5. Worksheet: Alpha Diversity

Emmi Mueller; Z620: Quantitative Biodiversity, Indiana University

20 January, 2019

OVERVIEW

In this exercise, we will explore aspects of local or site-specific diversity, also known as alpha (α) diversity. First we will quantify two of the fundamental components of (α) diversity: **richness** and **evenness**. From there, we will then discuss ways to integrate richness and evenness, which will include univariate metrics of diversity along with an investigation of the **species abundance distribution (SAD)**.

Directions:

1. In the Markdown version of this document in your cloned repo, change "Student Name" on line 3 (above) to your name.
2. Complete as much of the worksheet as possible during class.
3. Use the handout as a guide; it contains a more complete description of data sets along with the proper scripting needed to carry out the exercise.
4. Answer questions in the worksheet. Space for your answer is provided in this document and indicated by the ">" character. If you need a second paragraph be sure to start the first line with ">". You should notice that the answer is highlighted in green by RStudio (color may vary if you changed the editor theme).
5. Before you leave the classroom, **push** this file to your GitHub repo.
6. For the assignment portion of the worksheet, follow the directions at the bottom of this file.
7. When you are done, **Knit** the text and code into a PDF file.
8. After Knitting, submit the completed exercise by creating a **pull request** via GitHub. Your pull request should include this file `AlphaDiversity_Worskheet.Rmd` and the PDF output of `Knitr` (`AlphaDiversity_Worskheet.pdf`).

1) R SETUP

In the R code chunk below, please provide the code to: 1) Clear your R environment, 2) Print your current working directory, 3) Set your working directory to your `5.AlphaDiversity` folder, and 4) Load the `vegan` R package (be sure to install first if you haven't already).

```
rm(list=ls())
getwd()
```

```
## [1] "C:/Users/emmim/GitHub/QB2019_Mueller/2.Worksheets/5.AlphaDiversity"
```

```
setwd("~/GitHub/QB2019_Mueller/2.Worksheets/5.AlphaDiversity")
require("vegan")
```

```
## Loading required package: vegan
## Loading required package: permute
## Loading required package: lattice
## This is vegan 2.5-3
```

2) LOADING DATA

In the R code chunk below, do the following: 1) Load the BCI dataset, and 2) Display the structure of the dataset (if the structure is long, use the `max.level = 0` argument to show the basic information).

```
data(BCI)
str(BCI, max.level = 0)
```

```
## 'data.frame':    50 obs. of  225 variables:
##  - attr(*, "original.names")= chr  "Abarema.macradenium" "Acacia.melanoceras" "Acalypha.diversifolia
```

3) SPECIES RICHNESS

Species richness (S) refers to the number of species in a system or the number of species observed in a sample.

Observed richness

In the R code chunk below, do the following:

1. Write a function called `S.obs` to calculate observed richness
2. Use your function to determine the number of species in `site1` of the BCI data set, and
3. Compare the output of your function to the output of the `specnumber()` function in `vegan`.

```
site1 <- BCI[1,]

S.obs <- function(x = ""){
   rowSums(x > 0) * 1
   }

S.obs(site1)
```

```
##  1
## 93
```

```
specnumber(site1)
```

```
##  1
## 93
```

Question 1: Does `specnumber()` from `vegan` return the same value for observed richness in `site1` as our function `S.obs`? What is the species richness of the first four sites (i.e., rows) of the BCI matrix?

> ***Answer 1***: The answer from specnumber() and from S.obs() is the same. The first four sites have species richness of 93, 84, 90, and 94 respectively.

Coverage: How well did you sample your site?

In the R code chunk below, do the following:

1. Write a function to calculate Good's Coverage, and
2. Use that function to calculate coverage for all sites in the BCI matrix.

```
C <- function(x = ""){
  1 - (rowSums(x == 1) / rowSums(x))
}

C(BCI)
```

```
##         1         2         3         4         5         6         7
## 0.9308036 0.9287356 0.9200864 0.9468504 0.9287129 0.9174757 0.9326923
##         8         9        10        11        12        13        14
```

```
## 0.9443155 0.9095355 0.9275362 0.9152120 0.9071038 0.9242054 0.9132420
##        15        16        17        18        19        20        21
## 0.9350649 0.9267735 0.8950131 0.9193084 0.8891455 0.9114219 0.8946078
##        22        23        24        25        26        27        28
## 0.9066986 0.8705882 0.9030612 0.9095023 0.9115479 0.9088729 0.9198966
##        29        30        31        32        33        34        35
## 0.8983516 0.9221053 0.9382423 0.9411765 0.9220183 0.9239374 0.9267887
##        36        37        38        39        40        41        42
## 0.9186047 0.9379310 0.9306488 0.9268868 0.9386503 0.8880597 0.9299517
##        43        44        45        46        47        48        49
## 0.9140049 0.9168704 0.9234234 0.9348837 0.8847059 0.9228916 0.9086651
##        50
## 0.9143519
```

