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 15 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 0.01898 *
## 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)
```
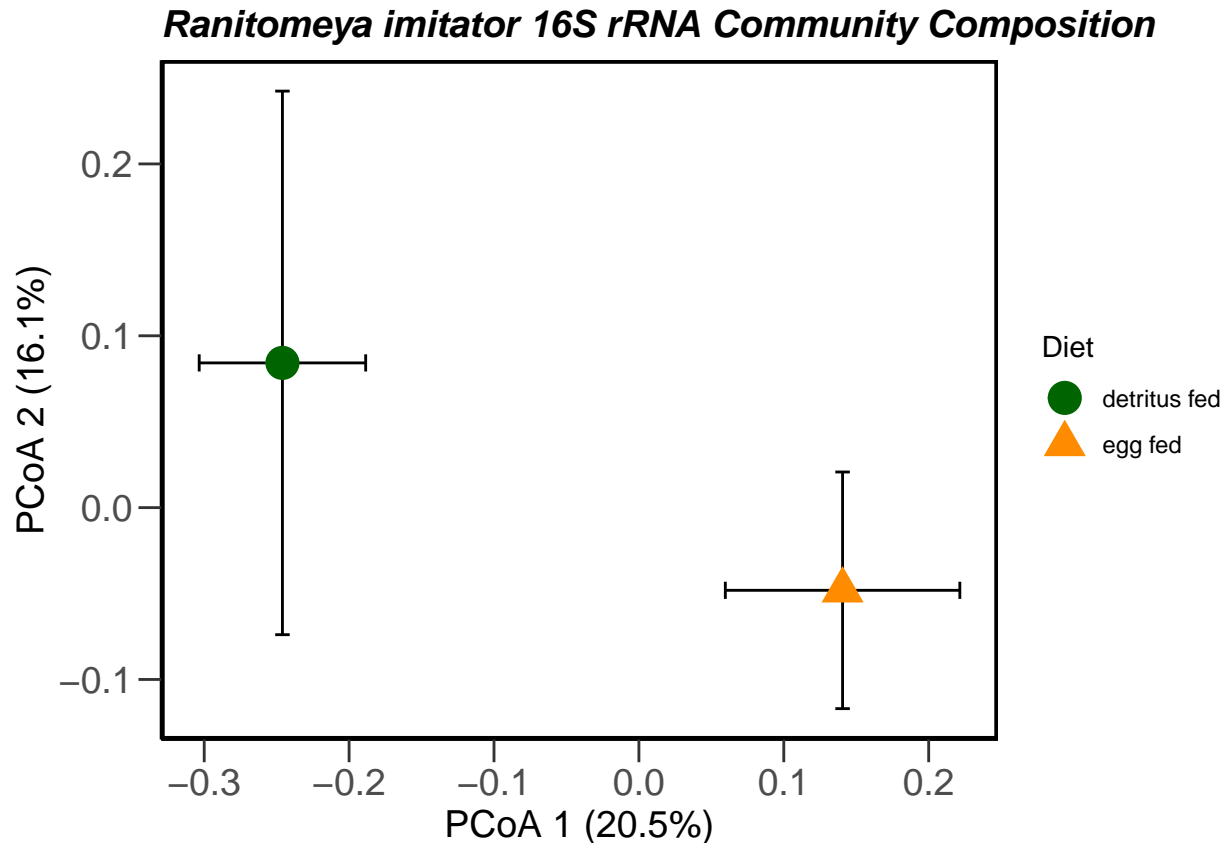
2

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

Bacterial community indicator species analysis - R. imitator diet

Simple Hypothesis Testing - R. variabilis

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

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

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

```
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 0.1209
## 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)
```
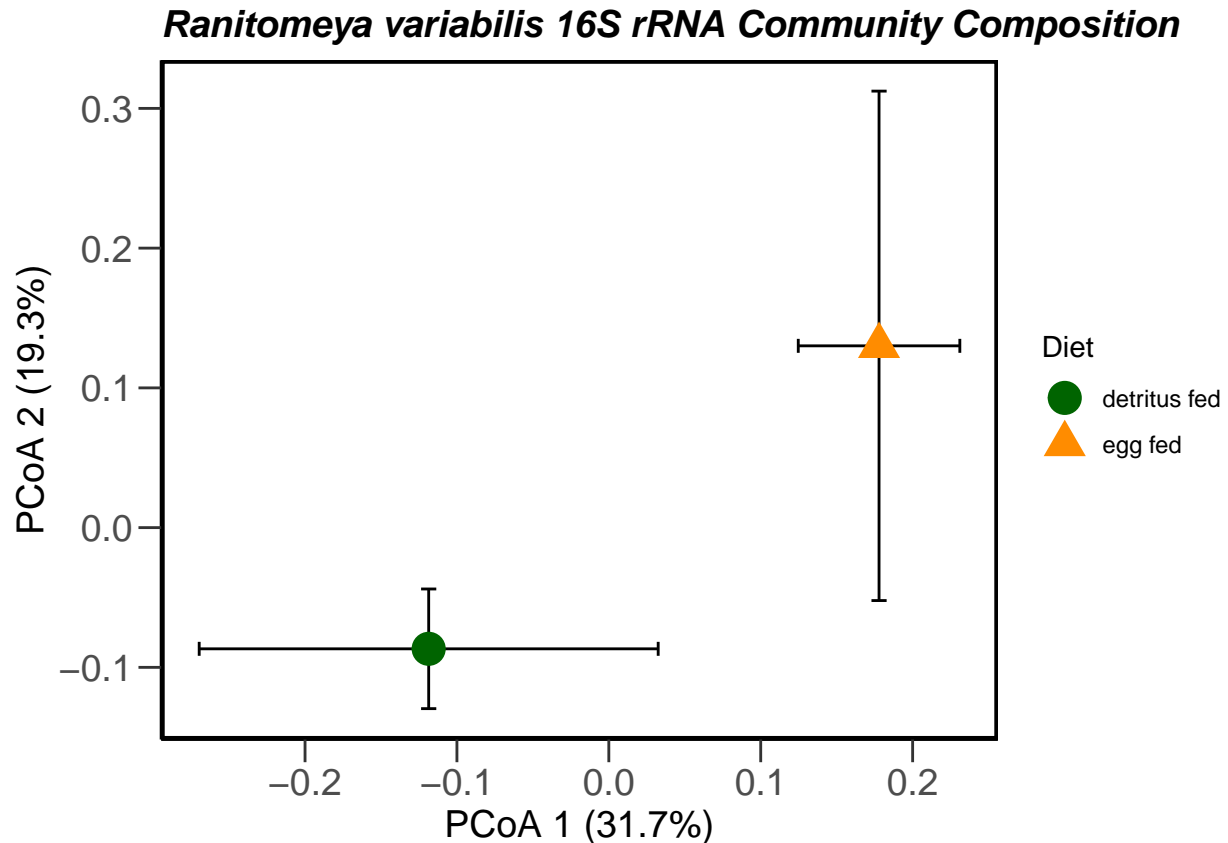
```r
# Combine
pcoa.cent.dataframe <- cbind(t(pcoa.centroids), t(pcoa.centroids.se))
colnames(pcoa.cent.dataframe) <- c("V1", "V2", "V1e", "V2e")
pcoa.cent.treats <- rownames(pcoa.cent.dataframe)

