CviMVP_Nursery_Hatchery_Analysis

2024-02-22

R Markdown

```r
#load necessary packages
library(tidyverse)
```

```
## -- Attaching core tidyverse packages ----------------------- tidyverse 2.0.0 --
## v dplyr     1.1.4      v readr     2.1.5
## v forcats   1.0.0      v stringr   1.5.1
## v ggplot2   3.5.1      v tibble    3.2.1
## v lubridate 1.9.3      v tidyr     1.3.1
## v purrr     1.0.2
## -- Conflicts ------------------------------------------- tidyverse_conflicts() --
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()    masks stats::lag()
## i Use the conflicted package (<http://conflicted.r-lib.org/>) to force all conflicts to become errors
```

```r
library(ggplot2)
library(gridExtra)
```

```
##
## Attaching package: 'gridExtra'
##
## The following object is masked from 'package:dplyr':
##
##     combine
```

```r
library(multcompView)
library(car) #upload package with Anova function
```

```
## Loading required package: carData
##
## Attaching package: 'car'
##
## The following object is masked from 'package:dplyr':
##
##     recode
##
## The following object is masked from 'package:purrr':
##
##     some
```

```r
library(cowplot)
```

```
##
## Attaching package: 'cowplot'
##
## The following object is masked from 'package:lubridate':
##
##     stamp
```

```r
#download data in csv format and view it

#download spawn treatment data
spawn <- read.csv("/Users/nicolemongillo/Desktop/GitHub/MVP_Chesapeake_VIMS_hatchery/data/CviMVP_Larvae_
#Examine data
spawn
```

```
##    SpawnTrt_Key    SpawnTrt_Label Tank_naming SpawnTrt_Timestamp
## 1      80D0CCF6         LA-SisLake      MVP-LA  5/9/2023 17:30:00
## 2      B1767257        TX-CapMatBay      MVP-TX  5/9/2023 14:30:00
## 3      334BC443           ME-HogIs      MVP-ME  5/9/2023 17:00:00
## 4      88C0ACD4           MA-PlumIs      MVP-MA  5/9/2023 16:00:00
## 5      7375C067           NH-GrtBay      MVP-NH  5/9/2023 16:30:00
## 6      0066D592       VA-JR-DeepWatSh      MVP-JR  5/9/2023 13:30:00
## 7      BD4409AE       VA-WV-EastShor      MVP-WV  5/9/2023 14:00:00
## 8      AE5E7C9A          FL-KingPlan      MVP-FL  5/9/2023 15:00:00
## 9      BA103554               LOLA    MVP-LOLA  5/9/2023 12:00:00
## 10     F30C9334               DEBY    MVP-DEBY  5/9/2023 13:00:00
## 11     F86F3D8B      Poly-WithOutcross  MVP-LARMIX  5/9/2023 18:00:00
## 12     A45B9C36      Poly-NoOutcross MVP-SEEDMIX
##                                    Egg_Label
## 1        Cvi-MVP-E_Eggs_LA_05-09-2023_formalin
## 2     Cvi-MVP-E_Eggs-CB_TX_05-09-2023_formalin
## 3        Cvi-MVP-E_Eggs_ME_05-09-2023_formalin
## 4        Cvi-MVP-E_Eggs_MA_05-09-2023_formalin
## 5                              no_eggs_sampled
## 6        Cvi-MVP-E_Eggs_JR_05-09-2023_formalin
## 7        Cvi-MVP-E_Eggs_WV_05-09-2023_formalin
## 8        Cvi-MVP-E_Eggs_FL_05-09-2023_formalin
## 9      Cvi-MVP-E_Eggs_LOLA_05-09-2023_formalin
## 10     Cvi-MVP-E_Eggs_DEBY_05-09-2023_formalin
## 11   Cvi-MVP-E_Eggs_LARMIX_05-09-2023_formalin
## 12
```

```r
summary(spawn)
```

```
##   SpawnTrt_Key       SpawnTrt_Label      Tank_naming        SpawnTrt_Timestamp
##  Length:12          Length:12          Length:12          Length:12
##  Class :character   Class :character   Class :character   Class :character
##  Mode  :character   Mode  :character   Mode  :character   Mode  :character
##   Egg_Label
##  Length:12
##  Class :character
##  Mode  :character
```

```r
#download group data
group <- read.csv("/Users/nicolemongillo/Desktop/GitHub/MVP_Chesapeake_VIMS_hatchery/data/CviMVP_Larvae_
summary(group)
```

```
##  SpawnTrt_Key      Tank_naming        Group_Day_Key      Group_Day_Label
##  Length:120        Length:120         Length:120         Length:120
##  Class :character  Class :character   Class :character   Class :character
##  Mode  :character  Mode  :character   Mode  :character   Mode  :character
##
##
##
##
##      Tank          Day_Treatment      Day_analysis            Day
##  Length:120        Length:120         Length:120         Min.   : 0.00
##  Class :character  Class :character   Class :character   1st Qu.: 0.00
##  Mode  :character  Mode  :character   Mode  :character   Median : 6.00
##                                                          Mean   :15.31
##                                                          3rd Qu.:19.00
##                                                          Max.   :78.00
##                                                          NA's   :9
##      Date          Filter_size_µm     Sample_count       Group_count
##  Length:120        Min.   : 35.00     Min.   :     0     Min.   :        0
##  Class :character  1st Qu.: 35.00     1st Qu.: 10000     1st Qu.:  287000
##  Mode  :character  Median : 48.00     Median : 30000     Median :  602000
##                    Mean   : 85.17     Mean   :100309     Mean   : 2726089
##                    3rd Qu.: 75.00     3rd Qu.:161250     3rd Qu.: 2912500
##                    Max.   :212.00     Max.   :600000     Max.   :15000000
##                    NA's   :38         NA's   :52         NA's   :30
##  Survival_rate_perc Vial_label        Notes
##  Min.   : 0.000     Mode:logical   Length:120
##  1st Qu.: 1.706     NA's:120       Class :character
##  Median : 5.346                    Mode  :character
##  Mean   : 7.880
##  3rd Qu.:11.250
##  Max.   :50.000
##  NA's   :52
```

