Antibiotic-altered gut microbiota explain plasticity in host memory and the covariation of pace-of-life traits in an aquatic snail

2024-04-12

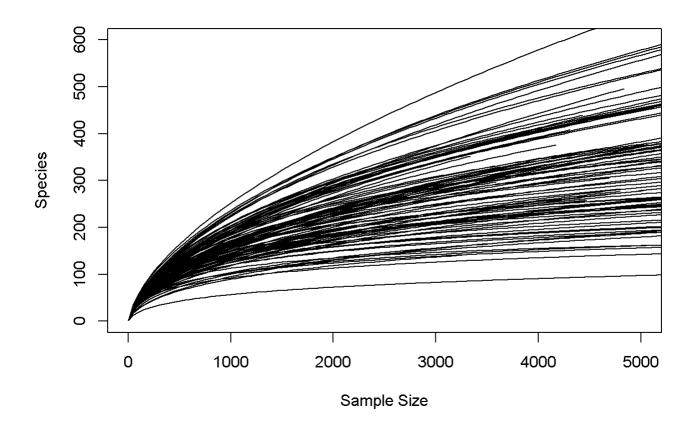
```
#load the following packages
library(vegan)
library(tidyverse)
library(phyloseq)
library(ggplot2)
library(dplyr)
library(lme4)
library(lmerTest)
library(metagenomeSeq)
setwd("F:/RWorkspace/Snail microbiome/snailMicrobiome Publication data")
taxonomy <- read.delim("rdp-classified_GD_taxTable.txt", header=TRUE)</pre>
metadata <- read.table("snail_metadata.txt", header=TRUE, row.names = "row.names") #before scal</pre>
ed data saved and alpha diversity
otus <- read.table("otu-table-centroids-iddef0-400bp.txt")</pre>
taxonomy <- data.frame(taxonomy[,-1], row.names=taxonomy[,1]) #set row.names</pre>
# Edit metadata for downstream analyses: invert the sign (-/+) for biteRateChange
# (more intuitive to interpret where higher values = better memory)
names(metadata)[names(metadata) == "biteRateChange"] <- "rawbiteRateChange"</pre>
metadata$biteRateChange = metadata$rawbiteRateChange*(-1)
##scale variables
metadata$scaledThig<-scale(metadata$Thigmotaxis)</pre>
metadata$scaledSpeed<-scale(metadata$Speed)</pre>
metadata$scaledBiteRate<-scale(metadata$biteRateChange)</pre>
metadata$scaledmetRate<-scale(metadata$metabolicRate)</pre>
names(metadata)
#phyloseq format
OTU = otu_table(otus, taxa_are_rows = TRUE)
TAX = tax table(as.matrix(taxonomy))
MET = sample data(metadata)
phyformat = phyloseq(OTU, TAX, MET)
# metadata with non-sequenced individuals and DMSO
#metadata<-read.csv("metadata_DMSO.csv",header=TRUE)</pre>
```

filtering steps

```
class(OTU) <- "matrix"</pre>
```

Warning in class(OTU) <- "matrix": Setting class(x) to "matrix" sets attribute ## to NULL; result will no longer be an S4 object

tab <- t(OTU)
rarecurve(tab, step=50, cex=0.5, label = FALSE, xlim=c(0, 5000), ylim=c(0,600))



```
#a read cut off of 1000 is sufficient and conservative.
snailPhyloFilt <- prune_samples(sample_sums(phyformat)>=1000, phyformat)
# sample 30 has been dropped
# if using metadata object downstream, must remove sample 30
#remove cyanobacteria
                                                   #, "","","")
filterPhyla = c("Cyanobacteriota")
snailPhyloFilt <- subset_taxa(snailPhyloFilt, !Phylum %in% filterPhyla)</pre>
#remove taxa w/ <0.005\% total abundance of total abundance of whole dataset recommeded by Boku
Lich
# taxa total abundance
taxa.abundances <- get_sample(snailPhyloFilt)</pre>
# dataset total abundance
total.abundance <- apply(taxa.abundances, 1,sum)</pre>
# combine
tt.abundances <- cbind(taxa.abundances,total.abundance)</pre>
cutoff <- sum(total.abundance)*0.00005</pre>
keep <- total.abundance>cutoff
snailPhyloFilt2 <- prune_taxa(keep,snailPhyloFilt)</pre>
reads<-sample_sums(snailPhyloFilt2)</pre>
reads<-as.data.frame(reads)</pre>
sum(reads$reads)
## [1] 1159694
mean(reads$reads)
## [1] 12337.17
min(reads$reads)
## [1] 1961
max(reads$reads)
## [1] 187835
# NORMALISATION Transform OTU counts, using Cumulative sum scaling from metagenomeseq package.
# convert phylo obj to metagenomeSeq object
SnailMGS <- phyloseq_to_metagenomeSeq(snailPhyloFilt2)</pre>
# normalization
p <- cumNormStatFast(SnailMGS)</pre>
```

Default value being used.

```
р
```

```
## [1] 0.5
```

```
SnailMGS <- cumNorm(SnailMGS, p =p)
#returns normalized factors for each sample
normFactors(SnailMGS)</pre>
```

```
##
     S10 L001
                 S11 L001
                              S12 L001
                                          S13 L001
                                                      S14 L001
                                                                   S15 L001
                                                                               S16 L001
##
          1531
                      1428
                                   845
                                               1178
                                                            792
                                                                        821
                                                                                     653
##
     S17_L001
                 S18_L001
                              S19_L001
                                           S1_L001
                                                      S20_L001
                                                                   S21_L001
                                                                               S22_L001
           844
                       573
                                                484
                                                                                     527
##
     S23_L001
                 S25_L001
                              S26_L001
                                          S27_L001
                                                       S28_L001
                                                                   S29_L001
                                                                                S2_L001
##
           205
                       288
                                    508
                                                490
                                                            202
                                                                                     450
     S31_L001
                 S32_L001
                                          S34_L001
                                                                               S37_L001
##
                              S33_L001
                                                       S35_L001
                                                                   S36_L001
                       396
                                                500
                                                            482
                                                                        199
                                                                                     322
##
           410
                                    351
##
     S38_L001
                 S39_L001
                               S3_L001
                                          S40_L001
                                                       S41_L001
                                                                   S42_L001
                                                                               S43_L001
##
           275
                       271
                                   277
                                                285
                                                            284
                                                                        471
                                                                                     248
     S44 L001
                 S45 L001
                              S46 L001
                                          S47 L001
                                                       S48 L001
                                                                   S49 L001
                                                                                S4 L001
##
##
           529
                       225
                                                257
                                                            331
                                                                        305
                                                                                     347
                                   120
##
     S50_L001
                 S51_L001
                              S52_L001
                                          S53_L001
                                                       S54_L001
                                                                   S55_L001
                                                                               S56_L001
                                                387
##
           323
                       276
                                    393
                                                            183
                                                                        279
                                                                                     177
     S57_L001
                                                                               S63_L001
                 S58_L001
                              S59_L001
                                           S5_L001
                                                      S61_L001
                                                                   S62_L001
##
##
           338
                       341
                                   354
                                                231
                                                            222
                                                                        233
                                                                                     190
##
     S64_L001
                 S65_L001
                              S66_L001
                                          S67_L001
                                                      S68_L001
                                                                   S69_L001
                                                                                S6_L001
##
           194
                       383
                                   156
                                                184
                                                            289
                                                                        196
                                                                                     222
##
     S70_L001
                 S71_L001
                              S72 L001
                                          S73_L001
                                                      S74_L001
                                                                   S75_L001
                                                                               S76 L001
           243
                                                405
                                                                        285
                                                                                     222
##
                        89
                                   349
                                                            332
##
     S77_L001
                 S78_L001
                              S79_L001
                                           S7_L001
                                                       S80_L001
                                                                   S81_L001
                                                                               S82_L001
##
                                                332
           356
                       226
                                   183
                                                            220
                                                                        138
                                                                                      86
##
     S83 L001
                 S85 L001
                              S86 L001
                                          S87 L001
                                                       S88 L001
                                                                   S89 L001
                                                                                S8 L001
##
           353
                       163
                                                240
                                                            230
                                                                        297
                                                                                     227
                                   319
     S90 L001
                                          S94 L001
                                                       S95 L001
                                                                               S97_L001
##
                 S92 L001
                              S93 L001
                                                                   S96 L001
                                                301
                                                                        173
##
           259
                       253
                                    339
                                                            147
                                                                                     183
##
      S9 L001 E5B55 L001 E5B56 L001
##
           173
```

```
# To export normalized count matrix
CSS.count.matrix.snail <- MRcounts(SnailMGS, norm = T)

# reintegrate w/ phyloseq
CSS.otu.snail <- otu_table(CSS.count.matrix.snail, taxa_are_rows = TRUE)
snailPhyloCSS <- merge_phyloseq(CSS.otu.snail,snailPhyloFilt2)

# snailPhyloCSS is normalised for beta diversity analysis
# snailPhyloFilt and snailPhyloFilt2 is not normalised for alpha diversity analysis and relative abundance analysis</pre>
```

preparing family and phylum for relative abundance

graphs

```
# collapse phyloseg object down to only contain Phyla level info ##
snailPhyloFilt2P <- tax_glom(snailPhyloFilt2, "Phylum", NArm = TRUE)</pre>
## transform to relative abundances
snailPhyloRA \leftarrow transform\_sample\_counts(snailPhyloFilt2P, function(x){x / sum(x)})
# special phyloseq psmelt function retuns dataframe w/ otu abundances, taxa names and sample da
motsnail <- psmelt(snailPhyloRA)</pre>
write.csv(motsnail, file = "otuAbundances_Taxa_Sample_Snail.csv")
phyloGlom = tax_glom(snailPhyloFilt2P, "Phylum")
glomTax = tax_table(phyloGlom)[,"Phylum"]
glomOTU = otu_table(phyloGlom)
glomTable = merge(glomOTU,glomTax,by=0,all=TRUE)
rownames(glomTable) = glomTable[,"Phylum"]
glomTable$Row.names = NULL
glomTable$Phylum = NULL
glomTable2 = glomTable / rep(colSums(glomTable), each = nrow(glomTable)) ###percentages
glomTable3 = as.data.frame(t(glomTable2))
write.csv(glomTable3, file = "RA_Phylum.csv")
SnailPhylumRA2023<-merge(glomTable3, metadata, by = 0)</pre>
write.csv(SnailPhylumRA2023, file = "metadata_PhylumRA.csv")
PhylumRA<-read.csv(file="metadata_PhylumRA.csv", header=TRUE)</pre>
# currently each column is a phylum with its abundance as values in cells. I want a column name
d Phylum and a column named Abundance
names(PhylumRA)
SnailPhylumRAGather<-gather(PhylumRA, 'Pseudomonadota', 'Verrucomicrobiota', 'Spirochaetota', 'B
acteria_unclassified' , 'Armatimonadota', 'Candidatus_Saccharibacteria',
 'Planctomycetota', 'Bacillota', 'Actinomycetota', 'Campylobacterota', 'Bacteroidota', 'Acid
obacteriota',
 'Chlamydiota'
                , key="Phylum", value = "Abundance")
snailMeans <- summarySE(SnailPhylumRAGather, measurevar="Abundance", groupvars=c("treatmentOrde</pre>
r", "Phylum"))
write.csv(snailMeans, file="RAPhylumMeans.csv")
snailMeans2 <- summarySE(SnailPhylumRAGather, measurevar="Abundance", groupvars="Phylum")</pre>
write.csv(snailMeans2, file="RAPhylumMeansOverall.csv")
# same again but at Family level
# collapse phyloseq object down to only contain Phyla level info ##
snailPhyloFilt3 <- tax_glom(snailPhyloFilt2, "Family", NArm = TRUE)</pre>
famGlom = tax_glom(snailPhyloFilt3, "Family")
glomTax = tax table(famGlom)[,"Family"]
glomOTU = otu table(famGlom)
glomTable = merge(glomOTU,glomTax,by=0,all=TRUE)
rownames(glomTable) = glomTable[,"Family"]
glomTable$Row.names = NULL
```

```
glomTable$family = NULL
glomTable<-subset(glomTable, select=-c(Family))</pre>
glomTable2 = glomTable / rep(colSums(glomTable), each = nrow(glomTable))
glomTable3 = as.data.frame(t(glomTable2))
write.csv(glomTable3, file = "RA_Family.csv")
SnailFamilyRA2023<-merge(glomTable3, metadata, by = 0)</pre>
write.csv(SnailFamilyRA2023, file = "metadata_FamilyRA.csv")
family<-rownames(glomTable2)</pre>
uniqueFamily<-unique(family)</pre>
SnailFamilyRAGather<-gather(SnailFamilyRA2023, uniqueFamily , key="Family", value = "Abundanc
e")
snailMeans <- summarySE(SnailFamilyRAGather, measurevar="Abundance", groupvars=c("treatmentOrde</pre>
r", "Family"))
write.csv(snailMeans, file="RAFamilyMeans.csv")
snailMeans2 <- summarySE(SnailFamilyRAGather, measurevar="Abundance", groupvars="Family")</pre>
write.csv(snailMeans2, file="RAFamilyMeansOverall.csv")
```

Beta Diversity

library(compositions)

```
# calc aitchison distance

clr.asvTable <- compositions::clr(snailPhyloCSS@otu_table)

#transform so columns are rows and vice versa
clr<-as.data.frame(clr.asvTable)
clr = t(clr)

aitchison.distances <- vegan::vegdist(clr, method = "euclid", na.rm=TRUE)
AD<-as.matrix(aitchison.distances)

# PERMUTATION TEST TO SEE IF VARIANCES DIFFER BY GROUPS
ADTreat<-vegan::betadisper(aitchison.distances, snailPhyloCSS@sam_data$treatmentOrder)
permutest(ADTreat, pairwise=FALSE, permutations=1000)</pre>
```