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

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

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

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

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

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

```
# 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

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

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

```
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.          5.12      0.482
## 3 variabilis detritus           851.       130.          4.14      0.295
## 4 variabilis egg fed            548         17.0         3.36      0.194
```

```
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. 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
```

```
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
##     0.02710      0.01907
```

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

```
##
## Call:
## lm(formula = simp.even ~ Diet, data = diversity.i)
##
## Residuals:
##    RiNEW021    RiNEW024    RiWAS001    RiNEW008    RiNEW006    RiNEW025    RiNEW023
## -0.0161132 -0.0101002 -0.0006164 -0.0145577  0.0151741  0.0235083  0.0027051
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) 0.027104   0.008259   3.282   0.0219 *
## Dietegg fed 0.019066   0.012616   1.511   0.1911
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.01652 on 5 degrees of freedom
## Multiple R-squared:  0.3136, Adjusted R-squared:  0.1763
## F-statistic: 2.284 on 1 and 5 DF,  p-value: 0.1911
```

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

```
##
## Call:
## lm(formula = shannon ~ Diet, data = diversity.i)
##
## Coefficients:
## (Intercept)  Dietegg fed
##      4.3643       0.7546
```

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

```
##
```

```
## Call:
## lm(formula = shannon ~ Diet, data = diversity.i)
##
## Residuals:
## RiNEW021 RiNEW024 RiWAS001 RiNEW008 RiNEW006 RiNEW025 RiNEW023
## -0.57530 -0.05965  0.91551 -0.71964 -0.19587  0.29072  0.34423
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)   4.3643     0.3108   14.04 3.29e-05 ***
## Dietegg fed   0.7546     0.4748    1.59    0.173
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.6216 on 5 degrees of freedom
## Multiple R-squared:  0.3357, Adjusted R-squared:  0.2028
## F-statistic: 2.527 on 1 and 5 DF,  p-value: 0.1728
```

```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
##     0.03692     -0.01888
```

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

```
##
## Call:
## lm(formula = simp.even ~ Diet, data = diversity.v)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.028076 -0.021134 -0.001622  0.008933  0.067270
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)  0.03692    0.01221   3.024   0.0165 *
## Dietegg fed -0.01888    0.01930  -0.978   0.3566
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.02991 on 8 degrees of freedom
## Multiple R-squared:  0.1069, Adjusted R-squared:  -0.004792
## F-statistic: 0.9571 on 1 and 8 DF,  p-value: 0.3566
```

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

```
##
## Call:
## lm(formula = shannon ~ Diet, data = diversity.v)
##
## Coefficients:
## (Intercept)  Dietegg fed
##      4.1420      -0.7837
```

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

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

```
## -0.90369 -0.38073 -0.03225  0.26843  1.01969
##
## Coefficients:
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept)    4.1420      0.2525  16.406 1.92e-07 ***
## Dietegg fed   -0.7837      0.3992  -1.963   0.0852 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.6184 on 8 degrees of freedom
## Multiple R-squared:  0.3251, Adjusted R-squared:  0.2408
## F-statistic: 3.854 on 1 and 8 DF,  p-value: 0.08523
```

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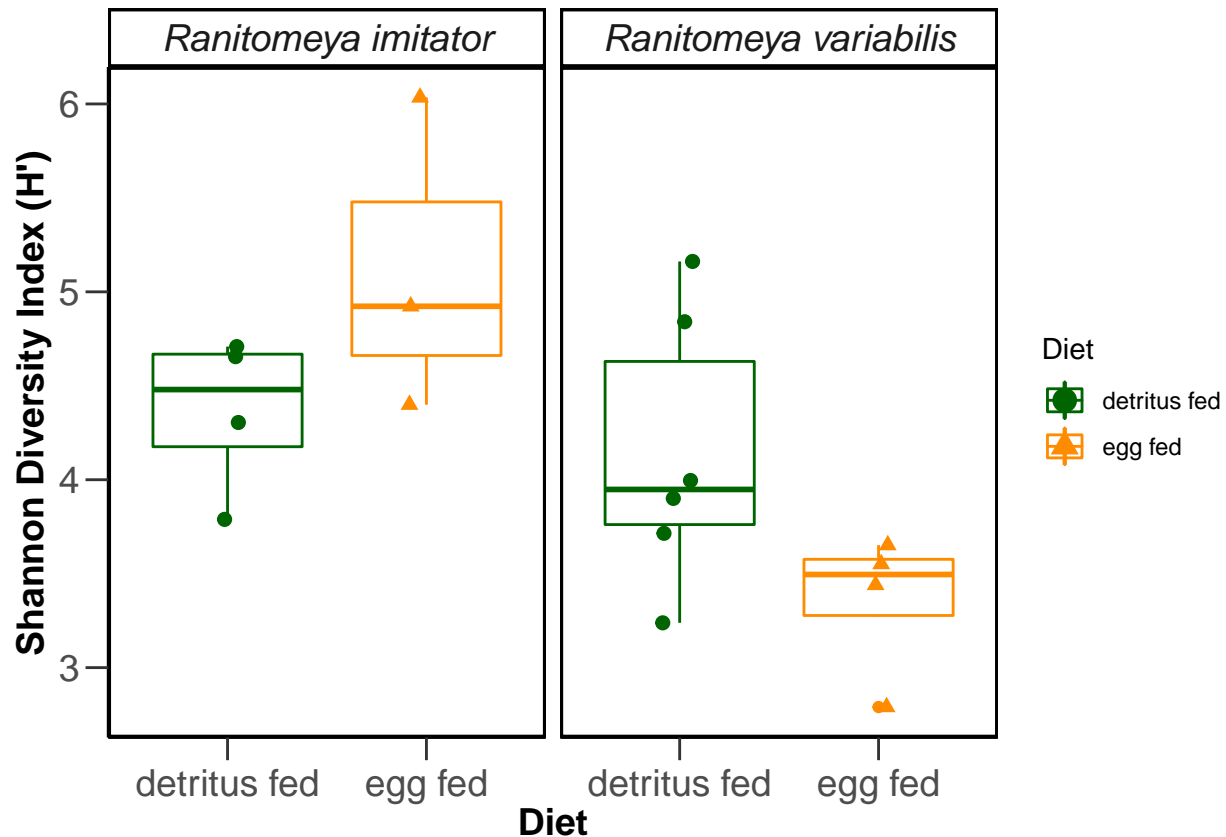
```
ggsave("../figures/shannon.pdf", plot=last_plot(), device=NULL, path=NULL, scale=1, width=7, height=5, c
```

