Comparative Analysis of Feeding Mode and Microbiome Composition in Poison Frog Tadpoles

Kayla Weinfurther, Adam Stuckert, Mario Muscarella, Ariane L. Peralta, Kyle Summers

Last updated on 11 July, 2022

Project Description: Fill out

Initial Setup

#Import Files ## Environmental Data

Bacterial Data

Eukaryote Data

```
# Import 18S OTU Data
euk.otu.in <- read.otu("../mothur/MicroEuk.final.shared")
```

Simple Hypothesis Testing - R. imitator

```
# R. imitator field egg-fed vs. field detritus-fed

otus.design.imitator <- subset(otus.design, Species == "imitator")
dim(otus.design.imitator)
```

```
## [1]   11 8277
```

```
design.final.i <- otus.design.imitator[,c(1:4)] # seaparate design file
otu.i <- otus.design.imitator[,-c(1:4)] # separate otu file

# OTU table - remove otus w/ < 2 occurrences across all sites
otu_removal <- otu.i[, which(colSums(otu.i) >= 2)]
dim(otu_removal) # 4696 OTUs
```

```
## [1]   11 4696
```

```r
otu.i <- otu.i[, which(colSums(otu.i) >= 2)]

# Make Relative Abundance Matrices
dataREL.i <- otu.i
for(i in 1:dim(otu.i)[1]){
  dataREL.i[i,] <- otu.i[i,]/sum(otu.i[i,])
}

#PERMANOVA
new.data.i <-cbind(design.final.i,dataREL.i)
adonis = adonis2(new.data.i[,-c(1:4)]~Diet, method = "bray", data = new.data.i, perm=1000, set.seed=42)
adonis
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 1000
##
## adonis2(formula = new.data.i[, -c(1:4)] ~ Diet, data = new.data.i, permutations = 1000, method = "bra
##           Df SumOfSqs     R2      F  Pr(>F)
## Diet       1   0.5298 0.1564 1.6685 0.01798 *
## Residual   9   2.8575 0.8436
## Total     10   3.3873 1.0000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
# Principal Coordinates Analysis
dataREL.dist <- vegdist(dataREL.i, method="bray")

pcoa <- cmdscale(dataREL.dist, k=3, eig=TRUE, add=FALSE)
  # Classical (Metric) Multidimensional Scaling; returns PCoA coordinates
  # eig=TRUE returns eigenvalues; k = # of dimensions to calculate

explainvar1b <- round(pcoa$eig[1] / sum(pcoa$eig), 3) * 100
explainvar2b <- round(pcoa$eig[2] / sum(pcoa$eig), 3) * 100
sum.eigb <- sum(explainvar1b, explainvar2b)

explainvar1b #20.5
```

```
## [1] 20.5
```

```r
explainvar2b #16.1
```

```
## [1] 16.1
```

```r
pcoa.groups <- paste(new.data.i$Diet, new.data.i$Species, sep = "_")
pcoa.points <- data.frame(pcoa$points, group = pcoa.groups)

# Calculate Centroids (mean and SE)
pcoa.L.centroids <- melt(pcoa.points, id="group", measure.vars = c("X1", "X2"))
pcoa.centroids <- acast(pcoa.L.centroids, variable ~ group, mean)
```

```r
pcoa.centroids.se <- acast(pcoa.L.centroids, variable ~ group, se)
pcoa.centroids.sd <- acast(pcoa.L.centroids, variable ~ group, sd)

# Combine
pcoa.cent.dataframe <- cbind(t(pcoa.centroids), t(pcoa.centroids.se))
colnames(pcoa.cent.dataframe) <- c("V1", "V2", "V1e", "V2e")
pcoa.cent.treats <- rownames(pcoa.cent.dataframe)

pcoa.col <- as.factor(sapply(strsplit(pcoa.cent.treats, "_"), `[`, 1))  # Diet

#Plot
df1a <- as.data.frame(pcoa.cent.dataframe)
plot1a <- ggplot(df1a, aes(x=V1, y=V2, colour=as.factor(pcoa.col), shape=as.factor(pcoa.col))) + theme_l
plot1a +
  theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank(), axis.line = element_lin
  theme(panel.background = element_blank()) +
  geom_errorbarh(aes(xmax=V1+V1e, xmin=V1-V1e, height=0.01), colour="black") +
  geom_errorbar(aes(ymax=V2+V2e, ymin=V2-V2e, width=0.01), colour="black") +
  geom_point(aes(colour=as.factor(pcoa.col)), size=5, stroke = 0.75, show.legend = TRUE) +
    scale_shape_manual(name = "Diet",
                       labels = c("detritus fed","egg fed"),
                       values = c(19, 17))+
  scale_colour_manual(name = "Diet",
                       labels = c("detritus fed","egg fed"),
                       values = c("darkgreen","darkorange")) +
  theme(axis.title = element_text(size=14), axis.text=element_text(size=14),
        axis.text.x = element_text(size=14),
        panel.border = element_rect(colour = "black", size=1.25)) +
  theme(axis.ticks.length=unit(0.3,"cm")) +
  xlab("PCoA 1 (20.5%)") + ylab("PCoA 2 (16.1%)") +
  ggtitle(label="Ranitomeya imitator 16S rRNA Community Composition") +
  theme(plot.title = element_text(color="black", size=14, face="bold.italic"))
```