Aitchinson Adonis by scaled continuous terms.

adonis2(aitchison.distances~snailPhyloCSS@sam_data\$treatmentOrder+snailPhyloCSS@sam_data\$scaled BiteRate+snailPhyloCSS@sam_data\$scaledSpeed + snailPhyloCSS@sam_data\$scaledThig+snailPhyloCSS@s am_data\$scaledmetRate, method = "euclidian", by= "terms")

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = aitchison.distances ~ snailPhyloCSS@sam_data$treatmentOrder + snailPhyloCS
S@sam_data$scaledBiteRate + snailPhyloCSS@sam_data$scaledSpeed + snailPhyloCSS@sam_data$scaledT
hig + snailPhyloCSS@sam_data$scaledmetRate, method = "euclidian", by = "terms")
                                       Df SumOfSqs
                                                        R2
                                                                F Pr(>F)
## snailPhyloCSS@sam data$treatmentOrder 3 4628 0.13418 4.6671 0.001 ***
## snailPhyloCSS@sam_data$scaledBiteRate 1
                                             325 0.00943 0.9842 0.428
## snailPhyloCSS@sam_data$scaledSpeed
                                              295 0.00856 0.8934 0.638
                                             412 0.01196 1.2478 0.116
## snailPhyloCSS@sam data$scaledThig
                                       1
## snailPhyloCSS@sam data$scaledmetRate 1
                                              403 0.01168 1.2186 0.146
                                       86
## Residual
                                             28425 0.82419
## Total
                                       93
                                             34488 1.00000
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
```

```
#jaccard distance

otu_table<-snailPhyloCSS@otu_table

otu_table = t(otu_table)
  jaccard <- vegdist(otu_table, method = "jaccard", binary = TRUE)

# PERMUTATION TEST TO SEE IF VARIANCES DIFFER BY GROUPS
JTreat<-vegan::betadisper(jaccard, snailPhyloCSS@sam_data$treatmentOrder)
permutest(JTreat, pairwise=FALSE, permutations=1000)</pre>
```

adonis2(jaccard~snailPhyloCSS@sam_data\$treatmentOrder+snailPhyloCSS@sam_data\$scaledBiteRate+sna
ilPhyloCSS@sam_data\$scaledSpeed + snailPhyloCSS@sam_data\$scaledThig+snailPhyloCSS@sam_data\$scal
edmetRate, method = "jaccard", by= "terms")

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
## adonis2(formula = jaccard ~ snailPhyloCSS@sam_data$treatmentOrder + snailPhyloCSS@sam_data$s
caledBiteRate + snailPhyloCSS@sam_data$scaledSpeed + snailPhyloCSS@sam_data$scaledThig + snailP
hyloCSS@sam_data$scaledmetRate, method = "jaccard", by = "terms")
                                     Df SumOfSqs
                                                           F Pr(>F)
## snailPhyloCSS@sam_data$treatmentOrder 3
                                        2.8983 0.18423 6.8760 0.001 ***
## snailPhyloCSS@sam_data$scaledBiteRate 1 0.1449 0.00921 1.0313 0.394
                                    1 0.2184 0.01388 1.5542 0.061 .
## snailPhyloCSS@sam data$scaledSpeed
## snailPhyloCSS@sam_data$scaledThig
                                    1 0.1938 0.01232 1.3792 0.090 .
86 12.0832 0.76807
## Residual
## Total
                                     93 15.7319 1.00000
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Alpha diversity

```
alphaDiversitySnails<-estimate_richness(snailPhyloFilt, split = TRUE, measures = NULL)
alphaDiversitySnails<-alphaDiversitySnails%>%
  select(Chao1, Shannon, Observed)
# add a-div dataframe to metadata df
metadataAlphaDiversity <- merge(metadata, alphaDiversitySnails, by="row.names")
names(metadataAlphaDiversity)</pre>
```

```
## [1] "Row.names"
                            "treatment"
                                                 "treatmentOrder"
## [4] "aquarium"
                            "extractionRound"
                                                 "Thigmotaxis"
## [7] "Speed"
                             "metabolicRate"
                                                 "Pretraining"
## [10] "Posttraining"
                            "rawbiteRateChange" "biteRateChange"
## [13] "scaledThig"
                            "scaledSpeed"
                                                 "scaledBiteRate"
## [16] "scaledmetRate"
                            "Chao1"
                                                 "Shannon"
## [19] "Observed"
```

```
write.table(metadataAlphaDiversity, file = "metadata_alphaDiversity.txt",sep="\t",row.names=FAL
SE)
```

clear environment and load packages and metadata for GLMM and pearsons corr tests

```
rm(list = ls(all.names = TRUE))
library(usdm)
library(MuMIn)
metadataFull<-read.csv(file="metadata_full.csv",header=TRUE)</pre>
```

Principal component and colinearity tests

```
#Speed and thigmotaxis are measures of exploration in the same test - candidate for principle
component of these two traits?
names(metadataFull)[names(metadataFull) == "biteRateChange"] <- "rawbiteRateChange"</pre>
metadataFull$biteRateChange = metadataFull$rawbiteRateChange*(-1)
##scale variables
metadataFull$scaledThig<-scale(metadataFull$Thigmotaxis)</pre>
metadataFull$scaledSpeed<-scale(metadataFull$Speed)</pre>
metadataFull$scaledBiteRate<-scale(metadataFull$biteRateChange)</pre>
metadataFull$scaledmetRate<-scale(metadataFull$metabolicRate)</pre>
names(metadataFull)
   [1] "uniqueID"
                                                  "Speed"
                             "Thigmotaxis"
## [4] "Pretraining"
                             "Posttraining"
                                                  "rawbiteRateChange"
## [7] "treatment"
                             "treatmentOrder"
                                                  "aquarium"
## [10] "metabolicRate"
                             "biteRateChange"
                                                  "scaledThig"
## [13] "scaledSpeed"
                             "scaledBiteRate"
                                                  "scaledmetRate"
PCA<-data.frame((metadataFull$Thigmotaxis), (metadataFull$Speed))</pre>
```

```
PCA<-data.frame((metadataFull$Thigmotaxis), (metadataFull$Speed))
pcaout <- prcomp(PCA, center = TRUE, scale = TRUE )

pcaout<-prcomp(PCA)
eigen<-pcaout$sdev^2
eigen</pre>
```

```
## [1] 0.08400490 0.03672284
```

```
#below 1 eigen values therefore no justification to analyse speed and thigmotaxis as a principl
e component

# check if variables are colinear before including all in GLMM:

df<-data.frame(metadataFull$scaledmetRate, metadataFull$scaledThig, metadataFull$scaledSpeed, m
etadataFull$scaledBiteRate)
vif(df)</pre>
```

```
## Variables VIF
## 1 metadataFull.scaledmetRate 1.151929
## 2 metadataFull.scaledThig 1.158081
## 3 metadataFull.scaledSpeed 1.109845
## 4 metadataFull.scaledBiteRate 1.098563
```

```
# all around 1 therefore no colinearity, can include in same model
# clean up environment
rm(PCA, pcaout, df, eigen, control)
```

Warning in rm(PCA, pcaout, df, eigen, control): object 'control' not found

Correlation tests with bootstrap

```
# credit to bootstrapping tutorial https://www.datawim.com/post/bootstrapping-correlation-coeff
icients-in-r/
library(dplyr)
library(tidyverse)
library(tidymodels)
library(rstatix)
library(ggpubr)
metadataFull<-read.csv(file="metadata_full.csv",header=TRUE)</pre>
names(metadataFull)[names(metadataFull) == "biteRateChange"] <- "rawbiteRateChange"</pre>
metadataFull$biteRateChange = metadataFull$rawbiteRateChange*(-1)
##thigmotaxis metabolic rate
point <- metadataFull %>%
  group_by(treatmentOrder) %>%
  cor_test(Thigmotaxis, metabolicRate, method = "pearson")
point
boot_corr <- metadataFull %>%
  nest(data = -c(treatmentOrder)) %>% # grouping the treatments
  mutate(boots = map(data, ~bootstraps(.x, times = 2000, apparent = FALSE))) %>% # defining boo
tstraps
  unnest(boots) %>% # un-nesting bootsrapped data lists
  mutate(correlations = map(splits, ~cor_test(Thigmotaxis, metabolicRate, data = analysis
((.)))) # performing correlation
corr <- boot_corr %>%
  unnest(correlations) %>% # unnesting tidied data frames
  select(-data, -splits, -id)
corr
#confidence intervals from bootstrap
CI <- corr %>%
  group_by(treatmentOrder) %>%
        summarise(lwr CI = quantile(cor, 0.025),
                  .estimate = median(cor),
                  upr CI = quantile(cor, 0.975))
##speed metabolic rate
point1 <- metadataFull %>%
  group_by(treatmentOrder) %>%
  cor_test(metabolicRate, Speed, method = "pearson")
point1
boot_corr <- metadataFull %>%
  nest(data = -c(treatmentOrder)) %>% # grouping the treatments
  mutate(boots = map(data, ~bootstraps(.x, times = 2000, apparent = FALSE))) %>% # defining boo
tstraps
  unnest(boots) %>% # un-nesting bootsrapped data lists
  mutate(correlations = map(splits, ~cor_test(Speed, metabolicRate, data = analysis((.))))) # p
```

```
erforming correlation
corr <- boot_corr %>%
  unnest(correlations) %>% # unnesting tidied data frames
  select(-data, -splits, -id)
#confidence intervals from bootstrap
CI_1 <- corr %>%
  group_by(treatmentOrder) %>%
        summarise(lwr_CI = quantile(cor, 0.025),
                  .estimate = median(cor),
                  upr_CI = quantile(cor, 0.975))
##biteRate Thigmotaxis
point2 <- metadataFull %>%
  group_by(treatmentOrder) %>%
  cor_test(biteRateChange, Thigmotaxis, method = "pearson")
point2
boot corr <- metadataFull %>%
  nest(data = -c(treatmentOrder)) %>% # grouping the treatments
  mutate(boots = map(data, ~bootstraps(.x, times = 2000, apparent = FALSE))) %>% # defining boo
tstraps
  unnest(boots) %>% # un-nesting bootsrapped data lists
  mutate(correlations = map(splits, ~cor_test(biteRateChange, Thigmotaxis, data = analysis
((.)))) # performing correlation
corr <- boot_corr %>%
  unnest(correlations) %>% # unnesting tidied data frames
  select(-data, -splits, -id)
#confidence intervals from bootstrap
CI_2 <- corr %>%
  group_by(treatmentOrder) %>%
        summarise(lwr_CI = quantile(cor, 0.025),
                  .estimate = median(cor),
                  upr_CI = quantile(cor, 0.975))
##biteRate Speed
point3 <- metadataFull %>%
  group_by(treatmentOrder) %>%
  cor test(biteRateChange, Speed, method = "pearson")
point3
boot corr <- metadataFull %>%
  nest(data = -c(treatmentOrder)) %>% # grouping the treatments
  mutate(boots = map(data, ~bootstraps(.x, times = 2000, apparent = FALSE))) %>% # defining boo
tstraps
  unnest(boots) %>% # un-nesting bootsrapped data lists
  mutate(correlations = map(splits, ~cor_test(biteRateChange, Speed, data = analysis((.))))) #
performing correlation
corr <- boot corr %>%
  unnest(correlations) %>% # unnesting tidied data frames
```

```
select(-data, -splits, -id)
#confidence intervals from bootstrap
CI_3 <- corr %>%
  group_by(treatmentOrder) %>%
        summarise(lwr_CI = quantile(cor, 0.025),
                  .estimate = median(cor),
                  upr_CI = quantile(cor, 0.975))
##biteRate metabolic rate
point4 <- metadataFull %>%
  group_by(treatmentOrder) %>%
  cor_test(biteRateChange, metabolicRate, method = "pearson")
point4
boot_corr <- metadataFull %>%
  nest(data = -c(treatmentOrder)) %>% # grouping the treatments
  mutate(boots = map(data, ~bootstraps(.x, times = 2000, apparent = FALSE))) %>% # defining boo
tstraps
  unnest(boots) %>% # un-nesting bootsrapped data lists
  mutate(correlations = map(splits, ~cor_test(biteRateChange, metabolicRate, data = analysis
((.)))) # performing correlation
corr <- boot corr %>%
  unnest(correlations) %>% # unnesting tidied data frames
  select(-data, -splits, -id)
#confidence intervals from bootstrap
CI_4 <- corr %>%
  group_by(treatmentOrder) %>%
        summarise(lwr_CI = quantile(cor, 0.025),
                  .estimate = median(cor),
                  upr_CI = quantile(cor, 0.975))
##Thiqmotaxis Speed
point5 <- metadataFull %>%
  group_by(treatmentOrder) %>%
  cor test(Thigmotaxis, Speed, method = "pearson")
point5
boot corr <- metadataFull %>%
  nest(data = -c(treatmentOrder)) %>% # grouping the treatments
  mutate(boots = map(data, ~bootstraps(.x, times = 2000, apparent = FALSE))) %>% # defining boo
tstraps
  unnest(boots) %>% # un-nesting bootsrapped data Lists
  mutate(correlations = map(splits, ~cor_test(Thigmotaxis, Speed, data = analysis((.))))) # per
forming correlation
corr <- boot corr %>%
  unnest(correlations) %>% # unnesting tidied data frames
  select(-data, -splits, -id)
#confidence intervals from bootstrap
CI_5 <- corr %>%
```

GLMMs

