

Antibiotic-altered gut microbiota explain host memory plasticity and disrupt pace-of-life trait covariation for an aquatic snail

Gabrielle Davidson, Ignacio Alvarez-Cienfuegos, and Sarah Dalesman

2024-04-25

```
#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)
metadata <- read.table("snail_metadata.txt", header=TRUE, row.names = "row.names") #before scaled data saved and alpha diversity
otus <- read.table("otu-table-centroids-iddef0-400bp.txt")

taxonomy <- data.frame(taxonomy[, -1], row.names=taxonomy[, 1]) #set row.names

# 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"
metadata$biteRateChange = metadata$rawbiteRateChange*(-1)

##scale variables

metadata$scaledThig<-scale(metadata$Thigmotaxis)
metadata$scaledSpeed<-scale(metadata$Speed)
metadata$scaledBiteRate<-scale(metadata$biteRateChange)
metadata$scaledmetRate<-scale(metadata$metabolicRate)
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

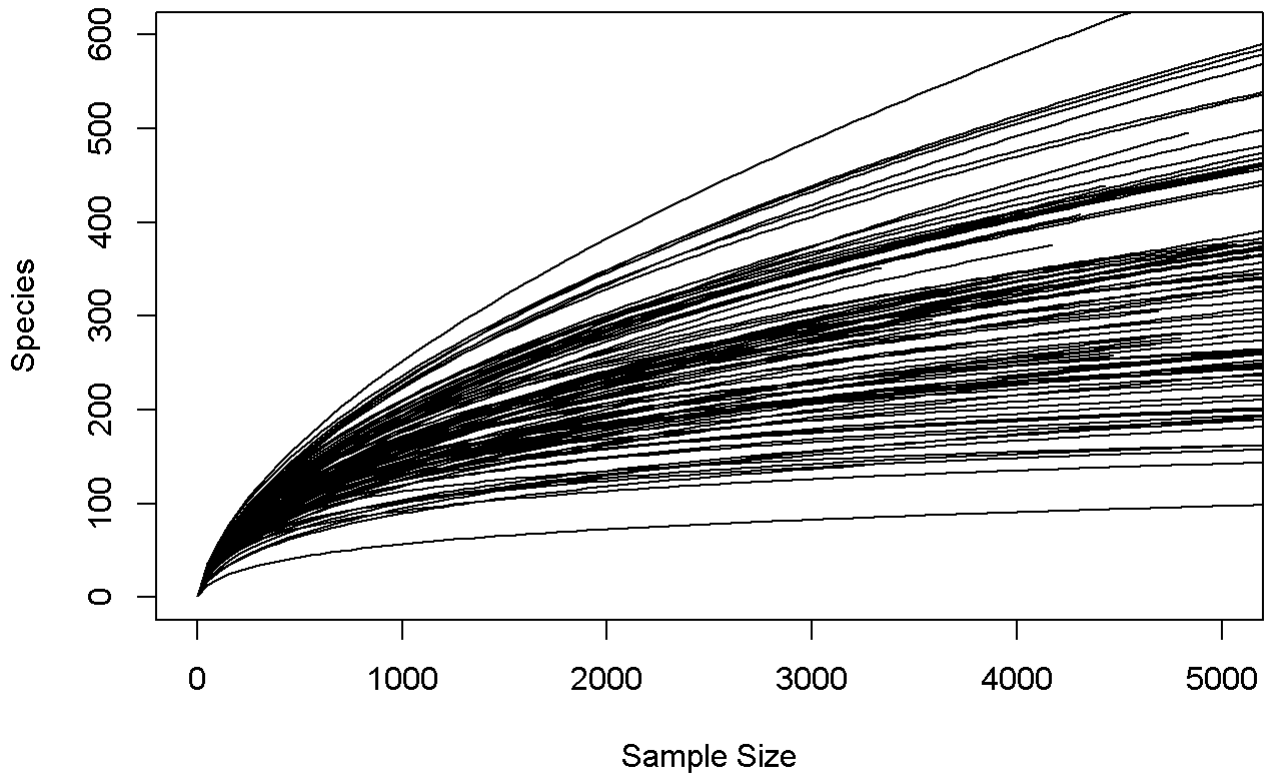
#metadata<-read.csv("metadata_DMSO.csv",header=TRUE)
```

filtering steps

```
class(OTU) <- "matrix"
```

```
## 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)

#remove taxa w/ <0.005\% total abundance of total abundance of whole dataset recommended by Boku
Lich

# taxa total abundance
taxa.abundances <- get_sample(snailPhyloFilt)
# dataset total abundance
total.abundance <- apply(taxa.abundances, 1,sum)
# combine
tt.abundances <- cbind(taxa.abundances,total.abundance)
cutoff <- sum(total.abundance)*0.00005
keep <- total.abundance>cutoff
snailPhyloFilt2 <- prune_taxa(keep,snailPhyloFilt)

reads<-sample_sums(snailPhyloFilt2)
reads<-as.data.frame(reads)
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)

# normalization
p <- cumNormStatFast(SnailMGS)
```

```
## Default value being used.
```

```
p
```