```
## 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, c
```

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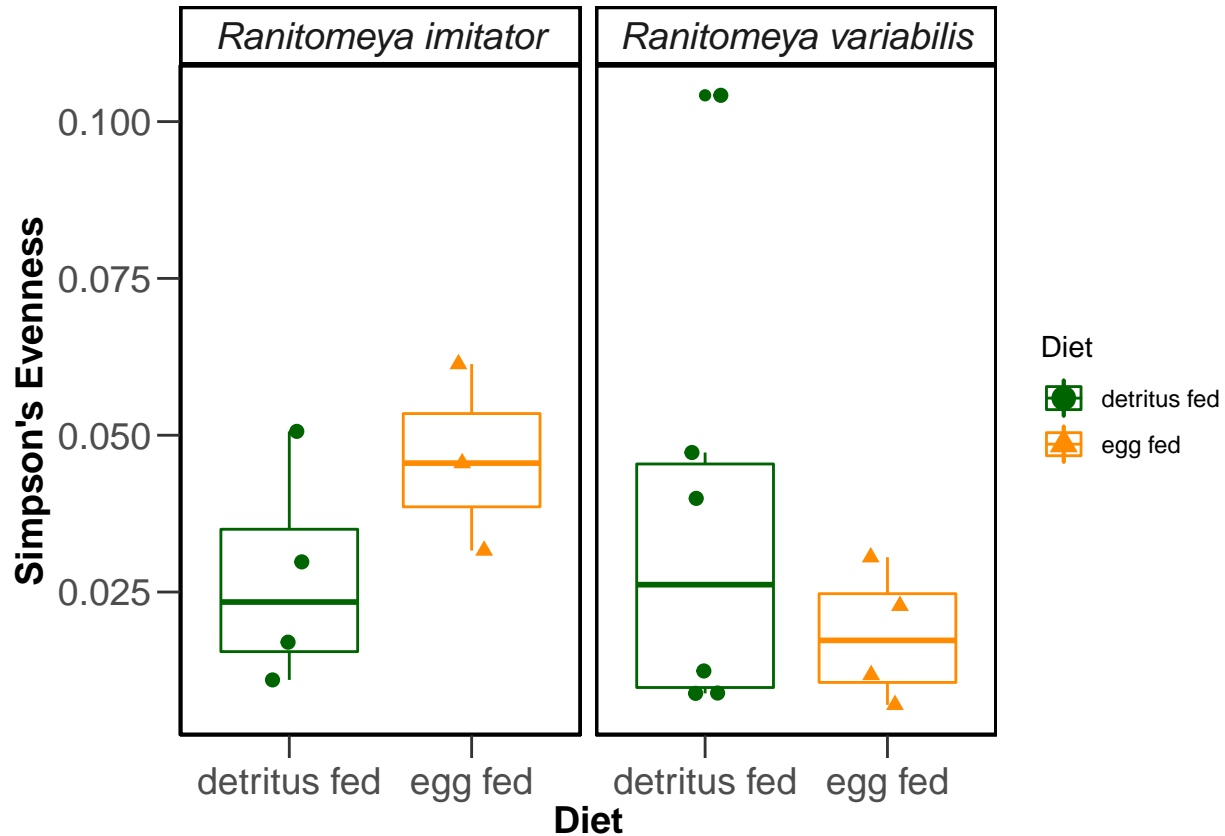
#Plot Evenness

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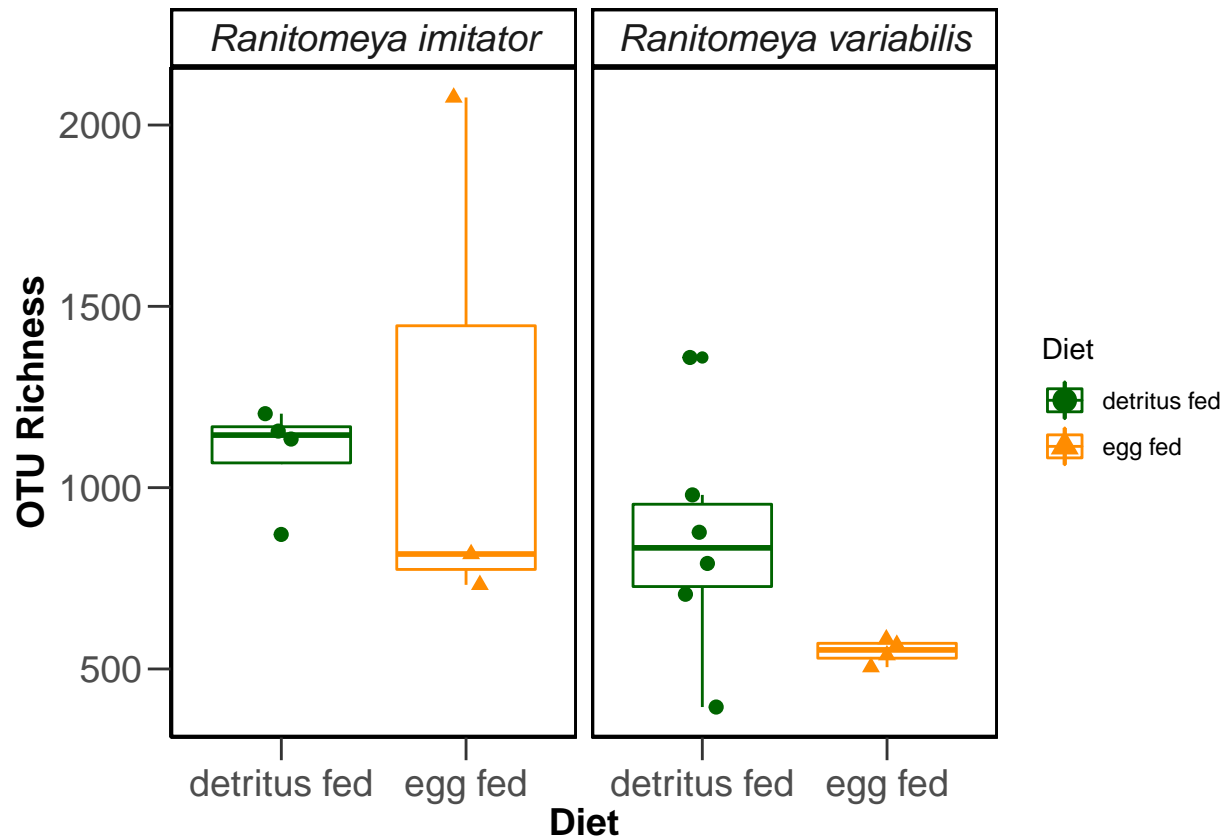
```
## Warning: Computation failed in 'stat_summary()':
##
## Computation failed in 'stat_summary()':
```