```r
#download larvae image length data
larvae_img <- read.csv("/Users/nicolemongillo/Desktop/GitHub/MVP_Chesapeake_VIMS_hatchery/data/CviMVP_La
summary(larvae_img)
```

```
##   Image_Key         Image_Label          Image_num       Num_Larvae_in_img
##  Length:7217        Length:7217        Min.   :  0.00     Min.   : 1.000
##  Class :character   Class :character   1st Qu.: 28.00     1st Qu.: 3.000
##  Mode  :character   Mode  :character   Median : 60.00     Median : 7.000
##                                        Mean   : 80.27     Mean   : 9.643
##                                        3rd Qu.:109.00     3rd Qu.:14.000
##                                        Max.   :362.00     Max.   :43.000
##                                        NA's   :3192       NA's   :1075
##     Ind_ID         Hinge_Length_µm    Shell_Length_µm    Group_Day_Key
##  Length:7217        Min.   :  30.28    Min.   :  26.59    Length:7217
##  Class :character   1st Qu.:  46.95    1st Qu.:  67.67    Class :character
##  Mode  :character   Median :  49.90    Median : 127.00    Mode  :character
```

```
##                       Mean   :  66.60   Mean   : 2419.62
##                       3rd Qu.:  53.08   3rd Qu.: 1138.00
##                       Max.   :52789.00  Max.   :28295.40
##                       NA's   :3992
##  Group_Day_Label    Img_Notes
##  Length:7217       Length:7217
##  Class :character   Class :character
##  Mode  :character   Mode  :character
##
##
##
##
```

```r
#make mega sheet with all length data

#join image data to group data in new dataframe

len_df <-group %>%
        left_join(larvae_img, by = "Group_Day_Key")

#remove some columns
len_analysis_df <- subset(len_df, select = -c(Ind_ID, Group_Day_Label.y, Image_Key, Img_Notes,SpawnTrt_

#subset late stage oyster data (days19-78)
latestage_df <- subset(len_analysis_df, Day_analysis == "49"| Day_analysis == "78"| Day_analysis == "15-

#a sample marked WV is erroneously in this data set- remove
latestage_df <- subset(latestage_df, Image_Label != "WV_06_200L_6")

#rename cols
colnames(latestage_df) <- c("group", "group_day_key", "group_day_label", "day_trt", "day_an", "day", "da

latestage_df$shell_length_mm <- (latestage_df$shell_length_um)/1000

head(latestage_df)
```

```
##       group group_day_key group_day_label day_trt day_an day       date
## 378 MVP-LA      7E8E995C      LA_19_Eyed 19_Eyed  15-21  19 2023-05-28
## 379 MVP-LA      7E8E995C      LA_19_Eyed 19_Eyed  15-21  19 2023-05-28
## 380 MVP-LA      7E8E995C      LA_19_Eyed 19_Eyed  15-21  19 2023-05-28
## 381 MVP-LA      7E8E995C      LA_19_Eyed 19_Eyed  15-21  19 2023-05-28
## 382 MVP-LA      7E8E995C      LA_19_Eyed 19_Eyed  15-21  19 2023-05-28
## 383 MVP-LA      7E8E995C      LA_19_Eyed 19_Eyed  15-21  19 2023-05-28
##        image_label shell_length_um shell_length_mm
## 378 TX_21_small_21        167.430        0.167430
## 379   LA_19_eyed_1        316.357        0.316357
## 380   LA_19_eyed_2        319.598        0.319598
## 381   LA_19_eyed_3        332.948        0.332948
## 382   LA_19_eyed_4        324.306        0.324306
## 383   LA_19_eyed_5        332.164        0.332164
```

```
#preliminary exploration of data
summary(latestage_df)
```

```
##     group          group_day_key      group_day_label        day_trt
##  Length:3191       Length:3191         Length:3191          Length:3191
##  Class :character  Class :character    Class :character     Class :character
##  Mode  :character  Mode  :character    Mode  :character     Mode  :character
##
##
##
##     day_an              day              date              image_label
##  Length:3191       Min.   :19.00     Length:3191          Length:3191
##  Class :character  1st Qu.:19.00     Class :character     Class :character
##  Mode  :character  Median :49.00     Mode  :character     Mode  :character
##                    Mean   :45.99
##                    3rd Qu.:78.00
##                    Max.   :78.00
##  shell_length_um   shell_length_mm
##  Min.   :  116.3   Min.   : 0.1163
##  1st Qu.:  301.2   1st Qu.: 0.3012
##  Median : 2916.5   Median : 2.9165
##  Mean   : 5370.4   Mean   : 5.3704
##  3rd Qu.:10453.2   3rd Qu.:10.4532
##  Max.   :28295.4   Max.   :28.2954
```