Ranitomeya imitator 16S rRNA Community Composition
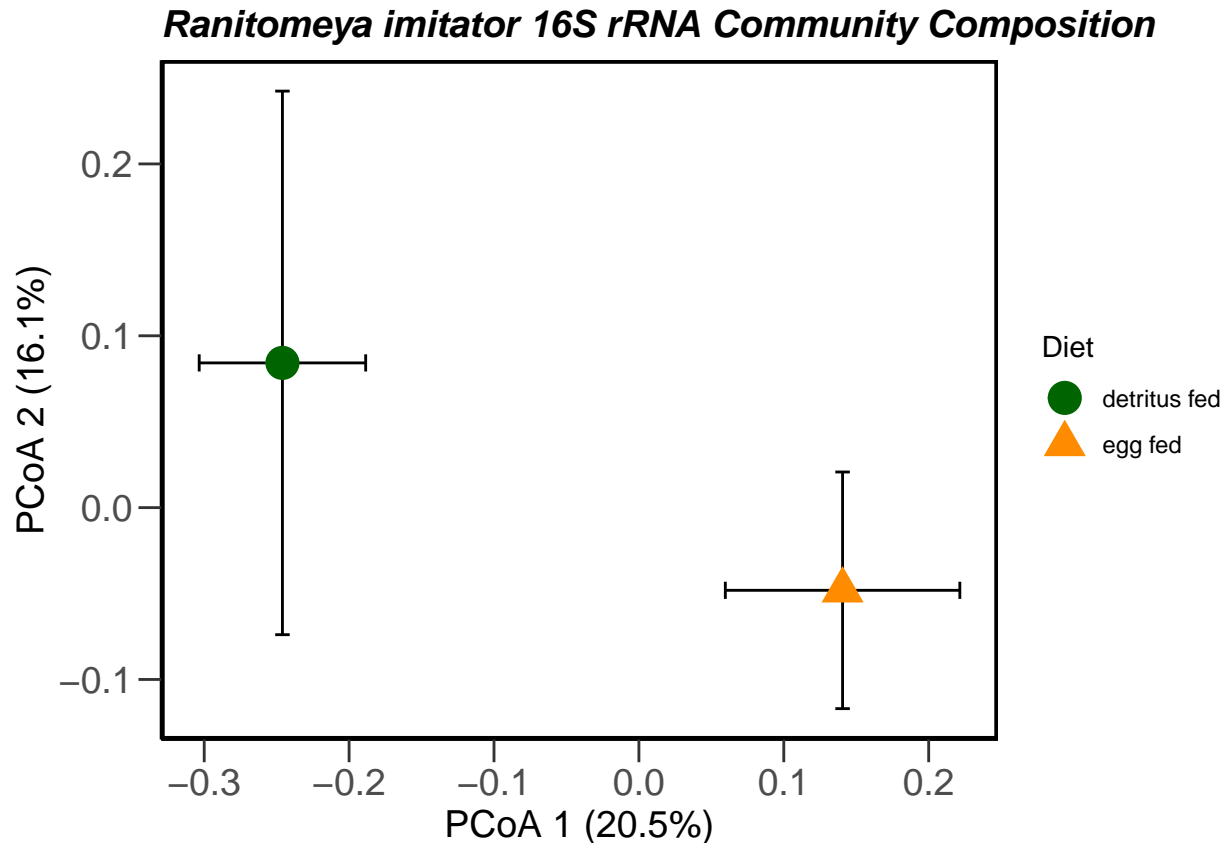

```
ggsave("../figures/Ordination_imitator_diet.pdf", plot=last_plot(), device=NULL, path=NULL, scale=1, wic
ggsave("../figures/Ordination_imitator_diet.png", plot=last_plot(), device=NULL, path=NULL, scale=1, wic
```

