ReCap.   Part I (Chapters 1,2,3,4), Part II (Ch 5, 6, 7)
ReCap   Part III (Ch 9, 10, 11)
ReCap   Multiple Regression (Ch 12)
13.1   Fixed Effects ANOVA (no interactive effects)
13.2   Fixed Effects ANOVA (interactive effects)
13.3   Fixed*Random Effects (Paired t-test)
13.4   Fixed*Random Effects (Randomized Block)
13.5   Fixed*Random Effects (Repeated Measures)
13.6   Nested Random Effects (Hierarchical ANOVA)
13.7   Random within Fixed (Hierarchical ANOVA)
13.8   More Than Two Factors (to be written)

Ch13.xls
Limpet Respiration

on chalk board

ReCap Part I (Chapters 1,2,3,4) Quantitative reasoning is based on models, including statistical analysis based on models.
ReCap Part II (Chapters 5,6,7)
ReCap (Ch 9, 10,11) The General Linear Model with a single explanatory variable.
ReCap (Ch 12) GLM with more than one regression variable (multiple regression)

Today: Two-way ANOVA.
One response variable Y as a function of two explanatory variables X_1 X_2. Both explanatory variables are categorical, on a nominal scale.

Wrap-up. General Linear Model with two classification variables,
     i.e. two explanatory variables on a nominal scale.

New concept, the interaction term.

GLM, applied to 2-way ANOVA in Sokal and Rohlf 1995 p332.

Background.
The intertidal zone shows clear patterns of vertical zonation. The accessibility of this habitat has resulted in a long history of field experimentation combined with laboratory studies. Here is an example of a laboratory study based on an observed pattern of abundance of two intertidal species. The data are from Box 11.2 in the Sokal and Rohlf (2012) textbook *Biometry*.

"As an undergraduate in an animal physiology course I thought it might be interesting to compare O_2 consumption in animals from a rocky intertidal area and a quiet area with a bay. The data in *Biometry* was an experiment to see if differences in O_2 consumption in more brackish water that the bay limpet might tolerate better." F.J. Rohlf (April 2017).

The bay limpet is *A digitalis.* Table 1 in Davis et al (2002) lists both *Colisella* (now *Lottia*) *scabra* and *Collisella digitalis* in San Diego Bay.
"Of taxa found both in the bay and on the open coast, five (*Littorina* snails, the limpet *Collisella scabra*, the brown alga *Egregia menziesii*, non-coralline red algae, and total seagrass) were significantly more abundant on the open coast than in the bay (ANOVA $P<0.05$)."
J.L.D. Davis, L.A. Levin, S.M. Walther. 2002. Artificial armored shorelines: sites for open-coast species in a southern California bay. *Marine Biology* 140: 1249–1262 DOI 10.1007/s00227-002-0779-8

1. Construct model

Data are: oxygen consumption (microliters per minute) / (mg dry weight) of two species of limpet, at three different salinities.

Response variable
Oxygen consumption. $V_{O2} = \mu l\ O_2\ min^{-1}\ mg^{-1}$ dry weight (ratio scale)

Explanatory variables are species and salinity levels.
Species. $X_{sp} = A.scabra, A.digitalis$ (two categories, nominal scale)

Salinity. X_{sal} = salinity in three categories: 100%, 75%, 50%
This quantity was measured on a ratio scale, but it is here reduced to nominal scale, of three categories.

Here is a table of variables.

	Variable			Response or	Categorical	Crossed or
Name	Symbol	Units	Scale	Explanatory	or Covariate	Nested
Limpet respiration	V_{02}	$\mu l\ O_2\ min^{-1}\ mg^{-1}$	ratio	response	N/A	N/A
Species	X_{Sp}	2 species	nominal	explanatory	categorical	N/A
Salinity	X_{Sal}	3 categories	nominal	explanatory	categorical	N/A
Sp by Salinity	$X_{SP\ x}\ X_{Sal}$	2 by 3 categories	nominal	explanatory	categorical	crossed

