Body Mass + Gosner Stage

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## R Markdown

This is an R Markdown document. Markdown is a simple formatting syntax for authoring HTML, PDF, and MS Word documents. For more details on using R Markdown see <http://rmarkdown.rstudio.com>.

When you click the **Knit** button a document will be generated that includes both content as well as the output of any embedded R code chunks within the document. You can embed an R code chunk like this:

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 1.5.0  
## 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

## Warning: package 'stringr' 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)  
library(emmeans)  
  
  
file.choose()

## [1] "C:\\R\\Aim 3 - Stress and the Microbiota\\Body-Mass-and-Gosner-Stage.docx"

df <-read.csv("C:\\Users\\kjeme\\OneDrive\\Desktop\\Woodley Lab\\Aim 3 - CORT & the Microbiota\\Aim 3 (Restarted)\\KE Data FIles\\Aim 3 - Body Mass + Gosner Stage\\Aim 3 - Body Mass and Gosner Stage.csv")  
df$Water\_Trtmt = factor(df$Water\_Trtmt)  
df$Stressor = factor(df$Stressor)  
df$Replicate = factor(df$Replicate)  
df$Trtmt\_Combo = factor(df$Trtmt\_Combo)  
df$Exp\_Days = factor(df$Exp\_Days)  
df$Gosner\_Stage = as.numeric(df$Gosner\_Stage)

## Warning: NAs introduced by coercion

shapiro.test(df$Body\_Mass)

##   
## Shapiro-Wilk normality test  
##   
## data: df$Body\_Mass  
## W = 0.98567, p-value = 0.1469

Pass.

leveneTest(df$Body\_Mass, df$Trtmt\_Combo, center = mean, na.rm = TRUE)

## Levene's Test for Homogeneity of Variance (center = mean: TRUE)  
## Df F value Pr(>F)   
## group 5 3.192 0.009326 \*\*  
## 136   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Fail.

shapiro.test(df$Log\_Body\_Mass)

##   
## Shapiro-Wilk normality test  
##   
## data: df$Log\_Body\_Mass  
## W = 0.98464, p-value = 0.1138

Pass with log.

leveneTest(df$Log\_Body\_Mass, df$Trtmt\_Combo, center = mean, na.rm = TRUE)

## Levene's Test for Homogeneity of Variance (center = mean: TRUE)  
## Df F value Pr(>F)  
## group 5 1.2509 0.2889  
## 136

Pass with log.

logbodymass\_glmm <- glmer(Log\_Body\_Mass~Water\_Trtmt\*Stressor + (1|Replicate), data = df, na.action = na.omit, family = "gaussian")

## Warning in glmer(Log\_Body\_Mass ~ Water\_Trtmt \* Stressor + (1 | Replicate), :  
## 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(logbodymass\_glmm)

## Analysis of Deviance Table (Type II Wald chisquare tests)  
##   
## Response: Log\_Body\_Mass  
## Chisq Df Pr(>Chisq)   
## Water\_Trtmt 9.5217 1 0.002031 \*\*  
## Stressor 5.8691 2 0.053156 .   
## Water\_Trtmt:Stressor 1.5296 2 0.465421   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Significant effect of water treatment, but none on Stressor.

emmeans(logbodymass\_glmm, list(pairwise ~ Stressor), adjust = "tukey")

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

## $`emmeans of Stressor`  
## Stressor emmean SE df lower.CL upper.CL  
## A 0.556 0.0181 17.5 0.518 0.594  
## B 0.539 0.0185 18.7 0.501 0.578  
## C 0.496 0.0181 17.5 0.458 0.534  
##   
## Results are averaged over the levels of: Water\_Trtmt   
## Degrees-of-freedom method: kenward-roger   
## Confidence level used: 0.95   
##   
## $`pairwise differences of Stressor`  
## 1 estimate SE df t.ratio p.value  
## A - B 0.0167 0.0259 18.1 0.646 0.7967  
## A - C 0.0601 0.0256 17.5 2.349 0.0749  
## B - C 0.0434 0.0259 18.1 1.675 0.2414  
##   
## Results are averaged over the levels of: Water\_Trtmt   
## Degrees-of-freedom method: kenward-roger   
## P value adjustment: tukey method for comparing a family of 3 estimates

This pairwise comparison (Tukey Post Hoc) found no differences between stressor treatments on body mass.

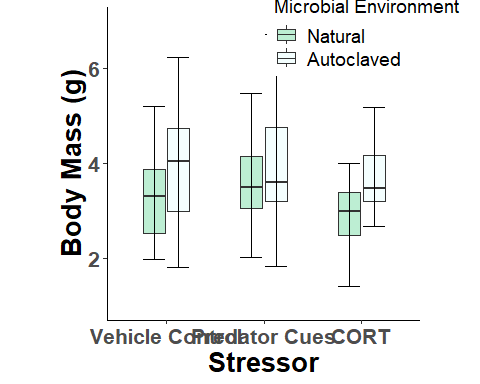
df %>%  
 drop\_na() %>%  
 group\_by(Water\_Trtmt) %>%  
 summarise\_at(vars(Body\_Mass), list(name = mean))

## # A tibble: 2 x 2  
## Water\_Trtmt name  
## <fct> <dbl>  
## 1 1 3.26  
## 2 2 3.89

Means of body mass within water treatments. Looks like autoclaved (2) tadpoles were ~16% larger.