Bacterial community indicator species analysis - R. imitator diet

Simple Hypothesis Testing - R. variabilis

```r
# R. variabilis lab egg-fed vs. field detritus-fed

otus.design.variabilis <- subset(otus.design, Species == "variabilis")
dim(otus.design.variabilis)
```

```
## [1]   10 8277
```

```r
design.final.v <- otus.design.variabilis[,c(1:4)] # seaparate design file
otu.v <- otus.design.variabilis[,-c(1:4)] # separate otu file

# OTU table - remove otus w/ < 2 occurrences across all sites
otu.v <- otu.v[, which(colSums(otu.v) >= 2)]
dim(otu.v) # 2940 OTUs
```

```
## [1]   10 2940
```

```r
# Make Relative Abundance Matrices
dataREL.v <- otu.v
for(i in 1:dim(otu.v)[1]){
  dataREL.v[i,] <- otu.v[i,]/sum(otu.v[i,])
}

#PERMANOVA
new.data.v <-cbind(design.final.v,dataREL.v)
adonis = adonis2(new.data.v[,-c(1:4)]~Diet, method = "bray", data = new.data.v, perm=1000, set.seed=42)
adonis
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 1000
##
## adonis2(formula = new.data.v[, -c(1:4)] ~ Diet, data = new.data.v, permutations = 1000, method = "bra
##          Df SumOfSqs     R2      F Pr(>F)
## Diet      1   0.4493 0.15314 1.4466 0.1219
## Residual  8   2.4847 0.84686
## Total     9   2.9340 1.00000
```

```r
# Principal Coordinates Analysis
dataREL.dist <- vegdist(dataREL.v, method="bray")

pcoa <- cmdscale(dataREL.dist, k=3, eig=TRUE, add=FALSE)
  # Classical (Metric) Multidimensional Scaling; returns PCoA coordinates
  # eig=TRUE returns eigenvalues; k = # of dimensions to calculate

explainvar1b <- round(pcoa$eig[1] / sum(pcoa$eig), 3) * 100
explainvar2b <- round(pcoa$eig[2] / sum(pcoa$eig), 3) * 100
sum.eigb <- sum(explainvar1b, explainvar2b)

explainvar1b #31.7
```

```
## [1] 31.7
```

```r
explainvar2b #19.3
```

```
## [1] 19.3
```

```r
pcoa.groups <- paste(new.data.v$Diet, new.data.v$Species, sep = "_")
pcoa.points <- data.frame(pcoa$points, group = pcoa.groups)

# Calculate Centroids (mean and SE)
pcoa.L.centroids <- melt(pcoa.points, id="group", measure.vars = c("X1", "X2"))