```
#treatments on behaviours

model4<-lmer(metabolicRate~ treatment + (1|aquarium), data = metadataFull)
summary(model4)</pre>
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: metabolicRate ~ treatment + (1 | aquarium)
     Data: metadataFull
##
##
## REML criterion at convergence: -42.2
##
## Scaled residuals:
##
       Min
                1Q Median
                                3Q
                                        Max
## -1.67373 -0.52076 0.00754 0.46166 2.49007
##
## Random effects:
## Groups
           Name
                     Variance Std.Dev.
##
  aquarium (Intercept) 0.01756 0.1325
## Residual
                      0.02236 0.1495
## Number of obs: 107, groups: aquarium, 49
##
## Fixed effects:
                                       df t value Pr(>|t|)
##
                Estimate Std. Error
## (Intercept)
                 ## treatmenthigh 0.10381 0.06346 66.30614 1.636
                                                   0.107
                  0.05787 0.06529 74.35471 0.886
## treatmentlow
                                                     0.378
## treatmentmedium 0.07914 0.06854 54.41979 1.155
                                                     0.253
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##
             (Intr) trtmnth trtmntl
## treatmnthgh -0.728
## treatmentlw -0.678 0.493
## treatmntmdm -0.702 0.511
                            0.476
```

```
model6<-lmer(biteRateChange~treatment+(1|aquarium), data = metadataFull)
summary(model6)</pre>
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: biteRateChange ~ treatment + (1 | aquarium)
      Data: metadataFull
##
##
## REML criterion at convergence: 745.9
##
## Scaled residuals:
##
       Min
                 1Q Median
                                  3Q
## -2.67315 -0.57976 0.02409 0.70177 2.44678
##
## Random effects:
## Groups
           Name
                       Variance Std.Dev.
## aquarium (Intercept) 1.008
                                1.004
                                8.429
## Residual
                       71.048
## Number of obs: 107, groups: aquarium, 49
##
## Fixed effects:
##
                  Estimate Std. Error
                                          df t value Pr(>|t|)
                             1.8222 48.9878 4.335 7.24e-05 ***
                   7.8986
## (Intercept)
                              2.3684 45.2299 -3.044 0.00388 **
## treatmenthigh -7.2096
## treatmentlow
                  0.2523
                             2.4768 46.5907 0.102 0.91929
## treatmentmedium -5.6741
                             2.4591 38.2166 -2.307 0.02654 *
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
## Correlation of Fixed Effects:
##
              (Intr) trtmnth trtmntl
## treatmnthgh -0.769
## treatmentlw -0.735 0.565
## treatmntmdm -0.741 0.570
                             0.544
```

```
model7<-lmer(Thigmotaxis~treatment+(1|aquarium), data = metadataFull)</pre>
```

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

summary(model7)

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: Thigmotaxis ~ treatment + (1 | aquarium)
     Data: metadataFull
##
##
## REML criterion at convergence: -21.9
##
## Scaled residuals:
       Min
                1Q Median
                                 3Q
## -2.96059 -0.54052 0.08709 0.74284 1.62195
##
## Random effects:
## Groups Name
                      Variance Std.Dev.
## aquarium (Intercept) 0.00000 0.0000
## Residual
                      0.04166 0.2041
## Number of obs: 107, groups: aquarium, 49
##
## Fixed effects:
##
                  Estimate Std. Error
                                           df t value Pr(>|t|)
                  ## (Intercept)
## treatmenthigh
                 0.03533 0.05653 103.00000
                                                0.625
                                                        0.533
## treatmentlow
                 0.04970 0.05913 103.00000 0.841
                                                        0.403
## treatmentmedium -0.02538 0.05862 103.00000 -0.433
                                                      0.666
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## Correlation of Fixed Effects:
##
              (Intr) trtmnth trtmntl
## treatmnthgh -0.770
## treatmentlw -0.736 0.567
## treatmntmdm -0.742 0.571
                            0.546
## optimizer (nloptwrap) convergence code: 0 (OK)
## boundary (singular) fit: see help('isSingular')
```

```
model8<-lmer(Speed~treatment+(1|aquarium), data = metadataFull)</pre>
```

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

```
summary(model8)
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: Speed ~ treatment + (1 | aquarium)
     Data: metadataFull
##
##
## REML criterion at convergence: 45.9
##
## Scaled residuals:
##
       Min
                 10 Median
                                   3Q
## -2.16107 -0.67061 -0.00073 0.63261 2.82908
##
## Random effects:
## Groups Name
                        Variance Std.Dev.
## aquarium (Intercept) 0.00000 0.0000
## Residual
                        0.08047 0.2837
## Number of obs: 107, groups: aquarium, 49
##
## Fixed effects:
##
                   Estimate Std. Error
                                             df t value Pr(>|t|)
                   0.65747 0.06048 103.00000 10.871
                                                          <2e-16 ***
## (Intercept)
## treatmenthigh
                  0.05829
                               0.07856 103.00000
                                                  0.742
                                                           0.460
## treatmentlow
                    0.02056 0.08217 103.00000 0.250
                                                           0.803
## treatmentmedium -0.03163 0.08147 103.00000 -0.388
                                                           0.699
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##
              (Intr) trtmnth trtmntl
## treatmnthgh -0.770
## treatmentlw -0.736 0.567
## treatmntmdm -0.742 0.571
                              0.546
## optimizer (nloptwrap) convergence code: 0 (OK)
## boundary (singular) fit: see help('isSingular')
#alpha diversity Shannon and Chao1
alphaDiversitySnails <-read.table("metadata_alphaDiversity.txt", row.names="Row.names", header
= TRUE)
# shannon
```

model1<-lmer(Shannon~scaledSpeed*treatmentOrder + scaledThig*treatmentOrder + scaledmetRate*tre
atmentOrder+ scaledBiteRate*treatmentOrder +treatmentOrder+ (1|aquarium), data=alphaDiversitySn</pre>

ails, na.action=na.fail)

summary(model1)

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula:
## Shannon ~ scaledSpeed * treatmentOrder + scaledThig * treatmentOrder +
      scaledmetRate * treatmentOrder + scaledBiteRate * treatmentOrder +
##
      treatmentOrder + (1 | aquarium)
##
##
      Data: alphaDiversitySnails
##
## REML criterion at convergence: 128.3
##
## Scaled residuals:
##
      Min
               10 Median
                               3Q
                                      Max
## -2.7813 -0.3677 0.1397 0.4820 1.8566
##
## Random effects:
## Groups
            Name
                        Variance Std.Dev.
   aquarium (Intercept) 0.04712 0.2171
   Residual
                        0.11361 0.3371
## Number of obs: 94, groups: aquarium, 46
##
## Fixed effects:
##
                                       Estimate Std. Error
                                                                 df t value
                                                 0.114882 51.991051 33.610
## (Intercept)
                                       3.861159
## scaledSpeed
                                       0.014103 0.087381 72.739137
                                                                      0.161
## treatmentOrderlow
                                       0.034565
                                                 0.159318 47.289894
                                                                      0.217
## treatmentOrdermedium
                                       0.356660 0.154331 44.927757
                                                                      2.311
## treatmentOrderxHigh
                                       0.253167
                                                 0.156532 44.732861
                                                                      1.617
## scaledThig
                                       0.353
## scaledmetRate
                                       0.125011
                                                 0.109043 73.921213
                                                                      1.146
## scaledBiteRate
                                      -0.098595
                                                 0.090266 72.847669 -1.092
## scaledSpeed:treatmentOrderlow
                                       0.062353
                                                 0.148094 64.376065
                                                                      0.421
## scaledSpeed:treatmentOrdermedium
                                       0.051362
                                                 0.121960 65.819225 0.421
## scaledSpeed:treatmentOrderxHigh
                                      -0.118113
                                                 0.125816 73.217396 -0.939
## treatmentOrderlow:scaledThig
                                       0.036196
                                                 0.143218 73.216544 0.253
## treatmentOrdermedium:scaledThig
                                       0.150332
                                                 0.150083 73.936446
                                                                      1.002
## treatmentOrderxHigh:scaledThig
                                                  0.145759 73.846197 -0.064
                                      -0.009395
## treatmentOrderlow:scaledmetRate
                                      -0.198571
                                                  0.139511 72.793567 -1.423
## treatmentOrdermedium:scaledmetRate
                                       0.058081
                                                  0.143865 72.873307
                                                                      0.404
## treatmentOrderxHigh:scaledmetRate
                                      -0.273563
                                                  0.160020 71.853472 -1.710
## treatmentOrderlow:scaledBiteRate
                                       0.156899
                                                  0.146560 69.677525 1.071
## treatmentOrdermedium:scaledBiteRate -0.023406
                                                  0.143772 72.157449 -0.163
## treatmentOrderxHigh:scaledBiteRate
                                       0.101478
                                                  0.129412 73.987235
                                                                      0.784
##
                                      Pr(>|t|)
                                        <2e-16 ***
## (Intercept)
## scaledSpeed
                                        0.8722
## treatmentOrderlow
                                        0.8292
## treatmentOrdermedium
                                        0.0255 *
## treatmentOrderxHigh
                                        0.1128
## scaledThig
                                        0.7249
## scaledmetRate
                                        0.2553
## scaledBiteRate
                                        0.2783
## scaledSpeed:treatmentOrderlow
                                        0.6751
## scaledSpeed:treatmentOrdermedium
                                        0.6750
## scaledSpeed:treatmentOrderxHigh
                                        0.3509
## treatmentOrderlow:scaledThig
                                        0.8012
```

```
## treatmentOrdermedium:scaledThig
                                        0.3198
## treatmentOrderxHigh:scaledThig
                                        0.9488
## treatmentOrderlow:scaledmetRate
                                        0.1589
## treatmentOrdermedium:scaledmetRate
                                        0.6876
## treatmentOrderxHigh:scaledmetRate
                                        0.0917 .
## treatmentOrderlow:scaledBiteRate
                                        0.2881
## treatmentOrdermedium:scaledBiteRate
                                        0.8711
## treatmentOrderxHigh:scaledBiteRate
                                        0.4355
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
## Correlation matrix not shown by default, as p = 20 > 12.
## Use print(x, correlation=TRUE) or
                     if you need it
##
      vcov(x)
dd<-dredge(model1, subset= ~treatmentOrder, evaluate=TRUE, rank=AICc)</pre>
## Warning in dredge(model1, subset = ~treatmentOrder, evaluate = TRUE, rank =
## AICc): comparing models fitted by REML
## Fixed term is "(Intercept)"
dd
```

```
### Global model call: lmer(formula = Shannon ~ scaledSpeed * treatmentOrder + scaledThig *
      treatmentOrder + scaledmetRate * treatmentOrder + scaledBiteRate *
##
      treatmentOrder + treatmentOrder + (1 | aquarium), data = alphaDiversitySnails,
##
      na.action = na.fail)
## ---
## Model selection table
      (Int) sBR scR scS scT trO sBR:trO scR:trO scS:trO
## 17 3.755
## 25 3.760
                                   0.07368
## 19 3.773
             0.04742
## 18 3.774 -0.03086
## 21 3.753
                           0.015240
## 26 3.774 -0.02404
                                   0.07208
           0.01933
## 27 3.766
                                   0.06701
## 29 3.760
                          -0.007105 0.07587
## 20 3.795 -0.03570 0.05055
## 23 3.771
                  0.04582 0.009522
## 83 3.817
                  0.18390
## 22 3.771 -0.02857 0.009464
## 281 3.766
                                   0.13780
## 28 3.784 -0.02714 0.02318
                                   0.06393
## 30 3.777 -0.02692 -0.012420 0.07555
## 91 3.804 0.13450
                                   0.07091
## 31 3.767 0.01950 -0.007846 0.06939
## 50 3.851 -0.14850
## 24 3.794 -0.03539 0.05006 0.001703
## 149 3.746
                           0.069630
## 84 3.829 -0.02513 0.17700
            0.18060 0.011570
## 87 3.814
## 282 3.787 -0.03583
                                   0.12580
## 283 3.773 0.02059
                                   0.12870
## 285 3.767
                         -0.009555 0.14470
## 157 3.760
                          0.021480 0.08079
## 58 3.835 -0.11830
                                   0.06231
## 32 3.788 -0.03063 0.02420 -0.014160 0.06758
## 92 3.814 -0.01813 0.13070
            0.13500 -0.005435 0.07257
## 95 3.805
## 52 3.858 -0.14070 0.03568
## 54 3.848 -0.14630
                           0.010900
## 151 3.763 0.04426 0.061550
                     0.065880
## 150 3.769 -0.03840
## 88 3.827 -0.02350 0.17550 0.006771
## 284 3.799 -0.04129 0.02831
                                   0.11150
## 286 3.790 -0.03739 -0.013390 0.13490
## 287 3.774 0.02049 -0.009634 0.13580
## 158 3.781 -0.03520
                           0.018530 0.07976
## 159 3.765 0.01680 0.021710 0.07525
## 60 3.839 -0.11740 0.01458
                                   0.05752
## 62 3.836 -0.11880
                          -0.007538 0.06469
## 96 3.816 -0.02043 0.13120 -0.009447 0.07194
## 152 3.793 -0.04488 0.04960 0.056270
## 56 3.856 -0.13990 0.03450 0.005422
            0.12370
## 347 3.802
                                   0.08671
## 116 3.880 -0.11510 0.15240
             0.17210 0.041890
## 215 3.809
```

