0.761368960	1.197559	0.9872190
HedgeSparrow-TreePipet	0.03142857	-0.948035627	1.010893	0.9999990

## Comparison

Population	Population	<i>t</i> -method		Tukey HSD	
		<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>
MeadowPipet	Wren	0.63	1.71	0.38	1.95
Robin	Wren	0.80	2.09	0.50	2.39
PiedWagtail	Wren	1.12	2.43	0.81	2.74
TreePipet	Wren	1.30	2.62	1.00	2.92
HedgeSparrow	Wren	1.32	2.66	1.01	2.97
Robin	MeadowPipet	-0.25	0.80	-0.49	1.04
PiedWagtail	MeadowPipet	0.07	1.14	-0.18	1.39
TreePipet	MeadowPipet	0.25	1.33	0.01	1.58
HedgeSparrow	MeadowPipet	0.27	1.37	0.02	1.63
PiedWagtail	Robin	-0.32	0.98	-0.62	1.28
TreePipet	Robin	-0.13	1.16	-0.43	1.46
HedgeSparrow	Robin	-0.11	1.21	-0.42	1.51
TreePipet	PiedWagtail	-0.47	0.84	-0.78	1.15
HedgeSparrow	PiedWagtail	-0.45	0.89	-0.76	1.20
HedgeSparrow	TreePipet	-0.64	0.70	-0.95	1.01

## Interpretation

- There is evidence that the population mean length of cuckoo bird eggs in wren nests is smaller than those of all other cuckoo bird populations.
- Other comparisons are difficult to interpret, as we are not confident in the order of means, even though we are confident about some differences.
- Note that the Tukey confidence intervals are noticeably wider.

## What you should know so far

You should know:

- how to complete a partially completed ANOVA table;
- how to fill an ANOVA table from summary statistics;
- how to find the pooled estimate of the common standard deviation;
- how to construct a confidence interval for the difference in two population means;
- why there may be a need to use a different method when constructing simultaneous confidence intervals.

## Linear Models

- ANOVA is an example of a *linear model*.
- In a linear model, a response variable  $Y$  is modeled as a mean plus error, where
  - ▶ the mean is a *linear function* of parameters and covariates;
  - ▶ the error is random normally distributed mean-zero variation.
- A linear function takes the form

$$\beta_0 + \beta_1 x_1 + \cdots + \beta_p x_p$$

where the  $\{\beta_i\}$  are parameters and the  $\{x_i\}$  are covariates.

## New Notation

- To accommodate multiple explanatory variables and prepare for other linear models such as regression, we will change notation.
  - ▶  $Y_i$  is the value of the response variable for the  $i$ th observation;
  - ▶  $n$  is the total number of observations;
  - ▶  $j(i)$  is the group of the  $i$ th observation;
  - ▶  $k$  is the number of groups.
- In this new notation:
  - ▶  $i = 1, \dots, n$  varies over all observations (and not groups);
  - ▶  $j = 1, \dots, k$  varies over groups (and not observations within groups);
  - ▶  $k$  and  $n$  mean the same thing as before.

## Linear Model

- A linear model takes the following form.

$$Y_i = \mu_{j(i)} + \varepsilon_i$$

where  $\varepsilon_i \sim N(0, \sigma^2)$  and  $\mu_{j(i)} = E(Y_i)$ .

- There are multiple ways to parameterize a one-way ANOVA model.
- Consider a toy example with  $k = 3$  groups with means 16, 20, and 21.

## First Parameterization

- One way to parameterize a one-way ANOVA model is to treat one group as a reference, and parameterize differences between the means of other groups and the reference group.
- If the first group is selected as the reference:
  - ▶  $\beta_0 = \mu_1$ ;
  - ▶  $\beta_1 = \mu_2 - \mu_1$ ;
  - ▶  $\beta_2 = \mu_3 - \mu_1$ .
- Using the example  $\mu_1 = 16$ ,  $\mu_2 = 20$ , and  $\mu_3 = 21$ , we have  $\beta_0 = 16$ ,  $\beta_1 = 4$  and  $\beta_2 = 5$ .
- Notice that the statement  
*the first mean is 16, the second mean is four larger than the first, and the third mean is five larger than the first*

is just a different way to convey the same information as

*the first mean is 16, the second is 20, and the third is 21.*

## First Parameterization (cont.)

- Define these covariates (here, indicator random variables):
  - ▶  $x_{1i}$  is 1 if the  $i$ th observation is in group 2 and be 0 if it is not.
  - ▶  $x_{2i}$  be 1 if the  $i$ th observation is in group 3 and be 0 if it is not.