pcoa.centroids <- acast(pcoa.L.centroids, variable ~ group, mean)
pcoa.centroids.se <- acast(pcoa.L.centroids, variable ~ group, se)
pcoa.centroids.sd <- acast(pcoa.L.centroids, variable ~ group, sd)
```

```r
# Combine
pcoa.cent.dataframe <- cbind(t(pcoa.centroids), t(pcoa.centroids.se))
colnames(pcoa.cent.dataframe) <- c("V1", "V2", "V1e", "V2e")
pcoa.cent.treats <- rownames(pcoa.cent.dataframe)

pcoa.col <- as.factor(sapply(strsplit(pcoa.cent.treats, "_"), `[`, 1))  # Diet

#Plot
df2a <- as.data.frame(pcoa.cent.dataframe)
plot1a <- ggplot(df2a, aes(x=V1, y=V2, colour=pcoa.col, shape=pcoa.col)) + theme_bw()
plot1a +
  theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank(), axis.line = element_line
  theme(panel.background = element_blank()) +
  geom_errorbarh(aes(xmax=V1+V1e, xmin=V1-V1e, height=0.01), colour="black") +
  geom_errorbar(aes(ymax=V2+V2e, ymin=V2-V2e, width=0.01), colour="black") +
  geom_point(aes(colour=as.factor(pcoa.col)), size=5, stroke = 0.75, show.legend = TRUE) +
  scale_shape_manual(name = "Diet",
                     labels = c("detritus fed","egg fed"),
                     values = c(19, 17))+
  scale_colour_manual(name = "Diet",
                      labels = c("detritus fed","egg fed"),
                      values = c("darkgreen","darkorange")) +
  theme(axis.title = element_text(size=14), axis.text=element_text(size=14),
        axis.text.x = element_text(size=14),
        panel.border = element_rect(colour = "black", size=1.25)) +
  theme(axis.ticks.length=unit(0.3,"cm")) +
  xlab("PCoA 1 (31.7%)") + ylab("PCoA 2 (19.3%)") +
  ggtitle(label="Ranitomeya variabilis 16S rRNA Community Composition") +
  theme(plot.title = element_text(color="black", size=14, face="bold.italic"))
```

Ranitomeya variabilis 16S rRNA Community Composition
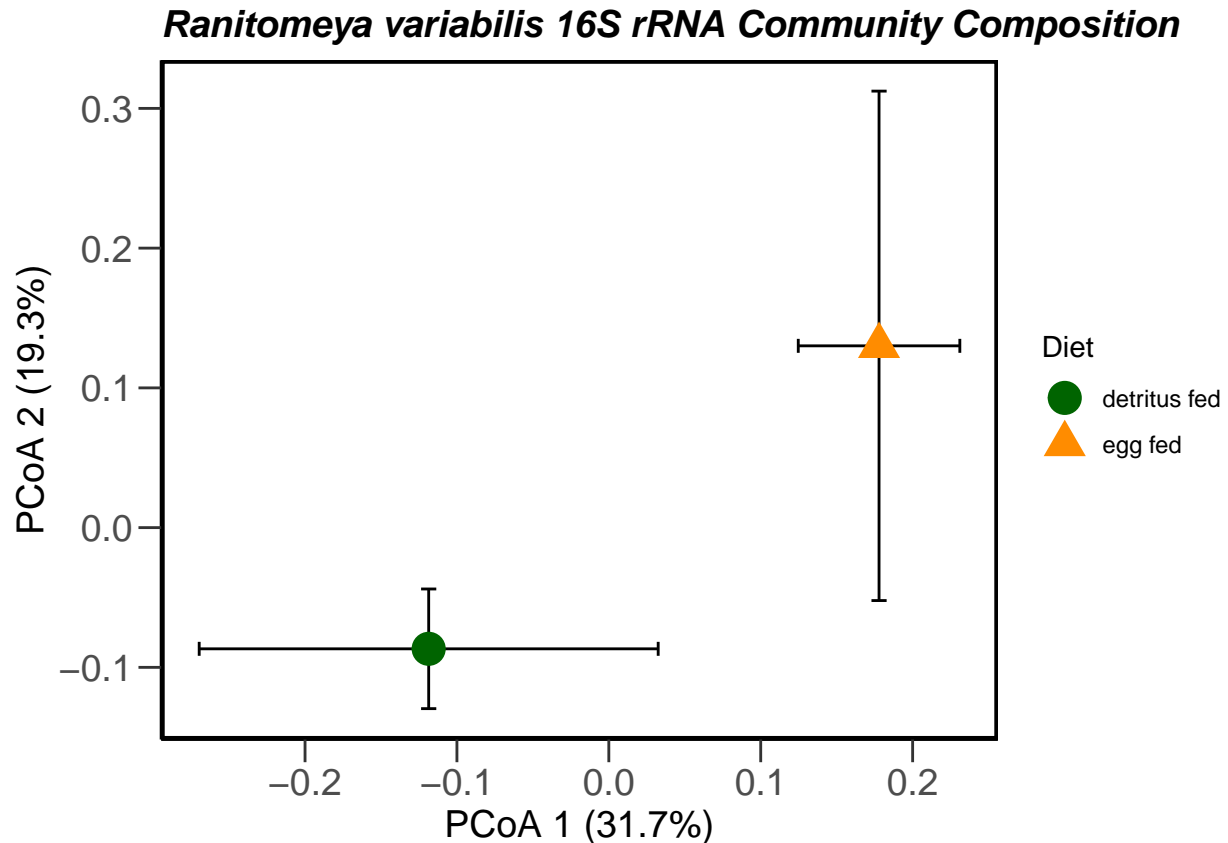

```
ggsave("../figures/Ordination_variabilis_diet.pdf", plot=last_plot(), device=NULL, path=NULL, scale=1,
ggsave("../figures/Ordination_variabilis_diet.png", plot=last_plot(), device=NULL, path=NULL, scale=1,
```