1. Construct model

<u>Verbal model.</u> Oxygen consumption depends on salinity and species

<u>Graphical model:</u>
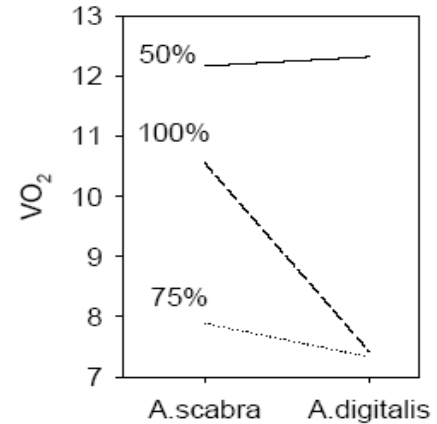Connect 3 means of each of two species.
 X-axis = Species.
 Y-axis = O_2 consumption, from 0 to 14
 Means: A. scabra 12.17 7.89 10.56
    A. digitalis 12.33 7.35 7.43

Plot suggests that effect of salinity on O_2
consumption depends on species of limpet.

<u>Formal Model.</u> Write the GLM

$$GLM: \quad VO_2 = \beta_o + \beta_{sp} \cdot X_{sp} + \beta_{sal} \cdot X_{sal} + \beta_{sp\ x\ sal} \cdot X_{sp}\ X_{sal} + \varepsilon$$
$$V_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk}$$

The model has been written using two forms of notation. One shows variables with population parameters. The other shows statistical notation where greek letters stand for fixed effects and subscripts track categories within a variable. The symbols in each of the two sets of notation have been aligned, to facilitate translation.

There are three explanatory terms, one for salinity, one for species, and one for interactive effects--the dependence of salinity effects on species. Graphical interpretation is that the shape of the relation of O_2 consumption to salinity in one species does not match the shape of the same relation in the other species. The interaction plot, using the means, suggests there is an interactive effect.

2. Execute analysis.
Place data in model format:
   Column with response variable oxygen consumption, labelled V_{O2}
   Column with explanatory variable, labelled Xsp (As or Ad)
   Column with explanatory variable, labelled Xsal (50, 75, 100)
      These are labels (categories), not numbers on ratio scale.

Code model statement in statistical package according to the GLM

$$VO_2 = \beta_o + \beta_{sp} \cdot X_{sp} + \beta_{sal} \cdot X_{sal} + \beta_{sp\ x\ sal} \cdot X_{sp} \cdot X_{sal} + \varepsilon$$

```
MTB> ANOVA 'VO2' = 'Xsp'  'Xsal'  'Xsp'*'Xsal'
MTB> GLM   'VO2' = 'Xsp'  'Xsal'  'Xsp'*'Xsal'
SUBC> fits c4;
SUBC> res c5.
```

2. Execute analysis.

Here are GLM parameters produced by a statistical package (SPlus)

```
              Value Std. Error    t value
(Intercept)  9.6195833  0.4462817  21.5549605    Grand mean
        SP -0.5887500  0.4462817  -1.3192342
      Sal1 -2.3181250  0.5465812  -4.2411359
      Sal2 -0.3122917  0.3155688  -0.9896152
    SPSal1 -0.1762500  0.5465812  -0.3224590
    SPSal2 -0.4887500  0.3155688  -1.5487907
```

The grand mean (intercept): $\beta_o = 48^{-1} \Sigma V = 48^{-1} \cdot 461.74 = 9.62$ µl min^{-1} mg^{-1}
The GLM parameters are contrasts relative to the intercept (grand mean).
The fitted values are the means for each level of each factor.
They can be calculated directly or from the parameters (intercept and contrasts).

$$\text{Mean}(_{sp=As}) = 24^{-1} \Sigma V_{sp=As} = 24^{-1} \cdot 245 = 10.21 \text{ µl min}^{-1} \text{ mg}^{-1}$$
$$9.62 + 0.59 = 10.21 \quad \text{(from parameters)}$$
$$\text{Mean}(_{sp=Ad}) = 24^{-1} \Sigma V_{sp=Ad} = 24^{-1} \cdot 216.74 = 9.03 \text{ µl min}^{-1} \text{ mg}^{-1}$$
$$9.62 - 0.59 = 9.03$$
$$\text{Mean}(V_{sal=50}) = 16^{-1} \Sigma V_{sal=50} = 16^{-1} \cdot 196 = 12.25 \text{ µl min}^{-1} \text{ mg}^{-1}$$
$$9.62 + 2.32 + 0.31 = 12.25$$
$$\text{Mean}(V_{sal=75}) = 16^{-1} \Sigma V_{sal=75} = 16^{-1} \cdot 121.82 = 7.61 \text{ µl min}^{-1} \text{ mg}^{-1}$$
$$9.62 - 2.32 + 0.31 = 7.61$$
$$\text{Mean}(V_{sal=100}) = 16^{-1} \Sigma V_{sal=100} = 16^{-1} \cdot 143.92 = 9.00 \text{ µl min}^{-1} \text{ mg}^{-1}$$
$$9.62 - 0.312 + 0.316 = 9.00$$

