Experiment 2 – Blackhead Eggs

## Experiment 2 Red Eggs–Overview

Here we examine the third developmental stage in eggs–blackhead (i.e., the headcapsule of the neonate larva is visible). The sexes are again examined separately because of expected differences in radiosensitivy.

library(readxl)  
# Load data and display first few rows  
df\_black <- read\_excel("Dataset.xlsx", sheet = "Blackhead\_400Gy")  
  
# Set x-ray dose as a factor (categorical)  
df\_black$dose <- as.factor(df\_black$dose)

Separate sexes

library(dplyr)  
# Isolate data for females and display the first few rows  
females <- df\_black %>%   
 filter(sex == "female")  
females

## # A tibble: 37 × 5  
## rep FemID sex dose sum\_blackhead  
## <chr> <chr> <chr> <fct> <dbl>  
## 1 c C2 female 0 6  
## 2 c C5 female 0 12  
## 3 e E1 female 0 3  
## 4 e E2 female 0 0  
## 5 e E3 female 0 0  
## 6 e E4 female 0 0  
## 7 e E5 female 0 0  
## 8 c C7 female 100 23  
## 9 c C9 female 100 0  
## 10 e E10 female 100 17  
## # ℹ 27 more rows

# Isolate data for males and display the first few rows  
males <- df\_black %>%   
 filter(sex == "male")  
males

## # A tibble: 37 × 5  
## rep FemID sex dose sum\_blackhead  
## <chr> <chr> <chr> <fct> <dbl>  
## 1 b B21 male 0 18  
## 2 b B22 male 0 38  
## 3 b B23 male 0 46  
## 4 b B24 male 0 60  
## 5 b B25 male 0 9  
## 6 d D1 male 0 0  
## 7 d D3 male 0 0  
## 8 d D4 male 0 7  
## 9 d D5 male 0 0  
## 10 b B1 male 100 39  
## # ℹ 27 more rows

## Summary and statistical analysis of red eggs from irradiated females

library(FSA) # for SE  
# n, mean, and SE by dose for females  
females %>%   
 group\_by(dose) %>%  
 summarise(nObs = sum(!is.na(sum\_blackhead)),  
 mn = mean(sum\_blackhead, na.rm = TRUE),  
 sem = se(sum\_blackhead),  
 pct\_sterile = 100\*(1-(sum(sum\_blackhead > 0)/nObs)))

## # A tibble: 5 × 5  
## dose nObs mn sem pct\_sterile  
## <fct> <int> <dbl> <dbl> <dbl>  
## 1 0 7 3 1.73 57.1  
## 2 100 4 10 5.90 50   
## 3 200 9 0.333 0.167 66.7  
## 4 300 8 0.875 0.515 62.5  
## 5 400 9 0.111 0.111 88.9

Looks like there are differences, although not necessarily in a logical order.

# Analysis of Deviance using GLM with negative binomial--females  
library(MASS)  
m1 <- MASS::glm.nb(sum\_blackhead ~ dose, data = females)  
summary(m1)

##   
## Call:  
## MASS::glm.nb(formula = sum\_blackhead ~ dose, data = females,   
## init.theta = 0.3610803238, link = log)  
##   
## Coefficients:  
## Estimate Std. Error z value Pr(>|z|)   
## (Intercept) 1.0986 0.6658 1.650 0.0989 .  
## dose100 1.2040 1.0773 1.118 0.2638   
## dose200 -2.1972 1.0413 -2.110 0.0349 \*  
## dose300 -1.2321 0.9656 -1.276 0.2019   
## dose400 -3.2958 1.3232 -2.491 0.0127 \*  
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## (Dispersion parameter for Negative Binomial(0.3611) family taken to be 1)  
##   
## Null deviance: 46.113 on 36 degrees of freedom  
## Residual deviance: 25.870 on 32 degrees of freedom  
## AIC: 106.13  
##   
## Number of Fisher Scoring iterations: 1  
##   
##   
## Theta: 0.361   
## Std. Err.: 0.166   
##   
## 2 x log-likelihood: -94.128

Examine model fit for females

# Calculate p-value under the chi-squared distribution  
dev <- deviance(m1)  
df <- df.residual(m1)  
p\_value <- pchisq(dev, df, lower.tail = FALSE)  
cat("Chi-square goodness-of-fit p-value:", p\_value, "\n")

## Chi-square goodness-of-fit p-value: 0.7693452

No indication of problem with model fit

library(car)  
# Model parameters with type II degrees of freedom  
Anova(m1)

## Analysis of Deviance Table (Type II tests)  
##   
## Response: sum\_blackhead  
## LR Chisq Df Pr(>Chisq)   
## dose 20.243 4 0.0004471 \*\*\*  
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Overall, there were significant differences. Proceeding to comparisons of multiple means.