```
## 288 3.803 -0.04302 0.02855 -0.014150 0.12110
## 160 3.791 -0.03868 0.02292 0.018600 0.07220
## 314 3.825 -0.09853
                                   0.10370
## 413 3.772
                          -0.020410 0.15010
## 64 3.840 -0.11790 0.01534 -0.008814 0.06009
## 124 3.853 -0.08828 0.10760
            0.12520 -0.002233 0.08451 +
## 223 3.807
## 348 3.823 -0.03645 0.12230
                                  0.07462 +
## 182 3.837 -0.13800 0.052120
## 351 3.801 0.12390 0.001415 0.08583 +
## 120 3.877 -0.11360 0.14970 0.010790
## 216 3.827 -0.03286 0.16360 0.039660
## 414 3.796 -0.04265
                      -0.013260 0.13100
## 316 3.833 -0.09810 0.02555 0.09270 +
## 318 3.826 -0.09735 -0.007068 0.10930
## 190 3.831 -0.11240
                           0.015950 0.06732
## 224 3.822 -0.02775 0.11910 -0.003415 0.08309
## 128 3.853 -0.08868 0.10810 -0.006084 0.07738
## 184 3.846 -0.13140 0.03462 0.047150
## 352 3.823 -0.03675 0.12150 -0.002383 0.07677
## 416 3.807 -0.04911 0.02932 -0.008116 0.11280
## 320 3.833 -0.09669 0.02584 -0.008290 0.09920
## 192 3.835 -0.11130 0.01432 0.016190 0.06301
## 380 3.858 -0.09606 0.12020 0.05463
## 248 3.871 -0.11000 0.14440 0.032570
## 446 3.828 -0.09794
                    -0.005017 0.10590 +
## 256 3.855 -0.08539 0.10410 -0.008733 0.08432 +
## 384 3.858 -0.09714 0.12190 0.006764 0.04899
## 480 3.830 -0.04340 0.12470 0.005244 0.06558
## 448 3.834 -0.09804 0.02548 -0.001296 0.09260
## 512 3.861 -0.09859 0.12500 0.014100 0.04016 +
##
      scT:trO df logLik AICc delta weight
## 17
             6 -49.802 112.6 0.00 0.746
## 25
              7 -50.386 116.1 3.50 0.129
## 19
             7 -51.441 118.2 5.61 0.045
## 18
              7 -51.751 118.8 6.23 0.033
## 21
             7 -52.019 119.3 6.77 0.025
             8 -52.427 122.5 9.98 0.005
## 26
## 27
             8 -52.461 122.6 10.05 0.005
## 29
             8 -52.624 122.9 10.37 0.004
## 20
             8 -53.311 124.3 11.75 0.002
## 23
             8 -53.695 125.1 12.51 0.001
## 83
            10 -51.271 125.2 12.62 0.001
## 22
             8 -53.982 125.7 13.09 0.001
## 281
          + 10 -52.646 127.9 15.37 0.000
## 28
             9 -54.453 129.0 16.48 0.000
## 30
             9 -54.612 129.4 16.80 0.000
## 91
             11 -52.101 129.4 16.85 0.000
## 31
             9 -54.693 129.5 16.96 0.000
## 50
             10 -53.928 130.5 17.94 0.000
## 24
             9 -55.556 131.3 18.68 0.000
             10 -54.531 131.7 19.14 0.000
## 149
## 84
             11 -53.297 131.8 19.24 0.000
## 87
             11 -53.533 132.3 19.72 0.000
```

```
## 282
             + 11 -54.491 134.2 21.63 0.000
             + 11 -54.705 134.6 22.06
## 283
                                        0.000
## 285
             + 11 -54.807 134.8 22.26
                                        0.000
## 157
               11 -55.006 135.2 22.66
                                        0.000
## 58
               11 -55.105 135.4 22.86
                                        0.000
## 32
               10 -56.621 135.9 23.32
                                        0.000
## 92
               12 -54.199 136.3 23.68
                                        0.000
## 95
               12 -54.370 136.6 24.02
                                        0.000
## 52
               11 -55.796 136.8 24.24
                                        0.000
## 54
               11 -56.149 137.5 24.95
                                        0.000
## 151
               11 -56.243 137.7 25.13
                                        0.000
## 150
               11 -56.341 137.9 25.33
                                        0.000
## 88
               12 -55.554 139.0 26.39
                                        0.000
## 284
             + 12 -56.450 140.8 28.18
                                        0.000
## 286
             + 12 -56.623 141.1 28.53
                                        0.000
## 287
             + 12 -56.862 141.6 29.01
                                        0.000
## 158
               12 -56.873 141.6 29.03
                                        0.000
## 159
               12 -57.074 142.0 29.43
                                        0.000
## 60
               12 -57.188 142.2 29.66
                                        0.000
## 62
               12 -57.307 142.5 29.89
                                        0.000
               13 -56.423 143.4 30.83
## 96
                                        0.000
               12 -57.931 143.7 31.14
## 152
                                        0.000
## 56
               12 -58.026 143.9 31.33
                                        0.000
## 347
             + 14 -55.421 144.2 31.59
                                        0.000
               14 -55.831 145.0 32.41
## 116
                                        0.000
## 215
               14 -56.293 145.9 33.33
                                        0.000
             + 13 -58.574 147.7 35.13
## 288
                                        0.000
## 160
               13 -58.875 148.3 35.73
                                        0.000
             + 14 -57.526 148.4 35.80
## 314
                                        0.000
## 413
             + 14 -57.554 148.4 35.85
                                        0.000
## 64
               13 -59.374 149.3 36.73
                                        0.000
## 124
               15 -56.616 149.4 36.82
                                        0.000
               15 -56.710 149.6 37.00
## 223
                                        0.000
## 348
             + 15 -57.247 150.6 38.08
                                        0.000
## 182
               14 -58.688 150.7 38.12
                                        0.000
## 351
             + 15 -57.601 151.4 38.79
                                        9.999
## 120
               15 -58.061 152.3 39.70
                                        0.000
## 216
               15 -58.188 152.5 39.96
                                        0.000
             + 15 -59.275 154.7 42.13
## 414
                                        0.000
             + 15 -59.498 155.1 42.58
## 316
                                        0.000
## 415
             + 15 -59.591 155.3 42.77
                                        0.000
## 318
             + 15 -59.663 155.5 42.91
                                        0.000
## 190
               15 -59.781 155.7 43.15
                                        0.000
## 224
               16 -58.680 156.4 43.86
                                        0.000
## 128
               16 -58.845 156.8 44.19
                                        0.000
## 184
               15 -60.554 157.3 44.69
                                        0.000
## 352
             + 16 -59.417 157.9 45.33
                                        0.000
## 416
             + 16 -61.188 161.4 48.87
                                        0.000
## 320
             + 16 -61.625 162.3 49.75
                                        0.000
## 192
               16 -61.828 162.7 50.15
                                        0.000
## 380
             + 18 -59.539 164.2 51.63
                                        0.000
## 479
             + 18 -59.996 165.1 52.54
                                        0.000
## 248
               18 -60.851 166.8 54.25
                                        0.000
## 446
             + 18 -62.298 169.7 57.15
                                        0.000
## 256
               19 -61.435 171.1 58.57
                                        0.000
## 384
             + 19 -61.679 171.6 59.06 0.000
```

```
ddAIC_S<-subset(dd, delta < 7)
ddAIC_S</pre>
```

```
### Global model call: lmer(formula = Shannon ~ scaledSpeed * treatmentOrder + scaledThig *
      treatmentOrder + scaledmetRate * treatmentOrder + scaledBiteRate *
      treatmentOrder + treatmentOrder + (1 | aquarium), data = alphaDiversitySnails,
##
      na.action = na.fail)
##
## ---
## Model selection table
      (Int)
                sBR
                        scR
                                scS
                                        scT trO df logLik AICc delta weight
## 17 3.755
                                              + 6 -49.802 112.6 0.00 0.762
## 25 3.760
                                    0.07368
                                            + 7 -50.386 116.1 3.50 0.132
## 19 3.773
                    0.04742
                                              + 7 -51.441 118.2 5.61 0.046
## 18 3.774 -0.03086
                                             + 7 -51.751 118.8 6.23 0.034
## 21 3.753
                            0.01524
                                             + 7 -52.019 119.3 6.77 0.026
## Models ranked by AICc(x)
## Random terms (all models):
   1 | aquarium
```

```
get.models(ddAIC_S, subset=TRUE)
```

```
## $\17\
## Linear mixed model fit by REML ['lmerModLmerTest']
## Formula: Shannon ~ treatmentOrder + (1 | aquarium)
      Data: alphaDiversitySnails
## REML criterion at convergence: 99.6048
## Random effects:
  Groups
                         Std.Dev.
             Name
   aquarium (Intercept) 0.2195
##
   Residual
                         0.3420
## Number of obs: 94, groups: aquarium, 46
## Fixed Effects:
##
            (Intercept)
                            treatmentOrderlow treatmentOrdermedium
                 3.7550
                                       0.1813
                                                              0.4614
##
##
   treatmentOrderxHigh
##
                 0.3066
##
## $\ 25\
## Linear mixed model fit by REML ['lmerModLmerTest']
## Formula: Shannon ~ scaledThig + treatmentOrder + (1 | aquarium)
      Data: alphaDiversitySnails
## REML criterion at convergence: 100.7712
## Random effects:
## Groups
                         Std.Dev.
             Name
## aquarium (Intercept) 0.2008
  Residual
                         0.3434
## Number of obs: 94, groups: aquarium, 46
## Fixed Effects:
##
            (Intercept)
                                   scaledThig
                                                  treatmentOrderlow
                3.75953
                                      0.07368
                                                             0.16574
##
## treatmentOrdermedium treatmentOrderxHigh
##
                0.47017
                                      0.28962
##
## $`19`
## Linear mixed model fit by REML ['lmerModLmerTest']
## Formula: Shannon ~ scaledmetRate + treatmentOrder + (1 | aquarium)
      Data: alphaDiversitySnails
## REML criterion at convergence: 102.8816
## Random effects:
## Groups
             Name
                         Std.Dev.
   aquarium (Intercept) 0.2290
   Residual
                         0.3379
## Number of obs: 94, groups: aquarium, 46
## Fixed Effects:
##
            (Intercept)
                                scaledmetRate
                                                  treatmentOrderlow
##
                3.77257
                                      0.04742
                                                             0.16943
## treatmentOrdermedium treatmentOrderxHigh
##
                0.43704
                                      0.27805
##
## $`18`
## Linear mixed model fit by REML ['lmerModLmerTest']
## Formula: Shannon ~ scaledBiteRate + treatmentOrder + (1 | aquarium)
##
      Data: alphaDiversitySnails
## REML criterion at convergence: 103.5021
## Random effects:
##
   Groups
                         Std.Dev.
             Name
```

```
## aquarium (Intercept) 0.2148
## Residual
                         0.3452
## Number of obs: 94, groups: aquarium, 46
## Fixed Effects:
##
            (Intercept)
                               scaledBiteRate
                                                  treatmentOrderlow
##
                3.77358
                                     -0.03086
                                                            0.17215
## treatmentOrdermedium treatmentOrderxHigh
                0.43791
##
                                      0.27159
##
## $\21\
## Linear mixed model fit by REML ['lmerModLmerTest']
## Formula: Shannon ~ scaledSpeed + treatmentOrder + (1 | aquarium)
      Data: alphaDiversitySnails
## REML criterion at convergence: 104.0373
## Random effects:
## Groups
            Name
                         Std.Dev.
## aquarium (Intercept) 0.2199
## Residual
                         0.3439
## Number of obs: 94, groups: aquarium, 46
## Fixed Effects:
                                                  treatmentOrderlow
##
            (Intercept)
                                  scaledSpeed
##
                3.75293
                                                            0.18290
                                      0.01524
## treatmentOrdermedium treatmentOrderxHigh
##
                0.46711
                                      0.30538
##
## attr(,"rank")
## function (x)
## do.call("rank", list(x))
## <environment: 0x0000024bc4c41868>
## attr(,"call")
## AICc(x)
## attr(,"class")
## [1] "function"
                      "rankFunction"
## attr(,"beta")
## [1] "none"
```

```
ddMAc<-model.avg(ddAIC_S, subset= delta <7)
summary(ddMAc)</pre>
```

```
##
## Call:
## model.avg(object = ddAIC_S, subset = delta < 7)</pre>
## Component model call:
## lmer(formula = Shannon ~ <5 unique rhs>, data = alphaDiversitySnails,
       na.action = na.fail)
##
## Component models:
##
     df logLik
                 AICc delta weight
     6 -49.80 112.57 0.00
                             0.76
## 45 7 -50.39 116.07 3.50
                             0.13
## 25 7 -51.44 118.18 5.61
                             0.05
## 15 7 -51.75 118.80 6.23
                             0.03
## 35 7 -52.02 119.34 6.77
                             0.03
##
## Term codes:
## scaledBiteRate scaledmetRate
                                  scaledSpeed
                                                 scaledThig treatmentOrder
                                            3
                                                          4
##
               1
                             2
                                                                         5
##
## Model-averaged coefficients:
## (full average)
##
                        Estimate Std. Error Adjusted SE z value Pr(>|z|)
## (Intercept)
                       3.7570045 0.0985995
                                             0.0999745 37.580 < 2e-16 ***
## treatmentOrderlow
                       0.1784445 0.1425961
                                              0.1445863 1.234 0.21714
## treatmentOrdermedium 0.4607763 0.1390980 0.1410369 3.267 0.00109 **
## treatmentOrderxHigh 0.3017943 0.1351256 0.1370051 2.203 0.02761 *
## scaledThig
                      0.0097428 0.0286678 0.0287661 0.339 0.73484
## scaledmetRate
                       0.0021830 0.0136882 0.0137798 0.158 0.87413
## scaledBiteRate
                      -0.0010418 0.0100162 0.0101139 0.103 0.91796
## scaledSpeed
                       0.0003936 0.0069620
                                              0.0070484
                                                         0.056 0.95547
##
## (conditional average)
                       Estimate Std. Error Adjusted SE z value Pr(>|z|)
## (Intercept)
                       3.75700
                                  0.09860
                                              0.09997 37.580 < 2e-16 ***
                       0.17844
                                              0.14459 1.234 0.21714
## treatmentOrderlow
                                  0.14260
## treatmentOrdermedium 0.46078
                                              0.14104
                                                       3.267 0.00109 **
                                  0.13910
## treatmentOrderxHigh 0.30179
                                  0.13513
                                              0.13701 2.203 0.02761 *
## scaledThig
                       0.07368
                                  0.03878
                                              0.03933 1.873 0.06102 .
## scaledmetRate
                       0.04742
                                  0.04387
                                              0.04449 1.066 0.28648
                                              0.04594
## scaledBiteRate
                       -0.03086
                                  0.04530
                                                       0.672 0.50164
## scaledSpeed
                       0.01524
                                  0.04062
                                              0.04120
                                                       0.370 0.71147
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
rm(dd, ddAIC S, ddMAc)
```