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

```
SnailMGS <- cumNorm(SnailMGS, p =p)
```

```
#returns normalized factors for each sample  
normFactors(SnailMGS)
```

```
## 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  
## 844 573 178 484 426 451 527  
## S23_L001 S25_L001 S26_L001 S27_L001 S28_L001 S29_L001 S2_L001  
## 205 288 508 490 202 346 450  
## S31_L001 S32_L001 S33_L001 S34_L001 S35_L001 S36_L001 S37_L001  
## 410 396 351 500 482 199 322  
## 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  
## 529 225 120 257 331 305 347  
## S50_L001 S51_L001 S52_L001 S53_L001 S54_L001 S55_L001 S56_L001  
## 323 276 393 387 183 279 177  
## S57_L001 S58_L001 S59_L001 S5_L001 S61_L001 S62_L001 S63_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 89 349 405 332 285 222  
## S77_L001 S78_L001 S79_L001 S7_L001 S80_L001 S81_L001 S82_L001  
## 356 226 183 332 220 138 86  
## S83_L001 S85_L001 S86_L001 S87_L001 S88_L001 S89_L001 S8_L001  
## 353 163 319 240 230 297 227  
## S90_L001 S92_L001 S93_L001 S94_L001 S95_L001 S96_L001 S97_L001  
## 259 253 339 301 147 173 183  
## S9_L001 E5B55_L001 E5B56_L001  
## 173 200 299
```

```
# 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
```

preparing family and phylum for relative abundance

graphs

```

# collapse phyloseq object down to only contain Phyla Level info ##
snailPhyloFilt2P <- tax_glom(snailPhyloFilt2, "Phylum", NArm = TRUE)

## transform to relative abundances

snailPhyloRA <- transform_sample_counts(snailPhyloFilt2P, function(x){x / sum(x)})
# special phyloseq psmelt function returns dataframe w/ otu abundances, taxa names and sample data
motsnail <- psmelt(snailPhyloRA)
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)
write.csv(SnailPhylumRA2023, file = "metadata_PhylumRA.csv")
PhylumRA<-read.csv(file="metadata_PhylumRA.csv", header=TRUE)

# currently each column is a phylum with its abundance as values in cells. I want a column named Phylum and a column named Abundance
names(PhylumRA)
SnailPhylumRAGather<-gather(PhylumRA, 'Pseudomonadota','Verrucomicrobiota', 'Spirochaetota', 'Bacteria_unclassified', 'Armatimonadota','Candidatus_Saccharibacteria', 'Planctomycetota', 'Bacillota', 'Actinomycetota', 'Campylobacterota', 'Bacteroidota', 'Acidobacteriota', 'Chlamydiota', key="Phylum", value = "Abundance")

snailMeans <- summarySE(SnailPhylumRAGather, measurevar="Abundance", groupvars=c("treatmentOrder", "Phylum"))
write.csv(snailMeans, file="RAPHylumMeans.csv")
snailMeans2 <- summarySE(SnailPhylumRAGather, measurevar="Abundance", groupvars="Phylum")
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)

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))
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)
write.csv(SnailFamilyRA2023, file = "metadata_FamilyRA.csv")

family<-rownames(glomTable2)
uniqueFamily<-unique(family)
SnailFamilyRAGather<-gather(SnailFamilyRA2023, uniqueFamily , key="Family", value = "Abundance")

snailMeans <- summarySE(SnailFamilyRAGather, measurevar="Abundance", groupvars=c("treatmentOrder", "Family"))
write.csv(snailMeans, file="RAFamilyMeans.csv")
snailMeans2 <- summarySE(SnailFamilyRAGather, measurevar="Abundance", groupvars="Family")
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)

```

```

##
## Permutation test for homogeneity of multivariate dispersions
## Permutation: free
## Number of permutations: 1000
##
## Response: Distances
##           Df Sum Sq Mean Sq      F N.Perm  Pr(>F)
## Groups    3 162.39  54.130  5.4192  1000 0.003996 **
## Residuals 90  898.97   9.989
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