- Then,

$$Y_i = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \varepsilon_i$$

- In this example the three means  $\{\mu_j\}$  are reparameterized with three parameters  $\{\beta_j\}$ .
- Notice:
  - ▶ if the  $i$ th observation is in group 1, then  $x_{1i} = 0$  and  $x_{2i} = 0$  so  $Y_i = \beta_0 + \varepsilon_i$ ;
  - ▶ if the  $i$ th observation is in group 2, then  $x_{1i} = 1$  and  $x_{2i} = 0$  so  $Y_i = \beta_0 + \beta_1 + \varepsilon_i$ ;
  - ▶ if the  $i$ th observation is in group 3, then  $x_{1i} = 0$  and  $x_{2i} = 1$  so  $Y_i = \beta_0 + \beta_2 + \varepsilon_i$ .

## lm() in R

- The previous parameterization is the default in R.
- Consider the cuckoo example again.

```
> cuckoo.lm = lm(eggLength ~ hostSpecies, data = cuckoo)
> summary(cuckoo.lm)
```

Call:

```
lm(formula = eggLength ~ hostSpecies, data = cuckoo)
```

Residuals:

```
      Min       1Q   Median       3Q      Max
-2.64889 -0.44889 -0.04889  0.55111  2.15111
```

Coefficients:

```
              Estimate Std. Error t value Pr(>|t|)
(Intercept)    21.1300    0.2348   90.004 < 2e-16 ***
hostSpeciesMeadowPipet  1.1689    0.2711   4.312 3.46e-05 ***
hostSpeciesRobin      1.4450    0.3268   4.422 2.25e-05 ***
hostSpeciesPiedWagtail 1.7733    0.3320   5.341 4.78e-07 ***
hostSpeciesTreePipet  1.9600    0.3320   5.903 3.74e-08 ***
hostSpeciesHedgeSparrow 1.9914    0.3379   5.894 3.91e-08 ***
---
```

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.9093 on 114 degrees of freedom

Multiple R-squared: 0.313, Adjusted R-squared: 0.2829

F-statistic: 10.39 on 5 and 114 DF, p-value: 3.152e-08

## lm() in R (cont.)

- In this formulation, the mean length of cuckoo birds laid in wren nests is the intercept  $\beta_0$ .
- This is estimated as 21.13, the wren group mean.
- The other parameters are differences between means of other groups and means of the wren group.
- For example, the meadow pipet mean group mean is 22.30, or 1.17 larger than the wren group mean.
- The summary contains inferences for six parameters.
- The first line tests  $H_0: \beta_0 = 0$ , or that the mean length of the eggs in the wren group is zero. This is biologically meaningless and overwhelmingly rejected.
- Each other row one of the pairwise comparisons between the wren group and the others.
- Each p-value is (much) less than 0.05, consistent with the 95% confidence intervals for these differences not containing 0.
- None of the other ten pairwise comparisons is shown, though.

## Confidence Intervals from the Summary

- We can construct some confidence intervals for population mean differences from this summary.
- The residual error 0.9093 on 114 degrees of freedom matches  $\sqrt{0.8267}$  from the ANOVA table.
- The standard error for the meadow pipet minus wren group mean difference is 0.2711 which matches

$$0.9093 \times \sqrt{\frac{1}{15} + \frac{1}{45}}$$

- The critical  $t$  quantile with 114 degrees of freedom for a 95% confidence interval is 1.98, so the margin of error is 0.54.
- Adding and subtracting this to the difference 1.17 results in the 95% confidence interval

$$0.63 < \mu_{\text{meadow pipet}} - \mu_{\text{wren}} < 1.71$$

- This matches the result from an earlier slide.
- This interval (and the others) do not compensate for multiple comparisons.

## Second Parameterization

- A second parameterization is that there is an overall mean  $\mu$  and a separate *treatment effect*  $\alpha_j$  for the  $j$ th group.
- Here,  $\mu_j = \mu + \alpha_j$  for  $j = 1, \dots, k$ .
- Each parameter  $\alpha_j$  now represents the difference between a group mean and an overall mean, not a difference between the group mean and a reference mean as in the first parameterization.
- As specified, this model is not well defined because the same set of population means can be represented by an infinite number of parameter values.
- For example, in the toy example with  $\mu_1 = 16$ ,  $\mu_2 = 20$ , and  $\mu_3 = 21$ , the parameterizations:
  - ▶  $\mu = 0$ ,  $\alpha_1 = 16$ ,  $\alpha_2 = 20$ , and  $\alpha_3 = 21$ ; and
  - ▶  $\mu = 19$ ,  $\alpha_1 = -3$ ,  $\alpha_2 = 1$ , and  $\alpha_3 = 2$ .

both simplify to the group means.

