Estimating Biodiversity on the VIRTUE Disc

Driving Question:
What observations and analysis can be performed with VIRTUE discs?

Once the VIRTUE discs have been collected from the water, what can be performed in the classroom? One option is to do a *qualitative analysis* of biodiversity and identify as many species to be found growing on the discs. This is suitable younger students who can be fascinated with observing the organisms using magnifying lenses, stereomicroscopes if available or with smart phones. The teacher can ask the pupils to draw the organisms or make models with clay.

For older students a *quantitative analysis* of biodiversity can be performed by calculating some basic *diversity indices* commonly used in ecological surveys. These indices will provide students with numbers they can use to make objective comparisons of discs.

The diversity indices, their definitions and their meanings:

1. **Species richness (S)** is a measure of the **number of species** found in a community, in our case, on the discs. This is obtained by just counting how many different organisms are found on the discs. Although very easy to measure species richness does not tell much about the abundances and relative abundances of the species on the discs.
   (The more species are found on the discs, the higher is the species richness)

2. **Simpson's Index of Diversity (D)** is a measure of both richness and the relative abundances of the species on the discs. Simpson's Index is also a measure of dominance.
   (D is usually between 0 and 1. The closer D is to 1, the higher is the diversity)

3. **Shannon-Wiener Index (H)** is like the Simpson's Index and also measures the richness and abundances of the species. From the Shannon-Wiener Index Evenness can be calculated.
   (The higher H is the higher is the diversity)

4. **Evenness (E)** complements species richness in that it gives us a picture of the relative abundances of the different species represented on the discs.
   (E is usually between 0 and 1. The closer E is to 1, then more species in the sample are equally common, the diversity is higher)

5. **Jaccard Index (J)** is used when comparing the species composition of two samples. This does not consider the abundances of the species but looks at how similar two samples are with regard species found.
   (The closer J is to 100 %, the more similar the samples are).

6. **Menhinick's Index (D_{Mn})** is also a measure of species richness, which also has the same shortcomings as species richness (S).
(The higher the D_{Mn} is the higher is the species richness)

7. **Effective Number of Species (ENS)** is a measure of true diversity with species number as its unit. It is the number of species in a community with equal abundances that would give the observed value of a diversity index, **D** or **H**. This is excellent for comparing the diversities of different samples. This corrects the non-linearity of **D** and **H**.
(The higher the ESN the more diverse is a community).

What index and Why?

This depends on what questions you want answered. The indices introduced here are quite simple and will not present any problems for a high school student from grade 8 and higher to calculate. The teacher should tell the students not to be intimidated by the formulas because these are actually simpler than they seem to appear.

Species Richness: Species richness (**S**) is the easiest parameter to measure. It is just the number of species found. This, however, does not tell much about the structure of the community. The same is true for the Menhinicks Index (D_{Mn}). For younger students, (**S**) may be the easiest and fastest "quantitative" way to analyse the discs.

Species Diversity: The Simpsons (**D**) and Shannon-Wiener (**H**) indices of diversity and the Effective Number of Species (**ENS**) are only meaningful if used for more than one sample. These are used to compare the diversities of at least two samples. **D** and **H** are both indices and they are just that, they "indicate" only the diversity and are not true diversities in themselves. True diversity can only be expressed with the **ENS**.
D and **H** are only linear if the species in the sample are all equally common. If the species are not equally common, it is not safe to assume that the diversity of a sample with a **D** or **H** of for example 2 is twice as diverse as a sample with **D** or **H** equal to one. In this case, it is more appropriate to calculate the **ENS**.

Comparing samples: If samples are to be compared **D**, **H** and **ENS** can be used and the Evenness (**E**) can tell something about the relative abundances of the species found. The Jaccard Index (**J**) shows how similar are the species found in two samples.

