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 (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] "/Users/eligraber/GitHub/QB2021_Graber/2.Worksheets/5.AlphaDiversity"
```

```
setwd("~/GitHub/QB2021_Graber/2.Worksheets/5.AlphaDiversity")
```

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)
max.level=0
```

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
```

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*: They return the same value of 93. As for species richness at the first for sites, it is 93, 84, 90, 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,])
```

```
##         1
## 0.9308036
```

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***: 0.8705882 to 0.9468504

> ***Answer 2b***: The number of individual species is equal to the number of total number of individuals sampled. There is only one of each species.

> ***Answer 2c***: ~7% of species were singletons.

> ***Answer 2d***: Coverage was reasonable as most species were observed at least a couple of times.

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

```
soilbac <- read.table("data/soilbac.txt", sep = "\t", header=TRUE, row.names =1)
soilbac.t <- as.data.frame(t(soilbac))
soilbac1 <- soilbac.t[1, ]
S.obs <- function (x="") {
   rowSums(x>0)*1
}
C <-function(x= ""){
   1-(rowSums(x== 1)/rowSums(x))
}
S.obs(soilbac1)
```

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

```
C(soilbac1)
```

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

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: 13310

   Answer 3b: 1074

   Answer 3c: There are more singletons in the KBS sample's coverage than the BCI sample showing that the BCI sample had better coverage.

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){
```

4

```
    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(soilbac1)
```

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

```
S.chao1(BCI[,])
```

```
##         1        2        3        4        5        6        7        8        9       10
## 1855.365 1846.365 1852.365 1856.365 1863.365 1847.365 1844.365 1850.365 1852.365 1856.365
##        11       12       13       14       15       16       17       18       19       20
## 1849.365 1846.365 1855.365 1860.365 1855.365 1855.365 1855.365 1851.365 1871.365 1862.365
##        21       22       23       24       25       26       27       28       29       30
## 1861.365 1853.365 1861.365 1857.365 1867.365 1853.365 1861.365 1847.365 1848.365 1859.365
##        31       32       33       34       35       36       37       38       39       40
## 1839.365 1850.365 1848.365 1854.365 1845.365 1854.365 1850.365 1844.365 1846.365 1842.365
##        41       42       43       44       45       46       47       48       49       50
## 1864.365 1849.365 1848.365 1843.365 1843.365 1848.365 1864.365 1853.365 1853.365 1855.365
```

```
S.chao2(1, soilbac1)
```

```
## T1_1
##  Inf
```

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

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

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

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

```
S.ace(BCI[,])
```

```
## [1] 8525.592
```

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***: Choa1 looks at the number of singletons and doubletons while ACE uses a threshold to consider the abundances of "rare" species. They do not seem to have consistent results. I would choose ACE as it has a more clear definition of "rare" species though this would really depend on the population in question (if the sample has may speies with few individuals ACE would ignore many of the sampled species.)

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')
```
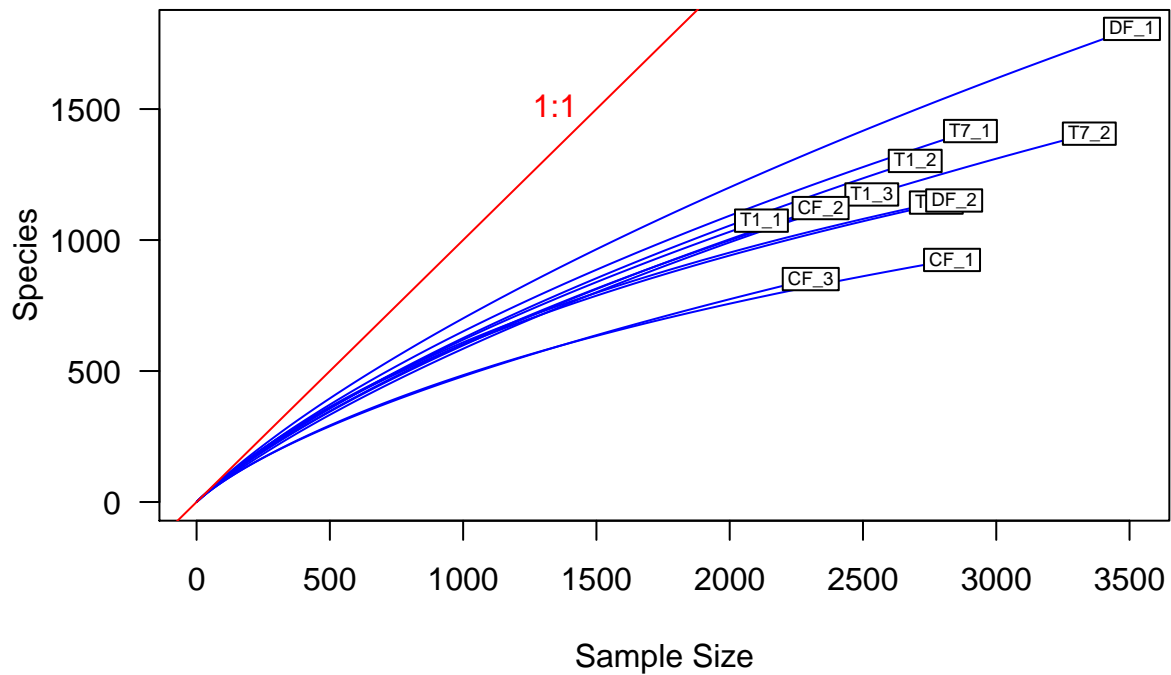