```
# Aitchinson Adonis by scaled continuous terms.
```

```
adonis2(aitchison.distances~snailPhyloCSS@sam_data$treatmentOrder+snailPhyloCSS@sam_data$scaledBiteRate+snailPhyloCSS@sam_data$scaledSpeed + snailPhyloCSS@sam_data$scaledThig+snailPhyloCSS@sam_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$treatmentOrder + snailPhyloCSS@sam_data$scaledBiteRate + snailPhyloCSS@sam_data$scaledSpeed + snailPhyloCSS@sam_data$scaledThig + 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 | 0.458 |
| ## snailPhyloCSS@sam_data\$scaledSpeed | 1 | 295 | 0.00856 | 0.8934 | 0.656 |
| ## snailPhyloCSS@sam_data\$scaledThig | 1 | 412 | 0.01196 | 1.2478 | 0.112 |
| ## snailPhyloCSS@sam_data\$scaledmetRate | 1 | 403 | 0.01168 | 1.2186 | 0.130 |
| ## Residual | 86 | 28425 | 0.82419 | | |
| ## Total | 93 | 34488 | 1.00000 | | |

```
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 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)
```

```
##
```

```
## Permutation test for homogeneity of multivariate dispersions
```

```
## Permutation: free
```

```
## Number of permutations: 1000
```

```
##
```

```
## Response: Distances
```

```
##
```

| | Df | Sum Sq | Mean Sq | F | N.Perm | Pr(>F) |
|--------------|----|---------|----------|--------|--------|--------------|
| ## Groups | 3 | 0.15856 | 0.052852 | 9.5348 | 1000 | 0.000999 *** |
| ## Residuals | 90 | 0.49888 | 0.005543 | | | |

```
## ---
```

```
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(jaccard~snailPhyloCSS@sam_data$treatmentOrder+snailPhyloCSS@sam_data$scaledBiteRate+snailPhyloCSS@sam_data$scaledSpeed + snailPhyloCSS@sam_data$scaledThig+snailPhyloCSS@sam_data$scaledmetRate, 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$treatmentOrder + snailPhyloCSS@sam_data$
caledBiteRate + snailPhyloCSS@sam_data$scaledSpeed + snailPhyloCSS@sam_data$scaledThig + snailP
hyloCSS@sam_data$scaledmetRate, method = "jaccard", by = "terms")
##
##              Df SumOfSqs      R2      F Pr(>F)
## snailPhyloCSS@sam_data$treatmentOrder  3   2.8983 0.18423 6.8760 0.001 ***
## snailPhyloCSS@sam_data$scaledBiteRate  1   0.1449 0.00921 1.0313 0.360
## snailPhyloCSS@sam_data$scaledSpeed    1   0.2184 0.01388 1.5542 0.044 *
## snailPhyloCSS@sam_data$scaledThig     1   0.1938 0.01232 1.3792 0.093 .
## snailPhyloCSS@sam_data$scaledmetRate  1   0.1934 0.01229 1.3762 0.100 .
## Residual                             86  12.0832 0.76807
## 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)
```

```
## [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=FALSE)
```

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)
```

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"
metadataFull$biteRateChange = metadataFull$rawbiteRateChange*(-1)
```

##scale variables

```
metadataFull$scaledThig<-scale(metadataFull$Thigmotaxis)
metadataFull$scaledSpeed<-scale(metadataFull$Speed)
metadataFull$scaledBiteRate<-scale(metadataFull$biteRateChange)
metadataFull$scaledmetRate<-scale(metadataFull$metabolicRate)
names(metadataFull)
```

```
## [1] "uniqueID"          "Thigmotaxis"         "Speed"
## [4] "Pretraining"         "Posttraining"        "rawbiteRateChange"
## [7] "treatment"           "treatmentOrder"      "aquarium"
## [10] "metabolicRate"       "biteRateChange"      "scaledThig"
## [13] "scaledSpeed"         "scaledBiteRate"      "scaledmetRate"
```

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

```
pcaout<-prcomp(PCA)
```

```
eigen<-pcaout$sdev^2
eigen
```

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

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

check if variables are colinear before including all in GLMM:

```
df<-data.frame(metadataFull$scaledmetRate, metadataFull$scaledThig, metadataFull$scaledSpeed, metadataFull$scaledBiteRate)
vif(df)
```

```
##           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-coefficients-in-r/
library(dplyr)
library(tidyverse)
library(tidymodels)
library(rstatix)
library(ggpubr)

metadataFull<-read.csv(file="metadata_full.csv",header=TRUE)
names(metadataFull)[names(metadataFull) == "biteRateChange"] <- "rawbiteRateChange"
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 bootstraps
  unnest(boots) %>% # un-nesting bootstrapped 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(corr, 0.025),
            .estimate = median(corr),
            upr_CI = quantile(corr, 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 bootstraps
  unnest(boots) %>% # un-nesting bootstrapped data lists
  mutate(correlations = map(splits, ~cor_test(Speed, metabolicRate, data = analysis((.)))))) # p