```
#prep data for ANOVA

#use grep function to make a vector of all indices in latestage_len with the phrase "small" in them, ig
small <- grep("small", latestage_df$group_day_label, ignore.case = TRUE)

small_img <- grep("small", latestage_df$image_label, ignore.case = TRUE)

#make new dataframe WITHOUT any indices saved in small, mean remaining data are everything except small
no_small_df <- latestage_df[-small, ]
no_small_df <- latestage_df[-small_img, ]

#rename rows
colnames(no_small_df) <- c("group", "group_day_key", "group_day_label", "day_trt", "day_an", "day", "da

#exclude "MVP" from group names to shorten them
no_small_df["group"][no_small_df["group"] == "MVP-DEBY"] <- "DEBY"
no_small_df["group"][no_small_df["group"] == "MVP-FL"] <- "FL"
no_small_df["group"][no_small_df["group"] == "MVP-JR"] <- "JR"
no_small_df["group"][no_small_df["group"] == "MVP-LA"] <- "LA"
no_small_df["group"][no_small_df["group"] == "MVP-LARMIX"] <- "LARMIX"
no_small_df["group"][no_small_df["group"] == "MVP-LOLA"] <- "LOLA"
no_small_df["group"][no_small_df["group"] == "MVP-ME"] <- "ME"
no_small_df["group"][no_small_df["group"] == "MVP-NH"] <- "NH"
no_small_df["group"][no_small_df["group"] == "MVP-TX"] <- "TX"

no_small_df <- no_small_df %>%
  mutate(group = group %>%
         fct_relevel("DEBY", "LOLA", "JR", "TX", "LA", "FL", "NH", "ME", "LARMIX"))
```

```
#save latestage_df as a csv file for future analyses
write.csv(no_small_df, "/Users/nicolemongillo/Desktop/GitHub/MVP_Chesapeake_VIMS_hatchery/data/latestage

#calculate mean oyster length per group per day using aggregate

#make standard error equation
se <- function(x){
  sd(x)/sqrt(length(x))
}

#find mean lengths of each group on each day
means_mm <- aggregate(shell_length_mm ~ group + day_an, FUN = mean, data = no_small_df)
colnames(means_mm) <- c("group", "days", "mean_length_mm")

#find standard error of length of each group on each day
se_mm <- aggregate(shell_length_mm ~ group + day_an, FUN = se, data = no_small_df)
colnames(se_mm) <- c("group", "days", "se_length_mm")


#Check if late stage oyster data (day 19 onward, small oysters excluded) match assumptions of ANOVA

len.lm <- lm(shell_length_mm ~ day_an + group, data = no_small_df) #fit to lm

len.lm.resid <- len.lm$residuals #save residuals

shapiro.test(len.lm.resid) #use Shapiro Wilk test to assess normality
```

```
##
##  Shapiro-Wilk normality test
##
## data:  len.lm.resid
## W = 0.95616, p-value < 2.2e-16
```

```
#plots to assess if data meet assumptions of ANOVA
par(mfrow = c(1,1))

#plot(len.lm)
#normality is a bit off, definite funnel in homoscedasticity plots, but KEL said to expect that what wi

#log10 transformation lm
loglen.lm <- lm(log(shell_length_mm) ~ day_an + group, data = no_small_df)
loglen.lm.resid <- loglen.lm$residuals

#plot(loglen.lm)
# I'm planning to use the log10 transformed data for the analysis, transformations helped get rid of so

#run ANOVA on log transformed length data
no_small_df$log10len <- log10(no_small_df$shell_length_mm)

len.anova3 <- Anova(lm(log10len ~ day_an + group + day_an:group, data = no_small_df), type = 3)

len.anova3
```

```
## Anova Table (Type III tests)
##
## Response: log10len
##                Sum Sq   Df   F value  Pr(>F)
## (Intercept)    23.625    1 1686.2240 <2e-16 ***
## day_an        146.720    2 5236.1278 <2e-16 ***
## group           0.106    8    0.9429 0.4795
## day_an:group    4.039   16   18.0175 <2e-16 ***
## Residuals      34.858 2488
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
summary(len.anova3)
```

```
##      Sum Sq                Df            F value            Pr(>F)
## Min.   :  0.1057   Min.   :   1   Min.   :   0.943   Min.   :0.0000
## 1st Qu.:  4.0389   1st Qu.:   2   1st Qu.:  13.749   1st Qu.:0.0000
## Median : 23.6245   Median :   8   Median : 852.121   Median :0.0000
## Mean   : 41.8693   Mean   : 503   Mean   :1735.328   Mean   :0.1199
## 3rd Qu.: 34.8577   3rd Qu.:  16   3rd Qu.:2573.700   3rd Qu.:0.1199
## Max.   :146.7197   Max.   :2488   Max.   :5236.128   Max.   :0.4795
##                                   NA's   :1          NA's   :1
```

```r
all_pval <- len.anova3$`Pr(>F)`

all_pval <- na.omit(all_pval)

#significant interactive effect of group and age (p < 2e-16), but no significant effect of group on its

#convert group and day from chr to factor
no_small_df$group <- as.factor(no_small_df$group)
no_small_df$day_an <- as.factor(no_small_df$day_an)
str(no_small_df)
```