3. Evaluate the model.
Plot residuals versus fits (cell means).

```
MTB > plot 'res' 'fits'
  res   -
                  *
        -                               *
    5.0+        *                                        *
        -     **
        -     22     *                    2
        -                                              *2
        -        *                                     *
    0.0+                                              **
        -     *2    2                 *               2
        -     32     *                2               3*
        -     *      2                *               *
        -       *                     *
   -5.0+
        -                                             *
        -
        -
        -
          +---------+---------+---------+---------+---------+------fits
         7.0       8.0       9.0      10.0      11.0      12.0
```

Straight line assumption. No line fitted in model, so skip this evaluation.

3. Evaluate the model.

Normal error model. Homogeneous errors?

   No systematic change in residuals with increase in fitted values (*i.e.* no cones) so residual homogeneous.

   Note there are only 6 fitted values, so the residual versus fit plot will consist of only 6 stacks of points. The stacks should be similar in vertical dispersion.

> One of the assumptions for the GLM is that the fitted and residuals values are not associated. In mathematical terms:
> $Y = \text{Model} + \text{Res}$
> $\text{Var}(Y) = \text{Var}(\text{Model} + \text{Res})$
> $\text{Var}(Y) = \text{Var}(\text{Model}) + \text{Var}(\text{Res}) + \text{Cov}(\text{Model},\text{Res})$
> If $\text{Cov}(\text{Model},\text{Res}) = 0$, then we can partition $\text{Var}(Y)$
> $\text{Var}(Y) = \text{Var}(\text{Model}) + \text{Var}(\text{Res})$
> If $\text{Cov}(\text{Model},\text{Res}) \neq 0$, then partitioning of $\text{Var}(Y)$ cannot be trusted.

Normal errors? The response variable deviates from normality, it is skewed by a few large values. The response variable and the residuals are clearly bimodal.

   "O_2 rate fluctuated over time seeming to reflect a memory of the tidal cycle but faded after a few days….The bimodality might reflect differences in time of day as I could not run the Warberg machine to measure O_2 for all of the specimens at the same time."
   F.J.Rohlf 2017.

```
MTB > hist 'res'
Histogram of res   N = 48
Midpoint    Count
     -6       1    *
     -5       0
     -4       2    **
     -3       5    *****
     -2      12    ************
     -1       8    ********
      0       2    **
      1       2    **
      2       3    ***
      3       7    *******
      4       2    **
      5       2    **
      6       1    *
      7       1    *
```

```
MTB > hist 'oxy'
Histogram of oxy   N = 48
Midpoint    Count
      4       3    ***
      6      12    ************
      8       6    ******
     10      11    ***********
     12       6    ******
     14       7    *******
     16       1    *
     18       2    **
```

Will this distort the estimate assuming a normal distribution? As a rule of thumb, distortion diminishes above $n = 30$.   In this analysis, n>30 but the residuals deviate substantially from normality so we will compare the estimates of Type I error from the F-distribution to the estimates by randomization.

<u>Sum(res) = 0</u>? Yes

3.   Evaluate the model.

<u>Independent residuals</u>? The order in which the specimens were run in the device to measure respiration is a potential source of non-independence. We have no information on the order. The lag plot for residuals in the order in which the data were presented show no pattern of association. The residuals were taken as independent.

```
MTB > let c8 = lag('res')
 MTB > plot c8 'res'
 C8    -
       -                  *
       -                        *
   5.0+                              *  *
       -             *    *
       -            *    ***    *              *        *
       -             *        *          *
       -                               **
   0.0+                   *
       -         *   ** * * *    *        *
       -            * * * * *         2    *     *   2        *
       -    *        *    *        *         *
       -                               2
  -5.0+
       -                          *
       -
       -
       -
          ------+---------+---------+---------+---------+---------+res
             -5.0      -2.5       0.0       2.5       5.0       7.5
        N* = 1
```