```

performing 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 boots
  tstraps
  unnest(boots) %>% # un-nesting bootstrapped 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 boots
  tstraps
  unnest(boots) %>% # un-nesting bootstrapped 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(corr, 0.025),
              .estimate = median(corr),
              upr_CI = quantile(corr, 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 boots
  tstraps
  unnest(boots) %>% # un-nesting bootstrapped 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(corr, 0.025),
              .estimate = median(corr),
              upr_CI = quantile(corr, 0.975))

##Thigmotaxis 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 boots
  tstraps
  unnest(boots) %>% # un-nesting bootstrapped data lists
  mutate(correlations = map(splits, ~cor_test(Thigmotaxis, Speed, data = analysis(
  (.)))))) # performing correlation

corr <- boot_corr %>%
  unnest(correlations) %>% # unnesting tidied data frames
  select(-data, -splits, -id)

#confidence intervals from bootstrap
CI_5 <- corr %>%

```

```

group_by(treatmentOrder) %>%
  summarise(lwr_CI = quantile(cor, 0.025),
            .estimate = median(cor),
            upr_CI = quantile(cor, 0.975))

pearsonsResults<-bind_rows(point, point5, point1, point2, point3, point4)
write.csv(pearsonsResults, file = "pearsonsCorrelationResults.csv")

```

GLMMs

```
#treatments on behaviours
```

```

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

```

```

## 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:
##              Estimate Std. Error    df t value Pr(>|t|)
## (Intercept)  0.39653   0.04810 68.97997  8.245 7.15e-12 ***
## treatmenthigh  0.10381   0.06346 66.30614  1.636  0.107
## treatmentlow  0.05787   0.06529 74.35471  0.886  0.378
## treatmentmedium 0.07914   0.06854 54.41979  1.155  0.253
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##              (Intr) trtmnth trtmntl
## treatmenthigh -0.728
## treatmentlow -0.678  0.493
## treatmentmedium -0.702  0.511  0.476

```

```

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

```

```
## 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      Max
## -2.67315 -0.57976  0.02409  0.70177  2.44678
##
## Random effects:
## Groups Name Variance Std.Dev.
## aquarium (Intercept) 1.008 1.004
## Residual 71.048 8.429
## Number of obs: 107, groups: aquarium, 49
##
## Fixed effects:
## Estimate Std. Error df t value Pr(>|t|)
## (Intercept) 7.8986 1.8222 48.9878 4.335 7.24e-05 ***
## treatmenthigh -7.2096 2.3684 45.2299 -3.044 0.00388 **
## 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 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
## (Intr) trtmnth trtmntl
## treatmenthigh -0.769
## treatmentlow -0.735 0.565
## treatmentmedium -0.741 0.570 0.544
```

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

```
## 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      Max
## -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)  0.56897    0.04352 103.00000  13.075 <2e-16 ***
## 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
## treatmenthigh -0.770
## treatmentlow -0.736  0.567
## treatmentmedium -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)
```

```
## 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       1Q   Median       3Q      Max
## -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|)
## (Intercept)  0.65747    0.06048 103.00000  10.871  <2e-16 ***
## 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
## treatmenthigh -0.770
## treatmentlw -0.736  0.567
## treatmentmdm -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*treatmentOrder+ scaledBiteRate*treatmentOrder +treatmentOrder+ (1|aquarium), data=alphaDiversitySnails, 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      1Q  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
## (Intercept)    3.861159   0.114882 51.991051  33.610
## 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      0.040158   0.113689 72.160650   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 -0.009395   0.145759 73.846197  -0.064
## 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|)
## (Intercept)    <2e-16 ***
## 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 '**' 0.01 '*' 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
```