Question 2: Answer the following questions about coverage:

a. What is the range of values that can be generated by Good's Coverage?
b. What would we conclude from Good's Coverage if n_i equaled N?
c. What portion of taxa in `site1` was represented by singletons?
d. Make some observations about coverage at the BCI plots.

   Answer 2a: The range of values should be between 0 and 1.

   Answer 2b: If n_i equaled N, the coverage is low (0) and the observed richness is entirely made of singletons.

   Answer 2c: Site 1 is represented by 6.9% singletons.

   Answer 2d: The coverage of the BCI plots is reasonably high. The lowest coverage is around 88%.

Estimated richness

In the R code chunk below, do the following:

1. Load the microbial dataset (located in the `5.AlphaDiversity/data` folder),

2. Transform and transpose the data as needed (see handout),

3. Create a new vector (`soilbac1`) by indexing the bacterial OTU abundances of any site in the dataset,

4. Calculate the observed richness at that particular site, and

5. Calculate coverage of that site

```r
soil <- read.table("./data/soilbac.txt", sep = "\t", header = TRUE, row.names = 1)
soilbac.t <- as.data.frame(t(soil))
soilbac1 <- soilbac.t[1,]
S.obs(soilbac1)
```

```
## T1_1
## 1074
```

```r
C(soilbac1)
```

```
##      T1_1
## 0.6479471
```

```r
rowSums(soilbac1)
```

```
## T1_1
## 2119
```

Question 3: Answer the following questions about the soil bacterial dataset.

a. How many sequences did we recover from the sample `soilbac1`, i.e. N?
b. What is the observed richness of `soilbac1`?
c. How does coverage compare between the BCI sample (`site1`) and the KBS sample (`soilbac1`)?

> ***Answer 3a***: There are 2119 sequences from saple soilbac1.
>
> ***Answer 3b***: The observed richness of soilbac1 is 1074.
>
> ***Answer 3c***: The coverage of the KBS sample is much lower than that of the BCI sample. There are many more singletons in the soil sample.

Richness estimators

In the R code chunk below, do the following:

1. Write a function to calculate **Chao1**,
2. Write a function to calculate **Chao2**,
3. Write a function to calculate **ACE**, and
4. Use these functions to estimate richness at `site1` and `soilbac1`.

```
S.chao1 <-  function(x =""){
  S.obs(x) + (sum(x ==1)^2) / (2 * sum(x ==2))
}

S.chao2 <- function(site ="", SbyS = ""){
  SbyS = as.data.frame(SbyS)
  x = SbyS[site, ]
  SbyS.pa <- (SbyS > 0) * 1
  Q1 = sum(colSums(SbyS.pa) ==1)
  Q2 = sum(colSums(SbyS.pa) ==2)
  S.chao2 = S.obs(x) + (Q1^2)/(2*Q2)
  return(S.chao2)
}


S.ace <- function(x = "", thresh = 10){
  x <- x[x>0]
  S.abund <- length(which(x > thresh))
  S.rare <- length(which(x <= thresh))
  singlt <- length(which(x ==1))
  N.rare <- sum(x[which(x <= thresh)])
  C.ace <- 1 - (singlt /N.rare)
  i <- c(1:thresh)
  count <- function(i,y){
    length(y[y==i])
  }
  a.1 <- sapply(i,count,x)
  f.1 <-(i*(i-1))*a.1
  G.ace <-(S.rare/C.ace)*(sum(f.1)/(N.rare*(N.rare-1)))
  S.ace <- S.abund + (S.rare/C.ace) + (singlt/C.ace) * max(G.ace,0)
  return(S.ace)
}

S.chao1(site1)
```

```
##        1
## 119.6944
```

```
S.chao2(1, BCI)
```

```
##        1
## 104.6053
```

```
S.ace(site1)
```

```
## [1] 159.3404
```

```
S.chao1(soilbac1)
```

```
##     T1_1
## 2628.514
```

```
S.chao2(1, soilbac.t)
```

```
##     T1_1
## 21055.39
```

```
S.ace(soilbac1)
```

```
## [1] 4465.983
```

Question 4: What is the difference between ACE and the Chao estimators? Do the estimators give consistent results? Which one would you choose to use and why?

