Wrapping up Ch 2

Testing for significant differences between groups for % with spike and oxyconformity

Exp. 1 (Dana, 24C) Embryos

> spike<-c(1,2,1)

> fish<-c(10,9,9)

> prop.test(spike,fish,correct=F)

3-sample test for equality of proportions without continuity correction

data: spike out of fish

X-squared = 0.68704, df = 2, p-value = 0.7093

alternative hypothesis: two.sided

sample estimates:

prop 1 prop 2 prop 3

0.1000000 0.2222222 0.1111111

> oxyconf<-c(4,2,3)

> fish1<-c(10,9,9)

> prop.test(oxyconf,fish1,correct=F)

3-sample test for equality of proportions without continuity correction

data: oxyconf out of fish1

X-squared = 0.695, df = 2, p-value = 0.7065

alternative hypothesis: two.sided

sample estimates:

prop 1 prop 2 prop 3

0.4000000 0.2222222 0.3333333

Exp. 2 (Flax, 22C) Embryos

> spike<-c(2,2,5)

> fish<-c(11,12,14)

> prop.test(spike,fish,correct=F)

3-sample test for equality of proportions without continuity correction

data: spike out of fish

X-squared = 1.5944, df = 2, p-value = 0.4506

alternative hypothesis: two.sided

sample estimates:

prop 1 prop 2 prop 3

0.1818182 0.1666667 0.3571429

> oxyconf<-c(0,1,10)

> fish1<-c(11,12,14)

> prop.test(oxyconf,fish1,correct=F)

3-sample test for equality of proportions without continuity correction

data: oxyconf out of fish1

X-squared = 18.936, df = 2, p-value = 7.729e-05

alternative hypothesis: two.sided

sample estimates:

prop 1 prop 2 prop 3

0.00000000 0.08333333 0.71428571

Exp. 1 (Dana, 24C) 2dph Larvae

> spike<-c(10,9,7)

> fish<-c(10,10,19)

> prop.test(spike,fish,correct=F)

3-sample test for equality of proportions without continuity correction

data: spike out of fish

X-squared = 2.5241, df = 2, p-value = 0.2831

alternative hypothesis: two.sided

sample estimates:

prop 1 prop 2 prop 3

1.0000000 0.9000000 0.7777778

Exp. 2 (Flax, 22C) 2dph Larvae

> spike<-c(6,10,7)

> fish<-c(9,12,13)

> prop.test(spike,fish,correct=F)

3-sample test for equality of proportions without continuity correction

data: spike out of fish

X-squared = 2.4844, df = 2, p-value = 0.2887

alternative hypothesis: two.sided

sample estimates:

prop 1 prop 2 prop 3

0.6666667 0.8333333 0.5384615

Exp. 1 (Dana, 24C) 5dph Larvae

> spike<-c(5,3,3)

> fish<-c(9,8,9)

> prop.test(spike,fish,correct=F)

3-sample test for equality of proportions without continuity correction

data: spike out of fish

X-squared = 1.0199, df = 2, p-value = 0.6005

alternative hypothesis: two.sided

sample estimates:

prop 1 prop 2 prop 3

0.5555556 0.3750000 0.3333333

Exp. 2 (Flax, 22C) 5dph Larvae

> spike<-c(6,9,4)

> fish<-c(7,12,10)

> prop.test(spike,fish,correct=F) #no, p=0.2831

3-sample test for equality of proportions without continuity correction

data: spike out of fish

X-squared = 4.6236, df = 2, p-value = 0.09908

alternative hypothesis: two.sided

sample estimates:

prop 1 prop 2 prop 3

0.8571429 0.7500000 0.4000000

OUTLIERS – do not recompute above things after removing outliers, because oxyconformity and spike presence are still valid.

Exp. 1 (Dana, 24C) embryos

* Pcrit
  + Passes both diagnostic tests.
  + Two slightly more influential points (12 and 23) identified with Cook’s distance but they aren’t that extreme.
  + Boxplot identifies one outlier in high CO2 group.
* RMR
  + Passes both diagnostic tests.
  + No outliers identified with Cook’s distance.
  + Boxplot identifies four outliers in high CO2 group but that leaves extremely narrow distribution for that group.

Exp. 2 (Flax, 22C) embryos

* Pcrit
  + Fails homogeneity test but passes with reciprocal transformation, but the transformations cause normality to fail.
  + Five outliers identified with Cook’s distance but they don’t match up to residuals well so probably fine. 9 and 14 are most influential. Removing 9 and 14 makes diagnostics worse.
  + Removing 11, 12, and 26 fixes diagnostics
  + Boxplot identifies no outliers.
  + Remove 11, 12, and 26.
* RMR
  + Fails both tests but sqrt transformation fixes it.
  + Two outliers identified with Cook’s distance (20 and 26)
  + These are also the greatest residuals.
  + Boxplot identifies one outlier in the ambient group, probably 20.
  + Remove 20 and 26. With sqrt transformation it passes diagnostic tests.

