Relative Brain Shape

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## R Markdown

This RMarkdown will contain start to finish analysis of Relative Brain Shape information compiled from the Emerson 1 Microbial Experiment (Aim 2). It is updated to be more streamlined and easier to knit the output and be placed into my data notebook. Note: This is updated for Manuscript Writing and all analysis was done in RStudio

**Relative Brain Shape**

library(lme4)

## Warning: package 'lme4' was built under R version 4.1.3

## Loading required package: Matrix

## Warning: package 'Matrix' was built under R version 4.1.3

library(car)

## Warning: package 'car' was built under R version 4.1.3

## Loading required package: carData

## Warning: package 'carData' was built under R version 4.1.3

library(readr)

## Warning: package 'readr' was built under R version 4.1.3

library(moments)

## Warning: package 'moments' was built under R version 4.1.3

library(psych)

## Warning: package 'psych' was built under R version 4.1.3

##   
## Attaching package: 'psych'

## The following object is masked from 'package:car':  
##   
## logit

library(pastecs)  
library(ggplot2)

## Warning: package 'ggplot2' was built under R version 4.1.3

##   
## Attaching package: 'ggplot2'

## The following objects are masked from 'package:psych':  
##   
## %+%, alpha

library(ggbiplot)

## Loading required package: plyr

## Warning: package 'plyr' was built under R version 4.1.3

## Loading required package: scales

## Warning: package 'scales' was built under R version 4.1.3

##   
## Attaching package: 'scales'

## The following objects are masked from 'package:psych':  
##   
## alpha, rescale

## The following object is masked from 'package:readr':  
##   
## col\_factor

## Loading required package: grid

library(tidyverse)

## -- Attaching packages --------------------------------------- tidyverse 1.3.1 --

## v tibble 3.1.7 v dplyr 1.0.9  
## v tidyr 1.2.0 v stringr 1.4.0  
## v purrr 0.3.4 v forcats 0.5.1

## Warning: package 'tibble' was built under R version 4.1.3

## Warning: package 'tidyr' was built under R version 4.1.3

## Warning: package 'dplyr' was built under R version 4.1.3

## -- Conflicts ------------------------------------------ tidyverse\_conflicts() --  
## x ggplot2::%+%() masks psych::%+%()  
## x scales::alpha() masks ggplot2::alpha(), psych::alpha()  
## x dplyr::arrange() masks plyr::arrange()  
## x scales::col\_factor() masks readr::col\_factor()  
## x purrr::compact() masks plyr::compact()  
## x dplyr::count() masks plyr::count()  
## x purrr::discard() masks scales::discard()  
## x tidyr::expand() masks Matrix::expand()  
## x tidyr::extract() masks pastecs::extract()  
## x dplyr::failwith() masks plyr::failwith()  
## x dplyr::filter() masks stats::filter()  
## x dplyr::first() masks pastecs::first()  
## x dplyr::id() masks plyr::id()  
## x dplyr::lag() masks stats::lag()  
## x dplyr::last() masks pastecs::last()  
## x dplyr::mutate() masks plyr::mutate()  
## x tidyr::pack() masks Matrix::pack()  
## x dplyr::recode() masks car::recode()  
## x dplyr::rename() masks plyr::rename()  
## x purrr::some() masks car::some()  
## x dplyr::summarise() masks plyr::summarise()  
## x dplyr::summarize() masks plyr::summarize()  
## x tidyr::unpack() masks Matrix::unpack()

library(modelbased)

## Warning: package 'modelbased' was built under R version 4.1.3

library(dplyr)  
  
file.choose()

## [1] "C:\\R\\Emerson-Microbial-1\\Relative Brain Shape.docx"

df <-read.csv("C:\\Users\\kjeme\\OneDrive\\Desktop\\Woodley Lab\\Aim 2 - Emerson Microbial 1\\Emersion Microbial Experiment 1 (2021)\\Emerson Microbial Exp1\_Batch1.csv")  
df$Microbial\_Trtmt = factor(df$Microbial\_Trtmt)  
df$Replicate = factor(df$Replicate)  
  
df$Log\_DL = as.numeric(df$Log\_DL)  
df$Log\_TW = as.numeric(df$Log\_TW)  
df$Log\_TL = as.numeric(df$Log\_TL)  
df$Log\_OTW = as.numeric(df$Log\_OTW)  
df$Log\_OTL = as.numeric(df$Log\_OTL)  
df$Log\_DW = as.numeric(df$Log\_DW)  
df$Log\_MW = as.numeric(df$Log\_MW)  
  
shapiro.test(df$Log\_DL)

##   
## Shapiro-Wilk normality test  
##   
## data: df$Log\_DL  
## W = 0.94309, p-value = 0.09165

shapiro.test(df$Log\_TW)

##   
## Shapiro-Wilk normality test  
##   
## data: df$Log\_TW  
## W = 0.97037, p-value = 0.5098

shapiro.test(df$Log\_TL)