Bacterial community indicator species analysis - R. variabilis diet

Diversity Metrics - Hypothesis Testing

```
# Rarefy Abundances (min abundance is 13520. We are sampling to 13500)
min(rowSums(otu))
```

```
## [1] 13520
```

```
otus.r <- rrarefy(otu, 13500)

# Fisher's Alpha
fisher <- fisher.alpha(otus.r)

# Species Richness
#richness <- rowSums((PWESdata.r >= 1))
richness <- rowSums((otu >= 1))
```

```r
# Shannon Diversity
shannon <- diversity(otus.r, "shannon")

# Simpson's Evenness
simp.even <- apply(otus.r, 1, simp_even)

#Pielou's evenness
J <- shannon/log(specnumber(otus.r[,-c(1:1)]))

#combined richness, diversity, evenness
diversity <- cbind(design.final,richness,shannon,simp.even,J)
#write.csv(diversity,"../data/diversity.bact.raw.csv")
```

Diversity Metrics - Hypothesis Testing - by species

```r
#summary table for bacterial diversity
summary <- diversity %>% group_by(Species, Diet) %>% summarise(mean.richness=mean(richness), se.richness
```

```
## 'summarise()' has grouped output by 'Species'. You can override using the
## '.groups' argument.
```

```r
print(summary)
```

```
## # A tibble: 4 x 6
## # Groups:   Species [2]
##   Species    Diet      mean.richness se.richness mean.shannon se.shannon
##   <chr>      <chr>            <dbl>       <dbl>        <dbl>      <dbl>
## 1 imitator   detritus         1091.        74.9         4.36      0.212
## 2 imitator   egg fed          1208.       435.          5.13      0.489
## 3 variabilis detritus          851.       130.          4.14      0.288
## 4 variabilis egg fed           548         17.0         3.34      0.200
```

```r
write.csv(summary,"../data/diversity.bact.summary.csv")

#diversity <- read.csv("../data/diversity.bact.summary.csv", row.names=1)

library(lmerTest)
```

```
## Loading required package: lme4
## Loading required package: Matrix
##
## Attaching package: 'Matrix'
##
## The following objects are masked from 'package:tidyr':
##
##     expand, pack, unpack
##
## The following object is masked from 'package:reshape':
##
```

```
##     expand
##
##
## Attaching package: 'lme4'
##
## The following object is masked from 'package:labdsv':
##
##     factorize
##
## The following object is masked from 'package:nlme':
##
##     lmList
##
##
## Attaching package: 'lmerTest'
##
## The following object is masked from 'package:lme4':
##
##     lmer
##
## The following object is masked from 'package:stats':
##
##     step
```

```r
# R. imitator
diversity.i <- subset(diversity, Species == "imitator")
richness.lm.i <- lm(richness ~ Diet, data = diversity.i)
richness.lm.i
```

```
##
## Call:
## lm(formula = richness ~ Diet, data = diversity.i)
##
## Coefficients:
## (Intercept)  Dietegg fed
##      1091.2        117.1
```

```r
summary(richness.lm.i)
```

```
##
## Call:
## lm(formula = richness ~ Diet, data = diversity.i)
##
## Residuals:
## RiNEW021 RiNEW024 RiWAS001 RiNEW008 RiNEW006 RiNEW025 RiNEW023
##   112.75    64.75   867.67  -391.33  -476.33  -220.25    42.75
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    1091.2      245.0   4.455  0.00667 **
## Dietegg fed     117.1      374.2   0.313  0.76699
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
## 
## Residual standard error: 489.9 on 5 degrees of freedom
## Multiple R-squared:  0.01921,    Adjusted R-squared:  -0.177
## F-statistic: 0.09791 on 1 and 5 DF,  p-value: 0.767
```

```
evenness.lm.i <- lm(simp.even ~ Diet, data = diversity.i)
evenness.lm.i
```

```
## 
## Call:
## lm(formula = simp.even ~ Diet, data = diversity.i)
## 
## Coefficients:
## (Intercept)  Dietegg fed
##     0.02727      0.01886
```

```
summary(evenness.lm.i)
```

```
## 
## Call:
## lm(formula = simp.even ~ Diet, data = diversity.i)
## 
## Residuals:
##   RiNEW021   RiNEW024   RiWAS001   RiNEW008   RiNEW006   RiNEW025   RiNEW023
## -0.0164567 -0.0096900  0.0005444 -0.0149893  0.0144449  0.0232009  0.0029458
## 
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) 0.027265   0.008202   3.324   0.0209 *
## Dietegg fed 0.018862   0.012528   1.506   0.1925
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## 
## Residual standard error: 0.0164 on 5 degrees of freedom
## Multiple R-squared:  0.3119, Adjusted R-squared:  0.1743
## F-statistic: 2.267 on 1 and 5 DF,  p-value: 0.1925
```

```
shannon.lm.i <- lm(shannon ~ Diet, data = diversity.i)
shannon.lm.i
```

```
## 
## Call:
## lm(formula = shannon ~ Diet, data = diversity.i)
## 
## Coefficients:
## (Intercept)  Dietegg fed
##      4.3638       0.7633
```

```
summary(shannon.lm.i)
```

```
## 
```

```
## Call:
## lm(formula = shannon ~ Diet, data = diversity.i)
##
## Residuals:
## RiNEW021 RiNEW024 RiWAS001 RiNEW008 RiNEW006 RiNEW025 RiNEW023
## -0.57748 -0.05393  0.93155 -0.72539 -0.20616  0.28070  0.35071
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)   4.3638     0.3142   13.89 3.48e-05 ***
## Dietegg fed   0.7633     0.4800    1.59    0.173
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.6284 on 5 degrees of freedom
## Multiple R-squared:  0.3359, Adjusted R-squared:  0.2031
## F-statistic: 2.529 on 1 and 5 DF,  p-value: 0.1726
```

```r
# R. variabilis
diversity.v <- subset(diversity, Species == "variabilis")
richness.lm.v <- lm(richness ~ Diet, data = diversity.v)
richness.lm.v
```

```
##
## Call:
## lm(formula = richness ~ Diet, data = diversity.v)
##
## Coefficients:
## (Intercept)  Dietegg fed
##       851.3       -303.3
```

```r
summary(richness.lm.v)
```

```
##
## Call:
## lm(formula = richness ~ Diet, data = diversity.v)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -456.33  -56.00    4.50   31.92  507.67
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)    851.3      103.2   8.248  3.5e-05 ***
## Dietegg fed   -303.3      163.2  -1.859      0.1
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 252.8 on 8 degrees of freedom
## Multiple R-squared:  0.3016, Adjusted R-squared:  0.2143
## F-statistic: 3.454 on 1 and 8 DF,  p-value: 0.1001
```

```
evenness.lm.v <- lm(simp.even ~ Diet, data = diversity.v)
evenness.lm.v
```

```
##
## Call:
## lm(formula = simp.even ~ Diet, data = diversity.v)
##
## Coefficients:
## (Intercept)  Dietegg fed
##     0.03680     -0.01904
```

```
summary(evenness.lm.v)
```

```
##
## Call:
## lm(formula = simp.even ~ Diet, data = diversity.v)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.027842 -0.021244 -0.001612  0.008954  0.067426
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)  0.03680    0.01220   3.017   0.0166 *
## Dietegg fed -0.01904    0.01929  -0.987   0.3525
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.02988 on 8 degrees of freedom
## Multiple R-squared:  0.1086, Adjusted R-squared:  -0.002851
## F-statistic: 0.9744 on 1 and 8 DF,  p-value: 0.3525
```

```
shannon.lm.v <- lm(shannon ~ Diet, data = diversity.v)
shannon.lm.v
```

```
##
## Call:
## lm(formula = shannon ~ Diet, data = diversity.v)
##
## Coefficients:
## (Intercept)  Dietegg fed
##      4.1385      -0.7966
```

```
summary(shannon.lm.v)
```

```
##
## Call:
## lm(formula = shannon ~ Diet, data = diversity.v)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
```

```
## -0.88268 -0.37186 -0.03636  0.26926  1.00642
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)   4.1385     0.2487  16.642 1.72e-07 ***
## Dietegg fed  -0.7966     0.3932  -2.026   0.0773 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.6091 on 8 degrees of freedom
## Multiple R-squared:  0.3391, Adjusted R-squared:  0.2565
## F-statistic: 4.105 on 1 and 8 DF,  p-value: 0.07734
```

