

## 5. Worksheet: Alpha Diversity

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### OVERVIEW

In this exercise, we will explore aspects of local or site-specific diversity, also known as alpha ( $\alpha$ ) diversity. First we will quantify two of the fundamental components of ( $\alpha$ ) 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] "/Users/Atalanta/GitHub/QB2023_Ritter/2.Worksheets/5.AlphaDiversity"
```

```
setwd("~/GitHub/QB2023_Ritter/2.Worksheets/5.AlphaDiversity")
require("vegan")
```

```
## Loading required package: vegan
```

```
## Loading required package: permute
```

```
## Loading required package: lattice
```

```
## This is vegan 2.6-4
```

## 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 [1:225] "Abarema.macradenium" "Acacia.melanoceras" "Acalypha.diversa"
```

## 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`.

```
S.obs <- function(x = ""){
  rowSums(x > 0) * 1
}
S.obs(BCI[1,])
```

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

```
specnumber(BCI[1,])
```

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

```
S.obs(BCI[1:4,])
```

```
## 1 2 3 4
## 93 84 90 94
```

**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:** My function and `specnumber()` return the same value; there are 93 species in site 1. The species richness of the first four sites are 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      8
## 0.9308036 0.9287356 0.9200864 0.9468504 0.9287129 0.9174757 0.9326923 0.9443155
##      9     10     11     12     13     14     15     16
## 0.9095355 0.9275362 0.9152120 0.9071038 0.9242054 0.9132420 0.9350649 0.9267735
##     17     18     19     20     21     22     23     24
## 0.8950131 0.9193084 0.8891455 0.9114219 0.8946078 0.9066986 0.8705882 0.9030612
##     25     26     27     28     29     30     31     32
## 0.9095023 0.9115479 0.9088729 0.9198966 0.8983516 0.9221053 0.9382423 0.9411765
##     33     34     35     36     37     38     39     40
## 0.9220183 0.9239374 0.9267887 0.9186047 0.9379310 0.9306488 0.9268868 0.9386503
##     41     42     43     44     45     46     47     48
## 0.8880597 0.9299517 0.9140049 0.9168704 0.9234234 0.9348837 0.8847059 0.9228916
##     49     50
## 0.9086651 0.9143519
```

**Question 2:** Answer the following questions about coverage:

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

**Answer 2a:** Good's Coverage gives you a proportion between 0 and 1.

**Answer 2b:** That would mean that every single individual sampled is a unique species.

**Answer 2c:** 0.0692

**Answer 2d:** The sites on average have ~90% of  $N$  belonging to species sampled more than once. I think this indicates that the BCI plots had high coverage, meaning they were well-sampled and caught rare species more than once.

## Estimated richness

In the R code chunk below, do the following:

- Load the microbial dataset (located in the `5.AlphaDiversity/data` folder),
- Transform and transpose the data as needed (see handout),
- Create a new vector (`soilbac1`) by indexing the bacterial OTU abundances of any site in the dataset,
- Calculate the observed richness at that particular site, and
- Calculate coverage of that site

```
soilbac <- read.table("data/soilbac.txt", sep = "\t", header = TRUE, row.names = 1)
soilbac.t <- as.data.frame(t(soilbac))
soilbac1 <- soilbac.t[2,]
S.obs(soilbac1)
```

```
## T1_2
```

```
## 1302
```

```
C(soilbac1)
```

```
## T1_2
```

```
## 0.6676558
```

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

- 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:** 50

**Answer 3b:** 1302

**Answer 3c:** The coverage of the KBS sample is a lot lower than the BCI sample, meaning a greater proportion of the species found in the KBS sample was made up of singletons.

## 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] # excludes zero-abundance taxa
  S.abund <- length(which(x > thresh)) # richness of abundant taxa
  S.rare <- length(which(x <= thresh)) # richness of rare taxa
  singlt <- length(which(x == 1)) # number of singleton taxa
  N.rare <- sum(x[which(x <= thresh)]) # abundance of rare individuals
  C.ace <- 1 - (singlt / N.rare) # coverage (prop non-singlt rare inds)
  i <- c(1:thresh) # threshold abundance range
  count <- function(i, y){ # counter to go through i range
    length(y[y == i])
  }
  a.1 <- sapply(i, count, x) # number of individuals in richness i richness classes
  f.1 <- (i * (i-1)) * a.1 # k(k-1)kf sensu Gotelli
  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)
}

# chao estimated richness of BCI site 1
S.chao1(BCI[1,])
```