##   
## Shapiro-Wilk normality test  
##   
## data: df$Log\_TL  
## W = 0.92215, p-value = 0.02378

Does not pass.

shapiro.test(df$Log\_OTW)

##   
## Shapiro-Wilk normality test  
##   
## data: df$Log\_OTW  
## W = 0.97102, p-value = 0.528

shapiro.test(df$Log\_OTL)

##   
## Shapiro-Wilk normality test  
##   
## data: df$Log\_OTL  
## W = 0.95226, p-value = 0.1671

shapiro.test(df$Log\_DW)

##   
## Shapiro-Wilk normality test  
##   
## data: df$Log\_DW  
## W = 0.97902, p-value = 0.7705

shapiro.test(df$Log\_MW)

##   
## Shapiro-Wilk normality test  
##   
## data: df$Log\_MW  
## W = 0.96525, p-value = 0.3795

Tests of Normality for all brain dimensions. Log\_TL only one that didnt pass.

leveneTest(df$Log\_DL, df$Microbial\_Trtmt, center = mean, na.rm = TRUE)

## Levene's Test for Homogeneity of Variance (center = mean: TRUE)  
## Df F value Pr(>F)  
## group 1 0.1173 0.7344  
## 30

leveneTest(df$Log\_TW, df$Microbial\_Trtmt, center = mean, na.rm = TRUE)

## Levene's Test for Homogeneity of Variance (center = mean: TRUE)  
## Df F value Pr(>F)   
## group 1 12.739 0.001228 \*\*  
## 30   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

leveneTest(df$Log\_TL, df$Microbial\_Trtmt, center = mean, na.rm = TRUE)

## Levene's Test for Homogeneity of Variance (center = mean: TRUE)  
## Df F value Pr(>F)   
## group 1 12.191 0.00151 \*\*  
## 30   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

leveneTest(df$Log\_OTW, df$Microbial\_Trtmt, center = mean, na.rm = TRUE)

## Levene's Test for Homogeneity of Variance (center = mean: TRUE)  
## Df F value Pr(>F)   
## group 1 5.9459 0.02089 \*  
## 30   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

leveneTest(df$Log\_OTL, df$Microbial\_Trtmt, center = mean, na.rm = TRUE)

## Levene's Test for Homogeneity of Variance (center = mean: TRUE)  
## Df F value Pr(>F)   
## group 1 3.0153 0.09274 .  
## 30   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

leveneTest(df$Log\_DW, df$Microbial\_Trtmt, center = mean, na.rm = TRUE)

## Levene's Test for Homogeneity of Variance (center = mean: TRUE)  
## Df F value Pr(>F)   
## group 1 3.377 0.07604 .  
## 30   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

leveneTest(df$Log\_MW, df$Microbial\_Trtmt, center = mean, na.rm = TRUE)

## Levene's Test for Homogeneity of Variance (center = mean: TRUE)  
## Df F value Pr(>F)   
## group 1 13.08 0.001082 \*\*  
## 30   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Tests homogeneity of variance. TW, TL, MW and OTW did not pass… Decide to continue.

man1 <- manova(cbind(Log\_DL, Log\_DW, Log\_MW, Log\_OTL, Log\_OTW, Log\_TL, Log\_TW)~Microbial\_Trtmt\*Log\_BrainMass, data = df)  
summary(man1)

## Df Pillai approx F num Df den Df Pr(>F)   
## Microbial\_Trtmt 1 0.63911 5.5658 7 22 0.0008652 \*\*\*  
## Log\_BrainMass 1 0.74787 9.3222 7 22 2.365e-05 \*\*\*  
## Microbial\_Trtmt:Log\_BrainMass 1 0.27795 1.2098 7 22 0.3386529   
## Residuals 28   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

MANCOVA to test homogeneity of slopes. We will look at how each specific brain dimension responded to both treatements, while maintaining relative brain mass as a covariate.

summary.aov(man1)