#Plot shannon diversity

```r
# Graphing Shannon Diversity
spp.labs <- c("Ranitomeya imitator", "Ranitomeya variabilis") #for facet labels
names(spp.labs) <- c("imitator", "variabilis")

p <- ggplot(diversity, aes(x=Diet, y=shannon, color=as.factor(Diet), shape=as.factor(Diet)))+ geom_boxpl
  geom_point(aes(color=factor(Diet)), size=2, position = position_jitterdodge()) +
  scale_shape_manual(name = "Diet",
                     labels = c("detritus fed","egg fed"),
                     values = c(19, 17))+
  scale_colour_manual(name = "Diet",
                      labels = c("detritus fed","egg fed"),
                      values = c("darkgreen","darkorange")) +
  stat_summary(fun.data=mean_cl_boot,size=0.75)
p1<-p+facet_wrap(~Species)+facet_grid(. ~ Species,labeller = labeller(Species=spp.labs)) + theme(strip.
p1 + theme_bw() +
    theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank(), axis.line
          =element_line(colour = "black")) +
    theme(axis.title=element_text(vjust=1,size=14,face="bold"),
          axis.text=element_text(size=14), axis.text.x = element_text(vjust=0.65, hjust=0.5,
          size=14), panel.border = element_rect(colour = "black",size=1)) +
    theme(axis.ticks.length=unit(0.3,"cm")) + labs(x = "Diet", y = "Shannon Diversity Index (H')") +
    theme(strip.text.x = element_text(size=14, face="italic"), strip.text.y =
          element_text(size=14, face="bold"), strip.background = element_rect(colour="black",
          fill="white", size=1)) +
          scale_x_discrete(breaks=c("detritus", "egg fed"), labels=c("detritus fed", "egg fed"))
```

```
## Warning: Computation failed in 'stat_summary()':
##
## Computation failed in 'stat_summary()':
```
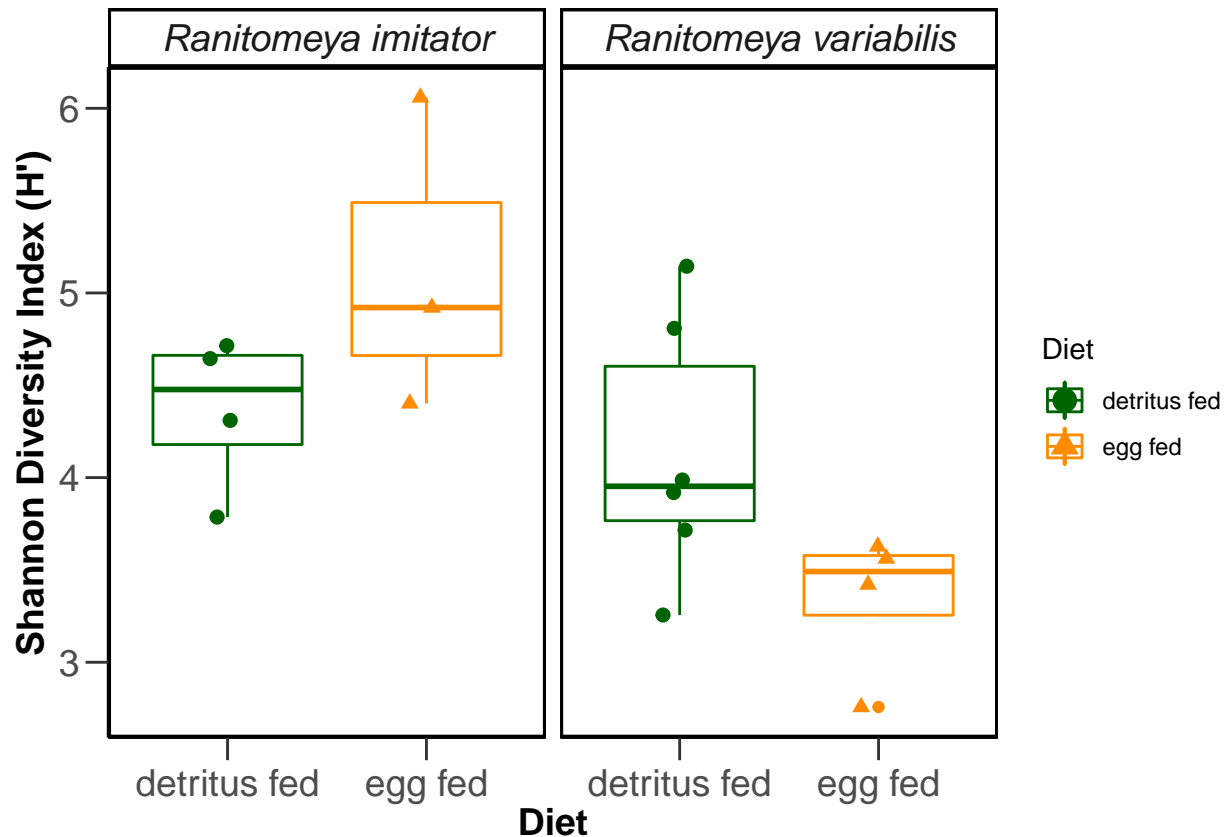
```
ggsave("../figures/shannon.pdf", plot=last_plot(), device=NULL, path=NULL, scale=1, width=7, height=5,
```

```
## Warning: Computation failed in 'stat_summary()':
##
## Computation failed in 'stat_summary()':
```

```
ggsave("../figures/shannon.png", plot=last_plot(), device=NULL, path=NULL, scale=1, width=7, height=5,
```

```