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

```

S.chao2("1", BCI)

##          1
## 104.6053
# chao estimated richness of KBS site 2
S.chao1(soilbac1)

##      T1_2
## 3195.434
S.chao2("T1_2", soilbac.t)

##      T1_2
## 21283.39
# ace for BCI site 1
S.ace(BCI[1,])

## [1] 159.3404
# ace for KBS site 2
S.ace(soilbac1)

## [1] 5211.927

```

**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 sets a threshold to look at abundance of other rare species, defining “rare” as taxa with 10 or fewer individuals, whereas CHAO uses the numbers of singletons and doubletons. It looks like ACE gives higher richness estimates than Chao, so I would probably use Chao to avoid overestimating.

## Rarefaction

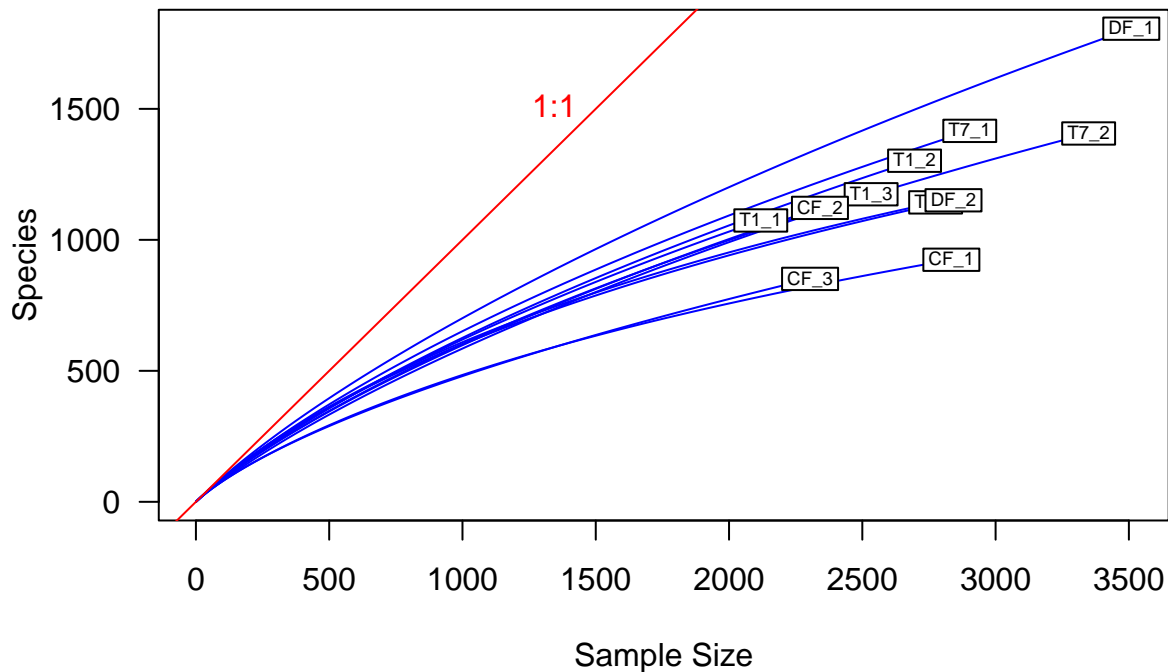
In the R code chunk below, please do the following:

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)) # min.N = smallest sample
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.ab = x[x > 0] # removing species with 0 abundances
  x.ab.ranked = x.ab[order(x.ab, decreasing = TRUE)] # order the vector from
  as.data.frame(lapply(x.ab.ranked, unlist))
  return(x.ab.ranked) # return ranked vector
}
```

Now, let us 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)”

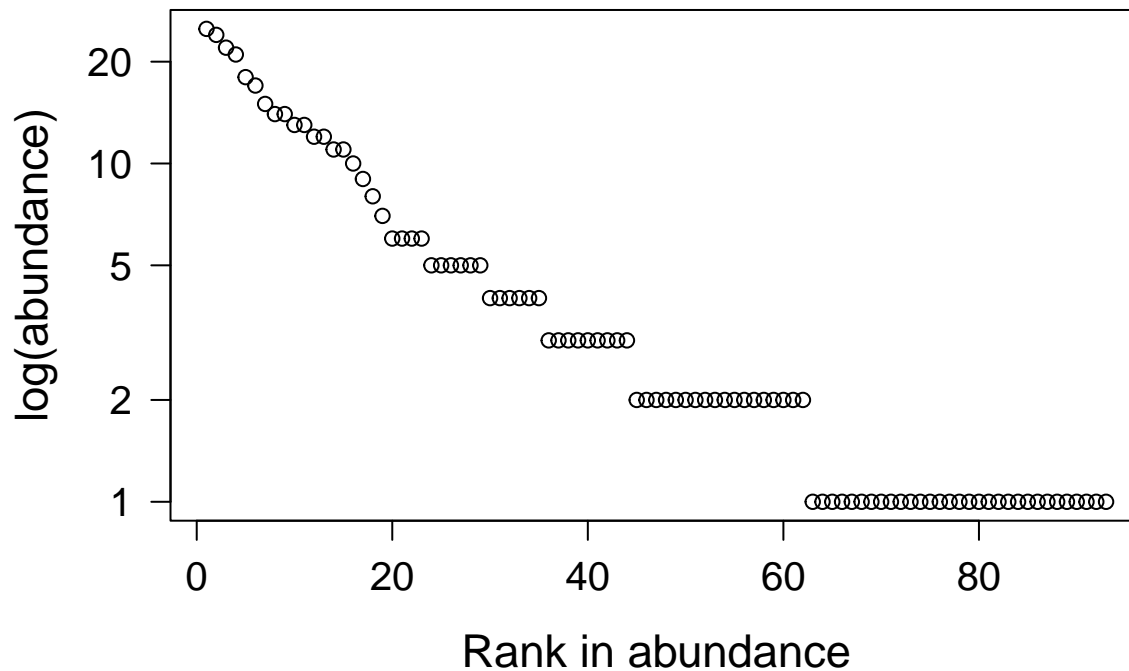
```

plot.new()
site1 <- BCI[1,]

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)))

```



**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-scaling allows us to easily visualize very large numbers with small numbers. This way, we can see how, for a few species, abundances are very high while most species are not very abundant at all.

Now that we have visualized unevenness, 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:** Simpson's evenness for site 1 is 0.42, less than Smith and Wilson's evenness of 0.51. This is probably because Simpson's evenness is biased by the most abundant species, so it would calculate the probability the next sampled individual is a different species as lower than if you accounted for that bias.

## 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".

```
# 1. Provide the code for calculating D (Simpson's diversity)
```

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

```
# 2. Calculate both the inverse (1/D) and 1 - D
```

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

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

```
D.sub
```

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

```
# 3. Compare this estimate with the output of `vegan's` diversity function using method = "simp"
```

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

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

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

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

### Fisher's $\alpha$

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

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

```
# fisher's alpha is asymptotically similar to inverse Simpson's
```

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

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

```
Fisher.BCI <- fisher.alpha(site1)  
Fisher.BCI
```

```
##      1
## 35.67297
```

**Question 7:** How is Fisher's  $\alpha$  different from  $E_{H'}$  and  $E_{var}$ ? What does Fisher's  $\alpha$  take into account that  $E_{H'}$  and  $E_{var}$  do not?

**Answer 7:** Fisher's alpha estimates diversity, where as Shannon's Diversity and Smith and Wilson's Diversity Index actually calculate diversity. Fisher's alpha accounts for sampling error; Shannon's Diversity and Smith and Wilson's do not.

## 6) HILL NUMBERS

Remember that we have learned about the advantages of Hill Numbers to measure and compare diversity among samples. We also learned to explore the effects of rare species in a community by examining diversity for a series of exponents  $q$ .

**Question 8:** Using `site1` of BCI and `vegan` package, a) calculate Hill numbers for  $q$  exponent 0, 1 and 2 (richness, exponential Shannon's entropy, and inverse Simpson's diversity). b) Interpret the effect of rare species in your community based on the response of diversity to increasing exponent  $q$ .