> break\_mod3<-aov((emb\_flaxo$Pcrit\_break)~emb\_flaxo$CO2\_level/factor(emb\_flaxo$Tank))

> summary(break\_mod3)

Df Sum Sq Mean Sq F value Pr(>F)

emb\_flaxo$CO2\_level 2 0.962 0.4809 0.922 0.418

emb\_flaxo$CO2\_level:factor(emb\_flaxo$Tank) 4 3.918 0.9794 1.878 0.163

Residuals 16 8.343 0.5214

14 observations deleted due to missingness

> flax\_emb\_mdl3<-aov(sqrt(emb\_flaxo$RMR)~emb\_flaxo$CO2\_level/factor(emb\_flaxo$Tank))

> summary(flax\_emb\_mdl3) #CO2 is significant p=4.46e-6

Df Sum Sq Mean Sq F value Pr(>F)

emb\_flaxo$CO2\_level 2 0.005003 0.0025013 23.215 1.64e-06 \*\*\*

emb\_flaxo$CO2\_level:factor(emb\_flaxo$Tank) 6 0.001126 0.0001877 1.742 0.151

Residuals 26 0.002801 0.0001077

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

2 observations deleted due to missingness

> TukeyHSD(flax\_emb\_mdl3)

Tukey multiple comparisons of means

95% family-wise confidence level

Fit: aov(formula = sqrt(emb\_flaxo$RMR) ~ emb\_flaxo$CO2\_level/factor(emb\_flaxo$Tank))

$`emb\_flaxo$CO2\_level`

diff lwr upr p adj

med-amb 0.009365312 -0.001678725 0.02040935 0.1079255

high-amb 0.028638299 0.017789057 0.03948754 0.0000017

high-med 0.019272986 0.008947397 0.02959858 0.0002501

Exp. 1 (Dana, 24C) 2dph Larvae

* Pcrit
  + Diagnostics passed
  + Influential points: 14, 22, 26, but they are pretty typical. If anything maybe 26 is drawing the mean or median down for high CO2.
  + Boxplot no outliers.
  + Residuals: 14 is kind of high but not that bad. Leave it be.
* RMR
  + Normality failed.
  + Influential points: 5 and 15. Both are ambient and kind of low.
  + Boxplot two outliers, not the same as the influential points.
  + Residuals: 5 and 15 pretty high.
  + Removing the outliers doesn’t help but the reciprocal transformation does.

Exp. 1 (Dana, 24C) 2dph Larvae

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  + Influential points: 14, 22, 26, but they are pretty typical. If anything maybe 26 is drawing the mean or median down for high CO2.
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* RMR
  + Normality failed.
  + Influential points: 5 and 15. Both are ambient and kind of low.
  + Boxplot two outliers, not the same as the influential points.
  + Residuals: 5 and 15 pretty high.
  + Removing the outliers doesn’t help but the reciprocal transformation does.

Exp. 2 (Flax, 22C) 2dph Larvae

* Pcrit
  + Diagnostics passed.
  + Influential points: 14, 30, and 32, with 14 and 32 being pretty high and the residual for 14 is high. All are from high CO2.
  + Boxplot detected no outliers.
  + Residuals high for 14.
  + Remove 14, 30, and 32 – it doesn’t affect the diagnostics.
* RMR
  + Normality passed but homogeneity failed, but fixed with reciprocal transformation.
  + Influential points: 10 and 19. Both are a little low, 10 is from high CO2 and 19 is from ambient.
  + Boxplot detected no outliers.
  + Residuals are high for both.
  + Remove outliers – it doesn’t affect the diagnostics, still need reciprocal transformation. A few higher RMR values give a slight skew.

> break\_mod3<-aov((lar\_flaxo$Pcrit\_break)~lar\_flaxo$CO2\_level/factor(lar\_flaxo$Tank))

> summary(break\_mod3)

Df Sum Sq Mean Sq F value Pr(>F)

lar\_flaxo$CO2\_level 2 0.202 0.1010 0.152 0.860

lar\_flaxo$CO2\_level:factor(lar\_flaxo$Tank) 5 6.822 1.3644 2.058 0.108

Residuals 23 15.251 0.6631

6 observations deleted due to missingness

> flax\_lar\_mdl3<-aov(1/(lar\_flaxo$RMR)~lar\_flaxo$CO2\_level/factor(lar\_flaxo$Tank))

