Post-hocs and planned comparisons

KEY

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## Import and summary of data

We will work eel larval development data. Import the “eels.csv” into a data frame called “eels”.

library(readxl)  
eels <- read\_excel("eels.xlsx")  
eels$Stage <- factor(eels$Stage)

To plot the means and 95% confidence intervals use summarySE() to get the summary statistics you need:

library(Rmisc)

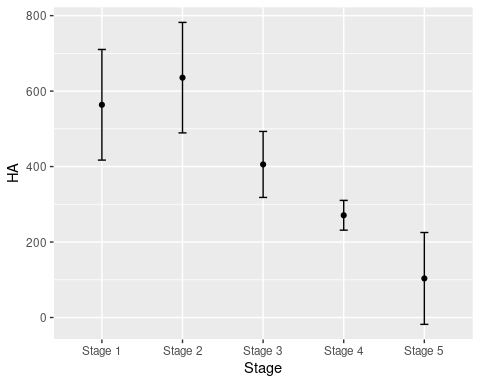
## Loading required package: lattice

## Loading required package: plyr

summarySE(eels, "HA","Stage") -> eels.sumstats

Then use the summary data set you created to plot the means and 95% confidence intervals using ggplot2:

library(ggplot2)  
ggplot(eels.sumstats, aes(x = Stage, y = HA)) + geom\_errorbar(aes(ymin = HA - ci, ymax = HA + ci), width = 0.1) + geom\_point()



#### Question: the stages are in their proper order on the plot. Stage is clearly an ordinal variable, but does that mean that R is considering Stage to be ordinal?

No, the categories are in alphabetical order, but the factor is not considered ordered at this point. The ordering will not be used in this first analysis.

## Fitting the linear model

Now run the lm() command to fit the model, using HA as the response variable and Stage as the predictor:

eels.ha.lm <- lm(HA ~ Stage, data = eels)  
anova(eels.ha.lm)

## Analysis of Variance Table  
##   
## Response: HA  
## Df Sum Sq Mean Sq F value Pr(>F)   
## Stage 4 747378 186844 35.331 1.805e-07 \*\*\*  
## Residuals 15 79327 5288   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

#### Question: is the Stage term significant? How do you know?

Yes, because the p-value for Stage is significant.

#### Question: can you tell at this point which stages differ?

No, only that there are at least two Stages have mean HA that differ.

Load the emmeans library:

library(emmeans)

## Tukey comparisons

Run the emmeans() command, from the emmeans library, for the Tukey procedure:

emmeans(eels.ha.lm, tukey ~ Stage) -> eels.ha.emm  
eels.ha.emm

## $emmeans  
## Stage emmean SE df lower.CL upper.CL  
## Stage 1 564 36.4 15 486.3 641  
## Stage 2 636 36.4 15 558.2 713  
## Stage 3 406 36.4 15 328.2 483  
## Stage 4 271 36.4 15 193.5 348  
## Stage 5 104 36.4 15 26.1 181  
##   
## Confidence level used: 0.95   
##   
## $contrasts  
## contrast estimate SE df t.ratio p.value  
## Stage 1 - Stage 2 -71.9 51.4 15 -1.397 0.6384  
## Stage 1 - Stage 3 158.1 51.4 15 3.075 0.0513  
## Stage 1 - Stage 4 292.8 51.4 15 5.695 0.0004  
## Stage 1 - Stage 5 460.2 51.4 15 8.950 <.0001  
## Stage 2 - Stage 3 230.0 51.4 15 4.472 0.0035  
## Stage 2 - Stage 4 364.7 51.4 15 7.092 <.0001  
## Stage 2 - Stage 5 532.1 51.4 15 10.348 <.0001  
## Stage 3 - Stage 4 134.7 51.4 15 2.620 0.1163  
## Stage 3 - Stage 5 302.1 51.4 15 5.876 0.0003  
## Stage 4 - Stage 5 167.4 51.4 15 3.256 0.0365  
##   
## P value adjustment: tukey method for comparing a family of 5 estimates

multcomp::cld(eels.ha.emm)

## Stage emmean SE df lower.CL upper.CL .group  
## Stage 5 104 36.4 15 26.1 181 1   
## Stage 4 271 36.4 15 193.5 348 2   
## Stage 3 406 36.4 15 328.2 483 23   
## Stage 1 564 36.4 15 486.3 641 34   
## Stage 2 636 36.4 15 558.2 713 4   
##   
## Confidence level used: 0.95   
## P value adjustment: tukey method for comparing a family of 5 estimates   
## significance level used: alpha = 0.05   
## NOTE: Compact letter displays can be misleading  
## because they show NON-findings rather than findings.  
## Consider using 'pairs()', 'pwpp()', or 'pwpm()' instead.