```
# q = 0, diversity is species richness
```

```
Q0_Hill <- specnumber(site1)
Q0_Hill
```

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

```
# q = 1, diversity is Exponential Shannon diversity
```

```
Q1_Hill <- exp(diversity(site1, index = "shannon"))
Q1_Hill
```

```
## [1] 55.6127
```

```
# q = 2, diversity is reciprocal of Simpson diversity
```

```
Q2_Hill <- 1 / SimpD(site1)
Q2_Hill
```

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

**Answer 8a:** see chunk above **Answer 8b:** As exponent  $q$  increases, diversity tends to decrease, so the effect of rare species in the community is decreasing.

## ###7) MOVING BEYOND UNIVARIATE METRICS OF $\alpha$ 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:

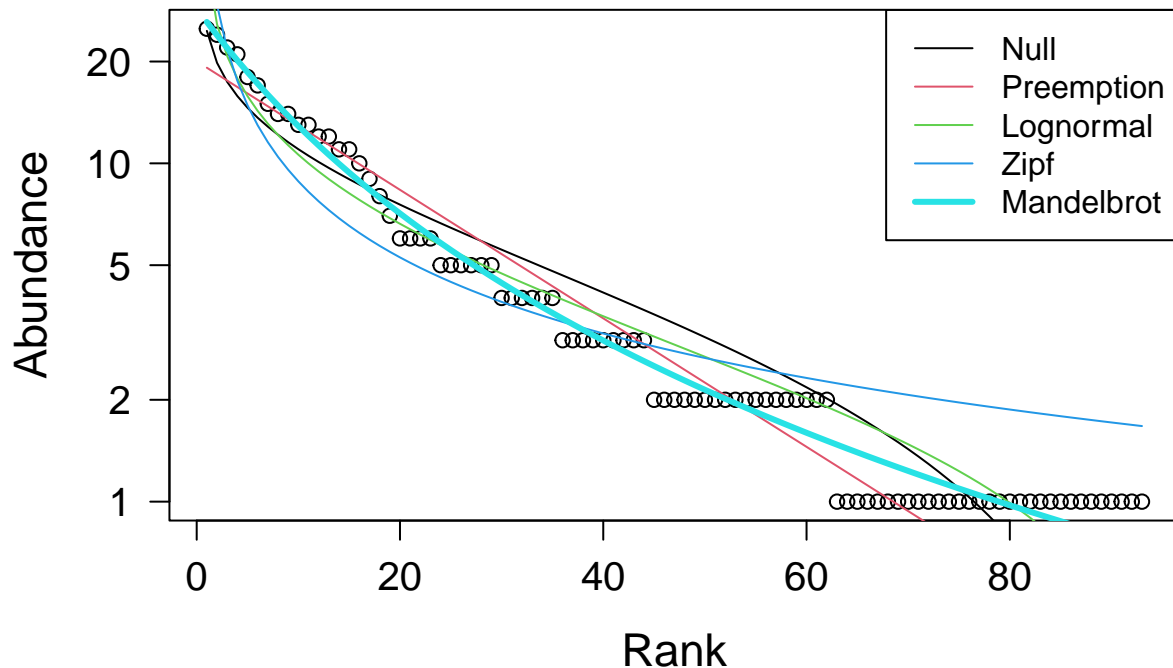
1. Use the `radfit()` function in the `vegan` package to fit the predictions of various species abundance models to the RAC of `site1` in BCI,

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 Mandelbrot model has the smallest Deviance value and appears to follow the pattern of the RAC the closest of all the models. It also has the lowest AIC and BIC values.

**Answer 9b:** Without knowing what the parameters are, we can't make conclusions about what factors are influencing this ecological community.

**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 preemption model assumes that as abundance increases, total resources

decreases/decays exponentially. **Answer 10b:** Because we log transformed abundance. log transforming an exponent cancels out the exponentiation, so you are left with a regular number.

**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 the model has, the better it'll fit the data. However, this doesn't necessarily mean that models with many parameters are "good" models. The point of modelling is to pare down the number of parameters you use while still being able to explain the results well.

## 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.