```
dd<-dredge(modell1, subset= ~treatmentOrder, evaluate=TRUE, rank=AICc)
```

```
## Warning in dredge(modell1, 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 tr0 sBR:tr0 scR:tr0 scS:tr0
## 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
## 27 3.766      0.01933          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
## 87 3.814      0.18060 0.011570
## 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          0.06948
## 95 3.805      0.13500 -0.005435 0.07257
## 52 3.858 -0.14070 0.03568
## 54 3.848 -0.14630          0.010900
## 151 3.763      0.04426 0.061550
## 150 3.769 -0.03840          0.065880
## 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
## 347 3.802      0.12370          0.08671
## 116 3.880 -0.11510 0.15240
## 215 3.809      0.17210 0.041890
```

| | | | | | | | | | | | |
|----|-----|---------|----------|---------|-----------|---------|--------|--|---|---|---|
| ## | 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 | | 0.07558 | + | | + | | + |
| ## | 223 | 3.807 | | 0.12520 | -0.002233 | 0.08451 | + | | | + | + |
| ## | 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 | + | | + | | |
| ## | 415 | 3.778 | | 0.01882 | -0.017920 | 0.14030 | + | | | | + |
| ## | 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 | + | | + | | + |
| ## | 479 | 3.806 | | 0.12450 | -0.001953 | 0.08558 | + | | | + | + |
| ## | 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:tr0 | 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 | | | | |
| ## | 26 | | 8 | -52.427 | 122.5 | 9.98 | 0.005 | | | | |
| ## | 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 | | | | |
| ## | 149 | | 10 | -54.531 | 131.7 | 19.14 | 0.000 | | | | |
| ## | 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 |
| ## 283 | + 11 | -54.705 | 134.6 | 22.06 | 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 |
| ## 96 | 13 | -56.423 | 143.4 | 30.83 | 0.000 |
| ## 152 | 12 | -57.931 | 143.7 | 31.14 | 0.000 |
| ## 56 | 12 | -58.026 | 143.9 | 31.33 | 0.000 |
| ## 347 | + 14 | -55.421 | 144.2 | 31.59 | 0.000 |
| ## 116 | 14 | -55.831 | 145.0 | 32.41 | 0.000 |
| ## 215 | 14 | -56.293 | 145.9 | 33.33 | 0.000 |
| ## 288 | + 13 | -58.574 | 147.7 | 35.13 | 0.000 |
| ## 160 | 13 | -58.875 | 148.3 | 35.73 | 0.000 |
| ## 314 | + 14 | -57.526 | 148.4 | 35.80 | 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 |
| ## 223 | 15 | -56.710 | 149.6 | 37.00 | 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 | 0.000 |
| ## 120 | 15 | -58.061 | 152.3 | 39.70 | 0.000 |
| ## 216 | 15 | -58.188 | 152.5 | 39.96 | 0.000 |
| ## 414 | + 15 | -59.275 | 154.7 | 42.13 | 0.000 |
| ## 316 | + 15 | -59.498 | 155.1 | 42.58 | 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 |

```
## 480      + 19 -61.697 171.7 59.09  0.000
## 448      + 19 -64.232 176.7 64.16  0.000
## 512      + 22 -64.143 186.5 73.97  0.000
## Models ranked by AICc(x)
## Random terms (all models):
##  1 | aquarium
```

```
ddAIC_S<-subset(dd, delta < 7)
ddAIC_S
```

```
## 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 tr0 df  logLik  AICc  delta  weight
## 17 3.755
## 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 Name Std.Dev.
## 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 Name Std.Dev.
## 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 Name Std.Dev.

```

```

## 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:
##      (Intercept)      scaledSpeed      treatmentOrderlow
##      3.75293          0.01524          0.18290
## treatmentOrdermedium treatmentOrderxHigh
##      0.46711          0.30538
##
## attr("rank")
## function (x)
## do.call("rank", list(x))
## <environment: 0x0000021a756aedb0>
## attr("call")
## AICc(x)
## attr("class")
## [1] "function"      "rankFunction"
## attr("beta")
## [1] "none"

```

```

ddMAc<-model.avg(ddAIC_S, subset= delta <7)
summary(ddMAc)

```

```
##
## Call:
## model.avg(object = ddAIC_S, subset = delta < 7)
##
## Component model call:
## lmer(formula = Shannon ~ <5 unique rhs>, data = alphaDiversitySnails,
##       na.action = na.fail)
##
## Component models:
##   df logLik  AICc delta weight
##  5   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
##                1                2                3                4                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 ***
## treatmentOrderlow  0.17844  0.14260  0.14459  1.234  0.21714
## treatmentOrdermedium 0.46078  0.13910  0.14104  3.267  0.00109 **
## 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
## scaledBiteRate    -0.03086  0.04530  0.04594  0.672  0.50164
## scaledSpeed       0.01524  0.04062  0.04120  0.370  0.71147
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
rm(dd, ddAIC_S, ddMAc)
```