Stress <- c("Vehicle Control", "Predator Cues", "CORT")  
#For labeling X axis  
  
ggplot(df, aes(x= factor(Stressor, level=c('B', 'A','C')), y = Body\_Mass, fill = Water\_Trtmt)) +  
 geom\_boxplot(width = 0.5, outlier.colour = "transparent", alpha =0.35) +  
 stat\_boxplot(geom = "errorbar", width = .5) +  
 theme\_classic() +  
 scale\_fill\_manual(values = c("seagreen3", "lightcyan"),  
 name = "Microbial Environment",  
 labels = c("Natural", "Autoclaved")) +  
 labs(x = "Stressor", y = "Body Mass (g)") +  
 theme(aspect.ratio = 1) +  
 coord\_cartesian(ylim = c(1, 7)) +  
 scale\_x\_discrete(labels = Stress)+  
 theme(axis.text = element\_text(face = "bold", size = 16)) +  
 theme(axis.title = element\_text(face = "bold", size = 20)) +  
 theme(legend.position = c(.83, .92)) +  
 theme(legend.title = element\_text(size = 14)) +  
 theme(legend.text = element\_text(size = 14))

## Warning: Removed 2 rows containing non-finite values (`stat\_boxplot()`).  
## Removed 2 rows containing non-finite values (`stat\_boxplot()`).



#\*\*GOSNER STAGE\*\*  
  
shapiro.test(df$Gosner\_Stage)

##   
## Shapiro-Wilk normality test  
##   
## data: df$Gosner\_Stage  
## W = 0.92039, p-value = 4.538e-07

leveneTest(df$Gosner\_Stage, df$Trtmt\_Combo, center = mean, na.rm = TRUE)

## Levene's Test for Homogeneity of Variance (center = mean: TRUE)  
## Df F value Pr(>F)   
## group 5 2.5286 0.03192 \*  
## 135   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Gosner\_glmm <- glmer(Gosner\_Stage~Water\_Trtmt\*Stressor + (1|Replicate), data = df, na.action = na.omit, family = "gaussian")

## Warning in glmer(Gosner\_Stage ~ Water\_Trtmt \* Stressor + (1 | Replicate), :  
## calling glmer() with family=gaussian (identity link) as a shortcut to lmer() is  
## deprecated; please call lmer() directly

Anova(Gosner\_glmm)

## Analysis of Deviance Table (Type II Wald chisquare tests)  
##   
## Response: Gosner\_Stage  
## Chisq Df Pr(>Chisq)   
## Water\_Trtmt 6.1261 1 0.01332 \*  
## Stressor 2.0002 2 0.36784   
## Water\_Trtmt:Stressor 3.2444 2 0.19747   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Significant effect of water treatment but not stressor.

emmeans(Gosner\_glmm, list(pairwise ~ Stressor), adjust = "tukey")

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

## $`emmeans of Stressor`  
## Stressor emmean SE df lower.CL upper.CL  
## A 37.7 0.217 17.9 37.3 38.2  
## B 37.5 0.219 18.4 37.0 37.9  
## C 37.3 0.215 17.4 36.9 37.8  
##   
## Results are averaged over the levels of: Water\_Trtmt   
## Degrees-of-freedom method: kenward-roger   
## Confidence level used: 0.95   
##   
## $`pairwise differences of Stressor`  
## 1 estimate SE df t.ratio p.value  
## A - B 0.297 0.309 18.2 0.963 0.6088  
## A - C 0.416 0.306 17.7 1.360 0.3821  
## B - C 0.119 0.307 17.9 0.386 0.9214  
##   
## Results are averaged over the levels of: Water\_Trtmt   
## Degrees-of-freedom method: kenward-roger   
## P value adjustment: tukey method for comparing a family of 3 estimates

No difference in stressor treatment according to this pairwise comparison

ggplot(df, aes(x= factor(Stressor, level=c('B', 'A','C')), y = Gosner\_Stage, fill = Water\_Trtmt)) +  
 geom\_boxplot(width = 0.5, outlier.colour = "transparent", alpha =0.35) +  
 stat\_boxplot(geom = "errorbar", width = .5) +  
 theme\_classic() +  
 scale\_fill\_manual(values = c("seagreen3", "lightcyan"),  
 name = "Microbial Environment",  
 labels = c("Natural", "Autoclaved")) +  
 labs(x = "Stressor", y = "Gosner Stage") +  
 theme(aspect.ratio = 1) +  
 coord\_cartesian(ylim = c(34, 42)) +  
 scale\_x\_discrete(labels = Stress)+  
 theme(axis.text = element\_text(face = "bold", size = 16)) +  
 theme(axis.title = element\_text(face = "bold", size = 20)) +  
 theme(legend.position = "none")

## Warning: Removed 3 rows containing non-finite values (`stat\_boxplot()`).  
## Removed 3 rows containing non-finite values (`stat\_boxplot()`).