> summary(flax\_lar\_mdl3) #CO2 is significant p=4.46e-6

Df Sum Sq Mean Sq F value Pr(>F)

lar\_flaxo$CO2\_level 2 8.87 4.434 1.244 0.306

lar\_flaxo$CO2\_level:factor(lar\_flaxo$Tank) 5 9.30 1.861 0.522 0.757

Residuals 24 85.56 3.565

5 observations deleted due to missingness

Exp. 1 (Dana, 24C) 5dph Larvae

* Pcrit
  + Diagnostics passed.
  + Influential points: 2, 3, 4, and 5. 2 and 5 are pretty low, 3 is pretty high, all four are high CO2.
  + Boxplot doesn’t identify outliers but high CO2 has much greater spread.
  + Residuals are a little high especially for 2 and 3.
  + If remove outliers diagnostics still pass.
  + Remove them all.
* RMR
  + Diagnostics passed
  + Influential points: 2, 4, 5, 8, 9.
  + Boxplot detects no outliers but high CO2 is pretty skewed.
  + Residuals are pretty high for all of these plus 3.
  + Remove them all, maybe leave 2 and 9 though because they are right near the line.

> summary(break\_mod3)

Df Sum Sq Mean Sq F value Pr(>F)

lrv\_danao$CO2\_level 2 0.8799 0.4399 4.25 0.0361 \*

lrv\_danao$CO2\_level:factor(lrv\_danao$Tank) 5 2.1015 0.4203 4.06 0.0173 \*

Residuals 14 1.4492 0.1035

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

5 observations deleted due to missingness

> TukeyHSD(break\_mod3)

Tukey multiple comparisons of means

95% family-wise confidence level

Fit: aov(formula = (lrv\_danao$Pcrit\_break) ~ lrv\_danao$CO2\_level/factor(lrv\_danao$Tank))

$`lrv\_danao$CO2\_level`

diff lwr upr p adj

med-amb -0.2897175 -0.6988867 0.11945163 0.1889045

high-amb -0.5047438 -0.9744242 -0.03506337 0.0347006

high-med -0.2150263 -0.6950761 0.26502363 0.4879901

> summary(dana\_lrv\_mdl3) #CO2 is significant p=4.46e-6

Df Sum Sq Mean Sq F value Pr(>F)

lrv\_danao$CO2\_level 2 0.01943 0.009715 3.783 0.0486 \*

lrv\_danao$CO2\_level:factor(lrv\_danao$Tank) 5 0.01434 0.002868 1.117 0.3956

Residuals 14 0.03595 0.002568

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

5 observations deleted due to missingness

> TukeyHSD(dana\_lrv\_mdl3)

Tukey multiple comparisons of means

95% family-wise confidence level

Fit: aov(formula = sqrt(lrv\_danao$RMR) ~ lrv\_danao$CO2\_level/factor(lrv\_danao$Tank))

$`lrv\_danao$CO2\_level`

diff lwr upr p adj

med-amb -0.060254244 -0.126572648 0.006064161 0.0773524

high-amb 0.003412437 -0.068219627 0.075044501 0.9914713

high-med 0.063666680 -0.007965384 0.135298744 0.0848326

Exp. 2 (Flax, 22C) 5dph Larvae

* Pcrit
  + Diagnostics passed
  + Influential point: 31, but it looks fine to me.
  + Boxplot detects no outliers
  + Residuals are not that high
  + Remove it.
* RMR
  + Diagnostics passed
  + Influential points: 4, 6, and 14. 4 and 6 are most influential by a lot. 4 and 6 are both in high CO2 but only 6 looks extreme to me. 14 is ambient and a little high.
  + Boxplot detected 3 outliers, one in med and two in high CO2.
  + Residuals aren’t that bad.
  + Remove it.

> summary(break\_mod3)

Df Sum Sq Mean Sq F value Pr(>F)

lrv\_flaxo$CO2\_level 2 2.720 1.3599 4.971 0.0177 \*

lrv\_flaxo$CO2\_level:factor(lrv\_flaxo$Tank) 5 3.148 0.6296 2.301 0.0834 .