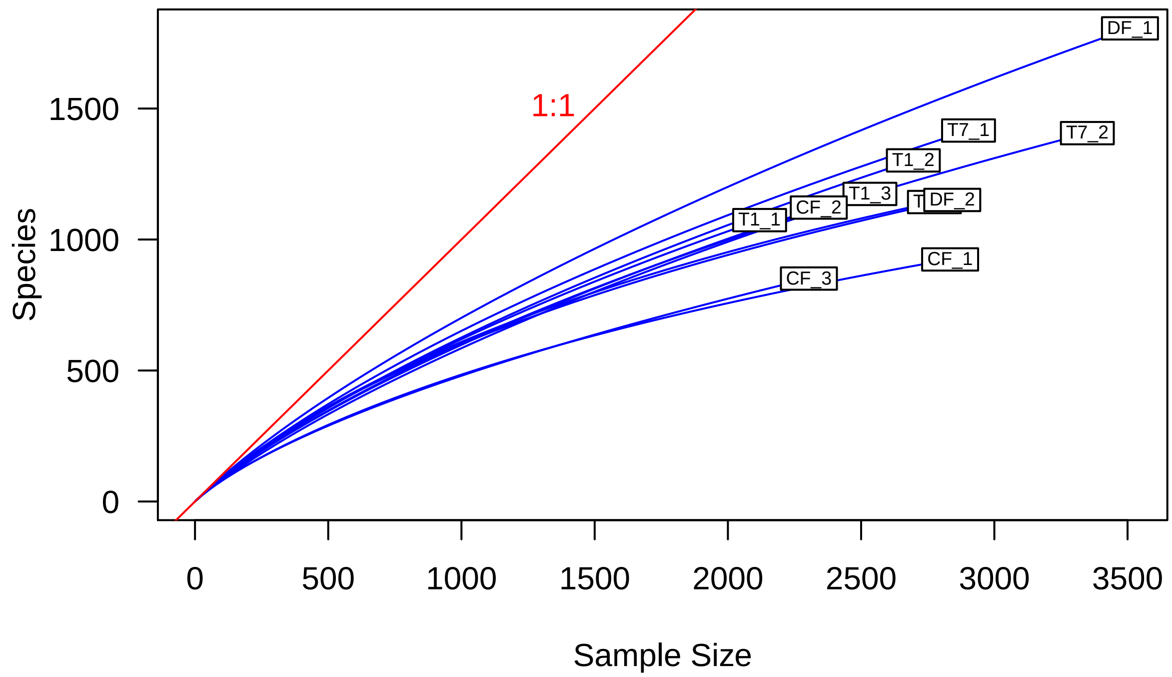
> ***Answer 4***: ACE is an abundance-based coverage estimator based on a cutoff of rare taxa (<10 individuals at a site) whereas Chao estimates are abundance-based and incidence-based estimators based on singletons and doubletons. The estimators do not give consistent results as Chao1 is based on the abundance of species and singletons and doubletons at a single site, Chao2 is based on abundance from a single site as well as presence/absence singletons and doubleton species within the entire SbyS matrix, ACE is based on the abundance of abundant taxa and of rare and singlet taxa while considering coverage. The estimator to be used in any particular case should be based on sampling effort as well as the distribution of species and likely the shape of the RAC for the community you are estimating. For communities with many rare species, ACE may underestimate abundance such as likely with the soilbac data. The estimates for the BCI data

Rarefaction

In the R code chunk below, please do the following:

1. Calculate observed richness for all samples in `soilbac`,
2. Determine the size of the smallest sample,
3. Use the `rarefy()` function to rarefy each sample to this level,
4. Plot the rarefaction results, and
5. Add the 1:1 line and label.

```
soilbac.S <- S.obs(soilbac.t)
min.N <- min(rowSums(soilbac.t))
S.rarefy <- rarefy(x = soilbac.t, sample = min.N, se = TRUE)
rarecurve(x = soilbac.t, step = 20, col = "blue", cex = 0.6, las = 1)
abline(0,1,col = 'red')
text(1500,1500, "1:1", pos = 2, col = 'red')
```



4) SPECIES EVENNESS

Here, we consider how abundance varies among species, that is, **species evenness**.