```
# chao1
```

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

```

## 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       1Q   Median       3Q      Max
## -1.7213 -0.6180 -0.1688  0.3949  3.4344
##
## Random effects:
## Groups   Name                Variance Std.Dev.
## aquarium (Intercept)  797.5    28.24
## Residual                96886.7  311.27
## Number of obs: 94, groups: aquarium, 46
##
## Fixed effects:
##              Estimate Std. Error    df t value
## (Intercept)    597.295    83.767  52.003   7.130
## scaledSpeed     -6.408    71.491  73.664  -0.090
## treatmentOrderlow -21.965   114.732  49.487  -0.191
## treatmentOrdermedium 170.050   109.707  44.012   1.550
## treatmentOrderxHigh  46.127   110.978  42.990   0.416
## 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  94.729  116.973  74.000   0.810
## treatmentOrderxHigh:scaledmetRate -123.250  124.655  69.739  -0.989
## treatmentOrderlow:scaledBiteRate  19.375  122.499  70.207   0.158
## treatmentOrdermedium:scaledBiteRate -91.362  116.991  73.884  -0.781
## treatmentOrderxHigh:scaledBiteRate  53.037  102.633  71.293   0.517
##
##              Pr(>|t|)
## (Intercept)    3.06e-09 ***
## 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

```

```
## treatmentOrderxHigh:scaledThig      0.834
## 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)
```

```
## 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
##   Residual                    95536     309.1
## Number of obs: 94, groups:  aquarium, 46
##
## Fixed effects:
##
##              Estimate Std. Error      df t value Pr(>|t|)
## (Intercept)    577.8050    73.5052  86.0000    7.861 1.01e-11 ***
## 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
## treatmentOrdermedium 187.7763  101.5574  86.0000    1.849  0.0679 .
## treatmentOrderxHigh 13.5906    101.7547  86.0000    0.134  0.8941
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##              (Intr) scldSp scldTh scldmR scldBRR trtmntOrdr1 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
## trtmntOrdr1 -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')
```

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

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

```
## Fixed term is "(Intercept)"
## boundary (singular) fit: see help('isSingular')
## boundary (singular) fit: see help('isSingular')
## boundary (singular) fit: see help('isSingular')
## boundary (singular) fit: see help('isSingular')
## boundary (singular) fit: see help('isSingular')
## boundary (singular) fit: see help('isSingular')
## boundary (singular) fit: see help('isSingular')
## 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  trtm0  df  logLik  AICc  delta  weight
## 32  577.8    0.3146 -35.75 20.50 50.89    + 10 -630.178 1283.0  0.00  0.898
## 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
## 24  577.1  -5.2600 -20.75 30.52    + 9  -635.696 1291.5  8.53  0.013
## 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
## 26  595.8  -9.6030           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
## 25  589.6           45.09    + 7  -644.271 1303.8 20.84  0.000
## 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           -15.31    + 7  -645.128 1305.6 22.55  0.000
## 17  582.2           + 6  -649.644 1312.3 29.25  0.000
## Models ranked by AICc(x)
## Random terms (all models):
## 1 | aquarium
```

```
ddAIC_C<-subset(dd, delta < 7)
ddAIC_C
```

```
## 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  trtm0  df  logLik  AICc  delta  weight
## 32  577.8  0.3146 -35.75  20.50  50.89   + 10 -630.178 1283.0  0.00  0.934
## 31  578.0           -35.70  20.44  50.86   + 9  -634.724 1289.6  6.58  0.035
## 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:
##           (Intercept)          scaledBiteRate          scaledmetRate
##           577.8050              0.3146              -35.7522
##           scaledSpeed          scaledThig          treatmentOrderlow
##           20.4986              50.8947              9.9219
## 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
##           578.028              -35.699              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: 0x0000021a0565e8f0>
## attr(,"call")
## AICc(x)
## attr(,"class")
## [1] "function"      "rankFunction"
## attr(,"beta")
## [1] "none"
```

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

```
##
## Call:
## model.avg(object = ddAIC_C, subset = delta < 7)
##
## 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  0.93
## 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
##              1              2              3              4              5
##
## Model-averaged coefficients:
## (full average)
##              Estimate Std. Error Adjusted SE z value Pr(>|z|)
## (Intercept)    578.0159   73.2970    74.3672   7.772 <2e-16 ***
## 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 .
## treatmentOrderxHigh 13.3642 101.3606   102.8407   0.130  0.8966
##
## (conditional average)
##              Estimate Std. Error Adjusted SE z value Pr(>|z|)
## (Intercept)    578.016   73.297    74.367   7.772 <2e-16 ***
## scaledBiteRate     0.162   37.659    38.209   0.004  0.9966
## scaledmetRate    -35.650   35.247    35.762   0.997  0.3188
## scaledSpeed      20.496   34.960    35.471   0.578  0.5634
## scaledThig       51.021   35.181    35.694   1.429  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 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# observed
```