##4) SPECIES EVENNESS Here, we consider how abundance varies among species, that is, **species evenness**.

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

```r
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)"

```r
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="Abundance",
     las=1, cex.lab =1.4, cex.axis= 1.25)
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)))
```
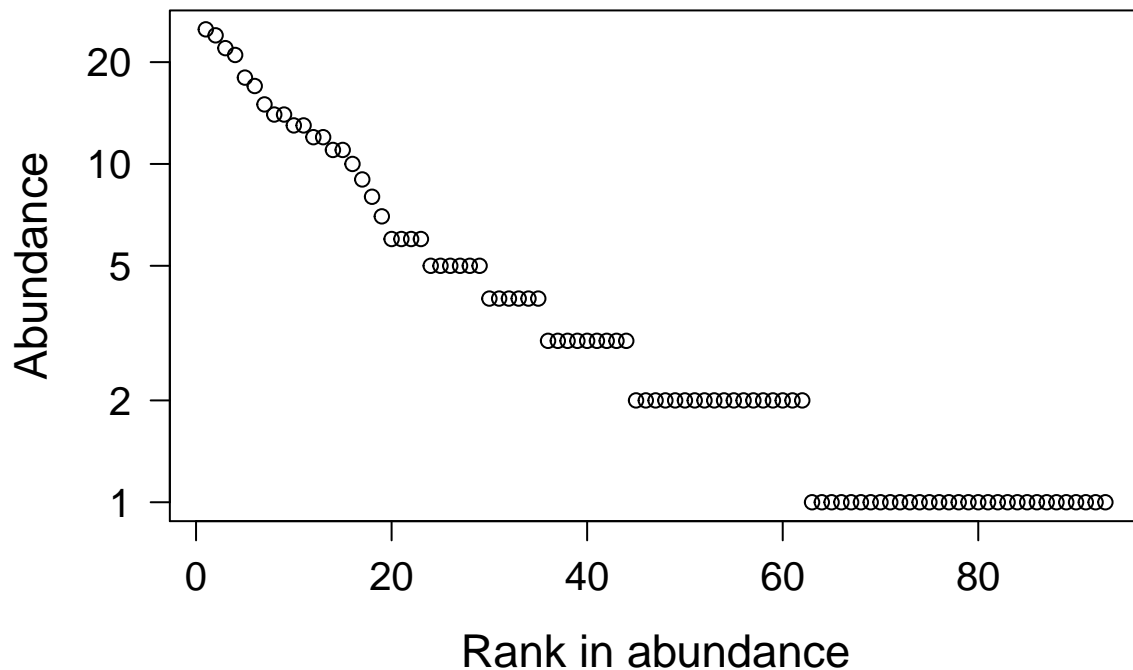
```
par <-opar
```

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**: It allows us to activley see how uneven (in this case, but could be how even in another case) the abundances of different species in area are in a more clear way than if the data was not modified.

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)
}

site1<- BCI[1,]
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***: Smith and Wilson's evenness tends to be skewed by the few most abundant species. Even with this skewedness they have relatively similar results, so they are not in exact agreement, but they do generally agree.

##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".