Visualizing evenness: the rank abundance curve (RAC)

One of the most common ways to visualize evenness is in a **rank-abundance curve** (sometime referred to as a rank-abundance distribution or Whittaker plot). An RAC can be constructed by ranking species from the most abundant to the least abundant without respect to species labels (and hence no worries about 'ties' in abundance).

In the R code chunk below, do the following:

1. Write a function to construct a RAC,
2. Be sure your function removes species that have zero abundances,
3. Order the vector (RAC) from greatest (most abundant) to least (least abundant), and
4. Return the ranked vector

```
RAC <- function(x = ""){
  x = as.vector(x)
  x.ab = x[x >0]
  x.ab.ranked = x.ab[order(x.ab, decreasing = TRUE)]
  return(x.ab.ranked)
}
```

Now, let's examine the RAC for `site1` of the BCI data set.

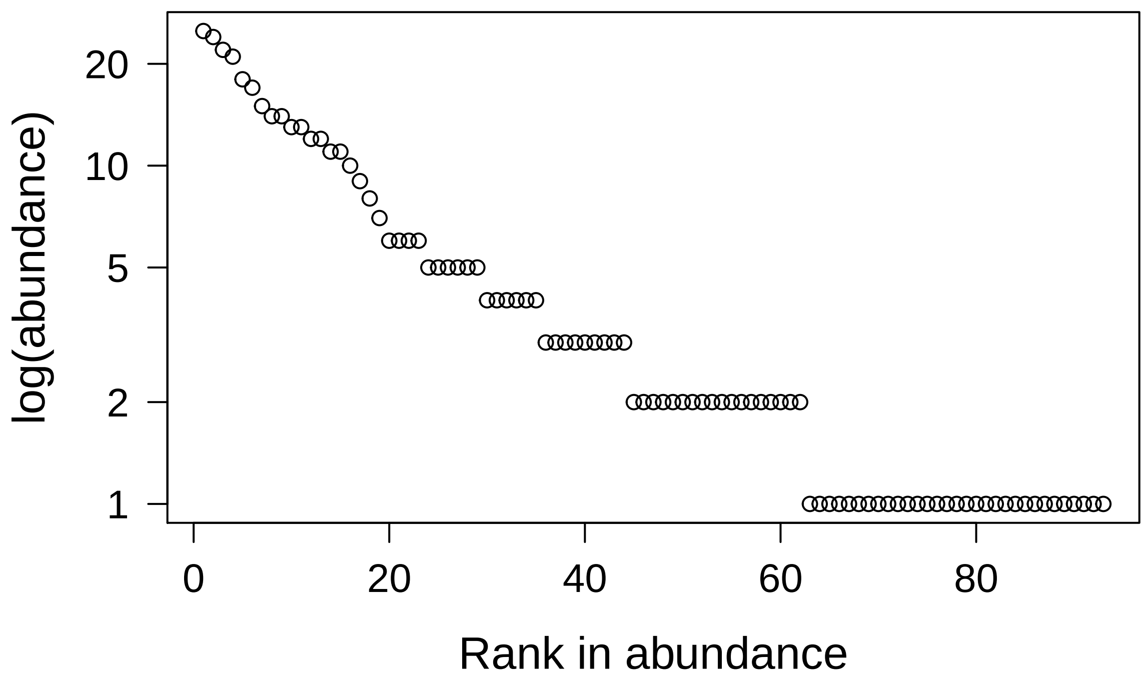
In the R code chunk below, do the following:

1. Create a sequence of ranks and plot the RAC with natural-log-transformed abundances,
2. Label the x-axis "Rank in abundance" and the y-axis "log(abundance)"

```
plot.new()

rac <- RAC(x = site1)
ranks <- as.vector(seq(1,length(rac)))
opar <- par(no.readonly = TRUE)
par(mar = c(5.1,5.1,4.1,2.1))
plot(ranks, log(rac), type = 'p', axes = F, xlab = "Rank in abundance", ylab = "log(abundance)", las = 1

box()
axis(side = 1, labels = T, cex.axis = 1.25)
axis(side = 2, las = 1, cex.axis = 1.25, labels = c(1,2,5,10,20), at = log(c(1,2,5,10,20)))
```




```
par <- opar
```

Question 5: What effect does visualizing species abundance data on a log-scaled axis have on how we interpret evenness in the RAC?