```
model3<-lmer(Observed~scaledSpeed*treatmentOrder + scaledThig*treatmentOrder + scaledmetRate*tr
eatmentOrder+ scaledBiteRate*treatmentOrder +treatmentOrder+ (1|aquarium), data=alphaDiversityS
nails, 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      1Q  Median      3Q      Max
## -2.0418 -0.6155 -0.1443  0.5071  2.6671
##
## Random effects:
## Groups Name          Variance Std.Dev.
## aquarium (Intercept) 1367      36.97
## Residual              14653     121.05
## Number of obs: 94, groups: aquarium, 46
##
## Fixed effects:
##
##              Estimate Std. Error   df t value
## (Intercept)    345.438    34.709 49.920   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  -3.100    40.905 73.412  -0.076
## treatmentOrderlow:scaledThig    20.727    47.110 72.660   0.440
## treatmentOrdermedium:scaledThig  26.742    48.801 73.996   0.548
## treatmentOrderxHigh:scaledThig -18.059    47.462 73.999  -0.380
## treatmentOrderlow:scaledmetRate -29.486    44.636 72.600  -0.661
## 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|)
## (Intercept)    1.92e-13 ***
## 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 '**' 0.01 '*' 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
```

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

```
## 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      1Q  Median      3Q      Max
## -1.7907 -0.7048 -0.1172  0.5325  2.6935
##
## Random effects:
##   Groups   Name                Variance Std.Dev.
##   aquarium (Intercept)  598.1    24.46
##   Residual                15627.7   125.01
## Number of obs: 94, groups:  aquarium, 46
##
## Fixed effects:
##              Estimate Std. Error      df t value Pr(>|t|)
## (Intercept)    338.1812   30.7308  51.5963  11.005 3.82e-15 ***
## scaledSpeed     10.1495   14.3928  85.7930   0.705  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 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##              (Intr) scldSp scldTh scldmR scldBR trtmntOrdr1 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
## trtmntOrdr1 -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)
```

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

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

```
dd
```

```
## 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  trtm0  df  logLik  AICc  delta  weight
## 32  338.2 -0.1211 -16.600 10.150 21.91   + 10 -553.806 1130.3  0.00  0.787
## 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
## 29  342.8          8.436 17.02   + 8  -561.714 1141.1 10.86  0.003
## 26  346.7 -4.8970          18.99   + 8  -561.775 1141.2 10.98  0.003
## 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
## 20  343.4 -6.0480 -6.746          + 8  -562.617 1142.9 12.67  0.001
## 25  343.8          19.39   + 7  -565.448 1146.2 15.94  0.000
## 21  340.8          13.280          + 7  -566.000 1147.3 17.04  0.000
## 18  346.0 -6.8750          + 7  -566.282 1147.9 17.60  0.000
## 19  339.6          -7.306          + 7  -566.325 1148.0 17.69  0.000
## 17  341.9          + 6  -570.004 1153.0 22.71  0.000
## Models ranked by AICc(x)
## Random terms (all models):
## 1 | aquarium
```

```
ddAIC_C<-subset(dd, delta < 7)
ddAIC_C
```

```
## 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  trtm0  df  logLik  AICc  delta  weight
## 32  338.2 -0.1211 -16.600 10.150 21.91   + 10 -553.806 1130.3  0.00  0.801
## 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
## 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
## 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             -0.1211             -16.6005
##           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   Name          Std.Dev.
## 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   Name          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
##          16.941                36.248                95.496
## 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   Name          Std.Dev.
## aquarium (Intercept)  33.85
## Residual                124.03
## Number of obs: 94, groups:  aquarium, 46
## Fixed Effects:
##          (Intercept)          scaledBiteRate          scaledmetRate
##          338.658                -2.115                -9.842
##          scaledSpeed      treatmentOrderlow  treatmentOrdermedium
##          14.606                43.549                100.875
## treatmentOrderxHigh
##          38.137
##
## attr("rank")
## function (x)
## do.call("rank", list(x))
## <environment: 0x0000021a080c5a60>
## attr("call")
## AICc(x)
## attr("class")
## [1] "function"      "rankFunction"
## attr("beta")
## [1] "none"

```