```
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

<<<<<

```
# Correct Sample IDs and Subset File - match up design file and otu files
missing <- setdiff(rownames(design), rownames(euk.otu.in))
design_final <- design[-(which(rownames(design) %in% missing)), ]
dim(design_final)
```

```
## [1] 23  4
```

```
euk.otu <- euk.otu.in
dim(euk.otu)
```

```
## [1]   23 4467
```

```
euk.otu <- euk.otu[match(rownames(design_final), rownames(euk.otu)),]
all.equal(rownames(euk.otu), rownames(design_final))
```

```
## [1] TRUE
```

```
euk.otus.design <- cbind(design_final,euk.otu)
euk.otus.design.sub <- subset(euk.otus.design, Location != "NA") #removed R. imitator/egg fed/lab
dim(euk.otus.design.sub)
```

```
## [1]   19 4471
```

```
euk.design.final <- euk.otus.design.sub[,c(1:4)] # seaparate design file after removinng NA
euk.otu <- euk.otus.design.sub[,-c(1:4)] # separate otu file after removinng NA

# OTU table - remove otus w/ < 2 occurrences across all sites
euk.otu <- euk.otu[, which(colSums(euk.otu) >= 2)]
dim(euk.otu) #went to 2113 OTUs
```

```
## [1]   19 2113
```

```
# Coverage
aa <- (rowSums(euk.otu))
aa
```

```
## RvSAJ030 RiNEW021 RvSAJ025 RvSAJ026 RvSAJ018 RvSAJ016 RvSAJ015 RiNEW024
##   160956    19027    28727    19611     9337    34077    44301     2667
## RvSAJ021 RiWAS001 RvSAJ020 RvSAJ032 RvSAJ014 RiNEW008 RiNEW006 RiNEW025
##    91970    51797    18323    18653    51721     4741     1285    17976
## RiNEW023 RvSAJ024 RiNEW003
##    12404    21670     4332
```

```
tadpole.euk.tax <- read.tax(taxonomy = "../mothur/MicroEuk.final.taxonomy",
                            format = "rdp", tax.levels = 6, col.tax = 3)
tadpole.euk.tax <- tadpole.euk.tax[which(tadpole.euk.tax$OTU %in% colnames(euk.otu)), ]
```

MicroEuk Transformation and Alpha Diversity

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

# Make Presence Absence Matrices
euk.dataPA <- (euk.otu > 0) * 1

# Richness
euk.R <- estimateR(euk.otu)

richness <- euk.R[2, ]

# Evenness
euk.Hinv <- diversity(euk.otu, "invsimpson")
euk.even <- euk.Hinv / euk.R[1, ]

# Shannon Diversity
shannon <- diversity(euk.otu, "shannon")

# Combine
euk.diversity <- cbind(euk.design.final,richness, euk.even, shannon)

library(lmerTest)
str(euk.diversity)
```

```
## 'data.frame':    19 obs. of  7 variables:
##  $ Project : chr  "FrogMicro" "FrogMicro" "FrogMicro" "FrogMicro" ...
##  $ Species : chr  "variabilis" "imitator" "variabilis" "variabilis" ...
##  $ Diet    : chr  "egg fed" "detritus" "detritus" "detritus" ...
##  $ Location: chr  "lab" "field" "field" "field" ...
##  $ richness: num  481 338 577 651 485 ...
##  $ euk.even: num  0.00473 0.01502 0.02671 0.01374 0.01129 ...
##  $ shannon : num  1.42 1.97 3.28 3.24 2.7 ...
```

```r
euk.diversity$Species <- as.factor(euk.diversity$Species)
euk.diversity$Diet <- as.factor(euk.diversity$Diet)
euk.diversity.i <- euk.diversity[which(euk.diversity$Species == "imitator"), ]
euk.diversity.v <- euk.diversity[which(euk.diversity$Species == "variabilis"), ]

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

```
##
## Call:
## lm(formula = richness ~ Diet, data = euk.diversity.i)
##
## Coefficients:
## (Intercept)  Dietegg fed
##       370.6       -136.8
```

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

```
##
## Call:
## lm(formula = richness ~ Diet, data = euk.diversity.i)
##
## Residuals:
##    Min    1Q Median    3Q    Max
## -97.12 -51.38 -20.02  19.43 192.58
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)   370.61      50.64   7.319 0.000332 ***
## Dietegg fed  -136.84      71.61  -1.911 0.104587
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 101.3 on 6 degrees of freedom
## Multiple R-squared:  0.3783, Adjusted R-squared:  0.2747
## F-statistic: 3.651 on 1 and 6 DF,  p-value: 0.1046
```

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

```
##
## Call:
## lm(formula = shannon ~ Diet, data = euk.diversity.i)
##
## Coefficients:
## (Intercept)  Dietegg fed
##       2.300       -0.113
```