> ***Answer 5***: Log transforming the species abundance data allows us to view the highly skewed distribution as less skewed. We are able to view the lower ranked abundances while still being able to observe the high ranked abundances.

Now that we have visualized unevennes, it is time to quantify it using Simpson's evenness ($E_{1/D}$) and Smith and Wilson's evenness index (E_{var}).

Simpson's evenness ($E_{1/D}$)

In the R code chunk below, do the following:

1. Write the function to calculate $E_{1/D}$, and
2. Calculate $E_{1/D}$ for `site1`.

```
SimpE <-function(x = ""){
  S <- S.obs(x)
  x = as.data.frame(x)
  D <- diversity(x, "inv")
  E <- (D)/S
  return(E)
}

SimpE(site1)
```

```
##         1
## 0.4238232
```

Smith and Wilson's evenness index (E_{var})

In the R code chunk below, please do the following:

1. Write the function to calculate E_{var},
2. Calculate E_{var} for `site1`, and
3. Compare $E_{1/D}$ and E_{var}.

```
Evar <- function(x){
  x <- as.vector(x[x>0])
  1- (2/pi)*atan(var(log(x)))
}

Evar(site1)
```

```
## [1] 0.5067211
```

Question 6: Compare estimates of evenness for `site1` of BCI using $E_{1/D}$ and E_{var}. Do they agree? If so, why? If not, why? What can you infer from the results.

> ***Answer 6***: E_{var} is higher than $E_{1/D}$ because it is based on the log tranformation of abundnace. It is more robust to large differences in the most abundant taxa and allows the lower ranked abundance taxa to be taken into account. From these results we can infer that the BCI data is right skewed. When comparing E_{var} and $E_{1/D}$ for the soilbac1 site you find that this data is even further right skewed than the BCI data.

5) INTEGRATING RICHNESS AND EVENNESS: DIVERSITY METRICS

So far, we have introduced two primary aspects of diversity, i.e., richness and evenness. Here, we will use popular indices to estimate diversity, which explicitly incorporate richness and evenness We will write our own diversity functions and compare them against the functions in `vegan`.

Shannon's diversity (a.k.a., Shannon's entropy)

In the R code chunk below, please do the following:

1. Provide the code for calculating H' (Shannon's diversity),

2. Compare this estimate with the output of `vegan`'s diversity function using method = "shannon".

```
ShanH <- function(x = ""){
  H = 0
  for (n_i in x){
    if(n_i >0){
      p = n_i / sum(x)
      H = H - p*log(p)
    }
  }
  return(H)
}

ShanH(site1)
```

```
## [1] 4.018412
```

```
diversity(site1, index = "shannon")
```

```
## [1] 4.018412
```

Simpson's diversity (or dominance)

In the R code chunk below, please do the following:

1. Provide the code for calculating D (Simpson's diversity),
2. Calculate both the inverse (1/D) and 1 - D,
3. Compare this estimate with the output of `vegan's` diversity function using method = "simp".

```
SimpD <- function(x=""){
  D = 0
  N=sum(x)
  for(n_i in x){
    D = D+(n_i^2)/(N^2)
  }
  return(D)
}

D.inv <- 1/SimpD(site1)
D.inv
```

```
## [1] 39.41555
```

```
D.sub <- 1- SimpD(site1)
D.sub
```

```
## [1] 0.9746293
```

```
diversity(site1, index = "inv")
```

```
## [1] 39.41555
```

```
diversity(site1, index = "simp")
```

```
## [1] 0.9746293
```

```
Evar(site1)
```

```
## [1] 0.5067211
```

Question 7: Compare estimates of evenness for `site1` of BCI using $E_{H'}$ and E_{var}. Do they agree? If so, why? If not, why? What can you infer from the results.

> ***Answer 7***: The estimates for E_{var} and $E_{H'}$ are different because one is a measure of just evenness at site1 (Smith and Wilson's evenness index) and one is an integrated measure of evenness and abundance (Shannon's Diversity).