Residuals 20 5.472 0.2736

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

5 observations deleted due to missingness

> TukeyHSD(break\_mod3)

Tukey multiple comparisons of means

95% family-wise confidence level

Fit: aov(formula = (lrv\_flaxo$Pcrit\_break) ~ lrv\_flaxo$CO2\_level/factor(lrv\_flaxo$Tank))

$`lrv\_flaxo$CO2\_level`

diff lwr upr p adj

med-amb -0.3432188 -0.9725799 0.2861423 0.3699804

high-amb -0.8168205 -1.4837078 -0.1499332 0.0149533

high-med -0.4736017 -1.0571280 0.1099247 0.1254461

> summary(flax\_lrv\_mdl3) #CO2 is significant p=4.46e-6

Df Sum Sq Mean Sq F value Pr(>F)

lrv\_flaxo$CO2\_level 2 0.05130 0.025648 14.82 8.41e-05 \*\*\*

lrv\_flaxo$CO2\_level:factor(lrv\_flaxo$Tank) 4 0.03075 0.007686 4.44 0.0088 \*\*

Residuals 22 0.03809 0.001731

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

4 observations deleted due to missingness

> TukeyHSD(flax\_lrv\_mdl3)

Tukey multiple comparisons of means

95% family-wise confidence level

Fit: aov(formula = sqrt(lrv\_flaxo$RMR) ~ lrv\_flaxo$CO2\_level/factor(lrv\_flaxo$Tank))

$`lrv\_flaxo$CO2\_level`

diff lwr upr p adj

med-amb 0.01806187 -0.03164724 0.06777097 0.6381412

high-amb -0.07582439 -0.12733234 -0.02431645 0.0034553

high-med -0.09388626 -0.13863899 -0.04913354 0.0000788

Plotting sample curves for typical, spike, and oxyconforming

Embryo Typical: E1P1D6, E1P2A1

2dph Typical: E2P1A5, E2P2B3

5dph Typical: E2P2A5, E2P2B3 (best?), E2P2C4, E2P2C6 (very good)

Embryo Spike: E2P1C2, E2P2A2 (also has a pretty clear Pcrit)

2dph Spike: E1P1A3, E1P1C1

Oxyconforming: E2P1B3, E2P1B4 (very good), E2P1C4, E2P2B1 (very good), E2P2B2 (very good), E2P2B4, E2P2B6, E1P1A5, E1P1B3

Summary of results for each treatment (and what’s new after outlier removal\*)

**Embryos**

RMR

* Exp. 1 (24C) – No significant effect of pCO2. Sqrt transformation, no outliers.
* Exp. 2 (22C) – **High pCO2 significantly higher** than ambient and moderate. Sqrt transformation required even after removing\* two outliers.

Pcrit

* Exp. 1 (24C) – No significant effect of pCO2. No outliers, no transformation.
* Exp. 2 (22C) – No significant effect of pCO2. Three outliers removed\*, no transformation.

Oxyconformity

* Exp. 1 (24C) – No significant effect of pCO2.
* Exp. 2 (22C) – **Significantly higher** **proportion in high pCO2**, with no oxyconformity in ambient.

**2dph Larvae**

RMR

* Exp. 1 (24C) – No significant effect of pCO2. No outliers, used reciprocal transformation.
* Exp. 2 (22C) – No significant effect of pCO2. Removed\* outliers, used reciprocal transformation.

Pcrit

* Exp. 1 (24C) – **High pCO2 significantly lower** than ambient. No outliers, no transformation.
* Exp. 2 (22C) – No significant effect of pCO2. Removed\* outliers, no transformation.

**5dph Larvae**

RMR

* Exp. 1 (24C) – **Significant effect of pCO2** in ANOVA but nothing in Tukey. Best guess is that it is from moderate pCO2 being a little lower than the other two. No transformation, some outliers were removed\*.
* Exp. 2 (22C) – **High pCO2 significantly lower** than ambient and moderate. Tank effect significant. No transformation, some outliers were removed\*.

Pcrit

* Exp. 1 (24C) – **High pCO2 significantly lower** than ambient. Tank effect significant. Removed\* four outliers, no transformation.
* Exp. 2 (22C) – **High pCO2 significantly lower** than ambient. Removed\* one outlier, no transformation.

Diagram

Description automatically generated

Diagram

Description automatically generated

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  |  | Ambient pCO2 | Moderate pCO2 | High pCO2 | *p*-value |
| Oxyconformity | Embryos | Exp. 1 (24°C) | 40.0% | 22.2% | 33.3% | 0.7065 |
| Exp. 2 (22°C) | 0.00% | 8.33% | 71.4% | **7.7e-5** |
| Low-DO increase | Embryos | Exp. 1 (24°C) | 10.0% | 22.2% | 11.1% | 0.71 |
| Exp. 2 (22°C) | 18.2% | 16.7% | 35.7% | 0.45 |
| 2dph Larvae | Exp. 1 (24°C) | 100% | 90.0% | 77.8% | 0.28 |
| Exp. 2 (22°C) | 66.7% | 83.3% | 53.8% | 0.29 |
| 5dph Larvae | Exp. 1 (24°C) | 55.6% | 37.5% | 33.3% | 0.60 |
| Exp. 2 (22°C) | 85.7% | 75.0% | 40.0% | 0.10 |