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

```
##
## Call:
## lm(formula = shannon ~ Diet, data = euk.diversity.i)
##
## Residuals:
##      Min      1Q   Median      3Q     Max
## -0.32885 -0.26099  0.00912  0.26395  0.31883
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)   2.3000     0.1464  15.707 4.22e-06 ***
## Dietegg fed  -0.1130     0.2071  -0.546    0.605
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2929 on 6 degrees of freedom
## Multiple R-squared:  0.04731,   Adjusted R-squared:  -0.1115
## F-statistic: 0.2979 on 1 and 6 DF,  p-value: 0.6049
```

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

```
##
## Call:
## lm(formula = euk.even ~ Diet, data = euk.diversity.i)
##
## Coefficients:
## (Intercept)  Dietegg fed
##     0.01794      0.01569
```

```
summary(euk.euk.even.lm.i)
```

```
##
## Call:
## lm(formula = euk.even ~ Diet, data = euk.diversity.i)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.021861 -0.004487 -0.003450  0.011285  0.015367
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept) 0.017935   0.006573   2.729   0.0343 *
## Dietegg fed 0.015690   0.009296   1.688   0.1424
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.01315 on 6 degrees of freedom
## Multiple R-squared:  0.322,  Adjusted R-squared:  0.209
## F-statistic: 2.849 on 1 and 6 DF,  p-value: 0.1424
```

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

```
##
## Call:
## lm(formula = richness ~ Diet, data = euk.diversity.v)
##
## Coefficients:
## (Intercept)  Dietegg fed
##      511.54       -78.64
```

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

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

```
## -241.84  -49.43   44.28   66.07  139.85
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)   511.54      46.18  11.078 1.52e-06 ***
## Dietegg fed   -78.64      68.49  -1.148    0.281
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 113.1 on 9 degrees of freedom
## Multiple R-squared:  0.1278, Adjusted R-squared:  0.03084
## F-statistic: 1.318 on 1 and 9 DF,  p-value: 0.2805
```

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

```
##
## Call:
## lm(formula = shannon ~ Diet, data = euk.diversity.v)
##
## Coefficients:
## (Intercept)  Dietegg fed
##      2.5596      -0.5879
```

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

```
##
## Call:
## lm(formula = shannon ~ Diet, data = euk.diversity.v)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -0.7818 -0.5533  0.1365  0.4670  0.7214
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)   2.5596     0.2403  10.651 2.11e-06 ***
## Dietegg fed  -0.5879     0.3564  -1.649    0.133
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.5886 on 9 degrees of freedom
## Multiple R-squared:  0.2321, Adjusted R-squared:  0.1468
## F-statistic: 2.721 on 1 and 9 DF,  p-value: 0.1335
```

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

```
##
## Call:
## lm(formula = euk.even ~ Diet, data = euk.diversity.v)
##
```

```
## Coefficients:
## (Intercept)  Dietegg fed
##    0.013388     -0.001146
```

```
summary(euk.euk.even.lm.v)
```

```
##
## Call:
## lm(formula = euk.even ~ Diet, data = euk.diversity.v)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.007515 -0.004832 -0.001324 -0.000139  0.014224
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)  0.013388   0.003117   4.295  0.00201 **
## Dietegg fed -0.001146   0.004624  -0.248  0.80986
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.007636 on 9 degrees of freedom
## Multiple R-squared:  0.006776,   Adjusted R-squared:  -0.1036
## F-statistic: 0.0614 on 1 and 9 DF,  p-value: 0.8099
```

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

# Graphing Richness
p <- ggplot(euk.diversity, aes(x=Diet, y=richness, color=as.factor(Diet),
                                shape=as.factor(Diet)))+ geom_boxplot() +
  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 = "18S 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()':
```
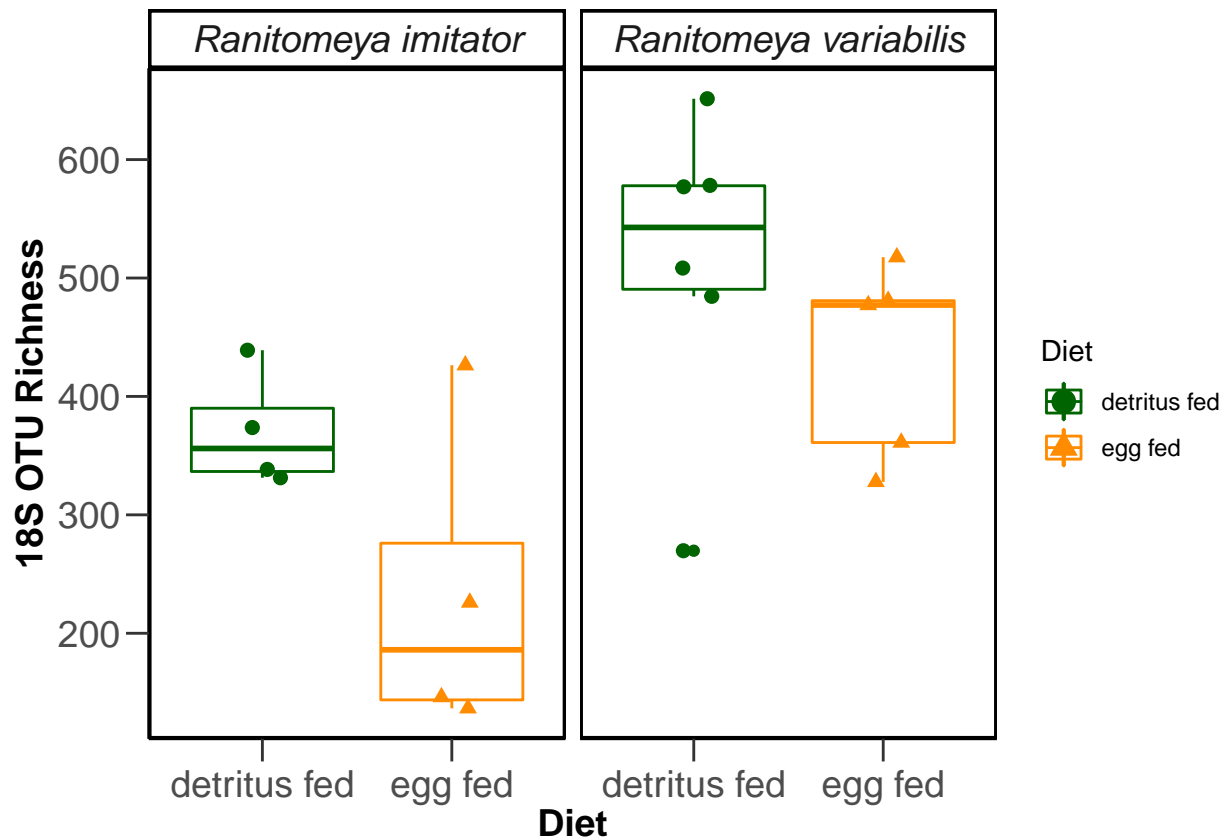