Index	Formula	Variables	How to interpret the values obtained
Species Richness (**S**)		total number of species found	High **S** means the sample is rich in species
Simpson's (**D**)	$1 - \left(\dfrac{\sum n(n-1)}{N(N-1)} \right)$	n = number of individuals for a given species N = total number of individuals of all species	**D** is usually between 0 and 1. The closer **D** is to 1, the higher is the diversity. This should be used to compare at least 2 samples.
Shannon-Wiener (**H**)	$\sum - (Pi * \ln Pi)$	Pi = fraction of the population made up of species i $Pi = {}^{n_i}/_N$ (calculate Pi for all species)	The higher **H** is the higher is the diversity. This should be used to compare at least 2 samples.
Evenness (**E**)	$\dfrac{H}{\ln(S)}$	**H** = Shannon-Wiener Index **S** = Species Richness	**E** is usually between 0 and 1. The closer **E** is to 1, then more species in the sample are equally common, that is, the abundances are similar.
Effective Number of Species (**ENS**)	$\dfrac{1}{(1-D)}$	**D** = Simpson's Index of Diversity	The higher the **ESN** the more diverse is a community. This should be used to compare at least 2 samples.
	$\exp(H)$	**H** = Shannon-Wiener Index	The higher the **ESN** the more diverse is a community. This should be used to compare at least 2 samples.
Menhinick (**D$_{Mn}$**)	$\dfrac{S}{\sqrt{N}}$	**S** = Species Richness N = total number of individuals of all species	The higher the **D$_{Mn}$** is the higher is the species richness

| Jaccard (J) | $\left(\dfrac{x \cap y}{x \cup y}\right) * 100$ | $x \cap y =$ number of species shared between samples $x \cup y = N =$ total number of species found | The closer **J** is to 100 %, the more similar the samples are in terms of species found. |

How to calculate the indices: The formulas at a glance

Calculations with real disc diversity

To be able to better appreciate application of the biodiversity indices, let us compare two discs, which have been deployed in the same location but at different times of the year. .

The photos of the discs below will be used as a template for learning how to make some estimates of biodiversity on the VIRTUE disc. The photo on the left was taken from a part of a VIRTUE disc that was suspended in the Baltimore Inner Harbor (Maryland, USA) for a period of 4 months (March-June) in 2018. The picture on the right was taken from the same location for a period of 7 months (March-September) in 2018.

Disc 1 suspended in the Baltimore inner harbour from March-June, 2018 at 1 meter depth

Disc 2 suspended in the Baltimore inner harbour from March-September, 2018 at 1 meter depth

Immediately, differences can be observed between the two samples by simply looking at the pictures. But how different are they? Calculation of the biodiversity can help make an objective interpretation.

The first step to do is to count the individual organisms for each species. This can be performed with a stereomicroscope or a good macro lens and camera (including a smart phone camera). If you can capture a good image then you may want to try counting with a program called ImageJ. You can choose the appropriate document for your purposes from this link: https://3.basecamp.com/3696266/buckets/3048171/vaults/849731534. Or examine the disc under a stereomicroscope and manually count the individuals of each species you can identify.

In the pictures below the individuals of each species have been counted and marked with dots. The colored dots represent the presence of an organism identified on the disc. For the purposes of this example we used the 5 most obvious organisms to identify. You may find other examples as you observe the sample. Here is color the key:
- Green- dark false mussel
- Blue- bryozoan (tube dwelling species)
- Pink- hydroid
- Orange- barnacle
- Yellow- whip mud worm (polychaete)
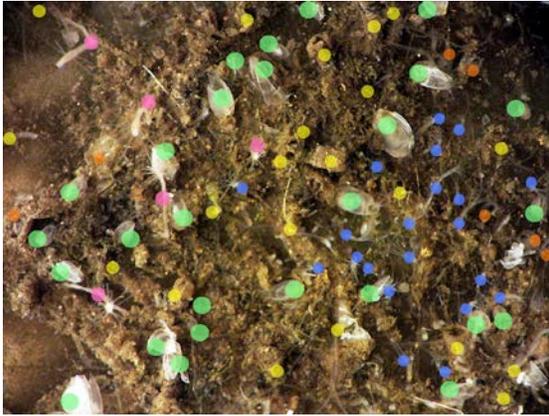
Next create a table for all your data.