```
rm(dd, ddAIC_S, ddMAc)

# chao1

model2<-lmer(Chao1~scaledSpeed*treatmentOrder + scaledThig*treatmentOrder + scaledmetRate*treat
mentOrder+ scaledBiteRate*treatmentOrder +treatmentOrder+ (1|aquarium), data=alphaDiversitySnai
ls, na.action=na.fail)
summary(model2)</pre>
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: Chao1 ~ scaledSpeed * treatmentOrder + scaledThig * treatmentOrder +
       scaledmetRate * treatmentOrder + scaledBiteRate * treatmentOrder +
##
##
       treatmentOrder + (1 | aquarium)
      Data: alphaDiversitySnails
##
##
## REML criterion at convergence: 1118.6
##
## Scaled residuals:
##
      Min
                10 Median
                                3Q
                                       Max
## -1.7213 -0.6180 -0.1688 0.3949 3.4344
##
## Random effects:
   Groups
                         Variance Std.Dev.
##
             Name
                           797.5
                                   28.24
##
   aquarium (Intercept)
    Residual
                         96886.7 311.27
## Number of obs: 94, groups: aquarium, 46
##
## Fixed effects:
##
                                       Estimate Std. Error
                                                                 df t value
## (Intercept)
                                                                     7.130
                                        597.295
                                                    83.767
                                                             52,003
                                                            73.664 -0.090
## scaledSpeed
                                         -6.408
                                                    71.491
## treatmentOrderlow
                                        -21.965
                                                   114.732
                                                             49.487 -0.191
## treatmentOrdermedium
                                        170.050
                                                   109.707
                                                             44.012 1.550
## treatmentOrderxHigh
                                                            42.990 0.416
                                         46.127
                                                  110.978
## scaledThig
                                         80.115
                                                    89.986
                                                            73.663 0.890
## scaledmetRate
                                         -9.905
                                                    87.632
                                                             73.983 -0.113
## scaledBiteRate
                                         -5.433
                                                    71.672
                                                             73.767 -0.076
## scaledSpeed:treatmentOrderlow
                                        -31.325
                                                   125.804
                                                             67.662 -0.249
## scaledSpeed:treatmentOrdermedium
                                        161.488
                                                   102.149
                                                             72.656
                                                                      1.581
## scaledSpeed:treatmentOrderxHigh
                                        -18.609
                                                   100.959
                                                             72.280 -0.184
## treatmentOrderlow:scaledThig
                                         26.832
                                                   117,500
                                                             72,770
                                                                      0.228
## treatmentOrdermedium:scaledThig
                                         19.322
                                                   120.979
                                                             73.959
                                                                      0.160
## treatmentOrderxHigh:scaledThig
                                        -24.745
                                                   117.652
                                                             73.925 -0.210
## treatmentOrderlow:scaledmetRate
                                        -46.855
                                                   110.141
                                                             72.956 -0.425
## treatmentOrdermedium:scaledmetRate
                                                             74.000
                                         94.729
                                                   116.973
                                                                      0.810
## treatmentOrderxHigh:scaledmetRate
                                       -123.250
                                                   124.655
                                                             69.739 -0.989
                                                             70.207
## treatmentOrderlow:scaledBiteRate
                                         19.375
                                                   122.499
                                                                      0.158
## treatmentOrdermedium:scaledBiteRate
                                        -91.362
                                                   116.991
                                                             73.884 -0.781
## treatmentOrderxHigh:scaledBiteRate
                                         53.037
                                                   102.633
                                                             71.293
                                                                      0.517
##
                                       Pr(>|t|)
                                       3.06e-09 ***
## (Intercept)
## scaledSpeed
                                          0.929
## treatmentOrderlow
                                          0.849
## treatmentOrdermedium
                                          0.128
## treatmentOrderxHigh
                                          0.680
## scaledThig
                                          0.376
## scaledmetRate
                                          0.910
## scaledBiteRate
                                          0.940
## scaledSpeed:treatmentOrderlow
                                          0.804
## scaledSpeed:treatmentOrdermedium
                                          0.118
## scaledSpeed:treatmentOrderxHigh
                                          0.854
## treatmentOrderlow:scaledThig
                                          0.820
## treatmentOrdermedium:scaledThig
                                          0.874
```

```
0.834
## treatmentOrderxHigh:scaledThig
## treatmentOrderlow:scaledmetRate
                                          0.672
## treatmentOrdermedium:scaledmetRate
                                          0.421
## treatmentOrderxHigh:scaledmetRate
                                          0.326
## treatmentOrderlow:scaledBiteRate
                                          0.875
## treatmentOrdermedium:scaledBiteRate
                                          0.437
## treatmentOrderxHigh:scaledBiteRate
                                          0.607
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
```

```
##
## Correlation matrix not shown by default, as p = 20 > 12.
## Use print(x, correlation=TRUE) or
## vcov(x) if you need it
```

model2<-lmer(Chao1~scaledSpeed + scaledThig + scaledmetRate+ scaledBiteRate +treatmentOrder+ (1
|aquarium), data=alphaDiversitySnails, na.action=na.fail)</pre>

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

```
summary(model2)
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: Chao1 ~ scaledSpeed + scaledThig + scaledmetRate + scaledBiteRate +
##
      treatmentOrder + (1 | aquarium)
##
     Data: alphaDiversitySnails
##
## REML criterion at convergence: 1260.4
##
## Scaled residuals:
##
      Min 1Q Median
                           3Q
                                   Max
## -1.4630 -0.6660 -0.1904 0.4812 3.5827
##
## Random effects:
## Groups Name
                      Variance Std.Dev.
## aquarium (Intercept)
                          0
                                0.0
                              309.1
## Residual
                      95536
## Number of obs: 94, groups: aquarium, 46
##
## Fixed effects:
                     Estimate Std. Error
                                           df t value Pr(>|t|)
##
                    577.8050 73.5052 86.0000 7.861 1.01e-11 ***
## (Intercept)
## scaledSpeed
                     20.4986 34.9952 86.0000 0.586 0.5596
## scaledThig
                     50.8947 35.2133 86.0000 1.445 0.1520
## scaledmetRate
                    -35.7522 35.2867 86.0000 -1.013 0.3138
## scaledBiteRate
                      0.3146 37.6821 86.0000 0.008 0.9934
## treatmentOrderlow
                      9.9219 96.9460 86.0000 0.102 0.9187
## treatmentOrderxHigh 13.5906 101.7547 86.0000 0.134 0.8941
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##
             (Intr) scldSp scldTh scldmR scldBR trtmntOrdrl trtmntOrdrm
## scaledSpeed -0.150
## scaledThig
              0.012 -0.196
## scaledmetRt 0.189 -0.154 -0.295
## scaledBitRt -0.364 0.212 0.109 -0.180
## trtmntOrdrl -0.703 0.082 -0.079 -0.065 0.133
## trtmntOrdrm -0.772 0.198 0.035 -0.203 0.378 0.525
## trtmntOrdrH -0.797 0.114 -0.012 -0.221 0.446 0.537
                                                        0.631
## optimizer (nloptwrap) convergence code: 0 (OK)
## boundary (singular) fit: see help('isSingular')
```

```
## Warning in dredge(model2, subset = ~treatmentOrder, evaluate = TRUE, rank =
## AICc): comparing models fitted by REML
```

dd<-dredge(model2, subset= ~treatmentOrder, evaluate=TRUE, rank=AICc)</pre>

```
## Fixed term is "(Intercept)"
## boundary (singular) fit: see help('isSingular')
```

dd

```
### Global model call: lmer(formula = Chao1 ~ scaledSpeed + scaledThig + scaledmetRate +
       scaledBiteRate + treatmentOrder + (1 | aquarium), data = alphaDiversitySnails,
##
##
      na.action = na.fail)
## ---
## Model selection table
      (Intrc)
                sclBR scldR scldS scldT trtmO df
                                                    logLik
                                                            AICc delta weight
##
       577.8
               0.3146 -35.75 20.50 50.89
                                            + 10 -630.178 1283.0 0.00 0.898
## 32
## 31
       578.0
                      -35.70 20.44 50.86
                                            + 9 -634.724 1289.6 6.58 0.033
## 28
       584.3 -4.3690 -32.57
                                   54.94
                                            + 9 -634.823 1289.8 6.78 0.030
## 30
       591.9 -6.5410
                             15.04 40.37
                                            + 9 -635.174 1290.5 7.48 0.021
       577.1 -5.2600 -20.75 30.52
                                             + 9 -635.696 1291.5 8.53 0.013
## 24
## 27
       581.3
                      -33.20
                                   55.58
                                            + 8 -639.348 1296.4 13.38 0.001
## 29
       587.5
                             16.20 40.73
                                            + 8 -639.719 1297.1 14.12 0.001
       595.8 -9.6030
## 26
                                   44.12
                                            + 8 -639.729 1297.2 14.14 0.001
## 23
       573.4
                      -21.48 31.68
                                             + 8 -640.251 1298.2 15.19 0.000
## 22
       586.0 -8.9420
                             25.76
                                             + 8 -640.327 1298.3 15.34 0.000
## 20
       586.8 -13.3700 -14.00
                                             + 8 -640.547 1298.8 15.78 0.000
                                            + 7 -644.271 1303.8 20.84 0.000
## 25
       589.6
                                   45.09
## 21
       580.2
                             27.51
                                             + 7 -644.885 1305.1 22.07
                                                                        0.000
## 18
       591.8 -15.1700
                                             + 7 -645.051 1305.4 22.40 0.000
## 19
       577.9
                                             + 7 -645.128 1305.6 22.55 0.000
                      -15.31
       582.2
                                             + 6 -649.644 1312.3 29.25 0.000
## 17
## Models ranked by AICc(x)
## Random terms (all models):
    1 | aquarium
##
```

```
ddAIC_C<-subset(dd, delta < 7)
ddAIC_C</pre>
```

```
## Global model call: lmer(formula = Chao1 ~ scaledSpeed + scaledThig + scaledmetRate +
      scaledBiteRate + treatmentOrder + (1 | aquarium), data = alphaDiversitySnails,
##
##
      na.action = na.fail)
## ---
## Model selection table
     (Intrc)
               sclBR scldR scldS scldT trtmO df
                                                  logLik
                                                          AICc delta weight
##
       577.8 0.3146 -35.75 20.50 50.89 + 10 -630.178 1283.0 0.00 0.934
## 32
                                        + 9 -634.724 1289.6 6.58 0.035
                     -35.70 20.44 50.86
## 31
       578.0
## 28
       584.3 -4.3690 -32.57
                                54.94 + 9 -634.823 1289.8 6.78 0.031
## Models ranked by AICc(x)
## Random terms (all models):
    1 | aquarium
```

```
get.models(ddAIC_C, subset=TRUE)
```

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

```
## $\32\
## Linear mixed model fit by REML ['lmerModLmerTest']
## Formula: Chao1 ~ scaledBiteRate + scaledmetRate + scaledSpeed + scaledThig +
       treatmentOrder + (1 | aquarium)
      Data: alphaDiversitySnails
##
## REML criterion at convergence: 1260.357
## Random effects:
  Groups
             Name
                         Std.Dev.
   aquarium (Intercept)
                           0.0
   Residual
                         309.1
## Number of obs: 94, groups: aquarium, 46
## Fixed Effects:
##
                              scaledBiteRate
            (Intercept)
                                                       scaledmetRate
               577.8050
                                                            -35.7522
##
                                       0.3146
##
            scaledSpeed
                                   scaledThig
                                                  treatmentOrderlow
                20.4986
                                                              9.9219
##
                                      50.8947
## treatmentOrdermedium treatmentOrderxHigh
               187.7763
##
                                      13.5906
## optimizer (nloptwrap) convergence code: 0 (OK); 0 optimizer warnings; 1 lme4 warnings
##
## $\31\
## Linear mixed model fit by REML ['lmerModLmerTest']
## Formula: Chao1 ~ scaledmetRate + scaledSpeed + scaledThig + treatmentOrder +
##
       (1 | aquarium)
##
      Data: alphaDiversitySnails
## REML criterion at convergence: 1269.447
## Random effects:
## Groups
             Name
                         Std.Dev.
## aquarium (Intercept)
                           0.0
## Residual
                         307.3
## Number of obs: 94, groups: aquarium, 46
## Fixed Effects:
##
            (Intercept)
                                scaledmetRate
                                                        scaledSpeed
##
                                      -35.699
                578.028
                                                              20.437
##
             scaledThig
                            treatmentOrderlow treatmentOrdermedium
##
                 50.863
                                        9.814
                                                             187.456
   treatmentOrderxHigh
##
##
                 13.212
## optimizer (nloptwrap) convergence code: 0 (OK); 0 optimizer warnings; 1 lme4 warnings
##
## $\28\
## Linear mixed model fit by REML ['lmerModLmerTest']
## Formula:
## Chao1 ~ scaledBiteRate + scaledmetRate + scaledThig + treatmentOrder +
##
       (1 | aquarium)
##
      Data: alphaDiversitySnails
## REML criterion at convergence: 1269.645
## Random effects:
## Groups
             Name
                         Std.Dev.
##
   aquarium (Intercept)
                           0.0
## Residual
                         307.9
## Number of obs: 94, groups: aquarium, 46
## Fixed Effects:
##
            (Intercept)
                               scaledBiteRate
                                                       scaledmetRate
##
                584,263
                                       -4.369
                                                             -32,570
```