```
ggsave("../figures/18Srichness.pdf", plot=last_plot(), device=NULL, path=NULL, scale=1, width=7, height=
```

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

```
ggsave("../figures/18Srichness.png", plot=last_plot(), device=NULL, path=NULL, scale=1, width=7, height=
```

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

```r
# Graphing Evenness
p <- ggplot(euk.diversity, aes(x=Diet, y=euk.even, color=as.factor(Diet),
                               shape=as.factor(Diet)))+ geom_boxplot() +
  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 = "18S OTU 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()`:
```
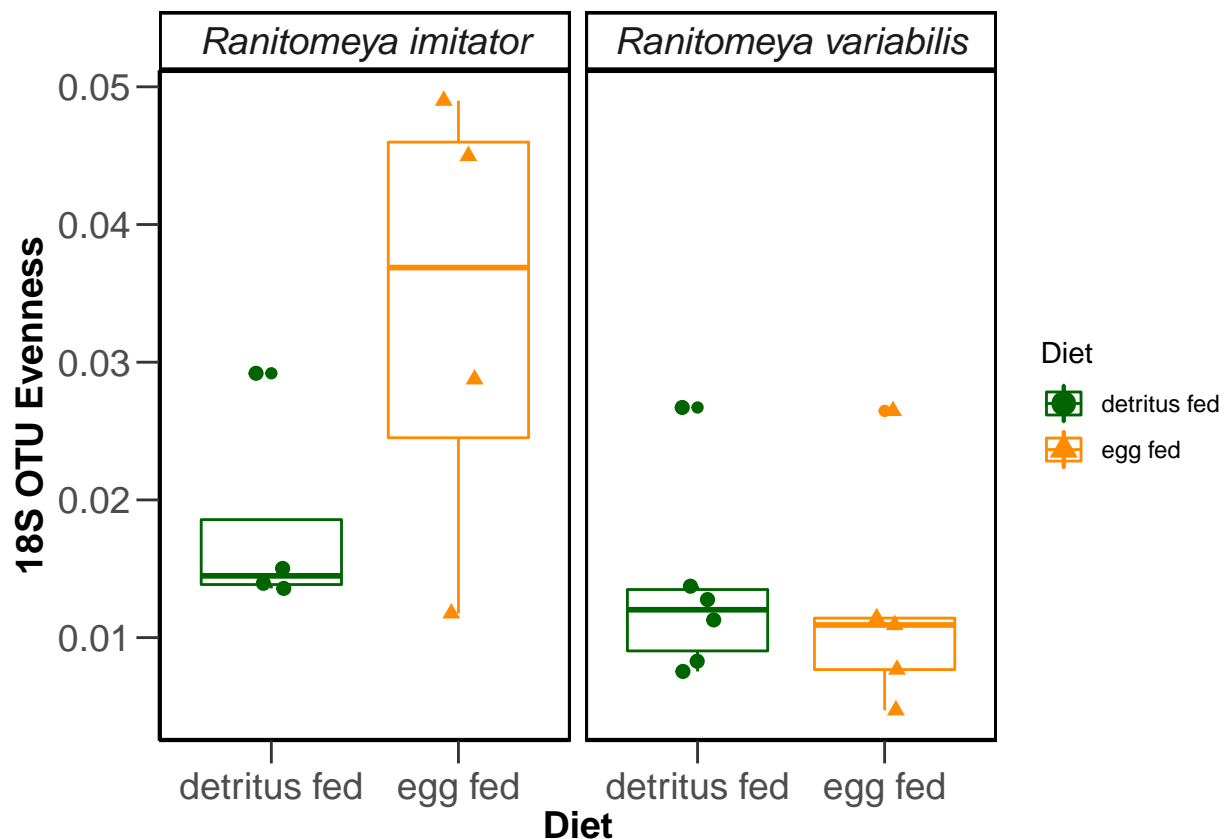


```
ggsave("../figures/18Sevenness.pdf", plot=last_plot(), device=NULL, path=NULL, scale=1, width=7, height=
```

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

```
ggsave("../figures/18Sevenness.png", plot=last_plot(), device=NULL, path=NULL, scale=1, width=7, height=
```

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