```
SimpD_finite <- function(x = ""){
  D = 0
  N = sum(x)
  for (n_i in x){
    D = D + ((n_i * (n_i-1)) / (N*(N-1)))
  }
  return(D)
}
site1_SimpD <- SimpD_finite(site1)
site1_SimpD
```

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

```
1 - site1_SimpD
```

```
## [1] 0.9768097
```

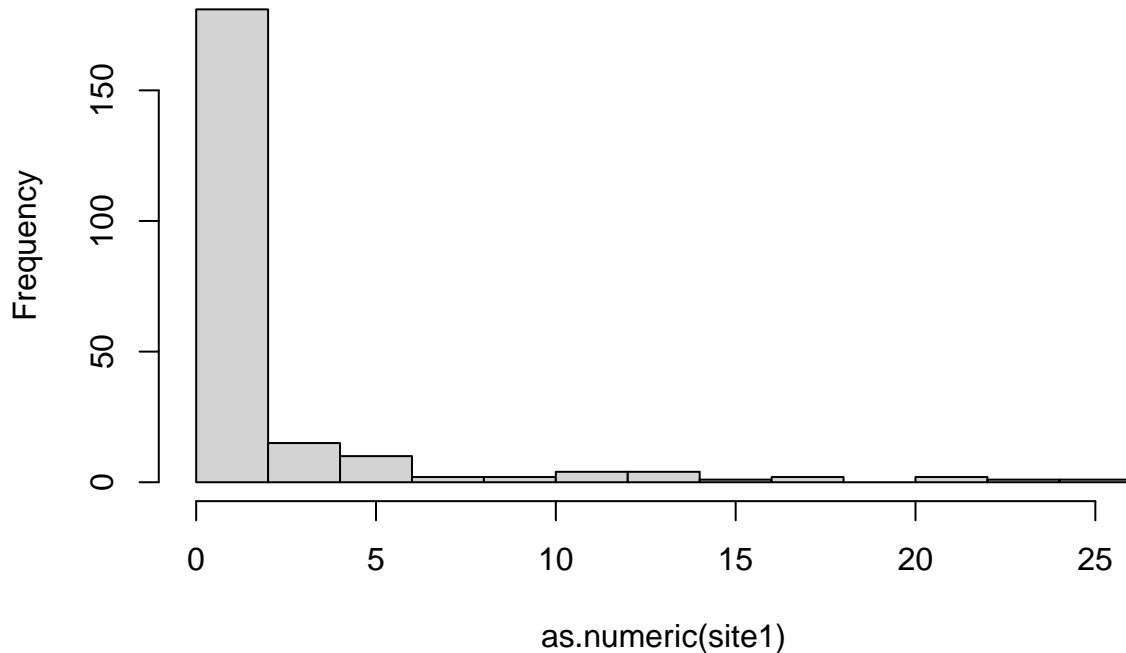
```
1 / site1_SimpD
```

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

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.

```
hist(as.numeric(site1))
```

## Histogram of as.numeric(site1)



> The histogram corroborates the RAC, showing that there is a highly skewed distribution of abundances. The lowest abundance rank has the highest frequency (i.e. there are many rare species) while high abundance ranks have low frequencies (i.e. there are few species that are very common).

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?

We are using Data from Perez-Correa et al. (2020) published on DRYAD, titled “Climate oscillation and alien species invasion influences oceanic seabird distribution.” There are 317 observations/sites. There are 4 species in the matrix: Wedge Tailed Shearwater, Red footed booby, White tern, and Brown Noddy.

## SUBMITTING YOUR ASSIGNMENT

Use Knitr to create a PDF of your completed 5.AlphaDiversity\_Worksheet.Rmd document, push it to GitHub, and create a pull request. Please make sure your updated repo include both the pdf and RMarkdown files.

Unless otherwise noted, this assignment is due on **Wednesday, January 25<sup>th</sup>, 2023 at 12:00 PM (noon)**.