```
## 'data.frame':    2515 obs. of  11 variables:
##  $ group          : Factor w/ 9 levels "DEBY","LOLA",..: 5 5 5 5 5 5 5 5 5 5 ...
##  $ group_day_key  : chr  "7E8E995C" "7E8E995C" "7E8E995C" "7E8E995C" ...
##  $ group_day_label: chr  "LA_19_Eyed" "LA_19_Eyed" "LA_19_Eyed" "LA_19_Eyed" ...
##  $ day_trt        : chr  "19_Eyed" "19_Eyed" "19_Eyed" "19_Eyed" ...
##  $ day_an         : Factor w/ 3 levels "15-21","49","78": 1 1 1 1 1 1 1 1 1 1 ...
##  $ day            : int  19 19 19 19 19 19 19 19 19 19 ...
##  $ date           : chr  "2023-05-28" "2023-05-28" "2023-05-28" "2023-05-28" ...
##  $ image_label    : chr  "LA_19_eyed_1" "LA_19_eyed_2" "LA_19_eyed_3" "LA_19_eyed_4" ...
##  $ shell_length_um: num  316 320 333 324 332 ...
##  $ shell_length_mm: num  0.316 0.32 0.333 0.324 0.332 ...
##  $ log10len       : num  -0.5 -0.495 -0.478 -0.489 -0.479 ...
```

```r
#subsetting based on oyster age (days)
eyed <- subset(no_small_df, day_an == "15-21")
seed <- subset(no_small_df, day_an == "49")
predep <- subset(no_small_df, day_an == "78")
```

```r
#make barplots of shell length vs group for each age/day

#barplot of shell length distributions pooled across all days
bp_all <- boxplot(no_small_df$shell_length_mm ~ no_small_df$group,
        main = "Shell Length Distribution In Each Group Days 19-78",
        xlab = "Group Label",
        ylab = "Shell Length mm")
```

Shell Length Distribution In Each Group Days 19–78
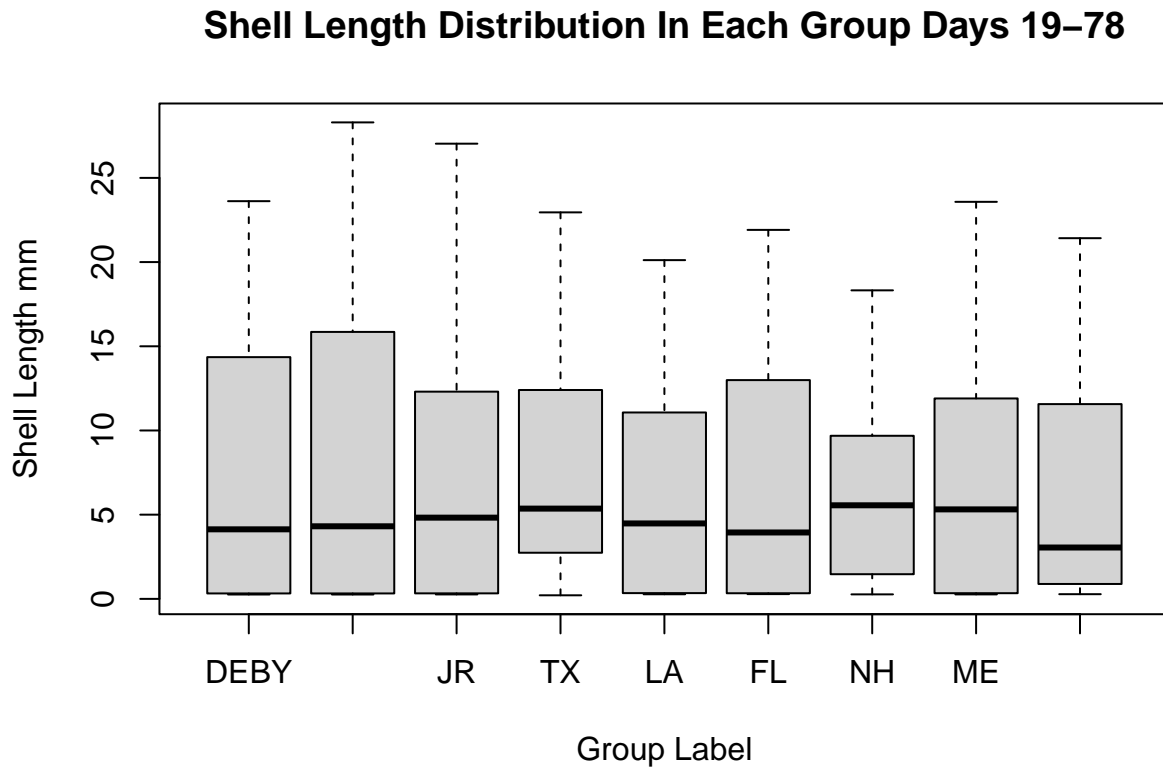

```r
#seed larvae shell length vs group
bp_seed <- boxplot(seed$shell_length_mm ~ seed$group,