```
# Graphing Shannon
p <- ggplot(euk.diversity, aes(x=Diet, y=shannon, color=as.factor(Diet),
                               shape=as.factor(Diet)))+ geom_boxplot() +
  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 = "18S 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()':
```
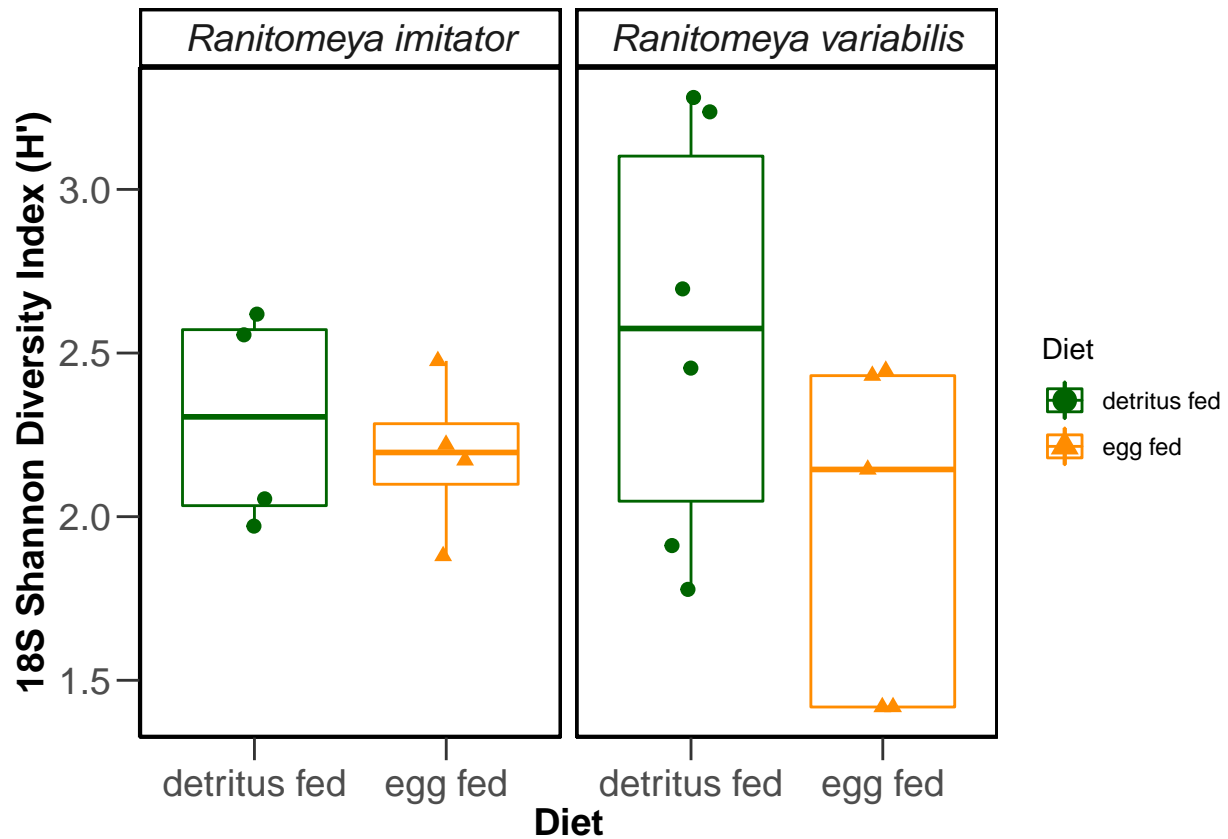
```
ggsave("../figures/18Sshannon.pdf", plot=last_plot(), device=NULL, path=NULL, scale=1, width=7, height=5
```

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

```
ggsave("../figures/18Sshannon.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()`:
```

Community Split by Species

```
#Ranitomeya variabilis
row.names(euk.dataREL) == row.names(euk.design.final)
```

```
##  [1] TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE
## [16] TRUE TRUE TRUE TRUE
```

```
# PERMANOVA
euk.dataREL.v <- euk.dataREL[which(euk.design.final$Species == "variabilis"), ]
euk.design.final.v <- euk.design.final[which(euk.design.final$Species == "variabilis"), ]
dataREL.dist <- vegdist(euk.dataREL.v, method="bray")

adonis.euk = adonis2(euk.dataREL.v~euk.design.final.v$Diet,
                     method = "bray", perm=1000)
adonis.euk
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 1000
##
## adonis2(formula = euk.dataREL.v ~ euk.design.final.v$Diet, permutations = 1000, method = "bray")
##                       Df SumOfSqs      R2      F Pr(>F)
## euk.design.final.v$Diet  1   0.5301 0.14347 1.5075 0.1419
## Residual                 9   3.1646 0.85653
## Total                   10   3.6946 1.00000
```

```
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 #33.9
```

```
## [1] 33.9
```

```
explainvar2b #18.4
```

```
## [1] 18.4
```

```
new.data <- euk.design.final[which(rownames(euk.design.final) %in% rownames(pcoa$points)), ]
pcoa.groups <- paste(new.data$Diet, new.data$Species, sep = "_")
pcoa.points <- data.frame(pcoa$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)

# 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 (33.9%)") + ylab("PCoA 2 (18.4%)") +
  ggtitle(label="Ranitomeya variabilis 18S rRNA Community Composition") +
  theme(plot.title = element_text(color="black", size=14, face="bold.italic"))
```


Ranitomeya variabilis 18S rRNA Community Composition
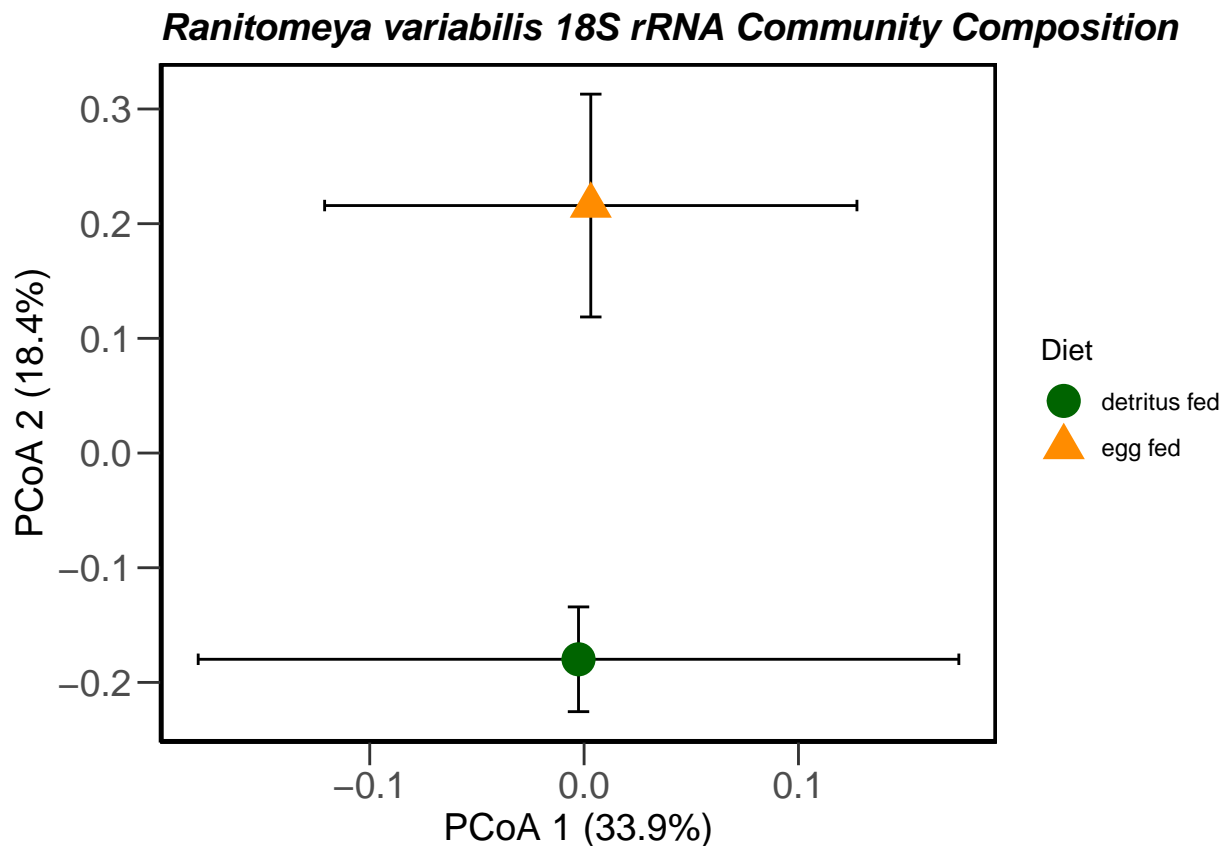
```
ggsave("../figures/18SOrdination_variabilis_diet.pdf", plot=last_plot(), device=NULL, path=NULL, scale=
ggsave("../figures/18SOrdination_variabilis_diet.png", plot=last_plot(), device=NULL, path=NULL, scale=