4. Partition df and SS according to the model.

Compute total degrees of freedom $df_{total} = n -1 = 48 -1 = 47$

Partition df*total* according to model, using rules

      two species, hence 2-1 = 1 df $df_{Sp} = 2 -1 = 1$

      three salinities, hence 3-1 = 2 df $df_{Sal} = 3 -1 = 2$

      $df_{Sp*Sal} = df_{Sp} \times df_{Sal}$ $df_{Sp*Sal} = 1 \times 2 = 2$

      $df_{res} = df_{total} - df_{Sp} - df_{Sal} - df_{Sp \, xSal}$ $dftotal = 47 -1 -2 -2 = 42$

Y	$=$	β_o	$+$	$\beta_{sp}X_{sp}$	$+$	$\beta_{sal}X_{sal}$	$+$	$\beta_{sp*sal}X_{sp*sal}$	$+$	ε
47 =		1	+	2	+	2	+	42
623.41 =		16.64	+	181.32	+	23.93	+	401.52

    Many packages have both GLM and ANOVA routines. These partition the variance according to the model (a two-way ANOVA for the limpet data). ANOVA routines require equal replication, as in the limpet example: 8 observations in each of 6 cells. GLM routines are more flexible, they allow unequal replication within the cells.

4. **Calculate *LR* from the unexplained variance 1-R^2**

   $1 - R^2 = (401.52/623.41) = 64.3\%$

   $LR = (0.644)^{-48/2} = 38512$

   The research hypothesis is for more likely than a simple one-parameter model, the mean. There is very strong evidence for the omnibus model with three terms. We continue to a listing of hypothesis pairs for each term in model.

5. **Define target of inference and whether the sample is representative.**

   When we draw conclusions from this sample, what is the population to which we are inferring? The population in this case is defined by a repeatable measurement protocol. The population parameters are those from averaging over thousands of hypothetical repetitions of the experiment. Inference is only to those salinities listed in the experimental protocol. Inference is only to the two species in the protocol. We will not infer to other limpet species.

   We are not inferring to an enumerable population, such as all limpets at the sites where the limpets were collected. We will assume that once the limpets were collected they were assigned randomly, or at least haphazardly, to one of the three salinities.

   "I am not sure how randomly the specimens were selected but at that point I had not yet had a statistics course." F.J. Rohlf 2017.

5. **Choose mode of inference.**

   We have several choices.
   -Evidential inference (Edwards 1972, Royall 1997),
   -Fiducial probability (Fisher 1935)
   -Priorist (Bayesian) inference (also called inverse probability),
   -Frequentist inference using Fisher sorting or decision theoretic hypothesis testing (Neyman and Pearson 1933).
   Fiducial probability (inverse probability without a prior probability) has not had a history of success. Priorist (Bayesian) inference requires a prior probability. Decision theoretic inference requires specification of a fixed Type I and Type II error, preferably based on relative costs of both types of error. Current texts and practice rely on fixed Type I error, with specification of Type II error in the case of prospective power analysis in experimental design. Decision theoretic inference produces a measure of uncertainty, it is not a measure of evidence (Royall 1997). Evidential inference is appropriate in the absence of a reason to control Type I error. We will estimate Type I error for comparison to the evidentialist conclusions.

6. **State test statistic, its distribution, and usage of Type I error.**

Test statistic	F-ratio
Distribution of test statistic	F-distribution
Type I error	Fisher sorting into high, moderate, and low.

6. List hypothesis pairs.

We focus first on the interaction term $\beta_{sp\ x\ sal}$ X_{sp} X_{sal}. If the factors have interactive effects on the response variable, then the observed difference in O_2 uptake due to one factor (between the two groups of animal) will depend on the other factor (salinity). If there are significant interactive effects then the differences in salinity cannot be interpreted unless we know the species.

The symbol $\beta_{sp\ x\ sal}$ stands for two parameters that together quantify the degree to which the effects of salinity on respiration depends on species. These two parameters are contrasts (differences).