        main = "Seed Larvae (Day 49) Shell Length",
        xlab = "Group Label",
        ylab = "Shell Length (mm)",
        ylim = c(0, 10))
```

Seed Larvae (Day 49) Shell Length
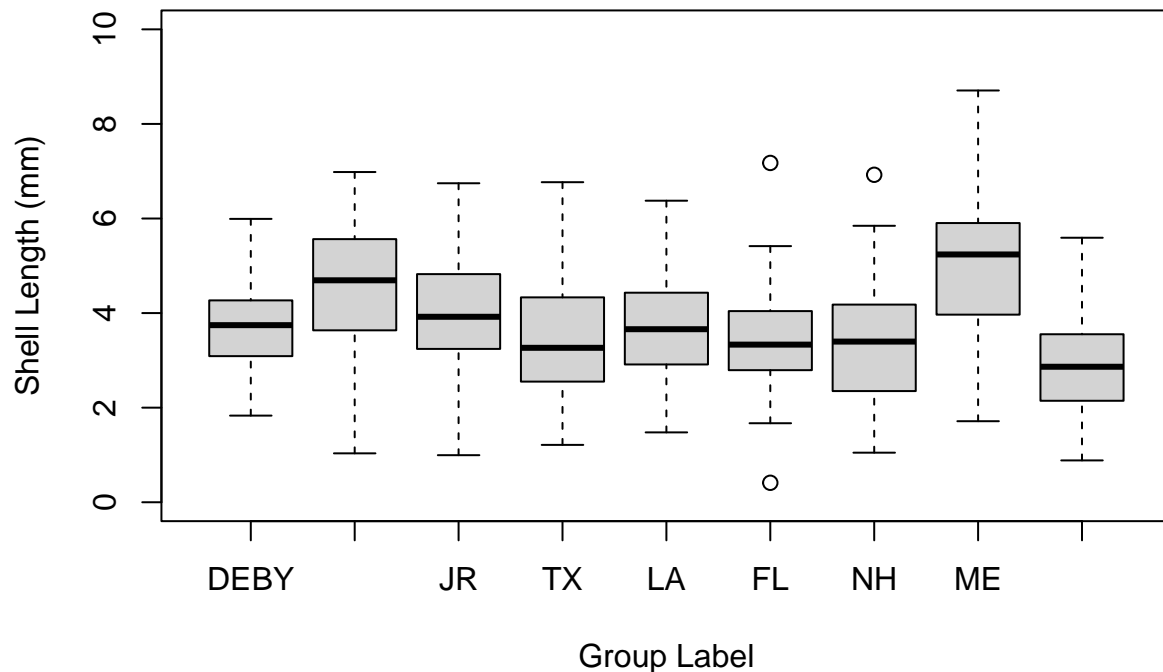
#Eyed ANOVA and Post-Hoc Comparison

```
#I want to run ANOVAs comparing shell length to group for each day separately

#ANOVA for eyed shell lengths
eyed$group <- as.factor(eyed$group)

eyed.lm <- lm(shell_length_mm ~ group, data = eyed) #fit to lm

eyed.lm.resid <- eyed.lm$residuals #save residuals

shapiro.test(eyed.lm.resid) #use Shapiro Wilk test to assess normality, p < 1.78 e-09,so not normal
```

```
##
##   Shapiro-Wilk normality test
##
## data:  eyed.lm.resid
## W = 0.97532, p-value = 1.789e-09
```

```
#plots to assess if data meet assumptions of ANOVA

#plot(eyed.lm)
#normality is a bit off, check log10 transformed lengths

#log10 transformation lm
logeyed.lm <- lm(log(shell_length_mm) ~ group, data = eyed)
```

9

```r
logeyed.lm.resid <- logeyed.lm$residuals


#plot(logeyed.lm)

#no real change in normality between transformed and non-transformed data, no outliers and homoscedasti


#run ANOVA on non transformed length data
eyed.anova3 <- Anova(lm(shell_length_mm ~ group, data = eyed), type = 3)
eyed.anova3 #p <2e-16, same as with other aov function
```

```
## Anova Table (Type III tests)
##
## Response: shell_length_mm
##              Sum Sq  Df   F value    Pr(>F)
## (Intercept) 8.7286    1 35862.955 < 2.2e-16 ***
## group       0.0548    8    28.139 < 2.2e-16 ***
## Residuals   0.1679  690
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