## Warning: Computation failed in 'stat_summary()':
##
## Computation failed in 'stat_summary()':
```
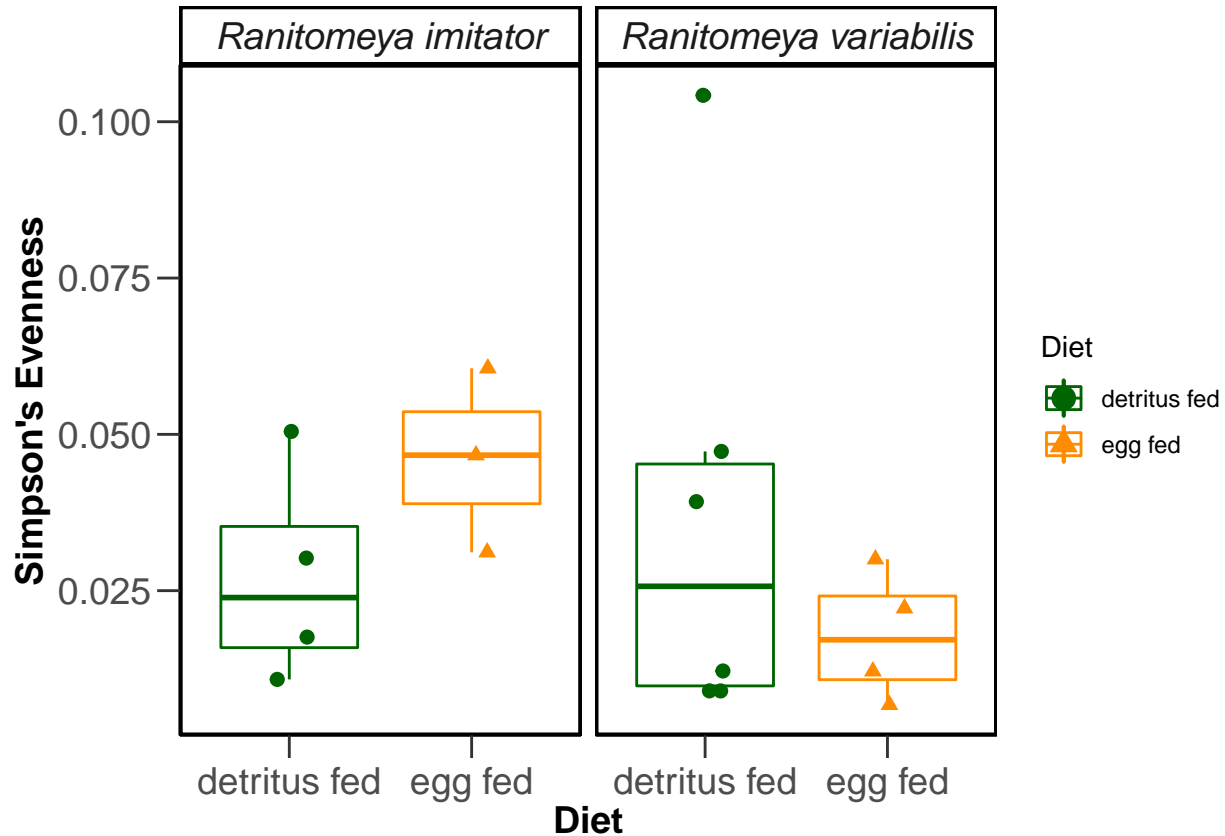
#Plot Evenness

```
# Graphing Simpson's Evenness
p <- ggplot(diversity, aes(x=Diet, y=simp.even, color=as.factor(Diet), shape=as.factor(Diet)))+ geom_box
  geom_point(aes(color=factor(Diet)), size=2, position = position_jitterdodge()) +
  scale_shape_manual(name = "Diet",
                     labels = c("detritus fed","egg fed"),
                     values = c(19, 17)) +
  scale_colour_manual(name = "Diet",
                      labels = c("detritus fed","egg fed"),
                      values = c("darkgreen","darkorange")) +
  stat_summary(fun.data=mean_cl_boot,size=0.75)
```

```
p1<-p+facet_wrap(~Species)+facet_grid(. ~ Species,labeller = labeller(Species=spp.labs)) + theme(strip.
p1 + theme_bw() +
    theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank(), axis.line
        =element_line(colour = "black")) +
    theme(axis.title=element_text(vjust=1,size=14,face="bold"),
        axis.text=element_text(size=14), axis.text.x = element_text(vjust=0.65, hjust=0.5,
        size=14), panel.border = element_rect(colour = "black",size=1)) +
    theme(axis.ticks.length=unit(0.3,"cm")) + labs(x = "Diet", y = "Simpson's Evenness") +
    theme(strip.text.x = element_text(size=14, face="italic"), strip.text.y =
        element_text(size=14, face="bold"), strip.background = element_rect(colour="black",
        fill="white", size=1)) +
        scale_x_discrete(breaks=c("detritus", "egg fed"), labels=c("detritus fed", "egg fed"))
```

```
## Warning: Computation failed in 'stat_summary()':
##
## Computation failed in 'stat_summary()':
```



```
ggsave("../figures/simp.even.pdf", plot=last_plot(), device=NULL, path=NULL, scale=1, width=7, height=5
```

```
## Warning: Computation failed in 'stat_summary()':
##
## Computation failed in 'stat_summary()':
```

```
ggsave("../figures/simp.even.png", plot=last_plot(), device=NULL, path=NULL, scale=1, width=7, height=5
```

```
## Warning: Computation failed in 'stat_summary()':
##
## Computation failed in 'stat_summary()':
```