```r
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)
}

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".

```r
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.sub <- 1-SimpD(site1)
diversity(site1, "inv")
```

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

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

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

Fisher's α

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

1. Provide the code for calculating Fisher's α,

2. Calculate Fisher's α 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 7: How is Fisher's α different from $E_{H'}$ and E_{var}? What does Fisher's α take into account that $E_{H'}$ and E_{var} do not?

> **Answer 7**: Fisher's alpha result is activley larger than EH and Evar. Fisher's alpha is estiamting diveristy instead of producing a metric allowing it to account for sampling errors.

##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)
plot.new()
plot(RACresults, las=1, cex.lab =1.4, cex.axis = 1.25)
```
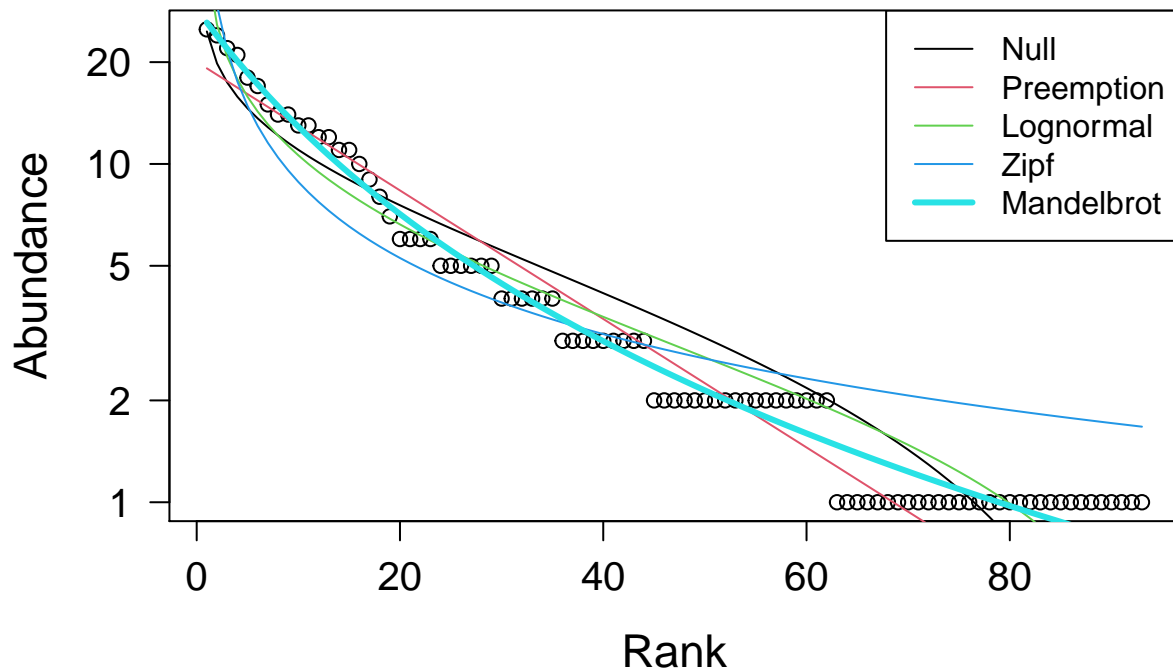
Question 8: Answer the following questions about the rank abundance curves: a) Based on the output of `radfit()` and plotting above, discuss which model best fits our rank-abundance curve for `site1`? b) Can we make any inferences about the forces, processes, and/or mechanisms influencing the structure of our system, e.g., an ecological community?

> *Answer 8a*: Mandelbrot seems to be the best fit as it follows the data most accurately. *Answer 8b*:Ecologically, there is more evenness among the highly abundant species because parameter 3 is positive. In turn, the more abundant a species is the more it is favored.

Question 9: 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 9a*: The abundance of a species directly correlates to the amount of resources they access.
> *Answer 9b*:It looks like a straight line because of the assumped correlation between abundance and resource consumption the coeefficient alpha has a decay rate for the abundance.

Question 10: 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 10*: It is important because the more parameters that are being accounted for the more specific the result is to the data set (which allows the result to be more accuarte than without the parameters).

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.

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.

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?

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, April 7$^{\text{th}}$, 2021 at 12:00 PM (noon)**.