## Response Log\_DL :  
## Df Sum Sq Mean Sq F value Pr(>F)   
## Microbial\_Trtmt 1 0.0094983 0.0094983 12.6410 0.001364 \*\*  
## Log\_BrainMass 1 0.0068908 0.0068908 9.1707 0.005237 \*\*  
## Microbial\_Trtmt:Log\_BrainMass 1 0.0002577 0.0002577 0.3430 0.562818   
## Residuals 28 0.0210388 0.0007514   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Response Log\_DW :  
## Df Sum Sq Mean Sq F value Pr(>F)   
## Microbial\_Trtmt 1 0.0022505 0.0022505 7.8116 0.009268 \*\*   
## Log\_BrainMass 1 0.0106412 0.0106412 36.9361 1.486e-06 \*\*\*  
## Microbial\_Trtmt:Log\_BrainMass 1 0.0000273 0.0000273 0.0949 0.760295   
## Residuals 28 0.0080667 0.0002881   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Response Log\_MW :  
## Df Sum Sq Mean Sq F value Pr(>F)   
## Microbial\_Trtmt 1 0.0020123 0.0020123 2.7933 0.105803   
## Log\_BrainMass 1 0.0296594 0.0296594 41.1690 6.015e-07 \*\*\*  
## Microbial\_Trtmt:Log\_BrainMass 1 0.0055675 0.0055675 7.7280 0.009608 \*\*   
## Residuals 28 0.0201720 0.0007204   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Response Log\_OTL :  
## Df Sum Sq Mean Sq F value Pr(>F)   
## Microbial\_Trtmt 1 0.0065502 0.0065502 21.0544 8.537e-05 \*\*\*  
## Log\_BrainMass 1 0.0103948 0.0103948 33.4120 3.311e-06 \*\*\*  
## Microbial\_Trtmt:Log\_BrainMass 1 0.0000847 0.0000847 0.2724 0.6059   
## Residuals 28 0.0087110 0.0003111   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Response Log\_OTW :  
## Df Sum Sq Mean Sq F value Pr(>F)   
## Microbial\_Trtmt 1 0.0070074 0.0070074 29.7880 7.946e-06 \*\*\*  
## Log\_BrainMass 1 0.0115715 0.0115715 49.1897 1.255e-07 \*\*\*  
## Microbial\_Trtmt:Log\_BrainMass 1 0.0000436 0.0000436 0.1853 0.6702   
## Residuals 28 0.0065868 0.0002352   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Response Log\_TL :  
## Df Sum Sq Mean Sq F value Pr(>F)   
## Microbial\_Trtmt 1 0.0041960 0.0041960 9.1482 0.005285 \*\*   
## Log\_BrainMass 1 0.0117082 0.0117082 25.5267 2.403e-05 \*\*\*  
## Microbial\_Trtmt:Log\_BrainMass 1 0.0007511 0.0007511 1.6377 0.211147   
## Residuals 28 0.0128427 0.0004587   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Response Log\_TW :  
## Df Sum Sq Mean Sq F value Pr(>F)   
## Microbial\_Trtmt 1 0.0025663 0.0025663 9.8949 0.003906 \*\*   
## Log\_BrainMass 1 0.0096835 0.0096835 37.3369 1.361e-06 \*\*\*  
## Microbial\_Trtmt:Log\_BrainMass 1 0.0000069 0.0000069 0.0268 0.871252   
## Residuals 28 0.0072620 0.0002594   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## 40 observations deleted due to missingness

Medulla Width had a significant interaction with the treatment and brain mass. This signifies the slopes of the line for these traits were not parallel amongst treatments. We will continue on and address this later. Now we will get our EMMs.

modelTW <- lm(Log\_TW ~ Microbial\_Trtmt\*Log\_BrainMass, data = df)  
means\_complexTW <- estimate\_means(modelTW)

## We selected `at = c("Microbial\_Trtmt")`.

## NOTE: Results may be misleading due to involvement in interactions

means\_complexTW$Mean

## [1] 0.2840861 0.2789279

EMMs for TW across treatments.

modelTL <- lm(Log\_TL ~ Microbial\_Trtmt\*Log\_BrainMass, data = df)  
means\_complexTL <- estimate\_means(modelTL)

## We selected `at = c("Microbial\_Trtmt")`.

## NOTE: Results may be misleading due to involvement in interactions

means\_complexTL$Mean

## [1] 0.2705873 0.2664782

EMMs for TL across treatments.

modelDW <- lm(Log\_DW ~ Microbial\_Trtmt\*Log\_BrainMass, data = df)  
means\_complexDW <- estimate\_means(modelDW)

## We selected `at = c("Microbial\_Trtmt")`.

## NOTE: Results may be misleading due to involvement in interactions

means\_complexDW$Mean

## [1] 0.2322600 0.2244235

EMMs for DW across treatments.

modelDL <- lm(Log\_DL ~ Microbial\_Trtmt\*Log\_BrainMass, data = df)  
means\_complexDL <- estimate\_means(modelDL)

## We selected `at = c("Microbial\_Trtmt")`.

## NOTE: Results may be misleading due to involvement in interactions

means\_complexDL$Mean

## [1] 0.2795277 0.2981876

EMMs for DL across treatments.

modelOTW <- lm(Log\_OTW ~ Microbial\_Trtmt\*Log\_BrainMass, data = df)  
means\_complexOTW <- estimate\_means(modelOTW)

## We selected `at = c("Microbial\_Trtmt")`.

## NOTE: Results may be misleading due to involvement in interactions

means\_complexOTW$Mean

## [1] 0.4522032 0.4574000

EMMs for OTW across treatments.

modelOTL <- lm(Log\_OTL ~ Microbial\_Trtmt\*Log\_BrainMass, data = df)  
means\_complexOTL <- estimate\_means(modelOTL)

## We selected `at = c("Microbial\_Trtmt")`.

## NOTE: Results may be misleading due to involvement in interactions

means\_complexOTL$Mean

## [1] 0.2819357 0.2887522

EMMs for OTL across treatments.

modelMW <- lm(Log\_MW ~ Microbial\_Trtmt\*Log\_BrainMass, data = df)  
means\_complexMW <- estimate\_means(modelMW)

## We selected `at = c("Microbial\_Trtmt")`.

## NOTE: Results may be misleading due to involvement in interactions

means\_complexMW$Mean

## [1] 0.1789755 0.1470028

EMMs for MW across treatments.

residuals <- man1$residuals  
man1$residuals

## Log\_DL Log\_DW Log\_MW Log\_OTL Log\_OTW  
## 1 0.026514403 -0.010263374 -0.0604485597 0.002228395 0.0075905350  
## 4 0.009122302 0.007143885 0.0009352518 -0.009949640 0.0040791367  
## 7 -0.037948747 -0.023261878 -0.0183494201 -0.024622334 -0.0117622522  
## 9 0.026051253 0.012738122 0.0186505799 0.004377666 -0.0007622522  
## 10 0.030336047 -0.001720750 0.0055651268 0.026683264 -0.0043924650  
## 12 -0.018306702 -0.008747823 -0.0403608482 -0.009643317 -0.0088981446  
## 13 -0.008138234 -0.006264497 0.0117270857 0.024616442 0.0028551253  
## 15 0.015935466 0.023738496 0.0169253648 0.006914983 -0.0198504489  
## 16 -0.014425786 -0.026072132 -0.0127188565 -0.026534457 -0.0223962514  
## 21 -0.017296109 -0.021260756 0.0084749345 -0.026010382 -0.0280268425  
## 25 0.019893378 -0.012258137 -0.0276015713 -0.004249158 0.0013557800  
## 31 -0.009064534 -0.013261504 -0.0410746352 0.001914983 -0.0068504489  
## 33 0.031472316 -0.004260007 0.0040245043 0.005064254 0.0047967639  
## 37 0.018051253 0.001738122 0.0136505799 -0.002622334 -0.0097622522  
## 38 0.005935466 -0.003261504 0.0309253648 -0.007085017 0.0131495511  
## 39 -0.082643472 -0.007259633 -0.0107007108 -0.003398429 0.0087085672  
## 40 -0.019783037 0.011954941 0.0542071185 0.017792124 0.0201094282  
## 43 -0.034180322 0.003738870 -0.0127998504 0.011452301 0.0200613543  
## 45 -0.005759259 0.001740741 0.0475740741 0.007138889 0.0116203704  
## 49 -0.010990834 -0.015258511 -0.0168763562 -0.021786476 -0.0165560232  
## 50 0.018167041 0.023737748 0.0383757950 -0.011159652 0.0243259446  
## 52 0.008787959 -0.014936766 -0.0060889814 -0.032901552 -0.0178678531  
## 53 0.020264294 0.011360470 0.0233430519 0.004663006 0.0241245740  
## 55 0.002124953 -0.014258885 -0.0191511410 0.011676207 0.0155321736  
## 56 0.021051253 0.026738122 0.0086505799 0.029377666 0.0112377478  
## 57 0.013703891 0.029739244 0.0174749345 -0.012010382 0.0029731575  
## 58 0.005978796 0.015306323 0.0054911019 0.024009845 0.0091132147  
## 61 -0.049717172 -0.032262626 -0.0058989899 -0.020696970 -0.0265858586  
## 62 0.015893378 0.017741863 0.0033984287 0.023750842 -0.0146442200  
## 63 0.028935466 0.013738496 -0.0160746352 -0.003085017 0.0031495511  
## 68 0.012009166 0.007741489 0.0091236438 0.008213524 0.0074439768  
## 70 -0.021973874 0.005711852 -0.0303729648 0.005880727 -0.0038716395  
## Log\_TL Log\_TW  
## 1 0.0108950617 0.0094012346  
## 4 0.0394964029 0.0212446043  
## 7 -0.0366526375 -0.0243709315  
## 9 0.0123473625 0.0156290685  
## 10 -0.0238326392 -0.0123542219  
## 12 -0.0205668307 -0.0140344566  
## 13 0.0090558361 0.0002303591  
## 15 -0.0020395623 0.0106860269  
## 16 -0.0376554335 -0.0451410451  
## 21 -0.0058134119 -0.0152000561  
## 25 0.0114781145 0.0041986532  
## 31 -0.0350395623 -0.0173139731  
## 33 -0.0055872615 0.0109138608  
## 37 0.0023473625 -0.0113709315  
## 38 -0.0010395623 0.0156860269  
## 39 0.0160258137 0.0159708193  
## 40 -0.0079212420 0.0085391897  
## 43 -0.0434264871 -0.0092570146  
## 45 0.0246388889 -0.0019722222  
## 49 0.0008650393 -0.0098583053  
## 50 0.0047342873 0.0065721100  
## 52 0.0040155244 -0.0157398713  
## 53 0.0343699356 0.0116864824  
## 55 0.0002519641 -0.0029152637  
## 56 -0.0026526375 -0.0013709315  
## 57 0.0271865881 -0.0002000561  
## 58 0.0039015524 0.0283260129  
## 61 0.0091212121 -0.0094848485  
## 62 -0.0125218855 -0.0008013468  
## 63 0.0239604377 0.0136860269  
## 68 -0.0081349607 0.0011416947  
## 70 0.0081927300 0.0174733056

Here are our residuals for each brain dimension measurement, generated by the MANCOVA. Below is code that can be used if this is the first time you are doing this analysis: # write.table(residuals, file = “EM1dfwithresidualsshape.csv”, sep = “,”) If you are doing this for the first time, this will give you a new data frame CSV file containing your individuals and their residuals. Once you have your residuals correctly assigned to each tadpole, we are going to Mass adjust those variables.

To do so, I am going to add a new column with EMM next to each column. Then, I am going to add a third column representing our mass adjusted brain mass next to the EMM column.I will add the Residuals for each brain dimension to the EMM based on treatment group and have the resulting number be our MA\_Brain Mass, in the original master CSV file. NOTE: IF a tadpole has any damage to any brain region, it was removed from the analysis. This could subsequently affect brain mass, which would affect how we control brain measurements for brain mass. So, not every brain has brain measurements, and thus will have an NA for each measurement. Now we can conduct PCA.

df.pca = df[,34:40]

This will now be our new working data frame containing all of our mass-adjusted brain dimensions.

KMO(df.pca)

## Kaiser-Meyer-Olkin factor adequacy  
## Call: KMO(r = df.pca)  
## Overall MSA = 0.61  
## MSA for each item =   
## MAV\_TW MAV\_TL MAV\_OTW MAV\_OTL MAV\_DW MAV\_DL MAV\_MW   
## 0.62 0.50 0.84 0.61 0.69 0.47 0.52

KMO is >0.5, so we pass.

cortest.bartlett(df.pca)

## R was not square, finding R from data

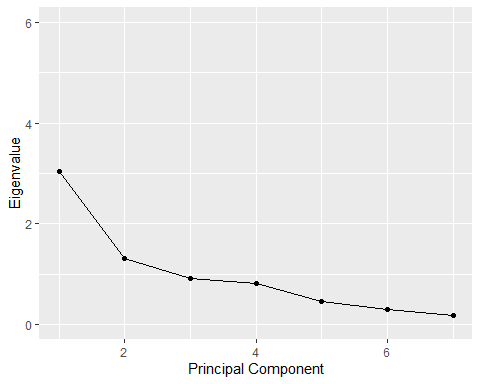
## $chisq  
## [1] 181.4568  
##   
## $p.value  
## [1] 1.544543e-27  
##   
## $df  
## [1] 21

Bartlett test must be <0.05, so we pass.

pca.p <- principal(df.pca, nfactors = 3, rotate = "varimax")

Output: Values = Eigenvalues, Scores = Factor Scores <https://www.rdocumentation.org/packages/psych/versions/2.1.6/topics/principal>

qplot(c(1:7), pca.p$values) +  
 geom\_line() +  
 xlab("Principal Component") +  
 ylab("Eigenvalue") +  
 ylim(0,6)



Screeplot for our rotated values. Will use 3 PCs. Need to add our factor scores to our df to conduct analysis

pca.p$values

## [1] 3.0401791 1.3100038 0.9197605 0.8051712 0.4597760 0.2897383 0.1753712

These are our eigenvalues.

pca.p$scores

## RC1 RC2 RC3  
## [1,] 0.05577294 0.85643383 -1.89005898  
## [2,] NA NA NA  
## [3,] NA NA NA  
## [4,] -0.17590802 1.88264009 -1.17385692  
## [5,] NA NA NA  
## [6,] NA NA NA  
## [7,] -1.39861010 -1.58843312 0.57199739  
## [8,] NA NA NA  
## [9,] 0.30189714 0.96607823 0.46954010  
## [10,] 1.42062485 -1.46807848 -1.22084141  
## [11,] NA NA NA  
## [12,] -0.30938667 -1.07474803 -1.54074689  
## [13,] 0.40414135 -0.11892623 0.67374881  
## [14,] NA NA NA  
## [15,] 0.19634184 0.34169512 0.86657045  
## [16,] -1.21004561 -2.17703438 -1.05485806  
## [17,] NA NA NA  
## [18,] NA NA NA  
## [19,] NA NA NA  
## [20,] NA NA NA  
## [21,] -1.93678188 -0.20970685 0.65531542  
## [22,] NA NA NA  
## [23,] NA NA NA  
## [24,] NA NA NA  
## [25,] -0.36923329 0.75673219 -0.95787451  
## [26,] NA NA NA  
## [27,] NA NA NA  
## [28,] NA NA NA  
## [29,] NA NA NA  
## [30,] NA NA NA  
## [31,] -0.12753162 -1.53846511 -0.44938735  
## [32,] NA NA NA  
## [33,] 0.42963702 0.25981624 -0.14727967  
## [34,] NA NA NA  
## [35,] NA NA NA  
## [36,] NA NA NA  
## [37,] -0.29253988 0.02374867 0.40059016  
## [38,] 0.02995742 0.49261833 1.04269736  
## [39,] -0.96092650 0.48297409 1.52305513  
## [40,] 1.38044709 -0.71339358 1.13501107  
## [41,] NA NA NA  
## [42,] NA NA NA  
## [43,] 0.93235745 -1.98334121 1.10336178  
## [44,] NA NA NA  
## [45,] 0.04189839 0.56716023 1.59297603  
## [46,] NA NA NA  
## [47,] NA NA NA  
## [48,] NA NA NA  
## [49,] -1.48862784 0.14962908 -0.05636809  
## [50,] 0.57390989 0.63312963 1.40136412  
## [51,] NA NA NA  
## [52,] -1.35424710 0.17057050 -1.44254220  
## [53,] 0.89388931 1.24909042 -0.70581627  
## [54,] NA NA NA  
## [55,] 0.35465315 -0.20415132 -0.34848614  
## [56,] 1.60370120 -0.34670490 0.62791614  
## [57,] -0.16375920 1.27092525 0.87720579  
## [58,] 1.48097235 0.34864021 -0.66420583  
## [59,] NA NA NA  
## [60,] NA NA NA  
## [61,] -2.34778561 0.11191904 0.64276252  
## [62,] 0.80533233 -0.55768960 0.49245101  
## [63,] 0.05277149 1.48516133 -0.51941735  
## [64,] NA NA NA  
## [65,] NA NA NA  
## [66,] NA NA NA  
## [67,] NA NA NA  
## [68,] 0.98320444 -0.45275866 -0.70061824  
## [69,] NA NA NA  
## [70,] 0.19387367 0.38446900 -1.20420539  
## [71,] NA NA NA  
## [72,] NA NA NA

These are our factor scores, aka our PC1, PC2 and PC3. These are what we will use to analyze PCA.

Factor <- pca.p$scores  
df <- cbind(df, Factor)

This ‘binds’ our new factor scores to our original data frame, so we can now conduct our analysis.

pca.p$loadings

##   
## Loadings:  
## RC1 RC2 RC3   
## MAV\_TW 0.426 0.750 0.173  
## MAV\_TL 0.957   
## MAV\_OTW 0.718 0.231   
## MAV\_OTL 0.898   
## MAV\_DW 0.661 0.407 0.383  
## MAV\_DL 0.531 0.290 -0.500  
## MAV\_MW 0.161 0.244 0.857  
##   
## RC1 RC2 RC3  
## SS loadings 2.247 1.848 1.175  
## Proportion Var 0.321 0.264 0.168  
## Cumulative Var 0.321 0.585 0.753

These are our loadings for each brain dimension for this PCA. ~.7 is the cut off point for loadings of dimensions onto PCs.

df$Microbial\_Trtmt = factor(df$Microbial\_Trtmt)  
df$Replicate = factor(df$Replicate)

Needed to turn columns into factors for analysis

pca1\_glmm <- glmer(RC1~Microbial\_Trtmt + (1|Replicate), data = df, na.action = na.omit, family = "gaussian")

## Warning in glmer(RC1 ~ Microbial\_Trtmt + (1 | Replicate), data = df, na.action =  
## na.omit, : calling glmer() with family=gaussian (identity link) as a shortcut to  
## lmer() is deprecated; please call lmer() directly

## boundary (singular) fit: see help('isSingular')

Anova(pca1\_glmm)

## Analysis of Deviance Table (Type II Wald chisquare tests)  
##   
## Response: RC1  
## Chisq Df Pr(>Chisq)  
## Microbial\_Trtmt 0.8276 1 0.363

No significant effect of treatment on PC1.

pca2\_glmm <- glmer(RC2~Microbial\_Trtmt + (1|Replicate), data = df, na.action = na.omit, family = "gaussian")

## Warning in glmer(RC2 ~ Microbial\_Trtmt + (1 | Replicate), data = df, na.action =  
## na.omit, : calling glmer() with family=gaussian (identity link) as a shortcut to  
## lmer() is deprecated; please call lmer() directly

## boundary (singular) fit: see help('isSingular')

Anova(pca2\_glmm)

## Analysis of Deviance Table (Type II Wald chisquare tests)  
##   
## Response: RC2  
## Chisq Df Pr(>Chisq)  
## Microbial\_Trtmt 0.2952 1 0.5869

No significant effect of treatment on PC2.

pca3\_glmm <- glmer(RC3~Microbial\_Trtmt + (1|Replicate), data = df, na.action = na.omit, family = "gaussian")

## Warning in glmer(RC3 ~ Microbial\_Trtmt + (1 | Replicate), data = df, na.action =  
## na.omit, : calling glmer() with family=gaussian (identity link) as a shortcut to  
## lmer() is deprecated; please call lmer() directly

Anova(pca3\_glmm)

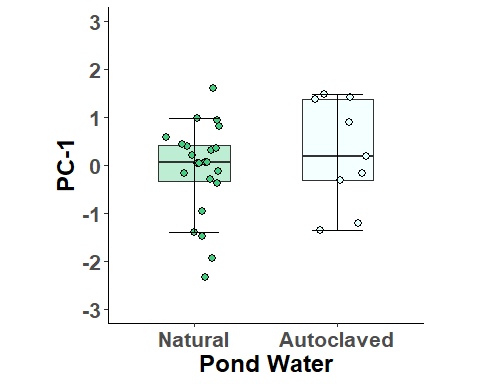
## Analysis of Deviance Table (Type II Wald chisquare tests)  
##   
## Response: RC3  
## Chisq Df Pr(>Chisq)   
## Microbial\_Trtmt 6.1021 1 0.0135 \*  
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Significant effect of treatment on PC3 (MW).

Pond\_Water <- c("Natural", "Autoclaved")  
  
ggplot(df, aes(x= Microbial\_Trtmt, y = RC1, fill = Microbial\_Trtmt)) +  
 geom\_boxplot(width = 0.5, outlier.colour = "transparent", alpha =0.35)+  
 geom\_jitter(width = .2, size = 2.5, shape = 21, color = "black") +  
 coord\_cartesian(ylim = c(-3, 3)) +  
 scale\_y\_continuous(breaks = seq(-3, 3, 1)) +  
 theme\_classic() +  
 stat\_boxplot(geom = "errorbar", width = .35) +  
 scale\_fill\_manual(values = c("seagreen3", "lightcyan"),  
 name = "Pond Water",  
 labels = c("Natural", "Autoclaved")) +  
 labs(x = "Pond Water", y = "PC-1") +  
 scale\_x\_discrete(labels = Pond\_Water) +  
 theme(aspect.ratio = 1) +  
 theme(axis.text = element\_text(face = "bold", size = 16)) +  
 theme(axis.title = element\_text(face = "bold", size = 18)) +  
 theme(legend.position = "none")

## Warning: Removed 40 rows containing non-finite values (stat\_boxplot).  
## Removed 40 rows containing non-finite values (stat\_boxplot).

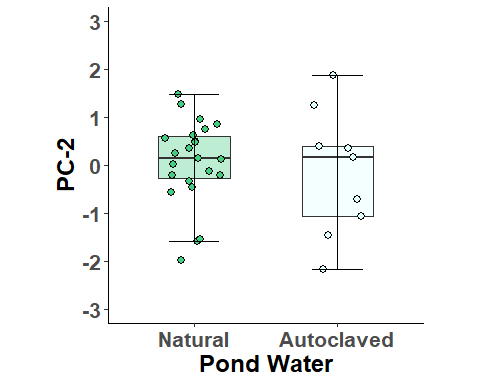
## Warning: Removed 40 rows containing missing values (geom\_point).



ggplot(df, aes(x= Microbial\_Trtmt, y = RC2, fill = Microbial\_Trtmt)) +  
 geom\_boxplot(width = 0.5, outlier.colour = "transparent", alpha =0.35)+  
 geom\_jitter(width = .2, size = 2.5, shape = 21, color = "black") +  
 coord\_cartesian(ylim = c(-3, 3)) +  
 scale\_y\_continuous(breaks = seq(-3, 3, 1)) +  
 theme\_classic() +  
 stat\_boxplot(geom = "errorbar", width = .35) +  
 scale\_fill\_manual(values = c("seagreen3", "lightcyan"),  
 name = "Pond Water",  
 labels = c("Natural", "Autoclaved")) +  
 labs(x = "Pond Water", y = "PC-2") +  
 scale\_x\_discrete(labels = Pond\_Water) +  
 theme(aspect.ratio = 1) +  
 theme(axis.text = element\_text(face = "bold", size = 16)) +  
 theme(axis.title = element\_text(face = "bold", size = 18)) +  
 theme(legend.position = "none")

## Warning: Removed 40 rows containing non-finite values (stat\_boxplot).  
## Removed 40 rows containing non-finite values (stat\_boxplot).