	Disc 1	Disc 2
Organisms found	Number of individuals (n)	
Dark false mussel (green)	27	39
Bryozoan (blue)	21	-
Hydroid (pink)	5	-
Barnacle (orange)	7	28
Whip mudworm (yellow)	21	-
Total Number of individuals (N)	81	67

There are some biodiversity estimates that are easy to know and others that require a bit more calculation.

The vast majority of biodiversity estimates are based on three basic parameters; **the number of species identified (S)**, **the number individuals of each species (n)**, and the **total number of organisms in a sample (N)**. Calculate the different variables needed in the formulas for the different indices. In the example below we calculated the variables for Disc 1.

Disc 1					
Organisms found	Number of individuals (n)	Pi n/N	log n (Pi)	Pi * log n (Pi)	n (n-1)
Dark false mussel (green)	27	0.33	-1.10	-0.37	728
Bryozoan (blue)	21	0.26	-1.35	-0.35	440
Hydroid (pink)	5	0.06	-2.79	-0.17	24
Barnacle (orange)	7	0.09	-2.45	-0.21	48
Whip mudworm (yellow)	21	0.26	-1.35	-0.35	440
Sum (Σ)	N= 81			-1.45	1680

Now calculate the indices for Disc1 using the Formulas given in Table 1. For demonstration purposes, we have calculated all the indices in this table. The tutorial on the Biofilms and Biodiversity website at Maryland Sea Grant can help with some of these calculations (we can include the others in the calculator once we update it in the near future) You may choose specific indices you want to use but be sure to use the same index for all samples being compared.

Disc 1				
Species Richness (S)				5
Simpson's (D)		$1 - \left(\dfrac{\sum n(n-1)}{N(N-1)} \right)$	$1 - \dfrac{1604}{81(81-1)}$	0.75
Shannon-Wiener (H)		$\sum - (Pi * lnPi)$	$- \quad (-1.45)$	1.45
Evenness (E)		$\dfrac{H}{\ln(S)}$	$\dfrac{1.45}{\ln(5)}$	0.90
Effective Number of Species (ENS)	Simpsons ENS$_{Si}$	$\dfrac{1}{(1-D)}$	$\dfrac{1}{(1-0.75)}$	4.0
	Shannon-Wiener ENS$_{Sh}$	exp (H)	exp (1.45)	4.26

Menhinick (D_{Mn})	$\dfrac{S}{\sqrt{N}}$	$\dfrac{5}{\sqrt{81}}$	0.55
Jaccard (J) **Disc 1 &Disc 2**	$\left(\dfrac{x \cap y}{x \cup y}\right) * 100$	$\left(\dfrac{2}{5}\right) * 100$	40%

Calculate the same values for Disc 2 (or all your other samples). The values calculated for Disc 1 and Disc 2 are summarized below:

Index		Disc 1	Disc 2
Species Richness (**S**)		5	2
Simpson's (**D**)		0.75	0.49
Shannon-Wiener (**H**)		1.45	0.68
Evenness (**E**)		0.90	0.98
Effective Number of Species (**ENS**)	Simpsons **ENS$_{Si}$**	4	1.96
	Shannon-Wiener **ENS$_{Sh}$**	4.26	1.97
Menhinick (**D$_{Mn)}$**		0.55	0.24
Jaccard (**J**)		40 %	

Remember, the estimates are simply a "snap shot" in time. As we gather more data by sampling over a period of time we can identify trends on the VIRTUE discs.

Analysis:
What do the numbers mean?

Species Richness and Evenness. It is quite obvious that Disc 1 has more species than Disc 2 even though Disc 2 has a longer exposure time than Disc 1 (3 months for Disc 1 against 6 months for Disc 2). This can be seen both the **S** and **D$_{Mn}$** values. **S** gives the

absolute number of species found in the discs while by using the D_{Mn} values one can conclude that Disc 1 has a species richness which is more than twice that of Disc 2. The evenness, **E,** of both discs are high at 0.9 and 0.98, respectively. The almost equal to 1 value of **E** for Disc 2 tells us that the 2 species found on the disc are almost equally common.