#### Question: which stages are different from one another?

Working from first to last stage, Stage 1 is different from Stage 4 and 5, Stage 2 is different from Stage 3, 4, and 5. Stage 3 is different from Stage 5. STage 4 is different from Stage 5.

## Dunnett’s comparisons

Dunnett’s comparisons are all groups against one. Usually the one that is compared against is a “control” group, and all the others are treatments of various kinds. We don’t have data like that, but since Stage 1 is set as the baseline group we can use Dunnett’s test to compare all of the other stages against Stage 1:

emmeans(eels.ha.lm, dunnett ~ Stage)

## $emmeans  
## Stage emmean SE df lower.CL upper.CL  
## Stage 1 564 36.4 15 486.3 641  
## Stage 2 636 36.4 15 558.2 713  
## Stage 3 406 36.4 15 328.2 483  
## Stage 4 271 36.4 15 193.5 348  
## Stage 5 104 36.4 15 26.1 181  
##   
## Confidence level used: 0.95   
##   
## $contrasts  
## contrast estimate SE df t.ratio p.value  
## Stage 2 - Stage 1 71.9 51.4 15 1.397 0.4594  
## Stage 3 - Stage 1 -158.1 51.4 15 -3.075 0.0265  
## Stage 4 - Stage 1 -292.8 51.4 15 -5.695 0.0002  
## Stage 5 - Stage 1 -460.2 51.4 15 -8.950 <.0001  
##   
## P value adjustment: dunnettx method for 4 tests

#### Question: at what stage did HA become different from Stage 1?

No, Stage 2 is not, but the rest of the stages are different from Stage 1.

## Sequential comparisons

Comparing each level to the previous one is done with the consec comparisons in emmeans:

eels.ha.consec <- emmeans(eels.ha.lm, consec ~ Stage)  
eels.ha.consec

## $emmeans  
## Stage emmean SE df lower.CL upper.CL  
## Stage 1 564 36.4 15 486.3 641  
## Stage 2 636 36.4 15 558.2 713  
## Stage 3 406 36.4 15 328.2 483  
## Stage 4 271 36.4 15 193.5 348  
## Stage 5 104 36.4 15 26.1 181  
##   
## Confidence level used: 0.95   
##   
## $contrasts  
## contrast estimate SE df t.ratio p.value  
## Stage 2 - Stage 1 71.9 51.4 15 1.397 0.4823  
## Stage 3 - Stage 2 -230.0 51.4 15 -4.472 0.0019  
## Stage 4 - Stage 3 -134.7 51.4 15 -2.620 0.0653  
## Stage 5 - Stage 4 -167.4 51.4 15 -3.256 0.0188  
##   
## P value adjustment: mvt method for 4 tests

#### Question: which stages are different from the previous one?

## Orthogonal polynomials - analyzing ordinal trends

R uses polynomial contrasts by default for ordered factors. If we make a version of the Stage variable that is an ordered factor, and then use it in a linear model, R will give us tests of the ordinal trends across the five stages.

Make a copy of Stage as an ordered factor:

eels$Stage.ordered <- factor(eels$Stage, ordered = T)

#### Question: refer to the polynomial weights in the instructions. These weights describe a pattern of change from first to last stage. How? Use Linear and Quadratic weights as an example.

Linear weights start at a negative value and increase by an equal amount across the levels. The quadratic term is a parabola that opens upward, so it starts at positive values, declines to a minimum, and then increases back to the same value as it started.

Fit a model using the ordered factor version of stage:

eels.op.lm <- lm(HA ~ Stage.ordered, data = eels)  
summary(eels.op.lm)

##   
## Call:  
## lm(formula = HA ~ Stage.ordered, data = eels)  
##   
## Residuals:  
## Min 1Q Median 3Q Max   
## -116.94 -43.12 12.68 30.44 122.39   
##   
## Coefficients:  
## Estimate Std. Error t value Pr(>|t|)   
## (Intercept) 395.94 16.26 24.349 1.79e-13 \*\*\*  
## Stage.ordered.L -406.41 36.36 -11.177 1.13e-08 \*\*\*  
## Stage.ordered.Q -102.44 36.36 -2.817 0.0130 \*   
## Stage.ordered.C 85.11 36.36 2.341 0.0335 \*   
## Stage.ordered^4 -62.74 36.36 -1.726 0.1050   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Residual standard error: 72.72 on 15 degrees of freedom  
## Multiple R-squared: 0.904, Adjusted R-squared: 0.8785   
## F-statistic: 35.33 on 4 and 15 DF, p-value: 1.805e-07