```
ddMAC<-model.avg(ddAIC_C, subset= delta <7)
summary(ddMAC)
```

```
##
## Call:
## model.avg(object = ddAIC_C, subset = delta < 7)
##
## 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  0.80
## 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
##           1           2           3           4           5
##
## Model-averaged coefficients:
## (full average)
##              Estimate Std. Error Adjusted SE z value Pr(>|z|)
## (Intercept)    338.6484   30.5820    31.0270  10.915 <2e-16 ***
## scaledBiteRate    -0.4298   14.9201    15.1371   0.028  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    14.6673    14.8684   1.431  0.1523
## treatmentOrderlow  39.0861   40.7301    41.3236   0.946  0.3442
## treatmentOrdermedium 104.6191  42.3674    42.9833   2.434  0.0149 *
## treatmentOrderxHigh  38.1000   42.3605    42.9766   0.887  0.3753
##
## (conditional average)
##              Estimate Std. Error Adjusted SE z value Pr(>|z|)
## (Intercept)    338.6484   30.5820    31.0270  10.915 <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
## scaledThig      21.8335   14.4452    14.6546   1.490  0.1363
## treatmentOrderlow  39.0861   40.7301    41.3236   0.946  0.3442
## treatmentOrdermedium 104.6191  42.3674    42.9833   2.434  0.0149 *
## treatmentOrderxHigh  38.1000   42.3605    42.9766   0.887  0.3753
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 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)
```

```
## 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)
```

```
## 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)
alphaDiversitySnails$Posttraining<-as.numeric(alphaDiversitySnails$Posttraining)
Alltraining2<-rowSums(alphaDiversitySnails[, c("Pretraining", "Posttraining")])

alphaDiversitySnails<-cbind(alphaDiversitySnails, Alltraining2)

model3<-lmer(Shannon~Alltraining2+ (1|aquarium), data=alphaDiversitySnails) ###t = 0.457 p =
0.649
summary(model3)
```

```
## 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:
## Groups Name Variance Std.Dev.
## 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)
```

```
## 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:
##   Min       1Q   Median       3Q      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)
```

```
## 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:
## Groups Name Variance Std.Dev.
## aquarium (Intercept) 2977 54.56
## Residual 14114 118.80
## Number of obs: 94, groups: aquarium, 46
##
## Fixed effects:
## Estimate Std. Error df t value Pr(>|t|)
## (Intercept) 379.6674 24.9718 82.9137 15.204 <2e-16 ***
## 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
```

```
model6<-lmer(Observed~Alltraining2+ (1|aquarium), data=alphaDiversitySnails) ###t = 0.457 p
=0.649
summary(model6)
```

```
## 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:
## Groups Name Variance Std.Dev.
## 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
```

```
## 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       1Q   Median       3Q      Max
## -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)    15.8571    1.3114 90.0000  12.092 <2e-16 ***
## treatmentOrderlow  -0.5714    1.8546 90.0000  -0.308  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) trtmntOrdr1 trtmntOrdrm
## trtmntOrdr1 -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)
```

```
## 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:
##      Min       1Q   Median       3Q      Max
## -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) 0.38788 0.03501 49.00000 11.078 6.03e-15 ***
## 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)
```

```
## 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)
```

```
## 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
## Residual 0.08754 0.2959
## Number of obs: 51, groups: aquarium, 25
##
## Fixed effects:
## Estimate Std. Error df t value Pr(>|t|)
## (Intercept) 0.65747 0.06308 49.00000 10.423 4.99e-14 ***
## 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(Thigmataxis~ treatmentOrder + (1|aquarium), data = metadataDMSO)
summary(model4)
```

```

## 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)    0.57505    0.05292 29.89423  10.867 6.65e-12 ***
## 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) ###
this has the scaled data for analyses, example in beta diversity
otus <- read.table("otu-table-centroids-iddef0-400bp.txt")

# 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)
#Assigning row names from 1st column
rownames(KO) <- KO[,1]
KO = subset(KO, select = -function. )
KO[] <- lapply(KO, as.integer)
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")
# 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)
meta$scaledSpeed<-scale(meta$Speed)
meta$scaledBiteRate<-scale(meta$biteRateChange)
meta$scaledmetRate<-scale(meta$metabolicRate)
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:
## [1] stats      graphics  grDevices  utils      datasets  methods   base
##
## 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      colorspace_2.1-0 XVector_0.38.0
## [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
## [46] biomformat_1.26.0 rhdf5_2.42.1     yaml_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

```



```
## [79] crayon_1.5.2          KernSmooth_2.23-21      utf8_1.2.3
## [82] tzdb_0.4.0              rmarkdown_2.22         locfit_1.5-9.8
## [85] grid_4.2.3              data.table_1.14.8      digest_0.6.31
## [88] numDeriv_2016.8-1.1    stats4_4.2.3           munsell_0.5.0
## [91] bslib_0.5.0
```