```
##
             scaledThig treatmentOrderlow treatmentOrdermedium
##
                 54.939
                                       5.253
                                                           175.981
## treatmentOrderxHigh
                 6.810
##
## optimizer (nloptwrap) convergence code: 0 (OK); 0 optimizer warnings; 1 lme4 warnings
##
## attr(,"rank")
## function (x)
## do.call("rank", list(x))
## <environment: 0x0000024bd17f0418>
## attr(,"call")
## AICc(x)
## attr(,"class")
                    "rankFunction"
## [1] "function"
## attr(,"beta")
## [1] "none"
```

```
ddMAc<-model.avg(ddAIC_C, subset= delta <7)
summary(ddMAc)</pre>
```

```
##
## Call:
## model.avg(object = ddAIC_C, subset = delta < 7)</pre>
## Component model call:
## lmer(formula = Chao1 ~ <3 unique rhs>, data = alphaDiversitySnails,
       na.action = na.fail)
##
## Component models:
##
        df logLik
                      AICc delta weight
## 12345 10 -630.18 1283.01 0.00
## 2345
         9 -634.72 1289.59 6.58
                                   0.03
## 1245
         9 -634.82 1289.79 6.78
                                  0.03
##
## Term codes:
## scaledBiteRate scaledmetRate
                                   scaledSpeed
                                                  scaledThig treatmentOrder
                                                           4
                                                                         5
##
               1
                              2
                                            3
##
## Model-averaged coefficients:
## (full average)
##
                       Estimate Std. Error Adjusted SE z value Pr(>|z|)
                                  73.2970
                                              74.3672 7.772
                                                               <2e-16 ***
## (Intercept)
                       578.0159
## scaledBiteRate
                         0.1563
                                  36.9993
                                              37.5396
                                                        0.004
                                                               0.9967
## scaledmetRate
                       -35.6503
                                  35.2473
                                              35.7619
                                                        0.997
                                                               0.3188
## scaledSpeed
                        19.8516
                                  34.5913
                                              35.0913
                                                        0.566
                                                               0.5716
## scaledThig
                        51.0208 35.1808
                                             35.6944 1.429
                                                               0.1529
## treatmentOrderlow
                         9.7713 96.8789
                                            98.2936 0.099 0.9208
## treatmentOrdermedium 187.3941 101.2342 102.7121 1.824
                                                               0.0681 .
                                                               0.8966
## treatmentOrderxHigh
                        13.3642 101.3606
                                             102.8407
                                                        0.130
##
## (conditional average)
##
                       Estimate Std. Error Adjusted SE z value Pr(>|z|)
## (Intercept)
                        578,016
                                   73,297
                                               74.367
                                                        7.772
                                                                <2e-16 ***
                                                        0.004
## scaledBiteRate
                          0.162
                                   37.659
                                               38.209
                                                               0.9966
## scaledmetRate
                        -35.650
                                   35.247
                                               35.762
                                                        0.997
                                                               0.3188
## scaledSpeed
                                  34.960
                                               35.471
                                                        0.578
                         20.496
                                                               0.5634
## scaledThig
                                               35.694
                                                        1.429
                         51.021
                                   35.181
                                                               0.1529
## treatmentOrderlow
                         9.771
                                  96.879
                                               98.294
                                                        0.099
                                                               0.9208
## treatmentOrdermedium 187.394
                                  101.234
                                              102.712
                                                        1.824
                                                               0.0681 .
## treatmentOrderxHigh
                         13.364
                                  101.361
                                              102.841
                                                        0.130
                                                               0.8966
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
```

observed

 $\label{localization} model 3 < -lmer (Observed \sim scaled Speed * treatment 0 rder + scaled Thig * treatment 0 rder + scaled met Rate * treatment 0 rder + scaled Bite Rate * treatment 0 rder + (1 | aquarium), data = alpha Diversity Snails, na.action = na.fail)$

summary(model3)

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula:
## Observed ~ scaledSpeed * treatmentOrder + scaledThig * treatmentOrder +
       scaledmetRate * treatmentOrder + scaledBiteRate * treatmentOrder +
##
       treatmentOrder + (1 | aquarium)
##
      Data: alphaDiversitySnails
##
##
## REML criterion at convergence: 984.2
##
## Scaled residuals:
##
      Min
               10 Median
                                3Q
                                       Max
## -2.0418 -0.6155 -0.1443 0.5071 2.6671
##
## Random effects:
                        Variance Std.Dev.
##
   Groups
            Name
   aquarium (Intercept) 1367
                                   36.97
   Residual
                        14653
                                  121.05
## Number of obs: 94, groups: aquarium, 46
##
## Fixed effects:
##
                                       Estimate Std. Error
                                                               df t value
                                                   34.709 49.920
## (Intercept)
                                        345.438
                                                                    9.952
## scaledSpeed
                                        -4.676
                                                   28.726 73.360 -0.163
## treatmentOrderlow
                                        23.603
                                                   47.666 46.433
                                                                    0.495
## treatmentOrdermedium
                                        99.481
                                                   45.792 41.817
                                                                    2.172
## treatmentOrderxHigh
                                        48.975
                                                   46.369 40.829
                                                                   1.056
## scaledThig
                                        30.454
                                                   36.399 73.274
                                                                    0.837
## scaledmetRate
                                         -2.386
                                                   35.319 73.998 -0.068
## scaledBiteRate
                                         1.873
                                                   28.975 73.492
                                                                    0.065
## scaledSpeed:treatmentOrderlow
                                        -3.665
                                                   50.038 65.848 -0.073
## scaledSpeed:treatmentOrdermedium
                                        78.948
                                                   40.880 70.515 1.931
## scaledSpeed:treatmentOrderxHigh
                                                   40.905 73.412 -0.076
                                        -3.100
## treatmentOrderlow:scaledThig
                                        20.727
                                                   47.110 72.660 0.440
## treatmentOrdermedium:scaledThig
                                        26.742
                                                   48.801 73.996
                                                                    0.548
## treatmentOrderxHigh:scaledThig
                                                   47.462 73.999 -0.380
                                        -18.059
## treatmentOrderlow:scaledmetRate
                                                   44.636 72.600 -0.661
                                        -29.486
## treatmentOrdermedium:scaledmetRate
                                        44.725
                                                   47.124 73.911
                                                                    0.949
## treatmentOrderxHigh:scaledmetRate
                                        -53.316
                                                   50.770 69.549 -1.050
## treatmentOrderlow:scaledBiteRate
                                        10.650
                                                   48.893 69.493
                                                                    0.218
## treatmentOrdermedium:scaledBiteRate
                                       -48.916
                                                   47.178 73.957 -1.037
## treatmentOrderxHigh:scaledBiteRate
                                         7.046
                                                   41.669 72.338
                                                                    0.169
##
                                       Pr(>|t|)
                                       1.92e-13 ***
## (Intercept)
## scaledSpeed
                                        0.8711
## treatmentOrderlow
                                        0.6228
## treatmentOrdermedium
                                        0.0355 *
## treatmentOrderxHigh
                                        0.2971
## scaledThig
                                        0.4055
## scaledmetRate
                                        0.9463
## scaledBiteRate
                                        0.9486
## scaledSpeed:treatmentOrderlow
                                        0.9418
## scaledSpeed:treatmentOrdermedium
                                        0.0575 .
## scaledSpeed:treatmentOrderxHigh
                                        0.9398
## treatmentOrderlow:scaledThig
                                        0.6613
```

```
## treatmentOrdermedium:scaledThig
                                       0.5853
## treatmentOrderxHigh:scaledThig
                                       0.7047
## treatmentOrderlow:scaledmetRate
                                       0.5110
## treatmentOrdermedium:scaledmetRate
                                       0.3457
## treatmentOrderxHigh:scaledmetRate
                                       0.2973
## treatmentOrderlow:scaledBiteRate
                                       0.8282
## treatmentOrdermedium:scaledBiteRate
                                       0.3032
## treatmentOrderxHigh:scaledBiteRate
                                       0.8662
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
```

```
##
## Correlation matrix not shown by default, as p = 20 > 12.
## Use print(x, correlation=TRUE) or
## vcov(x) if you need it
```

model3<-lmer(Observed~scaledSpeed + scaledThig + scaledmetRate+ scaledBiteRate +treatmentOrder+
(1|aquarium), data=alphaDiversitySnails, na.action=na.fail)
summary(model3)</pre>

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula:
## Observed ~ scaledSpeed + scaledThig + scaledmetRate + scaledBiteRate +
##
      treatmentOrder + (1 | aquarium)
     Data: alphaDiversitySnails
##
##
## REML criterion at convergence: 1107.6
##
## Scaled residuals:
##
      Min
             10 Median
                             3Q
                                   Max
## -1.7907 -0.7048 -0.1172 0.5325 2.6935
##
## Random effects:
## Groups
                      Variance Std.Dev.
          Name
## aquarium (Intercept) 598.1
                                24.46
                       15627.7 125.01
## Residual
## Number of obs: 94, groups: aquarium, 46
##
## Fixed effects:
##
                                             df t value Pr(>|t|)
                     Estimate Std. Error
## (Intercept)
                     10.1495 14.3928 85.7930 0.705
## scaledSpeed
                                                          0.4826
## scaledThig
                      21.9088 14.4597 84.6334 1.515
                                                         0.1335
## scaledmetRate
                     -16.6005 14.6652 69.0770 -1.132
                                                         0.2616
## scaledBiteRate
                      -0.1211 15.5019 85.9330 -0.008
                                                         0.9938
## treatmentOrderlow
                      39.2372 40.7496 39.1903 0.963
                                                         0.3415
## treatmentOrdermedium 105.4771 42.5884 44.2274 2.477
                                                         0.0172 *
## treatmentOrderxHigh 38.7349 42.6525 45.1717 0.908
                                                         0.3686
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
             (Intr) scldSp scldTh scldmR scldBR trtmntOrdrl trtmntOrdrm
## scaledSpeed -0.150
## scaledThig
             0.007 -0.201
## scaledmetRt 0.188 -0.144 -0.300
## scaledBitRt -0.359 0.214 0.106 -0.178
## trtmntOrdrl -0.701 0.082 -0.075 -0.064 0.132
## trtmntOrdrm -0.768 0.196 0.039 -0.202 0.369 0.522
## trtmntOrdrH -0.793 0.111 -0.007 -0.219 0.439 0.534
                                                          0.623
```

```
dd<-dredge(model3, subset= ~treatmentOrder, evaluate=TRUE, rank=AICc)</pre>
```

```
## Warning in dredge(model3, subset = ~treatmentOrder, evaluate = TRUE, rank =
## AICc): comparing models fitted by REML
```

```
## Fixed term is "(Intercept)"
```

```
### Global model call: lmer(formula = Observed ~ scaledSpeed + scaledThig + scaledmetRate +
      scaledBiteRate + treatmentOrder + (1 | aquarium), data = alphaDiversitySnails,
##
      na.action = na.fail)
## ---
## Model selection table
                       scldR scldS scldT trtmO df
                                                             AICc delta weight
##
     (Intrc)
               sclBR
                                                    logLik
       338.2 -0.1211 -16.600 10.150 21.91
                                             + 10 -553.806 1130.3 0.00 0.787
## 32
## 31
       338.1
                     -16.620 10.170 21.91
                                             + 9 -557.463 1135.1 4.81 0.071
## 28
       341.4 -2.4850 -15.110
                                   23.99
                                            + 9 -557.639 1135.4 5.16 0.060
## 30
       344.8 -3.1890
                             7.832 16.94
                                             + 9 -558.050 1136.2 5.98 0.040
## 24
      338.7 -2.1150 -9.842 14.610
                                             + 9 -558.517 1137.2 6.91 0.025
## 27
       339.8
                     -15.470
                                   24.31
                                            + 8 -561.283 1140.3 10.00 0.005
                                             + 8 -561.714 1141.1 10.86 0.003
## 29
       342.8
                              8.436 17.02
       346.7 -4.8970
                                             + 8 -561.775 1141.2 10.98 0.003
## 26
                                   18.99
## 23
       337.3
                     -10.110 15.070
                                             + 8 -562.184 1142.1 11.80 0.002
## 22
       343.0 -3.7230
                             12.530
                                             + 8 -562.328 1142.3 12.09 0.002
                                             + 8 -562.617 1142.9 12.67 0.001
## 20
       343.4 -6.0480 -6.746
## 25
      343.8
                                   19.39
                                             + 7 -565.448 1146.2 15.94 0.000
                                             + 7 -566.000 1147.3 17.04 0.000
## 21
       340.8
                             13.280
## 18
      346.0 -6.8750
                                             + 7 -566.282 1147.9 17.60 0.000
## 19
       339.6
                                             + 7 -566.325 1148.0 17.69 0.000
                      -7.306
                                            + 6 -570.004 1153.0 22.71 0.000
       341.9
## 17
## Models ranked by AICc(x)
## Random terms (all models):
    1 | aquarium
```

```
ddAIC_C<-subset(dd, delta < 7)
ddAIC_C</pre>
```

```
### Global model call: lmer(formula = Observed ~ scaledSpeed + scaledThig + scaledmetRate +
      scaledBiteRate + treatmentOrder + (1 | aquarium), data = alphaDiversitySnails,
##
      na.action = na.fail)
## ---
## Model selection table
     (Intrc)
               sclBR
                       scldR scldS scldT trtmO df
                                                    logLik
                                                            AICc delta weight
       338.2 -0.1211 -16.600 10.150 21.91
                                           + 10 -553.806 1130.3 0.00 0.801
## 32
## 31
       338.1
                     -16.620 10.170 21.91
                                           + 9 -557.463 1135.1 4.81 0.072
## 28 341.4 -2.4850 -15.110
                                   23.99
                                           + 9 -557.639 1135.4 5.16 0.061
      344.8 -3.1890
## 30
                             7.832 16.94
                                          + 9 -558.050 1136.2 5.98 0.040
      338.7 -2.1150 -9.842 14.610
                                           + 9 -558.517 1137.2 6.91 0.025
## 24
## Models ranked by AICc(x)
## Random terms (all models):
    1 | aquarium
##
```