library(emmeans)  
library(multcomp)  
# Means separation  
emm\_fem <- emmeans(m1, ~ dose, type = "response")  
multcomp::cld(emm\_fem, Letters = LETTERS, decreasing = TRUE)

## dose response SE df asymp.LCL asymp.UCL .group  
## 100 10.000 8.470 Inf 1.9013 52.60 A   
## 0 3.000 2.000 Inf 0.8136 11.06 AB   
## 300 0.875 0.612 Inf 0.2222 3.45 AB   
## 200 0.333 0.267 Inf 0.0694 1.60 B   
## 400 0.111 0.127 Inf 0.0118 1.05 B   
##   
## Confidence level used: 0.95   
## Intervals are back-transformed from the log scale   
## P value adjustment: tukey method for comparing a family of 5 estimates   
## Tests are performed on the log scale   
## significance level used: alpha = 0.05   
## NOTE: If two or more means share the same grouping symbol,  
## then we cannot show them to be different.  
## But we also did not show them to be the same.

## Summary and statistical analysis of red eggs from females mated with irradiated males

# n, mean, and SE by dose for females  
males %>%   
 group\_by(dose) %>%  
 summarise(nObs = sum(!is.na(sum\_blackhead)),  
 mn = mean(sum\_blackhead, na.rm = TRUE),  
 sem = se(sum\_blackhead),  
 pct\_sterile = 100\*(1-(sum(sum\_blackhead > 0)/nObs)))

## # A tibble: 5 × 5  
## dose nObs mn sem pct\_sterile  
## <fct> <int> <dbl> <dbl> <dbl>  
## 1 0 9 19.8 7.54 33.3  
## 2 100 7 40.1 19.8 42.9  
## 3 200 5 41.2 12.4 0   
## 4 300 7 2.71 1.23 28.6  
## 5 400 9 1 1 88.9

# Analysis of Deviance using GLM with negative binomial--males  
m2 <- MASS::glm.nb(sum\_blackhead ~ dose, data = males)  
summary(m2)

##   
## Call:  
## MASS::glm.nb(formula = sum\_blackhead ~ dose, data = males, init.theta = 0.3558404849,   
## link = log)  
##   
## Coefficients:  
## Estimate Std. Error z value Pr(>|z|)   
## (Intercept) 2.9846 0.5638 5.294 1.2e-07 \*\*\*  
## dose100 0.7079 0.8502 0.833 0.405081   
## dose200 0.7339 0.9406 0.780 0.435270   
## dose300 -1.9860 0.8786 -2.260 0.023796 \*   
## dose400 -2.9846 0.8609 -3.467 0.000527 \*\*\*  
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## (Dispersion parameter for Negative Binomial(0.3558) family taken to be 1)  
##   
## Null deviance: 57.840 on 36 degrees of freedom  
## Residual deviance: 37.584 on 32 degrees of freedom  
## AIC: 238.6  
##   
## Number of Fisher Scoring iterations: 1  
##   
##   
## Theta: 0.356   
## Std. Err.: 0.105   
##   
## 2 x log-likelihood: -226.596

Examine model fit for males

# Calculate p-value under the chi-squared distribution  
dev <- deviance(m2)  
df <- df.residual(m2)  
p\_value <- pchisq(dev, df, lower.tail = FALSE)  
cat("Chi-square goodness-of-fit p-value:", p\_value, "\n")

## Chi-square goodness-of-fit p-value: 0.228605

The residual deviance is not significantly different (P > 0.05) from the expected value for the residual degrees of freedom.

# Model parameters with type II degrees of freedom  
Anova(m2)

## Analysis of Deviance Table (Type II tests)  
##   
## Response: sum\_blackhead  
## LR Chisq Df Pr(>Chisq)   
## dose 20.255 4 0.0004447 \*\*\*  
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Overall, there were significant differences. Proceeding to comparisons of multiple means.

# Model parameters with type II degrees of freedom  
emm\_males <- emmeans(m2, ~ dose, type = "response")  
multcomp::cld(emm\_males, Letters = LETTERS, decreasing = TRUE)

## dose response SE df asymp.LCL asymp.UCL .group  
## 200 41.20 31.000 Inf 9.419 180.22 AB   
## 100 40.14 25.500 Inf 11.532 139.74 A   
## 0 19.78 11.200 Inf 6.550 59.71 AB   
## 300 2.71 1.830 Inf 0.725 10.17 BC   
## 400 1.00 0.651 Inf 0.279 3.58 C   
##   
## Confidence level used: 0.95   
## Intervals are back-transformed from the log scale   
## P value adjustment: tukey method for comparing a family of 5 estimates   
## Tests are performed on the log scale   
## significance level used: alpha = 0.05   
## NOTE: If two or more means share the same grouping symbol,  
## then we cannot show them to be different.  
## But we also did not show them to be the same.