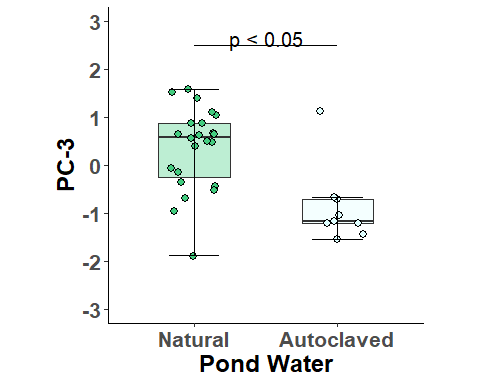
## Warning: Removed 40 rows containing missing values (geom\_point).



ggplot(df, aes(x= Microbial\_Trtmt, y = RC3, fill = Microbial\_Trtmt)) +  
 geom\_boxplot(width = 0.5, outlier.colour = "transparent", alpha =0.35) +  
 geom\_jitter(width = .2, size = 2.5, shape = 21, color = "black") +  
 coord\_cartesian(ylim = c(-3, 3)) +  
 scale\_y\_continuous(breaks = seq(-3, 3, 1)) +  
 annotate(x = 1, xend = 2, y = 2.5, yend = 2.5, geom = "segment") +  
 annotate(x=1.5, y= 2.65, label = "p < 0.05", geom = "text", size = 5.2) +  
 theme\_classic() +  
 stat\_boxplot(geom = "errorbar", width = .35) +  
 scale\_fill\_manual(values = c("seagreen3", "lightcyan"),  
 name = "Pond Water",  
 labels = c("Natural", "Autoclaved")) +  
 labs(x = "Pond Water", y = "PC-3") +  
 scale\_x\_discrete(labels = Pond\_Water) +  
 theme(aspect.ratio = 1) +  
 theme(axis.text = element\_text(face = "bold", size = 16)) +  
 theme(axis.title = element\_text(face = "bold", size = 18)) +  
 theme(legend.position = "none")

## Warning: Removed 40 rows containing non-finite values (stat\_boxplot).  
## Removed 40 rows containing non-finite values (stat\_boxplot).

## Warning: Removed 40 rows containing missing values (geom\_point).



Significant effect on PC3 (MW).

To better understand this change in PC3, we can evaluate the Mass-Adjusted MW information. Especially because the MANCOVA revealed that the slopes for this brain dimension were not homogenous across treatments.

MAVMW\_glmm <- glmer(MAV\_MW~Microbial\_Trtmt + (1|Replicate), data = df, na.action = na.omit, family = "gaussian")

## Warning in glmer(MAV\_MW ~ Microbial\_Trtmt + (1 | Replicate), data = df, :  
## calling glmer() with family=gaussian (identity link) as a shortcut to lmer() is  
## deprecated; please call lmer() directly

## boundary (singular) fit: see help('isSingular')

Anova(MAVMW\_glmm)

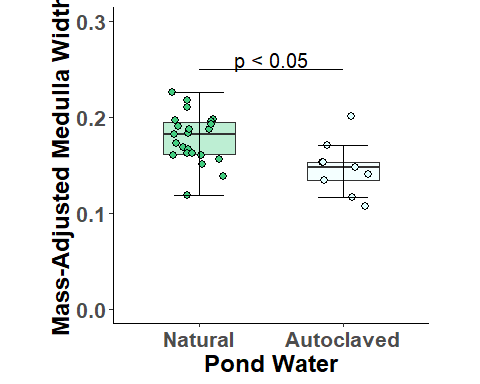
## Analysis of Deviance Table (Type II Wald chisquare tests)  
##   
## Response: MAV\_MW  
## Chisq Df Pr(>Chisq)   
## Microbial\_Trtmt 8.8253 1 0.002971 \*\*  
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Still significantly effected by treatment, regardless of PCA analysis.

ggplot(df, aes(x= Microbial\_Trtmt, y = MAV\_MW, fill = Microbial\_Trtmt)) +  
 geom\_boxplot(width = 0.5, outlier.colour = "transparent", alpha =0.35) +  
 geom\_jitter(width = .2, size = 2.5, shape = 21, color = "black") +  
 coord\_cartesian(ylim = c(0, .3)) +  
 scale\_y\_continuous(breaks = seq(0, .3, .1)) +  
 annotate(x = 1, xend = 2, y = .25, yend = .25, geom = "segment") +  
 annotate(x=1.5, y= .261, label = "p < 0.05", geom = "text", size = 5.2) +  
 theme\_classic() +  
 stat\_boxplot(geom = "errorbar", width = .35) +  
 scale\_fill\_manual(values = c("seagreen3", "lightcyan"),  
 name = "Pond Water",  
 labels = c("Natural", "Autoclaved")) +  
 labs(x = "Pond Water", y = "Mass-Adjusted Medulla Width") +  
 scale\_x\_discrete(labels = Pond\_Water) +  
 theme(aspect.ratio = 1) +  
 theme(axis.text = element\_text(face = "bold", size = 16)) +  
 theme(axis.title = element\_text(face = "bold", size = 18)) +  
 theme(legend.position = "none")

## Warning: Removed 40 rows containing non-finite values (stat\_boxplot).  
## Removed 40 rows containing non-finite values (stat\_boxplot).

## Warning: Removed 40 rows containing missing values (geom\_point).



Still see our same trend.