eyed.anova <- aov(lm(shell_length_mm ~ group, data = eyed))
eyed_anova_result <- as.matrix(summary(eyed.anova)) #significant p-value, p <2e-16

eyed_pval <- summary(eyed.anova)[[1]][["Pr(>F)"]][1]
print(eyed_pval)
```
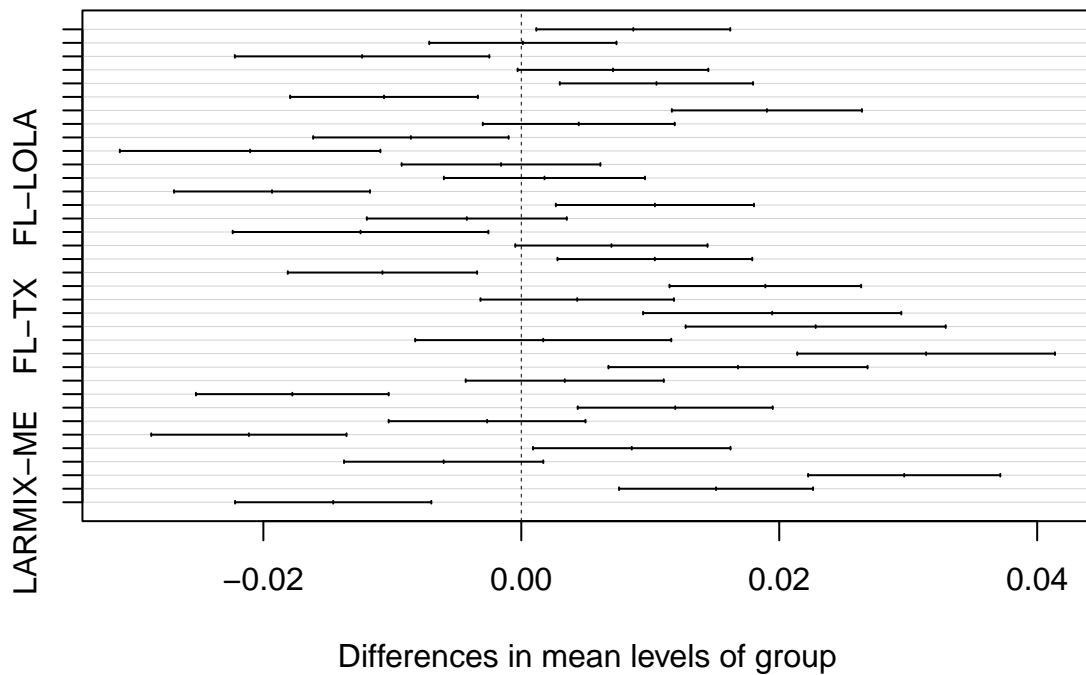
```
## [1] 5.270139e-38
```

```r
#post-hoc comparison- Tukey-Kramer test
eyed.tuk <- TukeyHSD(x=eyed.anova,conf.level = 0.95)

par(mfrow = c(1,1))

plot(eyed.tuk)
```

95% family–wise confidence level

Differences in mean levels of group

```
require(multcompView)

eyed_cld <- multcompLetters4(eyed.anova, eyed.tuk)

eyed_dt <- group_by(eyed, group) %>%
  summarise(m=mean(shell_length_mm)) %>%
  arrange(desc(m))

eyed_cld <- as.data.frame.list(eyed_cld$group)
eyed_dt$eyed_cld <- eyed_cld$Letters
```

##ANOVA for pre-deployment (day 78) shell lengths only

```
#Linera model and ANOVA for predep shell lengths
predep$group <- as.factor(predep$group)

predep.lm <- lm(shell_length_mm ~ group, data = predep) #fit to lm

predep.lm.resid <- predep.lm$residuals #save residuals

shapiro.test(predep.lm.resid) #use Shapiro Wilk test to assess normality
```

```
##
##  Shapiro-Wilk normality test
##
```

```
## data:  predep.lm.resid
## W = 0.9974, p-value = 0.08176

# p = 0.08176, fail to reject H0, data are normal

#plots to assess if data meet assumptions of 2-way ANOVA

#plot(predep.lm)

#this data looks good, normal, homoscedastic, no outliers

#I should use Anova function, but Tukey Kramer test won't run on an Anova object, only aov. Both functi