Species Diversity. Both the Shannon-Wiener **(H)** and the Simpsons **(D)** Indices point to a more diverse community on Disc 1 as compared to Disc 2.

Taking the Simpsons Index of 0.75 and 0.49 for Discs 1 and 2, respectively, we may conclude (wrongly) that there is roughly a 35% difference in diversity between Disc 1 and Disc 2. However, looking at the **ENS$_{Si}$** (Disc 1 = 4 and Disc 2= 1.96)**,** the decrease is actually around 50%. So there is an underestimation of the decrease in diversity.

The difference in the Shannon-Wiener Index for Disc 1(**H** = 1.45) and Disc 2 (**H** = 0.68) is 53% and the difference in the **ENS$_{Sh}$** (Disc 1 = 4.26 and Disc 2 = 1.97) is also about 54%. So taking the Shannon-Wiener Index in this case is more reliable than calculating only the Simpson's index.

The reliability of the Shannon-Wiener Index lies in the fact, that **H** considers all species present in the sample whereas, the Simpsons Index can sometimes disregard rare species or species with relatively low numbers.

Let us look at the **ENS, S** and the **E** of Disc 2. The **S** = 2 and the **E** = 0.98. The **E** says that the 2 species are almost equally common, so looking at both the **ENS** = 1,97$_{Sh}$ and 1.96$_{Si}$, these are almost 2 thus **ENS = S.**
(**ENS = S** if all the species are equally common). However, there is still a degree of dissimilarity in the values of the **ENS** and **S,** which means that there is some degree of dominance. The larger the differences in **S, ENS$_{Si}$** and **ENS$_{Sh}$**, are, the higher the degree of dominance. We can say from our results that the degree of dominance is higher in Disc 1 than in Disc 2, or some species in Disc 1 are more dominant than some other species.

Degree of Similarity. According to the Jaccard Index **(J)** the two discs are only moderately similar with regard to species composition. The degree of similarity is not very high.

Discussion of results: Baltimore Harbor is highly stressed with run-off and pollution and over-time the disc diversity has regularly represented a "Dominance Diversity" as illustrated by the colonization on the Disc 2 and dominance by mussels. This is where some fundamentals of ecology (Fundamentals of Ecology, by Odum, 1953) can be helpful in analysis.

Disc 1 can also be seen as the middle stage of colonization, the transition stage, after settling of the pioneer species, like bacteria and microalgae. During this stage more species are expected. During the latter stages of colonization, some species have asserted

themselves, displacing some of the species, which were not successful in adapting to the new environment. Competition for space ensues and the most successful species persist, in this case only the mussels and barnacles, leading to a lowering of species richness and, in turn, less diversity.

See an example representation below. The graphic (below) demonstrates how biodiversity data from the discs can be represented (dots from the counting estimates) using **S** (species richness) and **E** (Evenness). The graphic can help illustrate how biodiversity estimates over time can be used to judge the overall health of an environment.
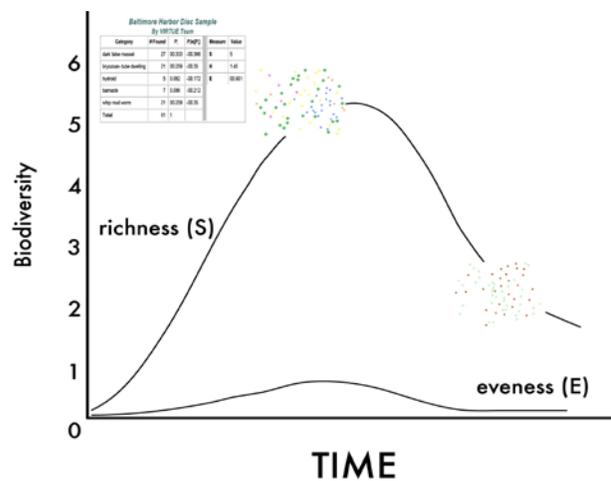
These data can be described further by using the Biofilms and Biodiversity website as a reference and other web resources:

World Register of Introduced Marine Species
http://www.marinespecies.org/introduced/wiki/Measurements_of_biodiversity

For on-line biodiversity calculations go to: