Relative Intestine Mass

Kyle Emerson

2023-11-02

## R Markdown for Relative Intestine Mass

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\\Relative-Intestine-Mass.docx"

df <- read.csv("C:\\Users\\kjeme\\OneDrive\\Desktop\\Woodley Lab\\Aim 3 - CORT & the Microbiota\\Aim 3 (Restarted)\\KE Data FIles\\Aim 3 - Mellott - Intestinal Dissections\\Aim 3 - Intestine Mass.csv")  
df$Water\_Trtmt = factor(df$Water\_Trtmt)  
df$Stressor = factor(df$Stressor)  
df$Trtmt\_Combo = factor(df$Trtmt\_Combo)  
df$Replicate = factor(df$Replicate)  
df$Log\_GI\_Mass = as.numeric(df$Log\_GI\_Mass)  
df$Log\_Liver\_Mass = as.numeric(df$Log\_Liver\_Mass)  
df$Liver\_Mass = as.numeric(df$Liver\_Mass)  
df$Log\_Pancreas\_Mass = as.numeric(df$Log\_Pancreas\_Mass)  
df$Pancreas\_Mass = as.numeric(df$Pancreas\_Mass)  
  
##Potential Removal of outlier AA303  
df <- df[-c(15),]  
  
  
shapiro.test(df$Log\_GI\_Mass)

##   
## Shapiro-Wilk normality test  
##   
## data: df$Log\_GI\_Mass  
## W = 0.99009, p-value = 0.4196

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

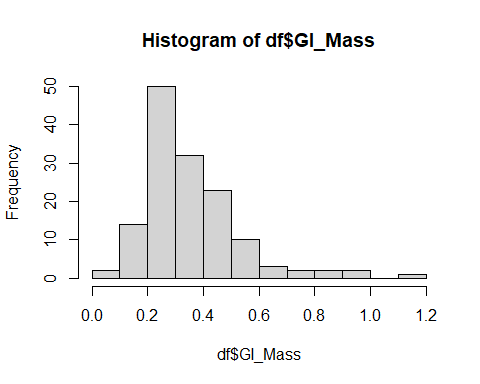
##   
## Shapiro-Wilk normality test  
##   
## data: df$Log\_Liver\_Mass  
## W = 0.98471, p-value = 0.207

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

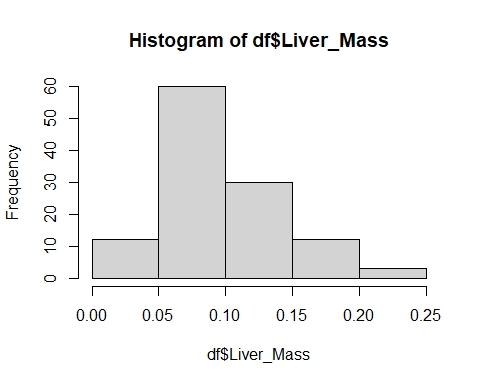
##   
## Shapiro-Wilk normality test  
##   
## data: df$Log\_Pancreas\_Mass  
## W = 0.96488, p-value = 0.003717

Data does not appear normal for pancreas, even when log transformed

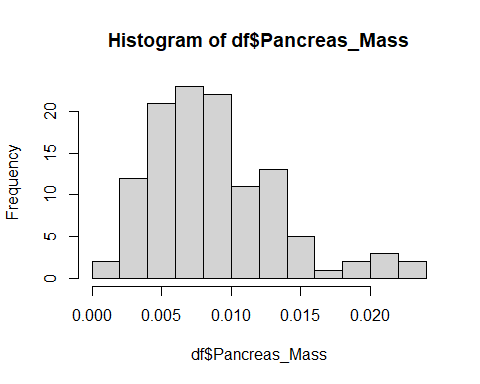
hist(df$GI\_Mass)



hist(df$Liver\_Mass)



hist(df$Pancreas\_Mass)



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

## Levene's Test for Homogeneity of Variance (center = mean: TRUE)  
## Df F value Pr(>F)   
## group 5 3.0501 0.01218 \*  
## 135   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Does not pass

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

## Levene's Test for Homogeneity of Variance (center = mean: TRUE)  
## Df F value Pr(>F)   
## group 5 2.5516 0.03167 \*  
## 111   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Does not pass

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

## Levene's Test for Homogeneity of Variance (center = mean: TRUE)  
## Df F value Pr(>F)  
## group 5 1.7981 0.119  
## 111

#GI Tract  
  
anovaGIMass <- aov(Log\_GI\_Mass~Water\_Trtmt\*Stressor\*Log\_Body\_Mass, data = df)  
summary(anovaGIMass)

## Df Sum Sq Mean Sq F value Pr(>F)   
## Water\_Trtmt 1 0.2756 0.2756 27.058 7.55e-07 \*\*\*  
## Stressor 2 0.3687 0.1844 18.098 1.18e-07 \*\*\*  
## Log\_Body\_Mass 1 3.0165 3.0165 296.105 < 2e-16 \*\*\*  
## Water\_Trtmt:Stressor 2 0.1428 0.0714 7.007 0.00129 \*\*   
## Water\_Trtmt:Log\_Body\_Mass 1 0.0696 0.0696 6.830 0.01003 \*   
## Stressor:Log\_Body\_Mass 2 0.0108 0.0054 0.531 0.58954   
## Water\_Trtmt:Stressor:Log\_Body\_Mass 2 0.0297 0.0149 1.459 0.23635   
## Residuals 129 1.3141 0.0102   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
## 2 observations deleted due to missingness

Pass, found no significant interaction between our treatments and body mass, we can say the slopes of lines for our trait (i.e. GI mass) were parallel among each trtmt group

anovaGIMass$residuals

## 1 2 3 4 5 6   
## -0.014551324 0.177636137 -0.076110209 0.114925395 0.106716588 0.117049048   
## 7 8 9 10 11 12   
## -0.014884137 -0.097098448 0.059646024 0.082919591 0.019458326 0.032904673   
## 13 14 16 17 18 19   
## -0.027110125 0.018103022 -0.098806751 -0.018987673 0.039174706 -0.028991154   
## 20 21 22 23 24 25   
## 0.035248935 -0.084404250 -0.150754793 -0.090092430 -0.101991151 0.069445237   
## 26 27 28 29 30 31   
## 0.222067127 0.128562557 0.168284966 0.149770272 0.139935292 -0.015359821   
## 32 33 34 35 36 37   
## 0.043909331 0.033735397 0.158657801 0.011867845 -0.060635999 0.031645857   
## 38 39 40 41 42 43   
## -0.053236112 0.006264087 0.058337483 -0.245397241 0.011289060 -0.092526989   
## 44 45 46 47 48 49   
## -0.183064019 -0.275819023 -0.222656345 -0.056188228 -0.028888533 0.062117473   
## 50 51 52 53 54 55   
## 0.099938713 -0.166124976 -0.035522531 0.062308508 -0.092325548 -0.025582988   
## 56 57 58 59 60 61   
## 0.069098267 0.055383924 -0.021334932 0.158740272 -0.027397163 -0.022603199   
## 62 63 64 65 66 67   
## 0.116478378 0.085910091 -0.153400430 -0.033592526 0.075036003 0.025962302   
## 68 69 70 71 72 73   
## 0.033712280 -0.137453035 -0.118914788 -0.019737380 0.009303284 0.041283265   
## 74 75 76 77 78 79   
## 0.074731121 0.083858461 0.161445349 -0.017059572 0.048047985 0.022471794   
## 80 81 82 83 84 85   
## -0.060884124 -0.030578483 0.011934587 0.007970296 -0.070210698 0.050128813   
## 86 87 88 89 90 91   
## -0.053750948 0.036124096 -0.124849624 0.022382639 -0.038793005 0.132393440   
## 92 93 94 95 96 97   
## 0.013636185 -0.070652925 -0.045004209 -0.103274442 -0.091350003 0.304323438   
## 98 99 100 101 102 103   
## -0.061027498 0.177967798 0.069464806 -0.058113434 0.056081366 0.140957649   
## 104 105 106 109 110 111   
## -0.010198821 -0.070341024 0.049408119 0.032889359 0.071814647 0.025755438   
## 112 113 114 115 116 117   
## 0.068566983 0.040572103 0.091721520 -0.120310467 -0.168625306 -0.028657019   
## 118 119 120 121 122 123   
## -0.092655511 -0.260176129 -0.259418017 -0.029166378 0.040755026 0.008849450   
## 124 125 126 127 128 129   
## 0.050683733 0.101922170 -0.024947346 -0.020343298 0.005337245 -0.059185413   
## 130 131 132 133 134 135   
## 0.017172899 -0.019363732 -0.060643391 -0.009199157 -0.083578271 -0.082332111   
## 136 137 138 139 140 141   
## -0.112206035 0.055712572 0.075928912 0.031484425 -0.002730198 0.030508082   
## 142 143 144   
## 0.057170789 -0.013206968 0.041376995

GIModel <- lm(Log\_GI\_Mass~Water\_Trtmt\*Stressor\*Log\_Body\_Mass, data = df)  
GI\_means\_complex <- estimate\_means(GIModel)

## We selected `at = c("Water\_Trtmt", "Stressor")`.

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

GI\_means\_complex

## Estimated Marginal Means  
##   
## Water\_Trtmt | Stressor | Mean | SE | 95% CI  
## ------------------------------------------------------  
## Autoclaved | A | -0.43 | 0.02 | [-0.48, -0.39]  
## Natural | A | -0.51 | 0.02 | [-0.56, -0.47]  
## Autoclaved | B | -0.49 | 0.02 | [-0.54, -0.45]  
## Natural | B | -0.48 | 0.02 | [-0.52, -0.43]  
## Autoclaved | C | -0.54 | 0.02 | [-0.58, -0.50]  
## Natural | C | -0.50 | 0.03 | [-0.56, -0.45]  
##   
## Marginal means estimated at Water\_Trtmt, Stressor

EMMs for the GI tract

GIresiduals <- anovaGIMass$residuals  
  
# Species\_ID <-df$Species\_ID  
# Log\_GI\_Mass <- df$Log\_GI\_Mass  
# Water\_Trtmt <- df$Water\_Trtmt  
# Stressor <-df$Stressor  
# Trtmt\_Combo <-df$Trtmt\_Combo  
# Replicate <- df$Replicate  
# df2 <- cbind(Species\_ID, Log\_GI\_Mass)  
# df2 <- cbind(df2, Water\_Trtmt)  
# df2 <- cbind(df2, Stressor)  
# df2 <- cbind(df2, Trtmt\_Combo)  
# df2 <- cbind(df2, Replicate)  
# w = complete.cases(df2)  
# df2 = df2[w,]  
# df2 <- cbind(df2, GIresiduals)  
# write.table(df2, file = "GImasswithresiduals.csv", sep = ",")

The above commented code is used for the first time you do this analysis to formulate a new .csv sheet with your residuals properly assigned to your species ID. From there, you add the residual for each animal to the EMM corresponding to the EMM (based on treatment combo) to achieve your Mass Adjusted Variable (i.e. GI tract).

###Liver  
anovaLiverMass <- aov(Log\_Liver\_Mass~Water\_Trtmt\*Stressor\*Log\_Body\_Mass, data = df)  
summary(anovaLiverMass)

## Df Sum Sq Mean Sq F value Pr(>F)   
## Water\_Trtmt 1 0.134 0.134 15.877 0.000125 \*\*\*  
## Stressor 2 0.455 0.227 26.928 3.63e-10 \*\*\*  
## Log\_Body\_Mass 1 3.286 3.286 389.103 < 2e-16 \*\*\*  
## Water\_Trtmt:Stressor 2 0.116 0.058 6.845 0.001606 \*\*   
## Water\_Trtmt:Log\_Body\_Mass 1 0.000 0.000 0.022 0.881763   
## Stressor:Log\_Body\_Mass 2 0.027 0.014 1.623 0.202281   
## Water\_Trtmt:Stressor:Log\_Body\_Mass 2 0.059 0.029 3.486 0.034216 \*   
## Residuals 105 0.887 0.008   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
## 26 observations deleted due to missingness

Slight fail

anovaLiverMass$residuals

## 2 3 4 5 6 8   
## -0.047223010 -0.086377388 0.067768356 -0.034103237 -0.054313711 -0.025738048   
## 9 10 11 12 14 16   
## -0.063583363 -0.072834526 -0.030067996 0.097906342 0.024546328 -0.015254577   
## 17 18 20 21 22 23   
## 0.044777096 -0.032216218 0.099306078 0.022793557 0.012562451 0.011371993   
## 24 26 27 28 29 30   
## 0.080679873 0.007133329 0.116221654 0.080481624 -0.030751809 0.077142443   
## 32 33 34 35 36 38   
## -0.016183159 0.043167290 -0.257041708 -0.033578019 0.003506855 0.052370004   
## 39 40 41 42 44 45   
## -0.060296329 -0.089212961 0.008293780 0.036410846 0.072859998 -0.025011699   
## 46 47 48 50 51 52   
## -0.100027289 0.094734265 0.019780885 -0.125527863 -0.131448968 -0.094104992   
## 53 54 56 57 58 59   
## 0.106134302 -0.142072990 -0.008491048 0.063285328 -0.044288921 -0.026255418   
## 60 62 63 64 65 66   
## -0.102142154 0.089741498 0.083329620 -0.091653604 0.082873439 -0.001005439   
## 68 69 70 71 72 74   
## 0.093841126 0.151573038 -0.055445739 0.095114911 0.056543876 -0.057094038   
## 75 76 77 78 80 81   
## -0.055983371 0.163397644 0.069063663 0.031670156 -0.067654230 -0.147701657   
## 82 83 84 86 87 88   
## -0.087922615 0.035343310 -0.102453252 -0.034490580 0.031623173 -0.089179943   
## 89 90 92 93 94 95   
## 0.025302428 0.103807153 -0.027404892 0.088303479 -0.053440688 0.083402445   
## 96 98 99 100 101 102   
## 0.091411817 -0.048770905 -0.323502600 0.058779320 -0.019012104 -0.166415665   
## 104 105 106 110 111 112   
## -0.051104301 -0.086237857 0.034944284 0.087788796 0.041009750 -0.004865824   
## 113 114 116 117 118 119   
## 0.210234310 0.094522587 0.078512426 -0.119665791 0.156512641 0.012518078   
## 120 122 123 124 125 126   
## 0.044752854 -0.023298139 0.065072808 0.104579212 0.063191751 -0.050656195   
## 128 129 130 131 132 134   
## -0.029070798 0.015632905 0.063620002 0.044410038 0.078227865 -0.197191381   
## 135 136 137 138 140 141   
## -0.114242408 -0.172023812 -0.035609108 0.001167176 0.009447189 0.059484909   
## 142 143 144   
## 0.024349713 0.012047039 0.080861232

LiverModel <- lm(Log\_Liver\_Mass~Water\_Trtmt\*Stressor\*Log\_Body\_Mass, data = df)  
Liver\_means\_complex <- estimate\_means(LiverModel)

## We selected `at = c("Water\_Trtmt", "Stressor")`.

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

Liver\_means\_complex

## Estimated Marginal Means  
##   
## Water\_Trtmt | Stressor | Mean | SE | 95% CI  
## ------------------------------------------------------  
## Autoclaved | A | -1.01 | 0.02 | [-1.05, -0.96]  
## Natural | A | -1.06 | 0.02 | [-1.10, -1.02]  
## Autoclaved | B | -1.04 | 0.02 | [-1.08, -1.00]  
## Natural | B | -1.03 | 0.02 | [-1.07, -0.98]  
## Autoclaved | C | -1.12 | 0.02 | [-1.16, -1.08]  
## Natural | C | -1.06 | 0.03 | [-1.11, -1.01]  
##   
## Marginal means estimated at Water\_Trtmt, Stressor

Liverresiduals <- anovaLiverMass$residuals  
  
# Species\_ID <-df$Species\_ID  
# Log\_Liver\_Mass <- df$Log\_Liver\_Mass  
# Water\_Trtmt <- df$Water\_Trtmt  
# Stressor <-df$Stressor  
# Trtmt\_Combo <-df$Trtmt\_Combo  
# Replicate <- df$Replicate  
# df3 <- cbind(Species\_ID, Log\_Liver\_Mass)  
# df3 <- cbind(df3, Water\_Trtmt)  
# df3 <- cbind(df3, Stressor)  
# df3 <- cbind(df3, Trtmt\_Combo)  
# df3 <- cbind(df3, Replicate)  
# w = complete.cases(df3)  
# df3 = df3[w,]  
# df3 <- cbind(df3, Liverresiduals)  
# write.table(df3, file = "Livermasswithresiduals.csv", sep = ",")

###Pancreas  
anovaPancreasMass <- aov(Log\_Pancreas\_Mass~Water\_Trtmt\*Stressor\*Log\_Body\_Mass, data = df)  
summary(anovaPancreasMass)

## Df Sum Sq Mean Sq F value Pr(>F)   
## Water\_Trtmt 1 0.1621 0.1621 6.715 0.0109 \*   
## Stressor 2 0.6828 0.3414 14.145 3.63e-06 \*\*\*  
## Log\_Body\_Mass 1 2.4611 2.4611 101.963 < 2e-16 \*\*\*  
## Water\_Trtmt:Stressor 2 0.0791 0.0396 1.639 0.1992   
## Water\_Trtmt:Log\_Body\_Mass 1 0.0239 0.0239 0.990 0.3220   
## Stressor:Log\_Body\_Mass 2 0.0144 0.0072 0.299 0.7422   
## Water\_Trtmt:Stressor:Log\_Body\_Mass 2 0.0113 0.0057 0.235 0.7911   
## Residuals 105 2.5344 0.0241   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
## 26 observations deleted due to missingness

Pass, found no significant interaction between our treatments and body mass, we can’t say the slopes of lines for our trait (i.e. Pancreas mass) were parallel among each trtmt group

anovaPancreasMass$residuals

## 2 3 4 5 6   
## 0.1987753781 -0.0486728855 0.1531954814 0.1309116995 0.1444069479   
## 8 9 10 11 12   
## -0.1648568308 -0.0009006278 -0.0062060655 0.0076863473 0.0161287362   
## 14 16 17 18 20   
## 0.0412739439 -0.2525254411 -0.0263788734 -0.0200095309 0.0763890305   
## 21 22 23 24 26   
## -0.0477708275 -0.1212165048 -0.1327369192 0.0525069418 0.2133784782   
## 27 28 29 30 32   
## 0.1911308506 0.3443306398 0.1335791135 0.1984629308 -0.0083567846   
## 33 34 35 36 38   
## 0.1044559823 -0.3690913518 0.1178138086 0.0749989302 -0.1830496972   
## 39 40 41 42 44   
## 0.1718790608 -0.2813582789 -0.2508068196 0.0179943126 -0.1294431102   
## 45 46 47 48 50   
## 0.0510624719 -0.2397754790 -0.1952892693 0.0380842114 0.0171748091   
## 51 52 53 54 56   
## 0.1450354497 0.0709318671 0.1751655539 -0.1552930371 -0.0366483667   
## 57 58 59 60 62   
## 0.0417950246 -0.0148203113 0.0016664364 -0.0156920668 0.0892431293   
## 63 64 65 66 68   
## 0.0138521773 -0.2574658454 0.0481915545 0.1192814996 0.0499499511   
## 69 70 71 72 74   
## -0.2262383520 -0.0257673979 0.0417396362 -0.0821017117 0.1318106293   
## 75 76 77 78 80   
## 0.1964244446 0.0916418029 0.1396197469 -0.0206049189 0.1070105716   
## 81 82 83 84 86   
## -0.0096476382 -0.0241677783 0.1039468987 -0.2648886920 -0.1455436559   
## 87 88 89 90 92   
## -0.0750181671 -0.0431304766 -0.0761851493 -0.0371559006 0.0230734502   
## 93 94 95 96 98   
## -0.0191053420 0.0100741438 -0.1999254529 0.1117714834 0.0819161152   
## 99 100 101 102 104   
## -0.5627506286 0.3945247551 0.1171767987 -0.0131722799 -0.0911595012   
## 105 106 110 111 112   
## -0.2371878442 0.1227485855 0.0108510079 0.1297605513 0.1727506650   
## 113 114 116 117 118   
## 0.1774223164 -0.0165501557 -0.1370230889 -0.1246772398 0.2021962971   
## 119 120 122 123 124   
## -0.0720745567 -0.1547517971 0.2378673371 0.1153577016 0.1023226808   
## 125 126 128 129 130   
## 0.1145877012 -0.1442161914 0.0518716274 0.0900576605 0.0317105965   
## 131 132 134 135 136   
## 0.1385927083 -0.0748044333 -0.3213575172 -0.0730671096 -0.0779731177   
## 137 138 140 141 142   
## -0.0443161768 -0.0818170939 0.0140145346 0.0220892271 -0.0693876035   
## 143 144   
## 0.0404725459 -0.0720050777

PancreasModel <- lm(Log\_Pancreas\_Mass~Water\_Trtmt\*Stressor\*Log\_Body\_Mass, data = df)  
Pancreas\_means\_complex <- estimate\_means(PancreasModel)

## We selected `at = c("Water\_Trtmt", "Stressor")`.

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

Pancreas\_means\_complex

## Estimated Marginal Means  
##   
## Water\_Trtmt | Stressor | Mean | SE | 95% CI  
## ------------------------------------------------------  
## Autoclaved | A | -2.00 | 0.04 | [-2.08, -1.93]  
## Natural | A | -2.07 | 0.04 | [-2.14, -2.00]  
## Autoclaved | B | -2.10 | 0.04 | [-2.17, -2.03]  
## Natural | B | -2.10 | 0.04 | [-2.17, -2.03]  
## Autoclaved | C | -2.15 | 0.03 | [-2.22, -2.08]  
## Natural | C | -2.09 | 0.05 | [-2.18, -2.00]  
##   
## Marginal means estimated at Water\_Trtmt, Stressor

Pancreasresiduals <- anovaPancreasMass$residuals  
  
# Species\_ID <-df$Species\_ID  
# Log\_Pancreas\_Mass <- df$Log\_Pancreas\_Mass  
# Water\_Trtmt <- df$Water\_Trtmt  
# Stressor <-df$Stressor  
# Trtmt\_Combo <-df$Trtmt\_Combo  
# Replicate <- df$Replicate  
# df4 <- cbind(Species\_ID, Log\_Pancreas\_Mass)  
# df4 <- cbind(df4, Water\_Trtmt)  
# df4 <- cbind(df4, Stressor)  
# df4 <- cbind(df4, Trtmt\_Combo)  
# df4 <- cbind(df4, Replicate)  
# w = complete.cases(df4)  
# df4 = df4[w,]  
# df4 <- cbind(df4, Pancreasresiduals)  
# write.table(df4, file = "Pancreasmasswithresiduals.csv", sep = ",")

#Anovas  
MAV\_GI\_glmm <- glmer(GI\_MAV~Water\_Trtmt\*Stressor + (1|Replicate), data = df, family = "gaussian")

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

Anova(MAV\_GI\_glmm)

## Analysis of Deviance Table (Type II Wald chisquare tests)  
##   
## Response: GI\_MAV  
## Chisq Df Pr(>Chisq)  
## Water\_Trtmt 0.0651 1 0.7985  
## Stressor 1.8381 2 0.3989  
## Water\_Trtmt:Stressor 2.9172 2 0.2326

NS

emmeans(MAV\_GI\_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 -0.475 0.0264 18.0 -0.530 -0.419  
## B -0.484 0.0265 18.2 -0.539 -0.428  
## C -0.522 0.0263 17.8 -0.578 -0.467  
##   
## 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 0.00902 0.0374 18.1 0.241 0.9684  
## A - C 0.04773 0.0373 17.9 1.280 0.4239  
## B - C 0.03870 0.0373 18.0 1.037 0.5641  
##   
## 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

N.S.

MAV\_Liver\_glmm <- glmer(Liver\_MAV~Water\_Trtmt\*Stressor + (1|Replicate), data = df, family = "gaussian")

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

Anova(MAV\_Liver\_glmm)

## Analysis of Deviance Table (Type II Wald chisquare tests)  
##   
## Response: Liver\_MAV  
## Chisq Df Pr(>Chisq)   
## Water\_Trtmt 0.0962 1 0.75638   
## Stressor 4.6399 2 0.09828 .  
## Water\_Trtmt:Stressor 3.6999 2 0.15724   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

emmeans(MAV\_Liver\_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 -1.03 0.0211 17.9 -1.08 -0.988  
## B -1.04 0.0212 18.4 -1.08 -0.992  
## C -1.09 0.0210 17.6 -1.13 -1.046  
##   
## 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 0.00387 0.0299 18.1 0.129 0.9908  
## A - C 0.05748 0.0297 17.8 1.934 0.1583  
## B - C 0.05361 0.0298 18.0 1.797 0.1988  
##   
## 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

NS

MAV\_Pancreas\_glmm <- glmer(Pancreas\_MAV~Water\_Trtmt\*Stressor + (1|Replicate), data = df, family = "gaussian")

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

Anova(MAV\_Pancreas\_glmm)

## Analysis of Deviance Table (Type II Wald chisquare tests)  
##   
## Response: Pancreas\_MAV  
## Chisq Df Pr(>Chisq)  
## Water\_Trtmt 0.0008 1 0.9776  
## Stressor 3.6776 2 0.1590  
## Water\_Trtmt:Stressor 1.9637 2 0.3746

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

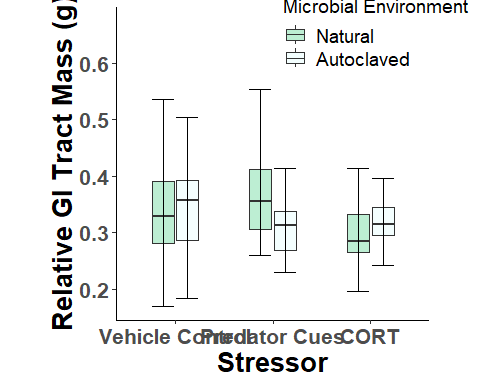
## NOTE: Results may be misleading due to involvement in interactions

## $`emmeans of Stressor`  
## Stressor emmean SE df lower.CL upper.CL  
## A -2.04 0.0325 17.9 -2.10 -1.97  
## B -2.10 0.0328 18.4 -2.17 -2.03  
## C -2.12 0.0323 17.5 -2.19 -2.05  
##   
## 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 0.0644 0.0462 18.2 1.395 0.3639  
## A - C 0.0847 0.0458 17.7 1.849 0.1827  
## B - C 0.0203 0.0460 17.9 0.441 0.8990  
##   
## 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

NS

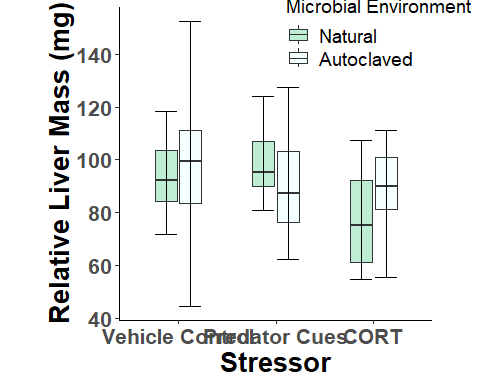
##Boxplots - GI Tract  
Stress <- c("Vehicle Control", "Predator Cues", "CORT")  
#For labeling X axis  
  
ggplot(df, aes(x= factor(Stressor, level=c('B', 'A','C')), y = Relative\_GI\_Mass\_g, fill = Water\_Trtmt)) +  
 geom\_boxplot(width = 0.5, outlier.colour = "transparent", alpha =0.35) +  
 stat\_boxplot(geom = "errorbar", width = .5) +  
 theme\_classic() +  
 scale\_fill\_manual(values = c("seagreen3", "lightcyan"),  
 name = "Microbial Environment",  
 labels = c("Natural", "Autoclaved")) +  
 labs(x = "Stressor", y = "Relative GI Tract Mass (g)") +  
 theme(aspect.ratio = 1) +  
 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()`).



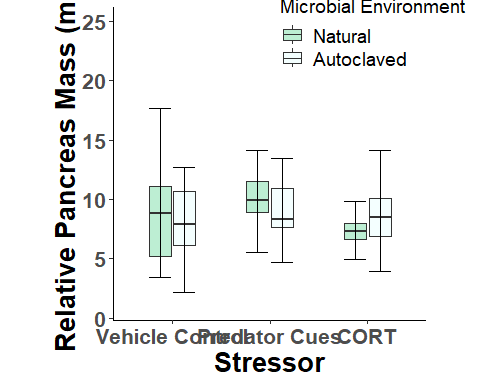
##Boxplots - Liver  
  
ggplot(df, aes(x= factor(Stressor, level=c('B', 'A','C')), y = Relative\_Liver\_Mass\_mg, fill = Water\_Trtmt)) +  
 geom\_boxplot(width = 0.5, outlier.colour = "transparent", alpha =0.35) +  
 stat\_boxplot(geom = "errorbar", width = .5) +  
 theme\_classic() +  
 scale\_fill\_manual(values = c("seagreen3", "lightcyan"),  
 name = "Microbial Environment",  
 labels = c("Natural", "Autoclaved")) +  
 labs(x = "Stressor", y = "Relative Liver Mass (mg)") +  
 theme(aspect.ratio = 1) +  
 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 26 rows containing non-finite values (`stat\_boxplot()`).  
## Removed 26 rows containing non-finite values (`stat\_boxplot()`).



##Boxplots - Pancreas  
ggplot(df, aes(x= factor(Stressor, level=c('B', 'A','C')), y = Relative\_Pancreas\_Mass\_mg, 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 = "Relative Pancreas Mass (mg)") +  
 theme(aspect.ratio = 1) +  
 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)) +  
 coord\_cartesian(ylim = c(1,25)) +  
 theme(legend.title = element\_text(size = 14)) +  
 theme(legend.text = element\_text(size = 14))

## Warning: Removed 26 rows containing non-finite values (`stat\_boxplot()`).  
## Removed 26 rows containing non-finite values (`stat\_boxplot()`).



##Looking at body mass vs liver mass  
ggplot(df, aes(x= Body\_Mass, y = Liver\_Mass)) +  
 geom\_point (size = 4)+  
 theme\_classic() +  
 labs(x = "Body", y = "Liver") +  
 theme(aspect.ratio = 1) +  
 theme(axis.text = element\_text(face = "bold", size = 16)) +  
 theme(axis.title = element\_text(face = "bold", size = 18)) +  
 theme(legend.title = element\_blank()) +  
 theme(legend.text = element\_text(size = 14))

## Warning: Removed 26 rows containing missing values (`geom\_point()`).