- This can be avoided by adding the constraint  $\sum_j \alpha_j = 0$ .
- Notice that in the toy example, three means are represented by four parameters, but adding a constraint means that only three of the four parameters are free.



## Quick summary

- A one-way analysis of variance model is fit in R using `lm()`.
- The results of this model fit can be summarized using `anova()` which displays an ANOVA table.
- The ANOVA table is a structured calculation of a test statistic for the null hypothesis  $H_0: \mu_1 = \dots = \mu_k$  with an  $F$  test.
- The results can also be summarized with `summary()` which displays estimated coefficients and standard errors for model parameters and  $t$ -tests for the hypotheses  $H_0: \beta_j = 0$ .
- The model parameters include  $k - 1$  of the pairwise differences, but not all of them.
- Standard errors for other differences may be found by hand  $\hat{\sigma}\sqrt{1/n_i + 1/n_j}$  or by changing the order of the levels in the factor.

## Cautions and Concerns

- One-way ANOVA assumes independent random sampling from different populations.
- The  $F$ -distribution of the test statistic assumes equal variances among populations and normality:
  - ▶ if not, the true sampling distribution is not exactly  $F$ ;
  - ▶ However, the method is robust to moderate deviations from equal variance;
  - ▶ and, the method is robust to moderate deviations from normality.
  - ▶ If the equal variance or normal assumptions (or both) are untenable, then the  $p$ -value could be found from the null distribution of the  $F$  statistic from a randomization test where groups are assigned in their given sizes at random.

## Extensions

- Linear models can be extended by adding additional explanatory variables.
- If all explanatory variables are factors, then the model is multi-way ANOVA.
- If all explanatory variables are quantitative, then the model is regression.
- If the levels of a factor are considered as *random draws from a population* instead of unknown fixed parameters, then the model is called a *random effects model*.
- Models with two or more explanatory variables can include parameters for *interactions*.
- If the response variable is not normal (or transformable to normal) and another distribution is more appropriate (such as binomial or Poisson), then we should consider instead a *generalized linear model*.

## Case Study

### Example

In a field experiment in the shallows of a small lake on Vancouver Island, biologists examined the relationship between fish abundance and the abundance and diversity of prey zooplankton. At five different locations in the lake, the biologists created three  $3\text{m} \times 3\text{m}$  regions: a control region which was open, a low fish abundance region in which 30 small fish were enclosed in a mesh cage, and a high fish abundance region in which 90 fish were enclosed in a mesh cage. After 13 days, the biologists summarized the abundance and diversity of zooplankton using an index called Levin's D, which depends both on the number of species found and their frequency.

Is there evidence that fish abundance affects zooplankton diversity?

# Data

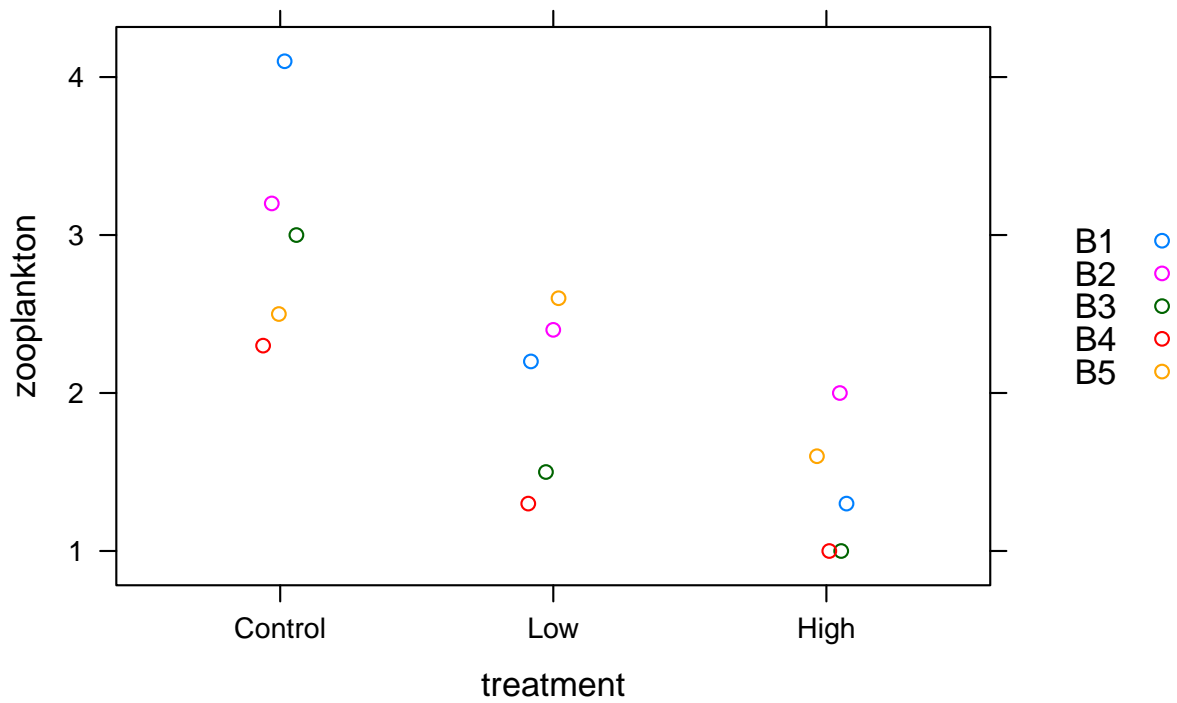
- Here is the data.