Fisher's $\boldsymbol{\alpha}$

In the R code chunk below, please do the following:

1. Provide the code for calculating Fisher's $\boldsymbol{\alpha}$,
2. Calculate Fisher's $\boldsymbol{\alpha}$ for `site1` of BCI.

```
rac <- as.vector(site1[site1 >0])
invD <-diversity(rac, "inv")
invD
```

```
## [1] 39.41555
```

```
Fisher <- fisher.alpha(rac)
Fisher
```

```
## [1] 35.67297
```

Question 8: How is Fisher's $\boldsymbol{\alpha}$ different from $E_{H'}$ and E_{var}? What does Fisher's $\boldsymbol{\alpha}$ take into account that $E_{H'}$ and E_{var} do not?

> ***Answer 8***: Fisher's $\boldsymbol{\alpha}$ fits the log transformed RAC with a log distribution using the parameter $\boldsymbol{\alpha}$. The parameter is then able to be used as a diversity index. This is different from $E_{H'}$ as Shannon's entropy does not log transform the data and therefore is less robust to abundant species and different from E_{var} as it takes into account both richness and evenness whereas $E_[var]$ is only based on evenness.

6) MOVING BEYOND UNIVARIATE METRICS OF α DIVERSITY

The diversity metrics that we just learned about attempt to integrate richness and evenness into a single, univariate metric. Although useful, information is invariably lost in this process. If we go back to the rank-abundance curve, we can retrieve additional information – and in some cases – make inferences about the processes influencing the structure of an ecological system.

Species abundance models

The RAC is a simple data structure that is both a vector of abundances. It is also a row in the site-by-species matrix (minus the zeros, i.e., absences).

Predicting the form of the RAC is the first test that any biodiversity theory must pass and there are no less than 20 models that have attempted to explain the uneven form of the RAC across ecological systems.

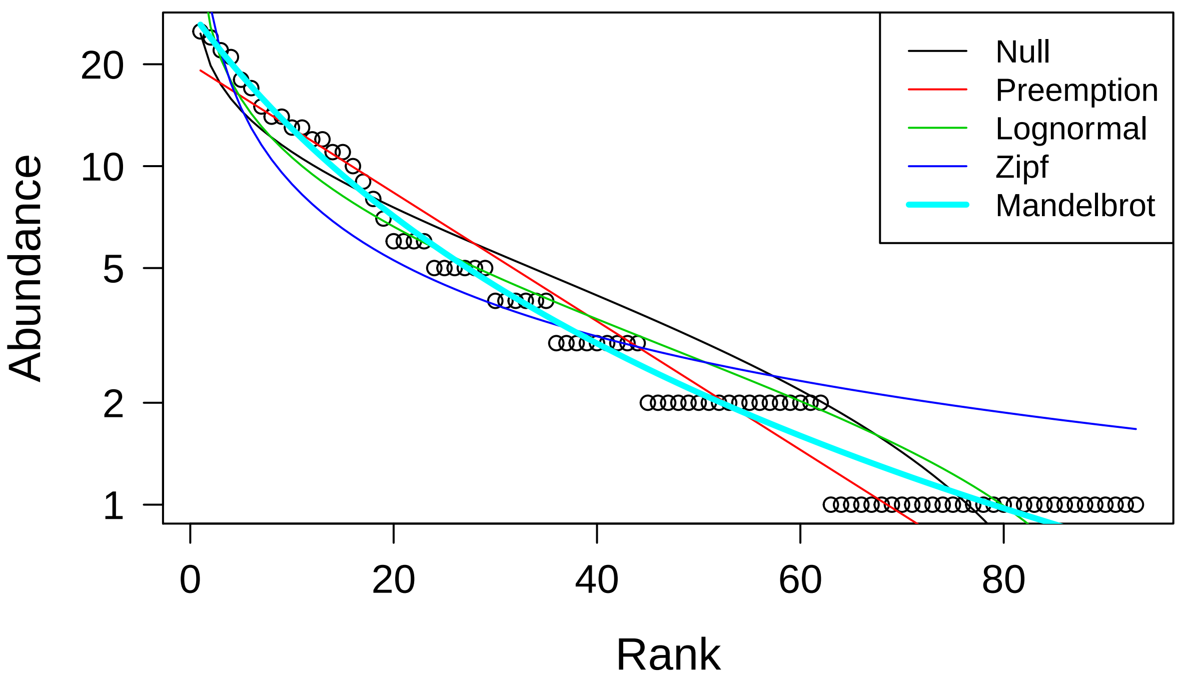
In the R code chunk below, please do the following:

1. Use the `radfit()` function in the `vegan` package to fit the predictions of various species abundance models to the RAC of `site1` in BCI,
2. Display the results of the `radfit()` function, and
3. Plot the results of the `radfit()` function using the code provided in the handout.

```
RACresults <- radfit(site1)
RACresults
```

```
##
## RAD models, family poisson
## No. of species 93, total abundance 448
##
##            par1      par2     par3    Deviance AIC      BIC
## Null                                  39.5261 315.4362 315.4362
## Preemption  0.042797                  21.8939 299.8041 302.3367
## Lognormal   1.0687    1.0186          25.1528 305.0629 310.1281
## Zipf        0.11033  -0.74705         61.0465 340.9567 346.0219
## Mandelbrot  100.52   -2.312    24.084   4.2271 286.1372 293.7350
```

```
plot.new()
plot(RACresults, las=1, cex.lab = 1.4, cex.axis = 1.25)
```



Question 9: Answer the following questions about the rank abundance curves: a) Based on the output of `radfit()` and plotting above, discuss which model best fits our rank-abundance curve for `site1`? b) Can we make any inferences about the forces, processes, and/or mechanisms influencing the structure of our system, e.g., an ecological community?

> ***Answer 9a***: The best fitting model is the Mandelbrot model based on the low BIC and AIC values compared to the other models. ***Answer 9b***: As the Mandelbrot model is best fitting we know that the abundance of a species in the RAC is inversely proportional to its rank in abundance. The large β value shows us that there is increased evenness amoung the highly abundant species in this site.

Question 10: Answer the following questions about the preemption model: a. What does the preemption model assume about the relationship between total abundance (N) and total resources that can be preempted? b. Why does the niche preemption model look like a straight line in the RAD plot?

Answer 10a: The niche preemption model assumes that the resources predicts the total abundance of organisms at a site and that each species has the same biomass output per resource. ***Answer 10b***: The niche preemption model looks like a straight line on a RAD plot because it is a geometric decay on non-log plot so when transformed, it becomes linear.

Question 11: Why is it important to account for the number of parameters a model uses when judging how well it explains a given set of data?

Answer 11: The more parameters that are used in a model, the better the model will fit. Overfitting models with too many parameters makes them non-generalizable and therefore, a balance must be found between number of parameters and fit of the model.

SYNTHESIS

1. As stated by Magurran (2004) the $D = \sum p_i^2$ derivation of Simpson's Diversity only applies to communities of infinite size. For anything but an infinitely large community, Simpson's Diversity index is calculated as $D = \sum \frac{n_i(n_i-1)}{N(N-1)}$. Assuming a finite community, calculate Simpson's D, 1 - D, and Simpson's inverse (i.e. 1/D) for `site 1` of the BCI site-by-species matrix.

```
SimpDfinite <- function(x = ""){
    D = 0
  N=sum(x)
  for(n_i in x){
    D = D+(n_i*(n_i-1))/(N*(N-1))
  }
  return(D)
}

SimpDfinite(site1)
```

```
## [1] 0.02319032
```

```
Dfinite.inv <- 1/SimpDfinite(site1)
Dfinite.sub <- SimpDfinite(site1)
Dfinite.inv
```

```
## [1] 43.12145
```

```
Dfinite.sub
```