predep.anova3 <- Anova(lm(shell_length_mm ~ group, data = predep))
predep.anova3

## Anova Table (Type II tests)
##
## Response: shell_length_mm
##             Sum Sq   Df F value    Pr(>F)
## group        4509.8    8  51.029 < 2.2e-16 ***
## Residuals 11765.3 1065
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

predep.anova <- aov(lm(shell_length_mm ~ group, data = predep))
summary(predep.anova)

##              Df Sum Sq Mean Sq F value Pr(>F)
## group          8   4510    563.7   51.03 <2e-16 ***
## Residuals   1065  11765     11.0
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

#extract p-value
predep_pval <- summary(predep.anova)[[1]][["Pr(>F)"]][1]
print(predep_pval)

## [1] 4.998853e-70

#post-hoc comparison- Tukey-Kramer test
predep.tuk <- TukeyHSD(x=predep.anova,conf.level = 0.95)

#extract significant letters from TK test and store
require(multcompView)

predep_cld <- multcompLetters4(predep.anova, predep.tuk)

predep_dt <- group_by(predep, group) %>%
  summarise(m=mean(shell_length_mm)) %>%
  arrange(desc(m))

predep_cld <- as.data.frame.list(predep_cld$group)
```

```
predep_dt$predep_cld <- predep_cld$Letters

#make shell length numeric in predep
predep$shell_length_mm <- as.numeric(predep$shell_length_mm)
```

#P Adjust

```
#adjust p-value to correct for multiple tests. Make vector of p-values from each ANOVA
p_values <- c(all_pval, eyed_pval, predep_pval)

p_values
```

```
## [1] 6.953098e-282  0.000000e+00  4.795296e-01  7.526768e-49  5.270139e-38
## [6]  4.998853e-70
```

```
p_adj <- p.adjust(p_values, method = "BH")

p_adj
```

```