Abundance	Location				
	1	2	3	4	5
Control	4.1	3.2	3.0	2.3	2.5
Low	2.2	2.4	1.5	1.3	2.6
High	1.3	2.0	1.0	1.0	1.6

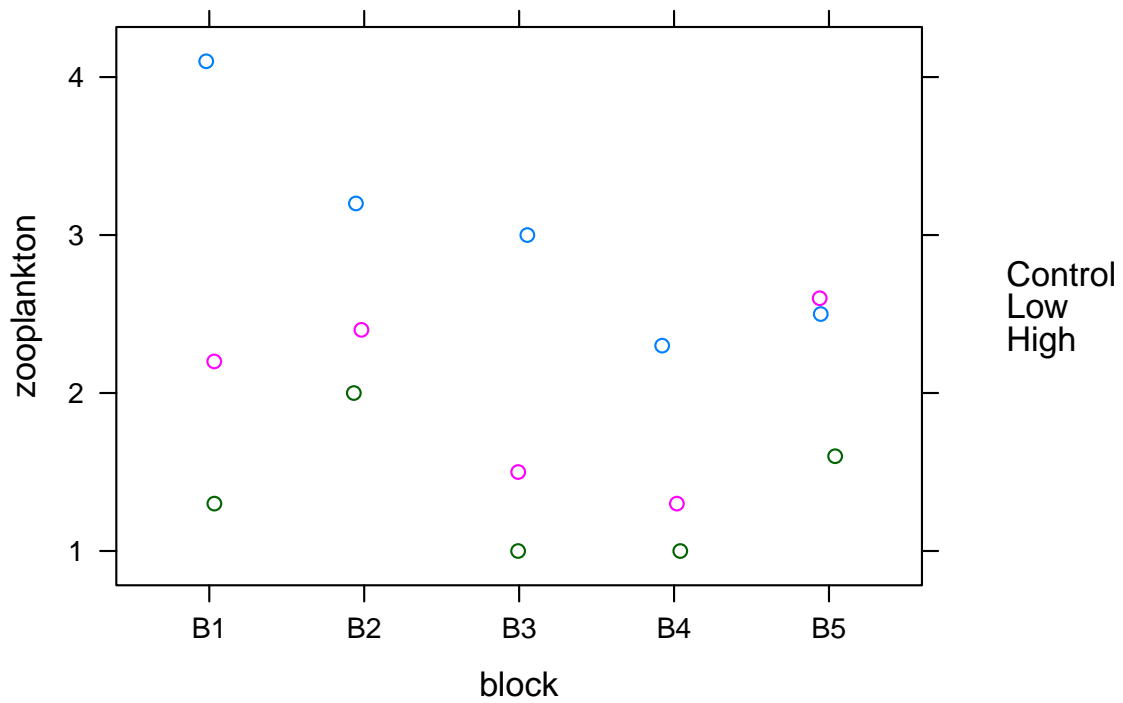
## Blocking

- It is not appropriate to treat this data as three independent samples of size five, as there are really a sample of five locations where each treatment is measured at each location.
- If there were only two treatments, this would be just like a *paired design*.
- In more generality with possibly more than two treatments, this is known as a *randomized block design*.
- Each treatment is applied to an individual region within each block (location on the lake).
- We model the diversity as a function of both location and fish abundance treatment.
- We care about differences in effects due to fish abundance, but wish to control for possible effects due to location.