```
get.models(ddAIC C, subset=TRUE)
```

```
## $\32\
## Linear mixed model fit by REML ['lmerModLmerTest']
## Formula:
## Observed ~ scaledBiteRate + scaledmetRate + scaledSpeed + scaledThig +
       treatmentOrder + (1 | aquarium)
##
      Data: alphaDiversitySnails
## REML criterion at convergence: 1107.611
## Random effects:
## Groups
             Name
                         Std.Dev.
## aquarium (Intercept) 24.46
## Residual
                         125.01
## Number of obs: 94, groups: aquarium, 46
## Fixed Effects:
            (Intercept)
##
                               scaledBiteRate
                                                      scaledmetRate
##
               338.1812
                                                            -16.6005
                                      -0.1211
##
            scaledSpeed
                                   scaledThig
                                                  treatmentOrderlow
                10.1495
                                      21.9088
                                                            39.2372
##
## treatmentOrdermedium treatmentOrderxHigh
               105.4771
##
                                      38.7349
##
## $\31\
## Linear mixed model fit by REML ['lmerModLmerTest']
## Formula:
## Observed ~ scaledmetRate + scaledSpeed + scaledThig + treatmentOrder +
##
       (1 | aquarium)
      Data: alphaDiversitySnails
##
## REML criterion at convergence: 1114.926
## Random effects:
## Groups
                         Std.Dev.
             Name
## aquarium (Intercept) 24.46
## Residual
                         124.27
## Number of obs: 94, groups: aquarium, 46
## Fixed Effects:
##
            (Intercept)
                              scaledmetRate
                                                        scaledSpeed
##
                 338.11
                                       -16.62
                                                               10.17
##
             scaledThig
                          treatmentOrderlow treatmentOrdermedium
##
                  21.91
                                        39.28
                                                              105.57
##
   treatmentOrderxHigh
##
                  38.86
##
## $\28\
## Linear mixed model fit by REML ['lmerModLmerTest']
## Formula:
## Observed ~ scaledBiteRate + scaledmetRate + scaledThig + treatmentOrder +
##
      (1 | aquarium)
##
      Data: alphaDiversitySnails
## REML criterion at convergence: 1115.278
## Random effects:
## Groups
             Name
                         Std.Dev.
##
  aquarium (Intercept) 23.48
## Residual
                         124.80
## Number of obs: 94, groups: aquarium, 46
## Fixed Effects:
##
            (Intercept)
                               scaledBiteRate
                                                      scaledmetRate
##
                341,361
                                       -2,485
                                                             -15.107
```

```
##
             scaledThig
                            treatmentOrderlow treatmentOrdermedium
                 23.988
                                        36.892
                                                               99.774
##
   treatmentOrderxHigh
##
                 35.469
##
##
## $\ 30\
## Linear mixed model fit by REML ['lmerModLmerTest']
## Formula:
## Observed ~ scaledBiteRate + scaledSpeed + scaledThig + treatmentOrder +
##
       (1 | aquarium)
##
      Data: alphaDiversitySnails
## REML criterion at convergence: 1116.1
## Random effects:
   Groups
                         Std.Dev.
   aquarium (Intercept) 25.9
##
   Residual
                         125.0
## Number of obs: 94, groups: aquarium, 46
## Fixed Effects:
##
            (Intercept)
                                scaledBiteRate
                                                          scaledSpeed
##
                344.818
                                        -3.189
                                                                7.832
##
             scaledThig
                             treatmentOrderlow treatmentOrdermedium
                                                               95,496
                 16.941
                                        36.248
##
##
   treatmentOrderxHigh
##
                 28.039
##
## $\ 24\
## Linear mixed model fit by REML ['lmerModLmerTest']
## Formula:
## Observed ~ scaledBiteRate + scaledmetRate + scaledSpeed + treatmentOrder +
##
       (1 | aquarium)
##
      Data: alphaDiversitySnails
## REML criterion at convergence: 1117.033
## Random effects:
   Groups
                         Std.Dev.
##
             Name
   aquarium (Intercept) 33.85
##
   Residual
                          124.03
##
## Number of obs: 94, groups: aquarium, 46
## Fixed Effects:
                                scaledBiteRate
                                                        scaledmetRate
##
            (Intercept)
                                                               -9.842
##
                338.658
                                        -2.115
##
            scaledSpeed
                             treatmentOrderlow treatmentOrdermedium
                                        43.549
                                                              100.875
##
                 14.606
   treatmentOrderxHigh
##
##
                 38.137
##
## attr(,"rank")
## function (x)
## do.call("rank", list(x))
## <environment: 0x0000024bd43397c0>
## attr(,"call")
## AICc(x)
## attr(,"class")
## [1] "function"
                      "rankFunction"
## attr(,"beta")
## [1] "none"
```

```
ddMAc<-model.avg(ddAIC_C, subset= delta <7)
summary(ddMAc)</pre>
```

```
##
## Call:
  model.avg(object = ddAIC_C, subset = delta < 7)</pre>
##
## Component model call:
  lmer(formula = Observed ~ <5 unique rhs>, data = alphaDiversitySnails,
##
        na.action = na.fail)
##
## Component models:
##
         df logLik
                      AICc delta weight
## 12345 10 -553.81 1130.26 0.00
## 2345
         9 -557.46 1135.07 4.81
                                    0.07
## 1245
         9 -557.64 1135.42 5.16
                                    0.06
## 1345
          9 -558.05 1136.24 5.98
                                    0.04
## 1235
          9 -558.52 1137.18 6.91
                                    0.03
##
## Term codes:
## scaledBiteRate scaledmetRate
                                    scaledSpeed
                                                    scaledThig treatmentOrder
##
                                              3
                                                             4
                                                                            5
## Model-averaged coefficients:
## (full average)
##
                        Estimate Std. Error Adjusted SE z value Pr(>|z|)
                                                31.0270 10.915
                        338.6484
                                    30.5820
                                                                  <2e-16 ***
## (Intercept)
## scaledBiteRate
                                    14.9201
                                                15.1371
                                                          0.028
                        -0.4298
                                                                  0.9773
## scaledmetRate
                        -15.6719
                                    14.7157
                                                14.9190
                                                          1.050
                                                                  0.2935
## scaledSpeed
                          9.5540
                                    14.1434
                                                14.3430
                                                          0.666
                                                                  0.5053
## scaledThig
                         21.2820
                                                14.8684 1.431
                                                                  0.1523
                                    14.6673
## treatmentOrderlow
                                    40.7301
                                                41.3236
                                                          0.946
                         39.0861
                                                                  0.3442
## treatmentOrdermedium 104.6191
                                    42.3674
                                                42.9833
                                                          2.434
                                                                  0.0149 *
                                                42.9766
                                                          0.887
## treatmentOrderxHigh
                         38.1000
                                    42.3605
                                                                  0.3753
##
## (conditional average)
##
                        Estimate Std. Error Adjusted SE z value Pr(>|z|)
                        338.6484
                                    30.5820
                                                31.0270 10.915
## (Intercept)
                                                                  <2e-16 ***
## scaledBiteRate
                        -0.4634
                                    15.4914
                                                15.7168
                                                          0.029
                                                                  0.9765
## scaledmetRate
                        -16.3295
                                    14.6595
                                                14.8721
                                                         1.098
                                                                  0.2722
## scaledSpeed
                        10.1719
                                    14.3766
                                                14.5856 0.697
                                                                  0.4856
                                                          1.490
## scaledThig
                         21.8335
                                    14.4452
                                                14.6546
                                                                  0.1363
## treatmentOrderlow
                         39.0861
                                    40.7301
                                                41.3236
                                                          0.946
                                                                  0.3442
## treatmentOrdermedium 104.6191
                                                42.9833
                                                          2.434
                                                                  0.0149 *
                                    42.3674
## treatmentOrderxHigh
                         38.1000
                                    42.3605
                                                42.9766
                                                          0.887
                                                                  0.3753
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
```

```
rm(dd, ddAIC_C, ddMAc, model1, model2, model3)
```

can carrot juice consumptions explain alpha

diversity or treatment on palatability?

```
###Can alpha diversity increase be explained by consumption of carrot juice?

model1<-lmer(Shannon~Pretraining+ (1|aquarium), data=alphaDiversitySnails)
summary(model1)</pre>
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: Shannon ~ Pretraining + (1 | aquarium)
      Data: alphaDiversitySnails
##
## REML criterion at convergence: 111.1
## Scaled residuals:
##
      Min
               1Q Median
                               3Q
                                      Max
## -3.5191 -0.3760 0.1103 0.5080 1.9157
##
## Random effects:
## Groups Name
                        Variance Std.Dev.
## aquarium (Intercept) 0.07545 0.2747
## Residual
                        0.11446 0.3383
## Number of obs: 94, groups: aquarium, 46
##
## Fixed effects:
##
               Estimate Std. Error
                                          df t value Pr(>|t|)
## (Intercept) 4.020000 0.117524 90.537245 34.206 <2e-16 ***
## Pretraining -0.001601   0.006802 78.056065   -0.235
                                                        0.815
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
              (Intr)
## Pretraining -0.885
```

```
model2<-lmer(Shannon~Posttraining+ (1|aquarium), data=alphaDiversitySnails)
summary(model2)</pre>
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: Shannon ~ Posttraining + (1 | aquarium)
      Data: alphaDiversitySnails
##
##
## REML criterion at convergence: 110
##
## Scaled residuals:
##
       Min
                1Q Median
                                3Q
                                       Max
## -3.5283 -0.3988 0.1410 0.5517 2.1217
##
## Random effects:
## Groups
           Name
                         Variance Std.Dev.
## aquarium (Intercept) 0.07167 0.2677
## Residual
                         0.11392 0.3375
## Number of obs: 94, groups: aquarium, 46
##
## Fixed effects:
##
                 Estimate Std. Error
                                            df t value Pr(>|t|)
## (Intercept) 3.915596 0.082080 85.340487 47.705
                                                         <2e-16 ***
## Posttraining 0.007075 0.005482 88.779165
                                                 1.291
                                                            0.2
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##
               (Intr)
## Posttrainng -0.755
alphaDiversitySnails$Pretraining<-as.numeric(alphaDiversitySnails$Pretraining)</pre>
alphaDiversitySnails$Posttraining<-as.numeric(alphaDiversitySnails$Posttraining)</pre>
Alltraining2<-rowSums(alphaDiversitySnails[ , c("Pretraining", "Posttraining")])
alphaDiversitySnails<-cbind(alphaDiversitySnails, Alltraining2)</pre>
```

 $model3<-lmer(Shannon\sim Alltraining2+(1|aquarium), data=alphaDiversitySnails) ###t = 0.457$

0.649

summary(model3)

p =

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: Shannon ~ Alltraining2 + (1 | aquarium)
     Data: alphaDiversitySnails
##
##
## REML criterion at convergence: 111.9
##
## Scaled residuals:
##
      Min
               1Q Median
                               3Q
                                      Max
## -3.6061 -0.3803 0.0951 0.5237 2.0603
##
## Random effects:
                       Variance Std.Dev.
## Groups Name
## aquarium (Intercept) 0.07689 0.2773
## Residual
                       0.11286 0.3360
## Number of obs: 94, groups: aquarium, 46
##
## Fixed effects:
                Estimate Std. Error
                                          df t value Pr(>|t|)
## (Intercept) 3.923283 0.112933 91.406926
                                               34.74 <2e-16 ***
## Alltraining2 0.002712 0.003713 80.793336
                                                0.73
                                                        0.467
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
              (Intr)
## Alltrainng2 -0.875
```

```
# observed

model4<-lmer(Observed~Pretraining+ (1|aquarium), data=alphaDiversitySnails)
summary(model4)</pre>
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: Observed ~ Pretraining + (1 | aquarium)
     Data: alphaDiversitySnails
##
##
## REML criterion at convergence: 1168
##
## Scaled residuals:
                               3Q
##
      Min
               1Q Median
                                      Max
## -1.7228 -0.7244 -0.1744 0.5999 2.5881
##
## Random effects:
## Groups Name
                       Variance Std.Dev.
## aquarium (Intercept) 2629
                                  51.27
## Residual
                       14311
                                 119.63
## Number of obs: 94, groups: aquarium, 46
##
## Fixed effects:
              Estimate Std. Error
                                      df t value Pr(>|t|)
## (Intercept) 409.985 37.191 91.796 11.024 <2e-16 ***
## Pretraining -1.629
                          2.228 86.621 -0.731
                                                    0.467
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
              (Intr)
## Pretraining -0.919
```

```
model5<-lmer(Observed~Posttraining+ (1|aquarium), data=alphaDiversitySnails)
summary(model5)</pre>
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: Observed ~ Posttraining + (1 | aquarium)
     Data: alphaDiversitySnails
##
##
## REML criterion at convergence: 1168.9
##
## Scaled residuals:
##
      Min
               1Q Median
                               3Q
                                      Max
## -1.7680 -0.7035 -0.1683 0.6490 2.6866
##
## Random effects:
                        Variance Std.Dev.
## Groups
           Name
## aquarium (Intercept) 2977
                                  54.56
## Residual
                                 118.80
                        14114
## Number of obs: 94, groups: aquarium, 46
##
## Fixed effects:
               Estimate Std. Error
                                         df t value Pr(>|t|)
                                                     <2e-16 ***
## (Intercept) 379.6674 24.9718 82.9137 15.204
## Posttraining 0.4585
                          1.7529 91.9983
                                              0.262
                                                       0.794
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
              (Intr)
## Posttrainng -0.802
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: Observed ~ Alltraining2 + (1 | aquarium)
     Data: alphaDiversitySnails
##
##
## REML criterion at convergence: 1169.6
##
## Scaled residuals:
##
      Min
               1Q Median
                               3Q
                                      Max
## -1.7438 -0.7264 -0.1789 0.5867 2.6285
##
## Random effects:
                       Variance Std.Dev.
## Groups Name
## aquarium (Intercept) 3034
                                  55.09
## Residual
                       14075
                                 118.64
## Number of obs: 94, groups: aquarium, 46
##
## Fixed effects:
               Estimate Std. Error
                                         df t value Pr(>|t|)
## (Intercept) 391.3135 35.7014 91.9865 10.961 <2e-16 ***
## Alltraining2 -0.2402 1.2119 88.5899 -0.198
                                                      0.843
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
              (Intr)
## Alltrainng2 -0.908
```