$D_{50\%}$ = Mean($V_{sal=50,\ sp=Ad}$) − Mean($V_{sal=50,\ sp=As}$) = 12.326 − 12.174 = 0.152
$D_{75\%}$ = Mean($V_{sal=75,\ sp=Ad}$) − Mean($V_{sal=75,\ sp=As}$) = 7.338 − 7.890 = − 0.552
$D_{100\%}$ = Mean($V_{sal=100,\ sp=Ad}$) − Mean($V_{sal=100,\ sp=As}$) = 7.429 − 10.561 = −3.132

$D_{75\%} - D_{50\%}$ = 0.152 − 0.552 = 0.704 Difference is small
$D_{100\%} - D_{50\%}$ = 0.152 − 3.032 = 3.284 Difference is large
   The contrast at 100% exceeds that at 75%

<u>Hypotheses for the interaction term.</u> The research hypothesis H_A is that $\beta_{sp\ x\ sal} \neq 0$
   H_A: $\beta_{sp\ x\ sal} \neq 0$
   H_o: $\beta_{sp\ x\ sal} = 0$

 Are there more specific hypotheses about the interaction term?
 No, because we have no information on which to base directional hypotheses.

If the parameter values are not zero, then there will be variance due to that term.
The H_A / H_o pairs equivalent to those listed above are:
   H_A: Var($\beta_{sp\ x\ sal}$) >0   or equivalently H_A: Var($\beta_{sp\ x\ sal} \cdot X_{sp} \cdot X_{sal}$) > 0
   H_o: Var($\beta_{sp\ x\ sal}$) = 0   or equivalently H_o: Var($\beta_{sp\ x\ sal} \cdot X_{sp} \cdot X_{sal}$) = 0

If the interaction term is not significant, then research hypotheses concerning each of the other terms in the model become of interest because we can interpret the effects of on factor (such as salinity) regardless of the effects of the other factor (species).

<u>Hypotheses for the species term.</u>
   H_A: $\mu_{sp=As} \neq \mu_{sp=Ad}$ The population means differ
   H_o: $\mu_{sp=As} = \mu_{sp=Ad}$ The population means do not differ
 These hypotheses are equivalent to
   H_A: $\beta_{Sp=Ad} \neq 0$
   H_o: $\beta_{Sp=Ad} = 0$
They are also equivalent to following H_A / H_o for parameters.
   H_A: Var(β_{sp}) > 0   There is variance present, due to species
   H_o: Var(β_{sp}) = 0   The is no variance due to species.
Are there more specific hypotheses about parameters ? No

6. List specific hypothesis pairs.

Hypotheses for the salinity term.

This is a fixed effects term so the contrast in means will be of interest.

H_A: $\mu_{Sal=50\%} \neq \mu_{Sal=75\%} \neq \mu_{Sal=100\%}$
   The population means differ among salinities.

H_A: $\mu_{Sal=50\%} = \mu_{Sal=75\%} = \mu_{Sal=100\%}$
   The population means do not differ

These hypotheses are equivalent to

H_A: $\beta_{Sal} \neq 0$
H_o: $\beta_{Sal} = 0$

The H_A H_o pair above is equivalent to the following hypotheses.

H_A: $Var(\beta_{sal}) > 0$ There is variance present, due to salinity.
H_o: $Var(\beta_{sal}) = 0$ There is no variance present, due to salinity.

Additional hypotheses for parameters in the source term ? Yes

H_A: $\mu_{100\%} \neq (1/2)(\mu_{75\%} + \mu_{50\%})$ Means at reduced salinity differ from unreduced.
H_o: $\mu_{100\%} = (1/2)(\mu_{75\%} + \mu_{50\%})$

7. ANOVA. Table Source, df, SS.

Transfer df and SS components from table (horizontal listing of the model) to the ANOVA table, which lists the model vertically.   Start with SS_{tot} at bottom, then add partitioned components SS_{sp} SS_{sal} $SS_{sp \times sal}$ SS_{res}

Source	df	SS
Sp	1	16.64
Sal	2	181.32
Sp◊Sal	2	23.93
Res	42	401.52
Total	47	623.41

7. ANOVA – Evidentialist. Calculate LR from SS

Calculate $SS_o = SS + SS_{resid}$ Calculate SS_o / SS_{res} Calculate $LR = (SS_o / SS_{res})^{n/2}$