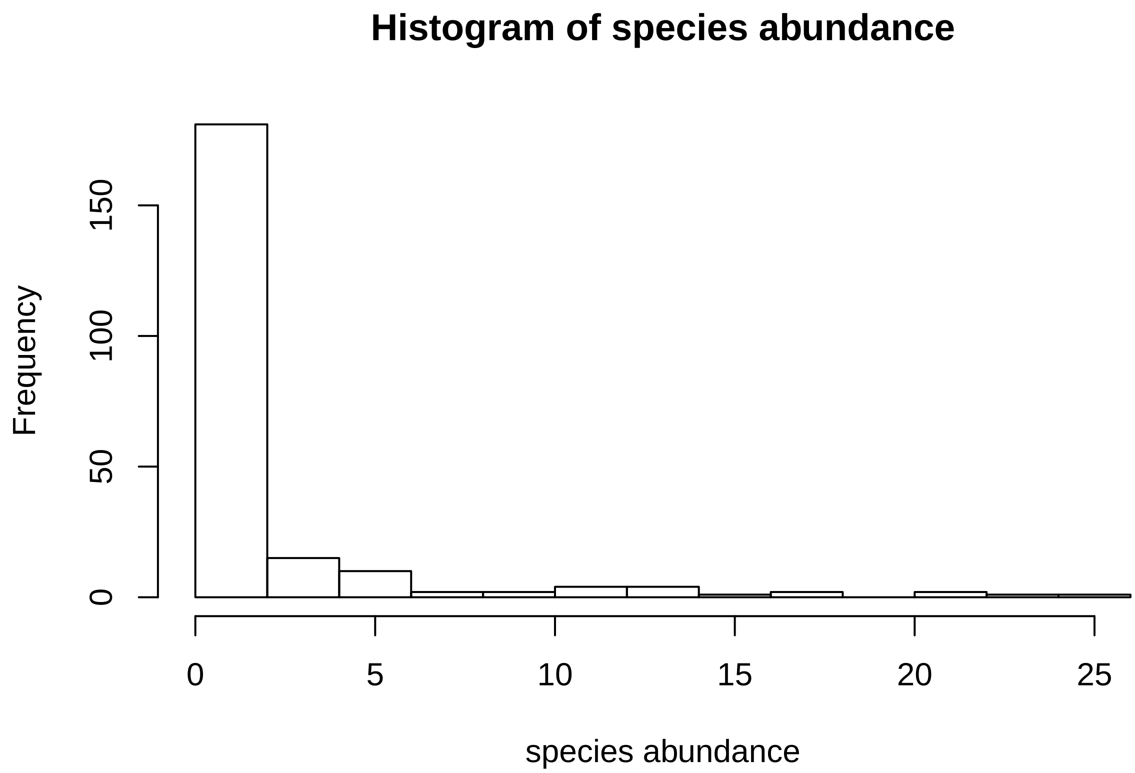
```
## [1] 0.02319032
```

2. Along with the rank-abundance curve (RAC), another way to visualize the distribution of abundance among species is with a histogram (a.k.a., frequency distribution) that shows the frequency of different abundance classes. For example, in a given sample, there may be 10 species represented by a single individual, 8 species with two individuals, 4 species with three individuals, and so on. In fact, the rank-abundance curve and the frequency distribution are the two most common ways to visualize the species-abundance distribution (SAD) and to test species abundance models and biodiversity theories. To address this homework question, use the R function **hist()** to plot the frequency distribution for `site 1` of the BCI site-by-species matrix, and describe the general pattern you see.

```
site1.df <- unlist(site1[1,])
site1.df <- as.vector(site1.df)
is.vector(site1.df)
```

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

```
hist(site1.df, main ="Histogram of species abundance", xlab = "species abundance")
```



>***Synthesis 2:*** The Histogram of species abundance shows that many species have 0 or a small number of individuals while very few species have many individuals.

3. We asked you to find a biodiversity dataset with your partner. This data could be one of your own or it could be something that you obtained from the literature. Load that dataset. How many sites are there? How many species are there in the entire site-by-species matrix? Any other interesting observations based on what you learned this week?

```
#install.packages("OTUtable")
require("OTUtable")
```

```
## Loading required package: OTUtable
```

```
data(otu_table)

str(otu_table, max.level=0)
```

```
## 'data.frame':    6208 obs. of  1387 variables:
```

> ***Synthesis 3:*** There are 6208 species and 1387 sites in this matrix. The 1387 sites come from the same 8 bogs at different times periods meaning that we will be able to clean up this matrix to a much smaller site-by-species matrix once we choose a time point to focus on.

SUBMITTING YOUR ASSIGNMENT

Use Knitr to create a PDF of your completed alpha_assignment.Rmd document, push it to GitHub, and create a pull request. Please make sure your updated repo include both the HTML and RMarkdown files.

Unless otherwise noted, this assignment is due on **Wednesday, January 23$^{\text{rd}}$, 2017 at 12:00 PM (noon)**.