## [1] 2.085929e-281  0.000000e+00  4.795296e-01  1.129015e-48  6.324167e-38
## [6]  9.997706e-70
```

```
eyed_box <- ggplot(data = eyed, aes(group, shell_length_mm))+
  geom_boxplot(size = .2)+
  stat_summary(fun.data = mean_se, geom = "errorbar", width = 0.2, linewidth = .2, color = "red")+
  ggtitle("Oyster Shell Lengths at Days 15-21 and Day 78 Across Broodstock Groups")+
  ylab("Days 15-21 Shell Length (mm)")+
  xlab("Broodstock Group")+
  ylim(c(0.2,.4))+
  geom_text(data = eyed_dt, size = 3, aes(label = eyed_cld, y = .35), vjust = -0.5)+
  labs(subtitle = "A: p < 0.001")+
  theme(axis.text.x = element_blank(), axis.ticks.x = element_blank(), title = element_text(size = 9),

eyed_box
```
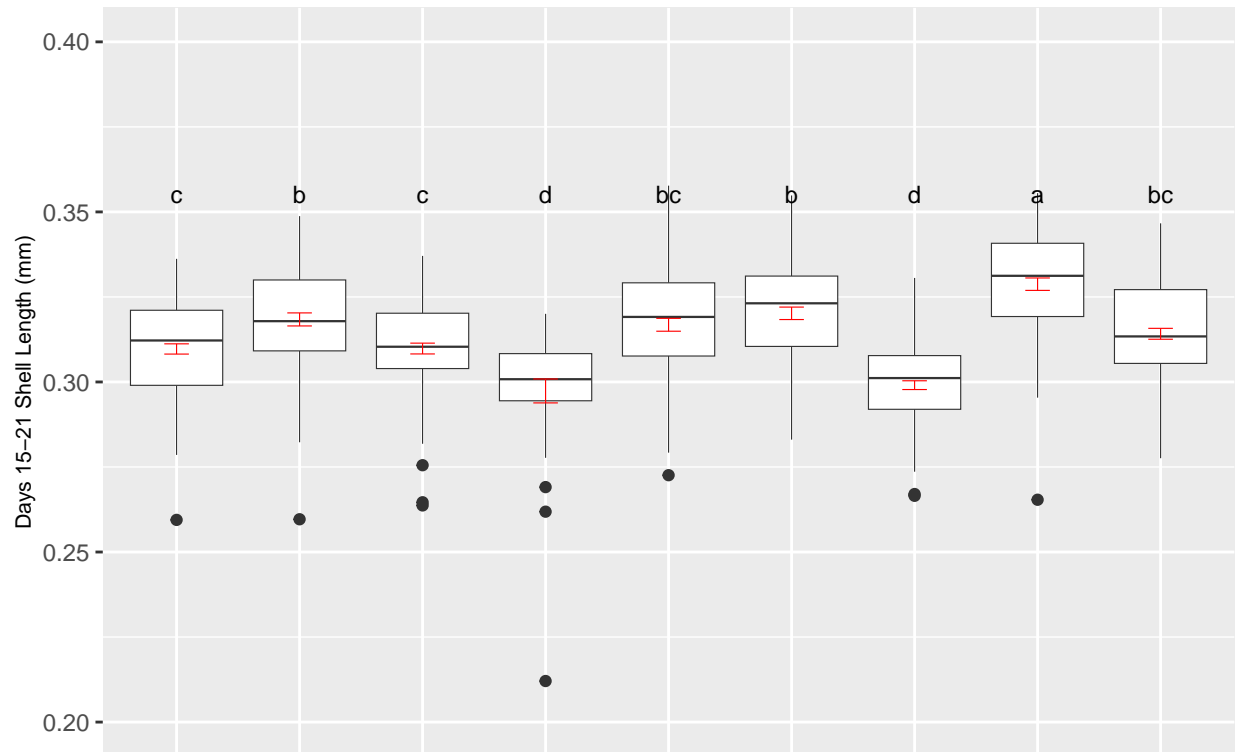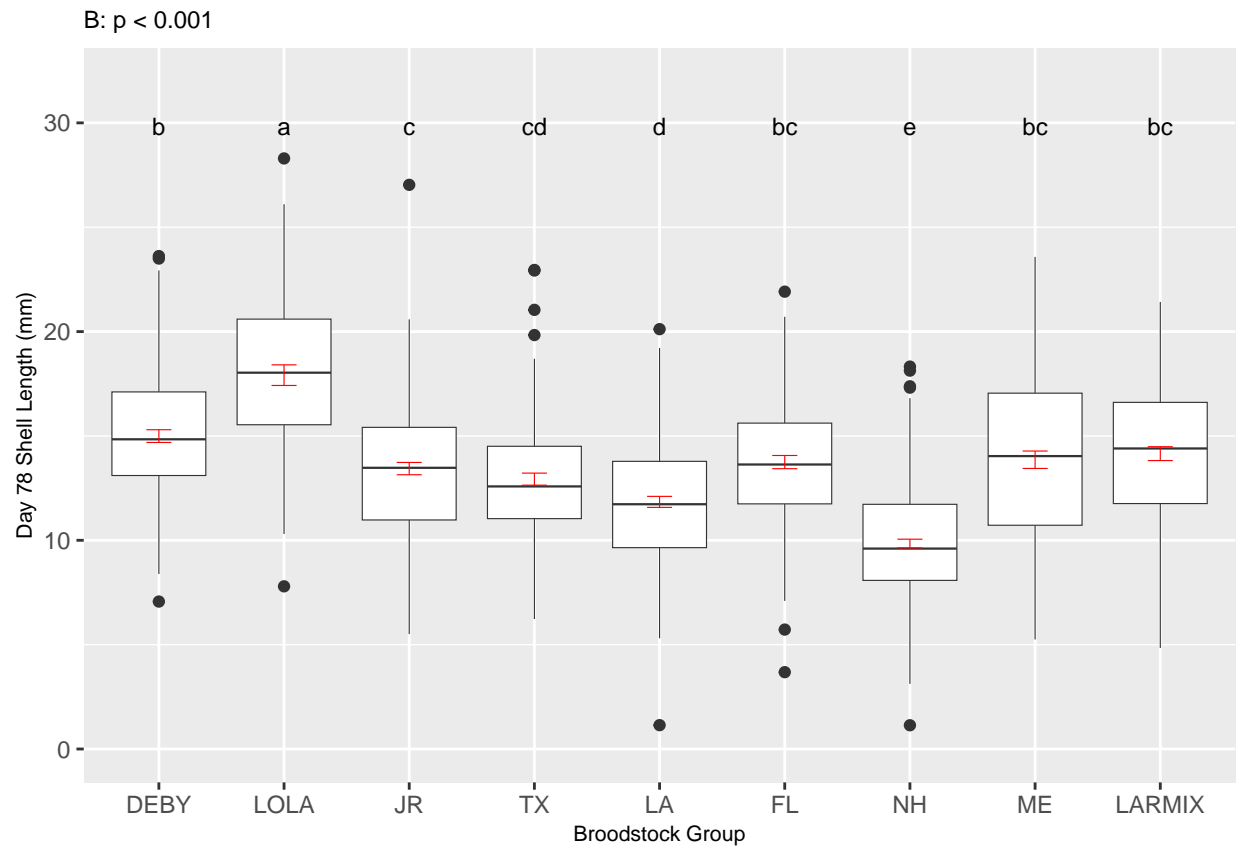
Oyster Shell Lengths at Days 15–21 and Day 78 Across Broodstock Groups
A: p < 0.001


```
#plot
predep_box <- ggplot(data = predep, aes(group, shell_length_mm))+
  geom_boxplot(size = .2)+
  stat_summary(fun.data = mean_se, geom = "errorbar", width = 0.2, linewidth = .2, color = "red")+
  ylab("Day 78 Shell Length (mm)")+
  xlab("Broodstock Group")+
  labs(subtitle = "B: p < 0.001")+
  ylim(c(0,32))+
  geom_text(data = predep_dt, size = 3, aes(label = predep_cld, y = 29, x = group), vjust = -0.5)+
  theme(plot.title = element_blank(), plot.subtitle = element_text(size = 9), axis.title.y = element_te

predep_box
```
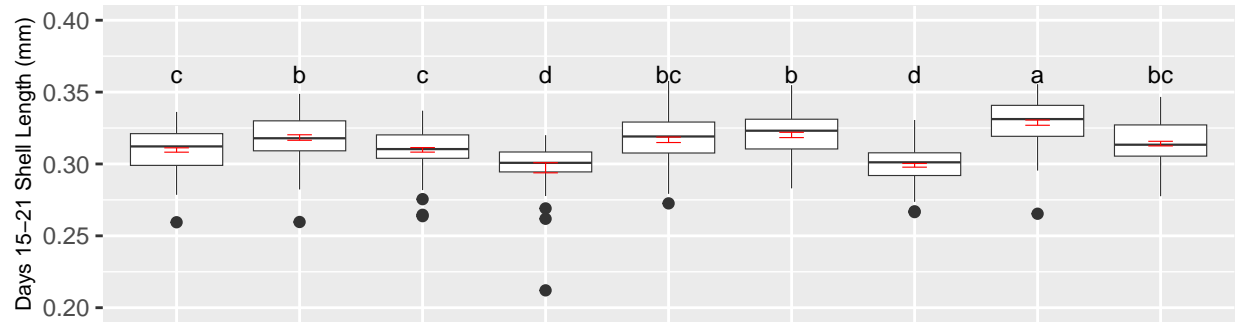
```
plot_grid(eyed_box, predep_box, ncol = 1, align = "v")
```

Oyster Shell Lengths at Days 15–21 and Day 78 Across Broodstock Groups