#Plot Richness

```
# Graphing Richness
p <- ggplot(diversity, aes(x=Diet, y=richness, color=as.factor(Diet), shape=as.factor(Diet)))+ geom_boxp
  geom_point(aes(color=factor(Diet)), size=2, position = position_jitterdodge()) +
  scale_shape_manual(name = "Diet",
                     labels = c("detritus fed","egg fed"),
                     values = c(19, 17)) +
  scale_colour_manual(name = "Diet",
                      labels = c("detritus fed","egg fed"),
                      values = c("darkgreen","darkorange")) +
  stat_summary(fun.data=mean_cl_boot,size=0.75)
p1=p+facet_wrap(~Species)+facet_grid(. ~ Species,labeller = labeller(Species=spp.labs))
p1 + theme_bw() +
    theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank(), axis.line
          =element_line(colour = "black")) +
    theme(axis.title=element_text(vjust=1,size=14,face="bold"),
          axis.text=element_text(size=14), axis.text.x = element_text(vjust=0.65, hjust=0.5,
          size=14), panel.border = element_rect(colour = "black",size=1)) +
    theme(axis.ticks.length=unit(0.3,"cm")) + labs(x = "Diet", y = "OTU Richness") +
    theme(strip.text.x = element_text(size=14, face="italic"), strip.text.y =
          element_text(size=14, face="bold"), strip.background = element_rect(colour="black",
          fill="white", size=1)) +
          scale_x_discrete(breaks=c("detritus", "egg fed"), labels=c("detritus fed", "egg fed"))
```

```
## Warning: Computation failed in 'stat_summary()':
##
## Computation failed in 'stat_summary()':
```
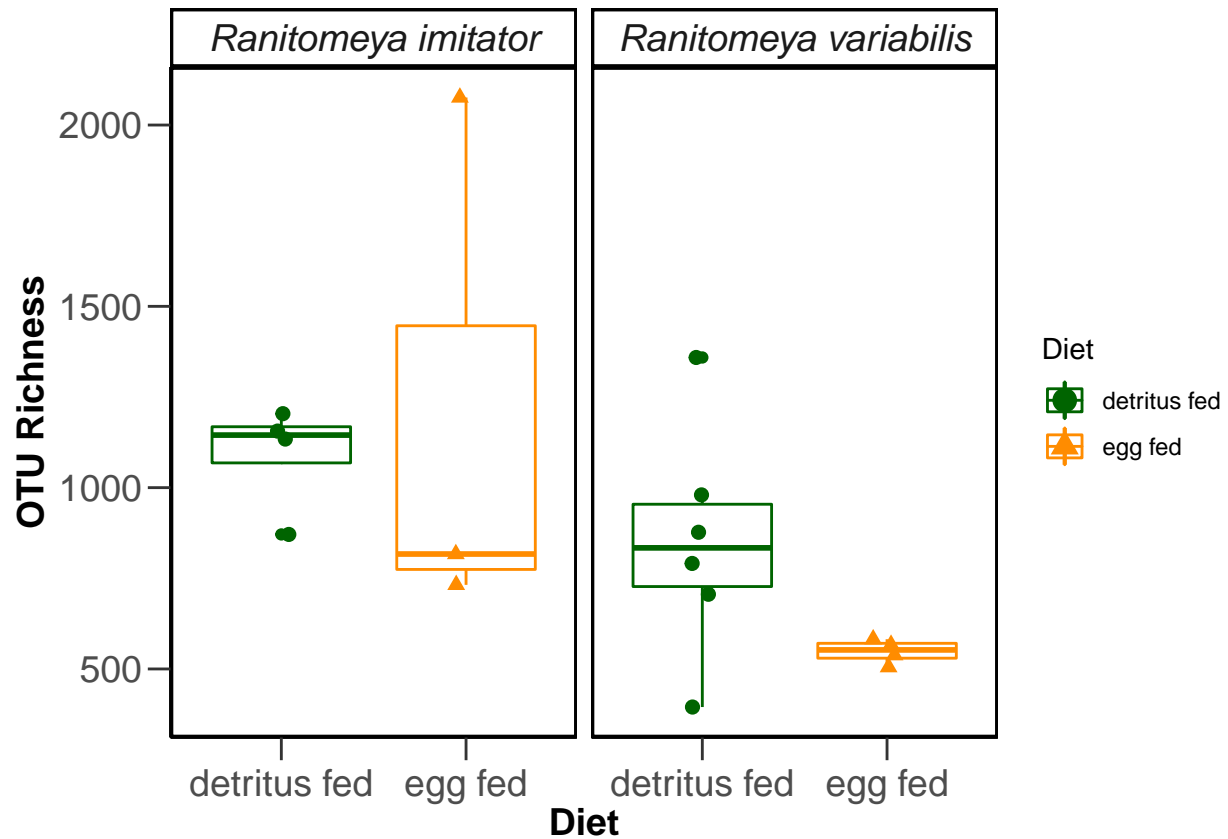
```
ggsave("../figures/richness.pdf", plot=last_plot(), device=NULL, path=NULL, scale=1, width=7, height=5,
```

```
## Warning: Computation failed in `stat_summary()`:
##
## Computation failed in `stat_summary()`:
```

```
ggsave("../figures/richness.png", plot=last_plot(), device=NULL, path=NULL, scale=1, width=7, height=5,
```

```
## Warning: Computation failed in `stat_summary()`:
##
## Computation failed in `stat_summary()`:
```

Bacterial community composition analysis - Species x Diet

Bacterial community indicator species analysis - Species x Diet

Micro Euks