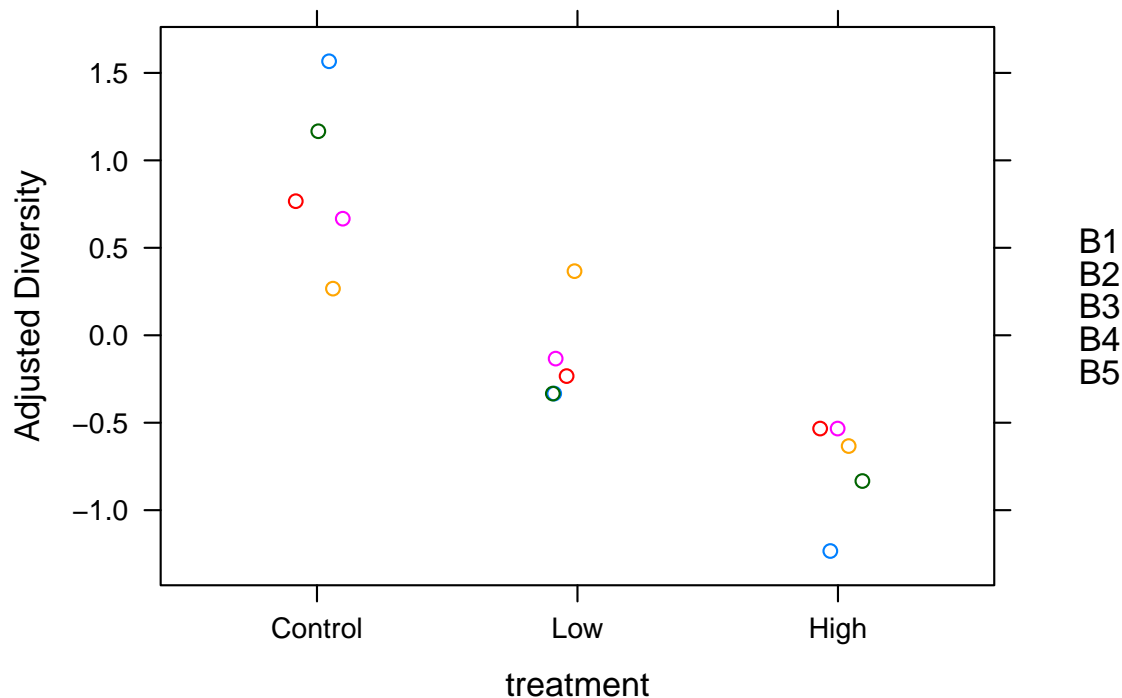
## Graphing the Data



## Displaying Block Effects



## Adjusted Data (subtract block mean)



## Two-way ANOVA Model

- We can test for a fish abundance effect while controlling for a possible block effect with a two-way ANOVA.
- The total sum of squares may be partitioned into sums of squares for treatment, block, and error.

$$SS_{\text{total}} = SS_{\text{treatment}} + SS_{\text{block}} + SS_{\text{error}}$$

- Treatment and block effects are tested separately with  $F$ -tests using ratios of corresponding mean square versus mean square error.
- Just like in one-way ANOVA, when the null hypothesis is true (no treatment effect or no block effect, respectively), then both the numerator and denominator mean squares are independent estimates of the individual variance  $\sigma^2$ .
- But, if the effects are not zero, the numerator is inflated. The ANOVA table contains two  $F$  statistics and two  $p$ -values.

## The data as a .csv file

```
treatment,zooplankton,block
Control,4.1,B1
Low,2.2,B1
High,1.3,B1
Control,3.2,B2
Low,2.4,B2
High,2,B2
Control,3,B3
Low,1.5,B3
High,1,B3
Control,2.3,B4
Low,1.3,B4
High,1,B4
Control,2.5,B5
Low,2.6,B5
High,1.6,B5
```

## Reading Data into R

```
> zoo = read.csv("zooplankton.csv")
> library(lattice)
> zoo$treatment = reorder(zoo$treatment, -1 * zoo$zooplankton)
```

- The reorder function orders the treatment variable from largest to smallest on the basis of mean zooplankton diversity measure (smallest to largest for negative diversity).
- This is not necessary, but puts the levels in the order Control, Low, High, which will make comparisons with the control group for both treatments easier to determine from output.

## ANOVA Table

```
> fit.1 = lm(zooplankton ~ treatment + block, data = zoo)
> anova(fit.1)
```

Analysis of Variance Table

Response: zooplankton

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
treatment	2	6.8573	3.4287	16.3660	0.001488 **
block	4	2.3400	0.5850	2.7924	0.101031
Residuals	8	1.6760	0.2095		

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

## Results

- There is very strong evidence of a treatment effect ( $p < 0.002$ ,  $F$ -test,  $F = 16.4$ ,  $df = 2, 8$ ).
- There is much weaker evidence of a block effect ( $p = 0.101$ ,  $F$ -test,  $df = 4, 8$ ).
- But the experiment was designed with blocks, so we keep block in the model, even though the estimated effect is not very significant.