##does treatment affect willingness to consume carrot juice?
model3b<-lmer(Pretraining~treatmentOrder+ (1|aquarium), data=alphaDiversitySnails) ###NO</pre>

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

summary(model3b)

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: Pretraining ~ treatmentOrder + (1 | aquarium)
     Data: alphaDiversitySnails
##
##
## REML criterion at convergence: 590.8
##
## Scaled residuals:
       Min
                10 Median
                                 3Q
## -2.35343 -0.71316 -0.02377 0.80552 2.29499
##
## Random effects:
## Groups Name
                       Variance Std.Dev.
   aquarium (Intercept) 0.00
                                0.000
## Residual
                       36.11
                                6.009
## Number of obs: 94, groups: aquarium, 46
##
## Fixed effects:
                      Estimate Std. Error
                                              df t value Pr(>|t|)
##
                      ## (Intercept)
                      -0.5714 1.8546 90.0000 -0.308
## treatmentOrderlow
                                                           0.759
## treatmentOrdermedium -0.6488
                                 1.7957 90.0000 -0.361
                                                           0.719
## treatmentOrderxHigh -0.7143
                                 1.7348 90.0000 -0.412
                                                           0.682
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##
              (Intr) trtmntOrdrl trtmntOrdrm
## trtmntOrdrl -0.707
## trtmntOrdrm -0.730 0.516
## trtmntOrdrH -0.756 0.535
                                 0.552
## optimizer (nloptwrap) convergence code: 0 (OK)
## boundary (singular) fit: see help('isSingular')
```

DMSO effect on behaviour

```
metadataDMSO<-read.csv(file="metadata_DMSO_control.csv",header=TRUE)
model1<-lmer(metabolicRate~ treatmentOrder + (1|aquarium), data = metadataDMSO)</pre>
```

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

```
summary(model1)
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: metabolicRate ~ treatmentOrder + (1 | aquarium)
     Data: metadataDMSO
##
##
## REML criterion at convergence: -31.5
##
## Scaled residuals:
                                 3Q
##
       Min
                1Q Median
## -2.15896 -0.56122 -0.00884 0.73184 2.24282
##
## Random effects:
## Groups Name
                      Variance Std.Dev.
## aquarium (Intercept) 0.00000 0.0000
## Residual
                       0.02697 0.1642
## Number of obs: 51, groups: aquarium, 25
##
## Fixed effects:
##
                    Estimate Std. Error
                                             df t value Pr(>|t|)
                     ## (Intercept)
## treatmentOrderDMSO 0.07677 0.04643 49.00000
                                                 1.654
                                                          0.105
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##
              (Intr)
## trtmntODMSO -0.754
## optimizer (nloptwrap) convergence code: 0 (OK)
## boundary (singular) fit: see help('isSingular')
```

```
model2<-lmer(biteRateChange~ treatmentOrder + (1|aquarium), data = metadataDMSO)
summary(model2)</pre>
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: biteRateChange ~ treatmentOrder + (1 | aquarium)
     Data: metadataDMSO
##
##
## REML criterion at convergence: 357.5
##
## Scaled residuals:
      Min
               1Q Median
                               3Q
                                      Max
## -2.4881 -0.3676 0.1694 0.5522 1.9817
##
## Random effects:
## Groups Name
                        Variance Std.Dev.
## aquarium (Intercept) 10.68
                                 3.269
## Residual
                        66.47
                                 8.153
## Number of obs: 51, groups: aquarium, 25
##
## Fixed effects:
##
                     Estimate Std. Error
                                             df t value Pr(>|t|)
## (Intercept)
                       -7.754
                                   1.990 24.895 -3.897 0.00065 ***
## treatmentOrderDMSO 1.749
                                   2.682 20.194 0.652 0.52158
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
              (Intr)
## trtmntODMSO -0.742
```

```
model3<-lmer(Speed~ treatmentOrder + (1|aquarium), data = metadataDMSO)</pre>
```

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

```
summary(model3)
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: Speed ~ treatmentOrder + (1 | aquarium)
     Data: metadataDMSO
##
##
## REML criterion at convergence: 26.2
##
## Scaled residuals:
##
      Min
               1Q Median
                              3Q
                                    Max
## -2.0720 -0.5661 -0.1021 0.5852 2.7125
##
## Random effects:
## Groups Name
                      Variance Std.Dev.
## aquarium (Intercept) 0.00000 0.0000
                       0.08754 0.2959
## Residual
## Number of obs: 51, groups: aquarium, 25
##
## Fixed effects:
##
                    Estimate Std. Error
                                             df t value Pr(>|t|)
                     ## (Intercept)
## treatmentOrderDMSO -0.12951 0.08365 49.00000 -1.548
                                                          0.128
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##
              (Intr)
## trtmntODMSO -0.754
## optimizer (nloptwrap) convergence code: 0 (OK)
## boundary (singular) fit: see help('isSingular')
```

```
model4<-lmer(Thigmotaxis~ treatmentOrder + (1|aquarium), data = metadataDMSO)
summary(model4)</pre>
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: Thigmotaxis ~ treatmentOrder + (1 | aquarium)
     Data: metadataDMSO
##
##
## REML criterion at convergence: 0.8
## Scaled residuals:
      Min
             1Q Median
                            3Q
                                   Max
## -2.4711 -0.5842 0.1184 0.6963 1.3237
##
## Random effects:
## Groups Name
                     Variance Std.Dev.
## aquarium (Intercept) 0.008798 0.0938
## Residual
                      0.044691 0.2114
## Number of obs: 51, groups: aquarium, 25
##
## Fixed effects:
##
                    Estimate Std. Error
                                         df t value Pr(>|t|)
                   ## (Intercept)
## treatmentOrderDMSO -0.06871 0.07150 25.34995 -0.961
                                                        0.346
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
             (Intr)
## trtmntODMSO -0.740
```

Differential Abundance at OTU level

```
rm(list = ls(all.names = TRUE))
library(Maaslin2)
metadata <-read.table("metadata_alphaDiversity.txt", row.names="Row.names", header = TRUE) ###</pre>
this has the scaled data for analyses, example in beta diversity
otus <- read.table("otu-table-centroids-iddef0-400bp.txt")</pre>
# OTU differential abundance analysis
fit_data = Maaslin2(
  input_data = otus,
  input_metadata = metadata,
  output = "Maaslin2_output_OTUs_AllFixedTerms",
  fixed_effects = c("treatmentOrder", "scaledBiteRate", "scaledThig", "scaledSpeed", "scaledmet
Rate"),
  random_effects = c("aquarium"),
  reference = c("treatmentOrder,control"),
  plot_scatter = FALSE)
KO <- read.table(file='KO_pred_metagenome_unstrat.tsv', header=TRUE)</pre>
#Assigning row names from 1st column
rownames(KO) \leftarrow KO[,1]
KO = subset(KO, select = -function. )
KO[] <- lapply(KO, as.integer)</pre>
PA <- read.table(file='path_abun_unstrat.tsv', header=TRUE)
#Assigning row names from 1st column
rownames(PA) <- PA[,1]
##remove the duplicate column
PA = subset(PA, select = -pathway )
##make values intergers
PA[] <- lapply(PA, as.integer)
#this metadata file has the full fastq sample names (not the shortened names) that were used in
the picrust analysis.
meta<-read.table(file="metadata_picrust.txt", header=TRUE, row.names = "row.names")</pre>
# Edit metadata for downstream analyses: invert the sign (-/+) for biteRateChange
# (more intuitive to interpret where higher values = better memory)
meta$biteRateChange = meta$biteRateChange*(-1)
##scale variables
meta$scaledThig<-scale(meta$Thigmotaxis)</pre>
meta$scaledSpeed<-scale(meta$Speed)</pre>
meta$scaledBiteRate<-scale(meta$biteRateChange)</pre>
meta$scaledmetRate<-scale(meta$metabolicRate)</pre>
names(meta)
fit data2 = Maaslin2(
```

```
input_data = KO,
  input_metadata = meta,
  output = "Maaslin2_output_KO_AllFixedTerms",
  fixed_effects = c("treatmentOrder", "scaledBiteRate", "scaledThig", "scaledSpeed", "scaledmet
Rate"),
  random_effects = c("aquarium"),
  reference = c("treatmentOrder,control"),
  plot_scatter = FALSE)
fit_data3 = Maaslin2(
  input_data = PA,
  input_metadata = meta,
  output = "Maaslin2_output_PA_AllFixedTerms",
  fixed_effects = c("treatmentOrder", "scaledBiteRate", "scaledThig", "scaledSpeed", "scaledmet
Rate"),
  random_effects = c("aquarium"),
  reference = c("treatmentOrder,control"),
  plot_scatter = FALSE)
```

sessionInfo()

```
## R version 4.2.3 (2023-03-15 ucrt)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 10 x64 (build 19045)
## Matrix products: default
##
## locale:
## [1] LC_COLLATE=English_United Kingdom.utf8
## [2] LC_CTYPE=English_United Kingdom.utf8
## [3] LC_MONETARY=English_United Kingdom.utf8
## [4] LC_NUMERIC=C
## [5] LC_TIME=English_United Kingdom.utf8
##
## attached base packages:
                 graphics grDevices utils
## [1] stats
                                               datasets methods
                                                                    hase
##
## other attached packages:
## [1] MuMIn_1.47.5
                             usdm_1.1-18
                                                   raster_3.6-20
## [4] sp_1.6-1
                             compositions_2.0-6
                                                   metagenomeSeq_1.40.0
## [7] RColorBrewer_1.1-3
                             glmnet_4.1-7
                                                   limma_3.54.2
## [10] Biobase_2.58.0
                             BiocGenerics_0.44.0 lmerTest_3.1-3
## [13] lme4_1.1-33
                             Matrix_1.5-3
                                                   phyloseq_1.42.0
## [16] lubridate_1.9.2
                             forcats_1.0.0
                                                   stringr_1.5.0
## [19] dplyr_1.1.2
                             purrr_1.0.1
                                                   readr_2.1.4
## [22] tidyr_1.3.0
                             tibble_3.2.1
                                                   ggplot2_3.4.2
## [25] tidyverse_2.0.0
                             vegan_2.6-4
                                                   lattice_0.20-45
## [28] permute_0.9-7
##
## loaded via a namespace (and not attached):
## [1] minqa_1.2.5
                                                       XVector_0.38.0
                               colorspace_2.1-0
## [4] rstudioapi_0.14
                               fansi_1.0.4
                                                       codetools_0.2-19
## [7] splines_4.2.3
                               cachem_1.0.8
                                                       robustbase_0.95-1
## [10] knitr 1.43
                               ade4 1.7-22
                                                       jsonlite_1.8.4
## [13] nloptr_2.0.3
                               cluster_2.1.4
                                                       compiler_4.2.3
## [16] fastmap_1.1.1
                               cli_3.6.1
                                                       htmltools_0.5.5
## [19] tools_4.2.3
                               igraph_1.4.2
                                                       gtable_0.3.3
## [22] glue_1.6.2
                               GenomeInfoDbData_1.2.9 reshape2_1.4.4
## [25] Rcpp_1.0.10
                               jquerylib_0.1.4
                                                      vctrs_0.6.1
## [28] Biostrings_2.66.0
                               rhdf5filters_1.10.1
                                                       multtest 2.54.0
## [31] ape 5.7-1
                               nlme 3.1-162
                                                       iterators 1.0.14
## [34] tensorA 0.36.2
                               xfun 0.39
                                                       timechange 0.2.0
## [37] lifecycle 1.0.3
                               gtools 3.9.4
                                                       terra 1.7-29
## [40] DEoptimR_1.0-14
                               zlibbioc_1.44.0
                                                       MASS_7.3-58.2
## [43] scales 1.2.1
                               hms 1.1.3
                                                       parallel 4.2.3
                               rhdf5_2.42.1
## [46] biomformat_1.26.0
                                                       yam1_2.3.7
## [49] sass_0.4.6
                               stringi_1.7.12
                                                       highr_0.10
## [52] S4Vectors_0.36.2
                               foreach_1.5.2
                                                       caTools_1.18.2
## [55] boot_1.3-28.1
                               shape_1.4.6
                                                       GenomeInfoDb 1.34.9
## [58] rlang_1.1.0
                               pkgconfig_2.0.3
                                                       bitops_1.0-7
## [61] matrixStats_1.0.0
                               Wrench_1.16.0
                                                       evaluate_0.21
## [64] Rhdf5lib 1.20.0
                               tidyselect 1.2.0
                                                       plyr 1.8.8
## [67] magrittr_2.0.3
                               R6_2.5.1
                                                       IRanges_2.32.0
## [70] gplots_3.1.3
                               generics_0.1.3
                                                       DBI_1.1.3
## [73] pillar_1.9.0
                               withr_2.5.0
                                                       mgcv_1.8-42
## [76] survival_3.5-5
                               RCurl_1.98-1.12
                                                       bayesm_3.1-5
```

|--|