#Ranitomeya imitator
row.names(euk.dataREL) == row.names(euk.design.final)
```

```
##  [1] TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE
## [16] TRUE TRUE TRUE TRUE
```

```
euk.dataREL.i <- euk.dataREL[which(euk.design.final$Species == "imitator"), ]
euk.design.final.i <- euk.design.final[which(euk.design.final$Species == "imitator"), ]
dataREL.dist <- vegdist(euk.dataREL.i, method="bray")

adonis.euk.i = adonis2(euk.dataREL.i~euk.design.final.i$Diet,
                    method = "bray", perm=1000)
adonis.euk.i
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 1000
##
## adonis2(formula = euk.dataREL.i ~ euk.design.final.i$Diet, permutations = 1000, method = "bray")
##                          Df SumOfSqs      R2     F  Pr(>F)
## euk.design.final.i$Diet  1  0.71953 0.32554 2.896 0.02697 *
## Residual                 6  1.49072 0.67446
## Total                    7  2.21025 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
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 #34.4
```

```
## [1] 34.4
```

```
explainvar2b #26.1
```

```
## [1] 26.1
```

```
new.data <- euk.design.final[which(rownames(euk.design.final) %in% rownames(pcoa$points)), ]
pcoa.groups <- paste(new.data$Diet, new.data$Species, sep = "_")
pcoa.points <- data.frame(pcoa$points, group = pcoa.groups)
```

```r
# 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)

# 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 (34.4%)") + ylab("PCoA 2 (26.1%)") +
  ggtitle(label="Ranitomeya imitator 18S rRNA Community Composition") +
  theme(plot.title = element_text(color="black", size=14, face="bold.italic"))
```

Ranitomeya imitator 18S rRNA Community Composition
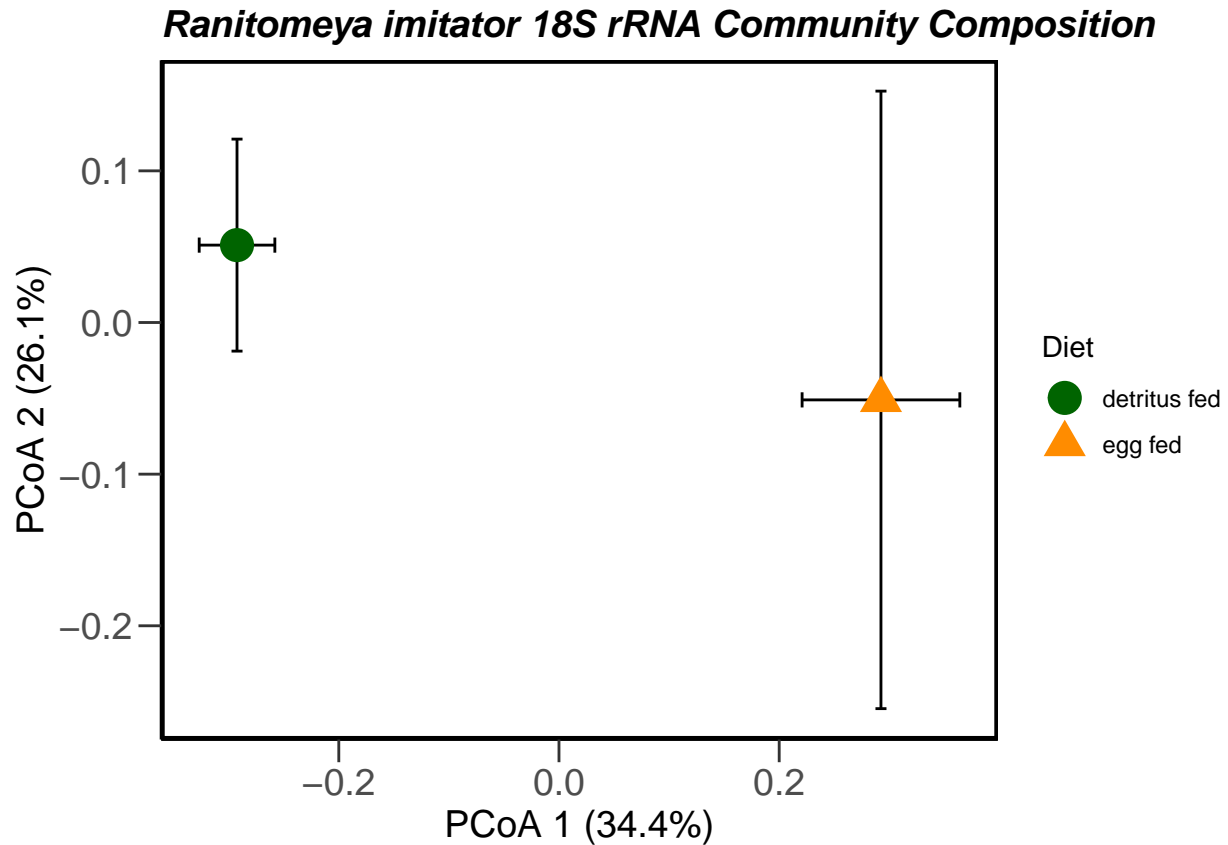
```
ggsave("../figures/18SOrdination_imitator_diet.pdf", plot=last_plot(), device=NULL, path=NULL, scale=1,
ggsave("../figures/18SOrdination_imitator_diet.png", plot=last_plot(), device=NULL, path=NULL, scale=1,
```

MicroEuk community composition analysis - Species x Diet

Otu3587 Abundance