Get the ANOVA table - it doesn’t look any different:

anova(eels.op.lm)

## Analysis of Variance Table  
##   
## Response: HA  
## Df Sum Sq Mean Sq F value Pr(>F)   
## Stage.ordered 4 747378 186844 35.331 1.805e-07 \*\*\*  
## Residuals 15 79327 5288   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Run Tukey tests on this ordinal stage variable:

emmeans(eels.op.lm, tukey ~ Stage.ordered)

## $emmeans  
## Stage.ordered emmean SE df lower.CL upper.CL  
## Stage 1 564 36.4 15 486.3 641  
## Stage 2 636 36.4 15 558.2 713  
## Stage 3 406 36.4 15 328.2 483  
## Stage 4 271 36.4 15 193.5 348  
## Stage 5 104 36.4 15 26.1 181  
##   
## Confidence level used: 0.95   
##   
## $contrasts  
## contrast estimate SE df t.ratio p.value  
## Stage 1 - Stage 2 -71.9 51.4 15 -1.397 0.6384  
## Stage 1 - Stage 3 158.1 51.4 15 3.075 0.0513  
## Stage 1 - Stage 4 292.8 51.4 15 5.695 0.0004  
## Stage 1 - Stage 5 460.2 51.4 15 8.950 <.0001  
## Stage 2 - Stage 3 230.0 51.4 15 4.472 0.0035  
## Stage 2 - Stage 4 364.7 51.4 15 7.092 <.0001  
## Stage 2 - Stage 5 532.1 51.4 15 10.348 <.0001  
## Stage 3 - Stage 4 134.7 51.4 15 2.620 0.1163  
## Stage 3 - Stage 5 302.1 51.4 15 5.876 0.0003  
## Stage 4 - Stage 5 167.4 51.4 15 3.256 0.0365  
##   
## P value adjustment: tukey method for comparing a family of 5 estimates

#### Question: are there any changes to these Tukey tests compared with the analysis with the unordered factor you did previously?

No, the comparisons of means are all the same.

The tests of polynomial trends are seen in the coefficient tests:

summary(eels.op.lm)

##   
## Call:  
## lm(formula = HA ~ Stage.ordered, data = eels)  
##   
## Residuals:  
## Min 1Q Median 3Q Max   
## -116.94 -43.12 12.68 30.44 122.39   
##   
## Coefficients:  
## Estimate Std. Error t value Pr(>|t|)   
## (Intercept) 395.94 16.26 24.349 1.79e-13 \*\*\*  
## Stage.ordered.L -406.41 36.36 -11.177 1.13e-08 \*\*\*  
## Stage.ordered.Q -102.44 36.36 -2.817 0.0130 \*   
## Stage.ordered.C 85.11 36.36 2.341 0.0335 \*   
## Stage.ordered^4 -62.74 36.36 -1.726 0.1050   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Residual standard error: 72.72 on 15 degrees of freedom  
## Multiple R-squared: 0.904, Adjusted R-squared: 0.8785   
## F-statistic: 35.33 on 4 and 15 DF, p-value: 1.805e-07

#### Question: which polynomial trends were statistically significant?

Linear, Quadratic, and Cubic trends were significant.

## Corrections for multiple p-values

We just completed an analysis of HA, but the data set includes measures of change in water and in neutral sugar as well. Fit a model using water as a response, and Stage as a predictor, then get the summary output:

lm(water ~ Stage, data = eels) -> water.lm  
summary(water.lm)

##   
## Call:  
## lm(formula = water ~ Stage, data = eels)  
##   
## Residuals:  
## Min 1Q Median 3Q Max   
## -4.9475 -1.9519 -0.8325 1.8881 6.6650   
##   
## Coefficients:  
## Estimate Std. Error t value Pr(>|t|)   
## (Intercept) 90.938 1.769 51.396 < 2e-16 \*\*\*  
## StageStage 2 -3.100 2.502 -1.239 0.23442   
## StageStage 3 -2.603 2.502 -1.040 0.31478   
## StageStage 4 -5.448 2.502 -2.177 0.04586 \*   
## StageStage 5 -9.915 2.502 -3.962 0.00125 \*\*   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Residual standard error: 3.539 on 15 degrees of freedom  
## Multiple R-squared: 0.5422, Adjusted R-squared: 0.4201   
## F-statistic: 4.442 on 4 and 15 DF, p-value: 0.01445