			SS_{resid}	SS_o			
Source	df	SS	$SS_{reduced}$	SS_{full}	SS_o/SS_{res}	LR	Evidence
sp	1	16.64	401.52	418.16	1.041	1	Inadequate
sal	2	181.32	401.52	582.84	1.452	24	Some
sp*sal	2	23.93	401.52	425.45	1.060	2	Inadequate
Residual	42	401.52					
Total	47	623.41					

Compute LR from SS for each term in the model

$LR_{sp} = [(SS_{sp} + SS_{res}) / SS_{res}]^{48/2} = [(16.64+401.52) / 401.52]^{48/2} = 1.41$

$LR_{sal} = [(SS_{sal} + SS_{res}) / SS_{res}]^{48/2} = [((181.32+401.52) / 401.52/]^{48/2} = 23.7$

$LR_{spXsal} = [(SS_{spXsal} + SS_{res}) / SS_{res}]^{48/2} = [(23.93+401.52) / 401.52]^{48/2} = 1.64$

There is inadequate evidence for an interactive effect, $LR < 20$
There is some evidence for a salinity effect, $LR > 20$
There is inadequate evidence for a species effect, $LR < 20$

7. ANOVA – Frequentist. Calculate MS, F, p

Source	df	SS adj	MS	F	p
sp	1	16.64	16.64	1.74	0.1942
sal	2	181.32	90.66	9.48	0.00
sp*sal	2	23.93	11.965	1.25	0.297
Residual	42	401.52	9.6		
Total	47	623.41			

Compute MS

$MS_{Sp} = SS_{Sp} / df_{Sp}$ $\quad = 16.64$

$MS_{Sal} = SS_{Sal} / df_{Sal}$ $\quad = 90.661$

$MS_{Sp*Sal} = SS_{Sp*Sal} / df_{sp \times sal}$ $\quad = 11.963$

$MS_{res} = SS_{res} / df_{res}$ $\quad = 9.56$

Compute F

Fixed effects for salinity and species, so all variance ratios taken relative to MS_{res}

$F = MS_{sp} / MS_{res} = 16.638 / 9.56 = 1.74$

$F = MS_{sal} / MS_{res} = 90.661 / 9.56 = 9.48$

$F = MS_{sp \times sal} / MS_{res} = 11.963 / 9.56 = 1.2$

Calculate Type I error from F-distribution.

$F_{2,42} = 1.251$ $\qquad$ $p = 0.297$ $\qquad$ interaction

$F_{1,42} = 1.74$ $\qquad$ $p = 0.194$ $\qquad$ species effect

$F_{2,42} = 9.483$ $\qquad$ $p = 0.0004$ $\qquad$ salinity effect

Draw picture of computational flow.

Statistical packages automatically partition df and SS, compute MS, compute F, compute p-value from F-statistic, and produce ANOVA table.

8. Decide whether to recompute Type I error.

The residuals were strongly bimodal, not normal.
$n > 30$ and so we judge that the Type I error rate from the F distribution will be approximately correct. We will check our judgement by computing the randomized p-value. To do this, we set up a control file and generate distributions based on a thousand randomizations.

Here are the results from 1000 random samples of the response variable.

$F_{2,42} = 1.251$ $p = 386/1000 = 0.39$ interaction term
$F_{1,42} = 1.74$ $p = 250/1000 = 0.25$ species effect
$F_{2,42} = 9.483$ $p = 1/1000 = 0.001$ salinity effect

How much did the p-values change?

interaction	$F = 1.25$	$p_{ran} = 0.39$	$p_{cdf} = 0.297$	$0.39 / 0.297 = 1.3$
species	$F = 1.74$	$p_{ran} = 0.25$	$p_{cdf} = 0.19$	$0.25 / 0.19 = 1.3$
salinity	$F = 9.48$	$p_{ran} < 0.001$	(poor estimate, only 1000 randomizations)	

9. Report statistical conclusion about terms in the model.

Start with interaction term.

There was inadequate evidence for an interactive effect, $LR < 20$
There was some evidence for a salinity effect, $LR > 20$
There was inadequate evidence for a species effect, $LR < 20$

Type I error was high for the interactive effect $p = 0.39$
We cannot reject the null hypothesis of not effect.

We proceed to main effects.
The next chapter shows an example where interactive effects are present, and we do not interpret the main effects in the 2 way analysis.

Remove interaction term if not significant ? This question arises in the context of a fixed tolerance for Type I error. In this example there is little to gain by doing this. Only a single degree of freedom moves to the residual term, with little effect on statistical power.

Species term.

A species effect was no more likely than no species effect.
We cannot dismiss the null hypothesis, not species effect.

Salinity term.

A salinity effect was more likely than no salinity effect.
We can dismiss the null hypothesis, no salinity effect.

10. Report and interpret parameters of biological interest.

There was some evidence that both species responded to reduce salinity.
At this point we could do planned comparisons .
A logical set of comparisons would be 100% versus 70% then 100% versus 50%.
We can undertake two comparisons.
Equivalently, we can examine the means and standard errors because as an approximation, two means differ significantly if the difference is greater than two standard errors.

$$\hat{\beta}_{sal=50} = 12.25 \; \mu l \; min^{-1} \; mg^{-1} \qquad st.err = 0.800 \; \mu l \; min^{-1} \; mg^{-1}$$
$$\hat{\beta}_{sal=75} = 7.61 \; \mu l \; min^{-1} \; mg^{-1} \qquad st.err = 0.669 \; \mu l \; min^{-1} \; mg^{-1}$$
$$\hat{\beta}_{sal=100} = 8.99 \; \mu l \; min^{-1} \; mg^{-1} \qquad st.err = 0.868 \; \mu l \; min^{-1} \; mg^{-1}$$

We can see that reduction to 50% salinity increased respiration, while reduction to 75% did not. We can draw this conclusion from inspection of the means and standard errors.

100% versus 75%   The difference is $8.99 - 7.61 = 1.38 \; \mu l \; min^{-1} \; mg^{-1}$.
Two standard errors are $0.669 + 0.868 = 1.54 \; \mu l \; min^{-1} \; mg^{-1}$.
The observed difference is less than two standard errors.

100% versus 50%   The difference is $8.99 - 12.25 = -3.26 \; \mu l \; min^{-1} \; mg^{-1}$.
Two standard errors are $0.669 + 0.800 = 1.47 \; \mu l \; min^{-1} \; mg^{-1}$.
The difference is greater than two standard errors.

This is an approximation, not an exact calculation, but it is quick and easy.

The result was surprising, compared to the expected increase in respiration with decrease in salinity.

The unexpected results together with the observed persistence of variation in O_2 rate following the tides suggest that the experiment be repeated to control for retained tidal variation in the open coast species, *L scabra*

* * *

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Royall, R. 1997. *Statistical Evidence*. Chapman and Hall

Appended material.
2. Execute analysis. – Fitted values.

The fitted values are the means for each of the 6 cells (sums/n in each cell)

$$\text{Mean}(V_{sal=50,\ sp=As}) = 8^{-1}\ \Sigma V_{(sal=50,\ sp=As)} = 8^{-1}\ \textcircled{} 97.39\ = 12.17\ \mu l\ min^{-1}\ mg^{-1}$$

	A.scabra	A.digitalis	Sums
50%	97.39	98.61	196.00
75%	63.12	58.70	121.82
100%	84.49	59.43	143.92
Sums	245.00	216.74	461.74

	A.scabra	A.digitalis	Means
50%	12.17	12.33	12.25
75%	7.89	7.34	7.61
100%	10.56	7.43	9.00
Means	10.21	9.03	9.62 $=\beta o$

	A.scabra	A.digitalis	Means–βo	βsal	
50%	2.55	2.71	2.63		
75%	-1.73	-2.28	-2.01	-2.3181	=(Mean@100%-βo)+(Mean@75%-βo)
100%	0.94	-2.19	-0.62	-0.3123	=(Mean@100%-βo)/2
Means–βo	0.59	-0.59			
				βsp	

	A.scabra	A.digitalis	Means–$\Sigma\beta$	βsal*Sp	
50%	-0.665	0.665	0.00		
75%	-0.313	0.313		-0.1762	=(Mean@100%–$\Sigma\beta$)+(Mean@75%–$\Sigma\beta$)
100%	0.977	-0.977		-0.4887	=(Mean@100%-$\Sigma\beta$)/2
Means - $\Sigma\beta$	0.00	0.00			

Here are the adjusted means.

$$\text{Mean}(_{sal=50}) - \hat{\beta}_0 = 12.25\ -\ 9.62 = 2.63\ \mu l\ min^{-1}\ mg^{-1}$$

$$\text{Mean}(_{sal=75}) - \hat{\beta}_0 = 7.61\ -\ 9.62 = -2.01\ \mu l\ min^{-1}\ mg^{-1}$$

$$\text{Mean}(_{sal=100}) - \hat{\beta}_0 = 9.00\ -\ 9.62 = -0.62\ \mu l\ min^{-1}\ mg^{-1}$$

$$\text{Mean}(_{sp=As}) - \hat{\beta}_0 = 10.21\ -\ 9.62 = 0.589\ \mu l\ min^{-1}\ mg^{-1}$$

$$\text{Mean}(_{sp=Ad}) - \hat{\beta}_0 = 9.03\ -\ 9.62 = -0.589\ \mu l\ min^{-1}\ mg^{-1}$$

$$\text{AdjMean}(_{sal=75,\ Sp=Ad}) = \text{Mean}(_{sal=75,\ Sp=Ad}) - \hat{\beta}_0\ -\ \text{Mean}(_{sal=75}) - \text{Mean}(_{sp=Ad})$$
$$= 7.34\ -\ 9.62\ (7.61\ -\ 9.62)\ -\ (9.03\ -\ 9.62) = 3.13\ \mu l\ min^{-1}\ mg^{-1}$$

$$\text{AdjMean}(_{sal=100,\ Sp=Ad}) = \text{Mean}(_{sal=100,\ Sp=Ad}) - \hat{\beta}_0\ -\text{Mean}(_{sal=100}) - \text{Mean}(_{sp=Ad})$$
$$= 7.43\ -\ 9.62\ -\ (9.00\ -\ 9.62)\ -\ (9.03\ -\ 9.62) = -0.977\ \mu l\ min^{-1}\ mg^{-1}$$

The GLM parameters are linear combinations of adjusted means.

$$\hat{\beta}_{sal=100} = (\text{Mean}(V_{sal=75}) - \hat{\beta}_0) + (\text{Mean}(V_{sal=100}) - \hat{\beta}_0)/2$$
$$= -2.01 - 0.62 / 2 = -2.31 \ \mu l \ min^{-1} \ mg^{-1}$$

$$\hat{\beta}_{sal=75} = (\text{Mean}(V_{sal=100}) - \hat{\beta}_0)/2 = -3.26 \ \mu l \ min^{-1} \ mg^{-1}$$

$$\hat{\beta}_{Sp=Ad} = \text{Mean}(V_{sp=Ad}) - \hat{\beta}_0 = -0.589 \ \mu l \ min^{-1} \ mg^{-1}$$

$$\hat{\beta}_{Ad*100} = \text{AdjMean}(V_{sal=100,Sp=Ad})/2 = -0.977/2 = -0.489 \ min^{-1} \ mg^{-1}$$

$$\hat{\beta}_{Ad*75} = \text{AdjMean}(V_{sal=75,\ Sp=Ad}) + \text{AdjMean}(_{sal=100,Sp=Ad})/2$$
$$= 3.13 - 0.489 = -0.176 \ \mu l \ min^{-1} \ mg^{-1}$$

8. Recompute Type I error by randomization.

Minitab code

```
sample 48 'oxy' c7
unstack c7 c31 c32 c33;
subscripts 'sal'.
let k31 = mean(c31)
let k32 = mean(c32)
let k33 = mean(c33)
set c8
(k31 k32 k33)16
end
unstack c7 c34 c35;
subscripts 'sp'.
let k34 = mean(c34)
let k35 = mean(c35)
set c9
(k34 k35)24
end
let k1 = stdev(c7)*stdev(c7)*47
let k2 = stdev('sp')*stdev('sp')*47
let k3 = stdev('sal')*stdev('sal')*47
let k4 = stdev('fits')*stdev('fits')*47
let k5 = stdev('res')*stdev('res')*47
let k8 = stdev(c8)*stdev(c8)*47
let k9 = stdev(c9)*stdev(c9)*47
let k10 = k4 - k8 - k9
let k15 = (k8/k5)*(42/2)               # F sal
let k16 = (k9/k5)*(42/1)               # F sp
let k17 = (k10/k5)*(42/2)              # F sal*sp
stack c15 k15 c15
stack c16 k16 c16
stack c17 k17 c17
```