## Additional Information

- There are 15 total observations, so 14 total degrees of freedom.
- There were 3 treatments, so 2 degrees of freedom for treatment.
- There were 5 blocks, so 4 degrees of freedom used for blocks.
- The remaining  $14 - 2 - 4 = 8$  degrees of freedom are for error.
- The square root of the mean square error,  $\sqrt{0.2095} = 0.458$  is the estimate of  $\sigma$ , the common deviation of an observation from its expected value after considering both treatment and block effects.

## Another Summary of the Model

```
> summary(fit.1)

Call:
lm(formula = zooplankton ~ treatment + block, data = zoo)

Residuals:
    Min       1Q   Median       3Q      Max
-0.62  -0.20  -0.08   0.22   0.68

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept)  3.420e+00  3.127e-01  10.938 4.33e-06 ***
treatmentLow -1.020e+00  2.895e-01  -3.524 0.007805 **
treatmentHigh -1.640e+00  2.895e-01  -5.665 0.000473 ***
blockB2       1.254e-15  3.737e-01  3.36e-15 1.000000
blockB3      -7.000e-01  3.737e-01  -1.873 0.097945 .
blockB4      -1.000e+00  3.737e-01  -2.676 0.028108 *
blockB5      -3.000e-01  3.737e-01  -0.803 0.445316
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.4577 on 8 degrees of freedom
Multiple R-squared:  0.8459, Adjusted R-squared:  0.7303
F-statistic: 7.317 on 6 and 8 DF,  p-value: 0.006513
```



## Comparisons

- The previous summary displays inferences for the seven model parameters for the expected values.
- There were 15 observations and 8 degrees of freedom for estimating error, and so there were  $15 - 8 = 7$  parameters used to specify the expected values of the 15 observations.
- In this parameterization, the intercept is the expected diversity level in block B1 for the control group. Its test is biologically irrelevant (the diversity is not zero in this setting).
- The remaining six parameters compare the low and high treatments to the control (averaging across blocks) and blocks B2 through B5 against block B1 (averaging across treatments).
- We see that both Low and High treatments have significantly lower means than the control ( $-1.02 \pm 0.29$  and  $-1.64 \pm 0.29$ , mean  $\pm$  SE, respectively).
- Block B4 has a lower average than Block B1.
- None of these p-values controls for multiple testing.

## Model Parameterization

$$Y_i = \mu + \alpha_{j(i)} + \beta_{k(i)} + \varepsilon_i$$

for:

- 1  $i = 1, \dots, n = 15$ , which indexes the observation;
  - 2  $j = 1, \dots, 3$ , for the treatment with  $\alpha_1 = 0$  for the control group;
  - 3  $k = 1, \dots, 5$ , for the block with  $\beta_1 = 0$  for block B1;
  - 4  $\mu$  is the intercept (expected value for control in block B1);
  - 5 and  $\varepsilon_i \sim \text{i.i.d. } N(0, \sigma^2)$ .
- The 7 free parameters for the mean are  $\mu, \alpha_2, \alpha_3, \beta_2, \beta_3, \beta_4$ , and  $\beta_5$ .
  - In this balanced design, the parameter estimates are simple.
  - The estimated effect for the Low group is the difference between the means of the Low (2.00) and the Control (3.02) groups.
  - The intercept is estimated by  
(Grand Mean)+(Control Mean–Grand Mean)+(B1 Mean–Grand Mean)
  - For unbalanced designs, estimated parameter values are not as simple.

## Random Effects Models

$$Y_i = \mu + \alpha_{j(i)} + \beta_{k(i)} + \varepsilon_i$$

- 1  $i = 1, \dots, n = 15$ , which indexes the observation;
  - 2  $j = 1, \dots, 3$ , for the treatment with  $\alpha_1 = 0$  for the control group;
  - 3  $k = 1, \dots, 5$ , for the block;
  - 4 and  $\beta_k \sim \text{i.i.d. } N(0, \sigma_\beta^2)$ .
  - 5 and  $\varepsilon_i \sim \text{i.i.d. } N(0, \sigma^2)$ .
- Another possible model is to treat the blocks not as four fixed differences, but as five draws from a normal distribution with mean zero.
  - Such a model is called a *random effects model*.
  - Next semester will study this type of model in detail.

## Fitted Values and Residuals

- The two-way ANOVA model is

$$Y_i = \mu + \alpha_{j(i)} + \beta_{k(i)} + \varepsilon_i$$

- Given data, the parameters may be estimated yielding this expression for *fitted values*.

$$\hat{Y}_i = \hat{\mu} + \hat{\alpha}_{j(i)} + \hat{\beta}_{k(i)}$$

- A *residual* is the difference between the observed value  $Y_i$  and the fitted value  $\hat{Y}_i$ .

$$\text{residual}_i = Y_i - \hat{Y}_i$$

## Parameter Estimation

- Values for estimates of the parameters may be found by the method of *least squares* which chooses estimates that minimize the sum of the squared residuals.
- Values may also be estimated by *maximum likelihood*, which selects values so that the likelihood of the data is as large as possible. (Here, likelihood is the product of normal densities.)
- The least squares and maximum likelihood estimates of parameters for the means are identical for ANOVA models (and also for all linear models with a normal response variable).
- The maximum likelihood estimate for the variance  $\sigma^2$  differs from the conventional unbiased estimate.

$$\widehat{\sigma^2}_{\text{unbiased}} = \frac{\text{sum of squared residuals}}{n - \# \text{ of parameters for means}}$$

$$\widehat{\sigma^2}_{\text{ML}} = \frac{\text{sum of squared residuals}}{n}$$

## Example

- Here are the observed values, the fitted values, and the residuals.

		Location					
		Abundance	1	2	3	4	5
Observed Values	Control		4.1	3.2	3.0	2.3	2.5
	Low		2.2	2.4	1.5	1.3	2.6
	High		1.3	2.0	1.0	1.0	1.6
		Location					
		Abundance	1	2	3	4	5
Fitted Values	Control		3.42	3.42	2.72	2.42	3.12
	Low		2.40	2.40	1.70	1.40	2.10
	High		1.78	1.78	1.08	0.78	1.48
		Location					
		Abundance	1	2	3	4	5
Residuals	Control		0.68	-0.22	0.28	-0.12	-0.62
	Low		-0.20	0.00	-0.20	-0.10	0.50
	High		-0.48	0.22	-0.08	0.22	0.12

## More on Fitted Values

- Each fitted value comes from the estimated parameters.

		Location				
Abundance		1	2	3	4	5
$\mu$	Control	3.42	3.42	3.42	3.42	3.42
	Low	3.42	3.42	3.42	3.42	3.42
	High	3.42	3.42	3.42	3.42	3.42

		Location				
Abundance		1	2	3	4	5
$\alpha_j$	Control	0	0	0	0	0
	Low	-1.02	-1.02	-1.02	-1.02	-1.02
	High	-1.64	-1.64	-1.64	-1.64	-1.64

		Location				
Abundance		1	2	3	4	5
$\beta_k$	Control	0	0	-0.7	-1.0	-0.3
	Low	0	0	-0.7	-1.0	-0.3
	High	0	0	-0.7	-1.0	-0.3

## Comments

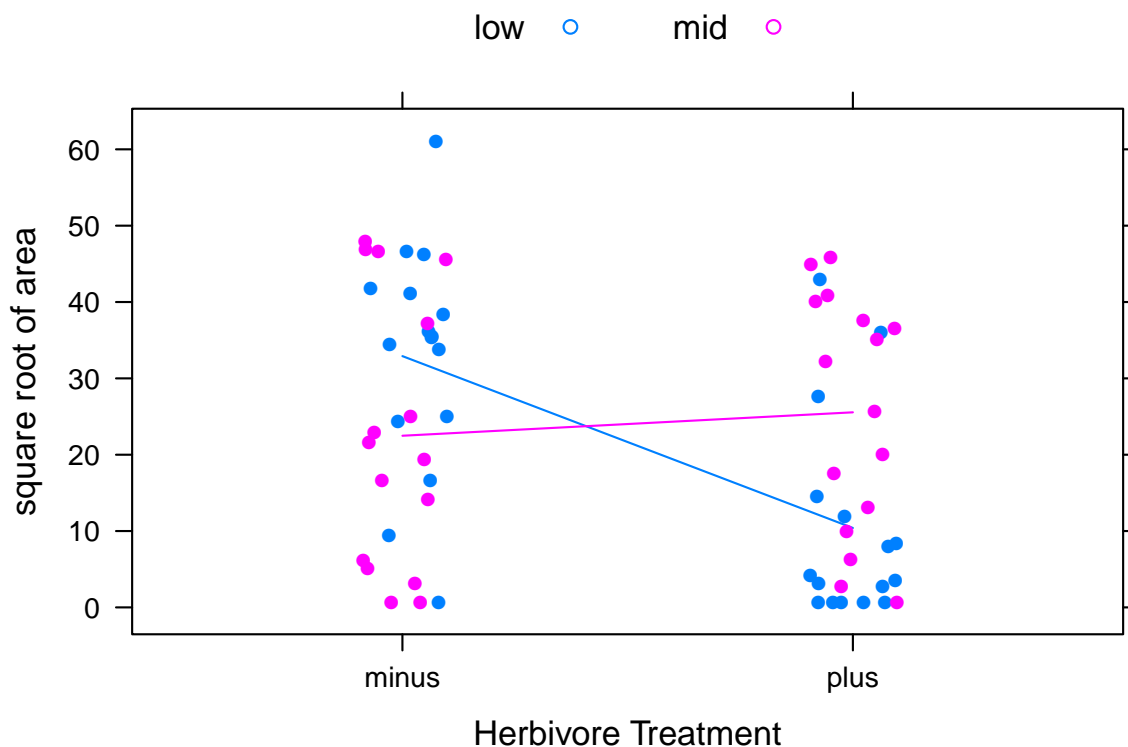
- Notice that in this two-way ANOVA model, effects of treatment and block (or any to factors in a different context) *are additive*.
- Rows of fitted values differ by constant amounts and columns of fitted values differ by constant amounts.
- The effect of the low treatment *is modeled as being the same* within each block.
- If the true means do not follow such a relationship (if there is an interaction between treatment and block), the model we have been studying will not capture the deviation.
- However, to model an interaction, we need *more than one observation at each treatment/block combination*, which we do not have in this example.

## Example

### Example

- We saw data from Example 18-3 earlier in the semester. The following plot shows the response (area of red algae) in an experiment in the intertidal habitat of coastal Washington on the basis of two factors.
- Experimental location is either just above the low tidal zone or midway between the low and high tidal zones, and each location is either accessible to herbivores or not.
- The plot indicates that the herbivore treatment has little effect at mid tidal zones, but that excluding herbivores results in larger algal growth at low tidal zones.
- We next show how to test this formally with ANOVA.

## Interaction Plot



## ANOVA with Interaction

- The two-way ANOVA model with an interaction is

$$Y_i = \mu + \alpha_{j(i)} + \beta_{k(i)} + (\alpha\beta)_{j(i),k(i)} + \varepsilon_i$$

- - 1  $i = 1, \dots, n$  indexes the observation;
  - 2  $j$  indexes one factor, let  $\alpha_1 = 0$ ;
  - 3  $k$  indexes a second factor, let  $\beta_1 = 0$ ;
  - 4  $(\alpha\beta)_{jk}$  are interaction parameters where the value is 0 is either  $j = 1$  or  $k = 1$ .
  - 5  $\mu$  is the intercept
  - 6 and  $\varepsilon_i \sim \text{i.i.d. } N(0, \sigma^2)$ .
- In this model, each treatment combination has its own mean, but the parameters give the same information in a different format.

## Example Data

```
height,herbivores,sqrtarea
low,minus,9.40
low,minus,34.4
low,minus,46.6
...
mid,plus,40.1
mid,plus,40.8
mid,plus,44.8
```

## Fitting a model with an interaction term in R

```
> algae = read.csv("algae.csv")
> algae.int = lm(sqrtarea ~ height * herbivores, data = algae)
```

- The expression `height*herbivores` means include main effects for both `height` and `herbivores` and also include an interaction term.
- The same model could be expressed less succinctly as follows:  
`sqrtarea ~ height + herbivores + height:herbivores`

## Summary

- The summary shows all estimated parameter values and tests.
- There are four treatment combinations and four parameters.
- Combining parameters returns the fitted values which are simply sample means for each treatment combination.

```
> summary(algae.int)

Call:
lm(formula = sqrtarea ~ height * herbivores, data = algae)

Residuals:
    Min       1Q   Median       3Q      Max
-32.2074  -9.6966  -0.3949  11.2076  32.5818

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept)      32.915      3.856   8.537 5.98e-12 ***
heightmid        -10.431      5.453  -1.913 0.060519 .
herbivoresplus   -22.511      5.453  -4.128 0.000115 ***
heightmid:herbivoresplus  25.578      7.711   3.317 0.001549 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 15.42 on 60 degrees of freedom
Multiple R-squared:  0.2281, Adjusted R-squared:  0.1896
F-statistic: 5.912 on 3 and 60 DF,  p-value: 0.001329
```

## ANOVA Table

- The ANOVA table tests three hypotheses.

```
> anova(algae.int)
```

```
Analysis of Variance Table
```

```
Response: sqrtarea
```

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
height	1	89.0	88.97	0.3741	0.543096
herbivores	1	1512.2	1512.18	6.3579	0.014360 *
height:herbivores	1	2617.0	2616.96	11.0029	0.001549 **
Residuals	60	14270.5	237.84		

```
---
```

```
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```