Fit a model using NS as a response, and get the summary output:

lm(NS ~ Stage, data = eels) -> ns.lm  
summary(ns.lm)

##   
## Call:  
## lm(formula = NS ~ Stage, data = eels)  
##   
## Residuals:  
## Min 1Q Median 3Q Max   
## -3.2575 -1.0269 0.2263 0.7138 2.4450   
##   
## Coefficients:  
## Estimate Std. Error t value Pr(>|t|)   
## (Intercept) 4.7075 0.8413 5.595 5.11e-05 \*\*\*  
## StageStage 2 3.9275 1.1898 3.301 0.004851 \*\*   
## StageStage 3 4.8900 1.1898 4.110 0.000928 \*\*\*  
## StageStage 4 9.1325 1.1898 7.675 1.43e-06 \*\*\*  
## StageStage 5 13.1975 1.1898 11.092 1.26e-08 \*\*\*  
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Residual standard error: 1.683 on 15 degrees of freedom  
## Multiple R-squared: 0.9064, Adjusted R-squared: 0.8815   
## F-statistic: 36.33 on 4 and 15 DF, p-value: 1.496e-07

#### Question: according to the model-level omnibus test (i.e. the p-value at the bottom of the summary output), do NS and water change during development? How do you know?

Yes they do, both have p-values less than 0.05.

Calculate the adjusted alpha levels:

0.05/3

## [1] 0.01666667

1 - (1 - 0.05)^(1/3)

## [1] 0.01695243

#### Question: are any of the variables no longer statistically significant at either of these adjusted alpha levels?

All are still statistically significant after adjustment, but water is much closer to the adjusted alpha level.

## Optional: post-hoc comparisons of your choice, and orthogonal contrasts

We need a matrix that defines comparisons between sequential stages.

s1 <- c(1,0,0,0,0)  
s2 <- c(0,1,0,0,0)  
s3 <- c(0,0,1,0,0)  
s4 <- c(0,0,0,1,0)  
s5 <- c(0,0,0,0,1)

Define the contrasts you wish to make:

list(s1s2.vs.s4s5 = (s1+s2)/2 - (s4+s5)/2, s1.vs.s2s3s4s5 = s1 - (s2+s3+s4+s5)/4) -> custom.contrasts

Now get the multiple comparisons for this set of custom contrasts:

emmeans(eels.ha.lm, ~Stage) -> eels.ha.emm  
  
contrast(eels.ha.emm, custom.contrasts, adjust = "mvt")

## contrast estimate SE df t.ratio p.value  
## s1s2.vs.s4s5 412 36.4 15 11.344 <.0001  
## s1.vs.s2s3s4s5 210 40.7 15 5.162 0.0002  
##   
## P value adjustment: mvt method for 2 tests

#### Question: which comparisons are significant?

Both are significant.

#### Question: why was it necessary to adjust for multiple comparisons with these contrasts?

These comparisons are not independent.

## Orthogonal contrasts

We will use the fourth set of possible orthogonal contrasts, which compares each stage against the mean of those that follow.

Contrast.1 <- s1 - (s2+s3+s4+s5)/4  
Contrast.2 <- (s2+s3)/2 - (s4+s5)/2  
Contrast.3 <- (s2+s4)/2 - (s3+s5)/2  
Contrast.4 <- (s3+s4)/2 - (s2+s5)/2

Make a list of each of the four independent contrasts:

list(s1.vs.s2s3s4s5 = Contrast.1, s2s3.vs.s4s5 = Contrast.2, s2s4.vs.s3s5 = Contrast.3, s3s4.vs.s2s5 = Contrast.4) -> orthog.contrasts

Test these contrasts for significance:

contrast(eels.ha.emm, orthog.contrasts)

## contrast estimate SE df t.ratio p.value  
## s1.vs.s2s3s4s5 209.8 40.7 15 5.162 0.0001  
## s2s3.vs.s4s5 333.4 36.4 15 9.170 <.0001  
## s2s4.vs.s3s5 198.7 36.4 15 5.464 0.0001  
## s3s4.vs.s2s5 -31.3 36.4 15 -0.860 0.4033

#### Question: which comparisons were significant?

All of the contrasts except for the last one, which compared the mean of Stage 3 and Stage 4 to the mean of Stage 2 and Stage 5, were significant.

**Question: why was it not necessary to adjust for multiple comparisons, even though there are four contrasts being tested?**

The contrasts are independent this time, so they do not need to be corrected for multiple comparisons.