Student Worksheet on Succession

The concept of ecological succession addresses the changes in a community's species composition and structure over time. In the VIRTUE-s project, we observe succession on discs placed in an aquatic environment (the sea, a lake or a river): after some time, the first organisms settle on the discs and - depending on season and geographical location - more organisms arrive and begin to compete with the first settlers for resources like light, space or food. This process is called primary succession.[1]

This worksheet is an exercise on how you can study succession using the VIRTUE discs. You will learn how to visualise and interpret the results obtained in an example project.

The worksheet will

- formulate the questions of the study,
- provide you with some background on the methods used (which you may need for the interpretation of the results later on),
- and give you the data recorded in the experiment.

Your task will be

- to visualize the data,
- to interpret them (using some questions and hints to help you on the way), and
- to find additional information on the internet if needed.

Particularly the last point is important, because - different from classroom teaching - in "virtual" teaching we explicitly encourage you to find and use resources from the web to deal with your assignments.

I. Goals of the study

In this worksheet we use data from a student project that took place in 2018 in a temperate location. The goals of this project were:

1. to observe the chronological order of the appearance of organisms on the discs;
2. to record the changes of community structure and biodiversity on the discs over time;
3. to determine if there are differences in the development of a community on the upper and lower side of a disc.

The same questions will be addressed here based on the data recorded by the students in that project.

[1] In contrast, secondary succession is observed in communities which have been subjected to a disturbance like a forest wild fire or complete deforestation. This can be likened to a disc which previously harbored organisms but dried out because it was taken out of the water for a long time, resulting in the death of all the original organisms growing on it.

II. Materials used and Method of Analysis

To provide some background on the experimental procedure, this section describes the materials and the methods used. Note that some of this information may become important in sections IV and V when we attempt to interpret the data.

II.1 Materials employed for the project

- Discs and racks
- Buckets and deep dishes
- Thermometer
- Refractometer (for measuring salinity)
- Kitchen scale (for measuring weight)
- Cameras; Microscope cameras
- Stereo microscopes
- Self-constructed PVC counting grids
- Protocol sheets

II.2 Rack Assembly

In the project, it was decided that the experiment should run for several weeks, and a new disc was to be deployed every week. Thus, the construction of the racks had to take this into account.

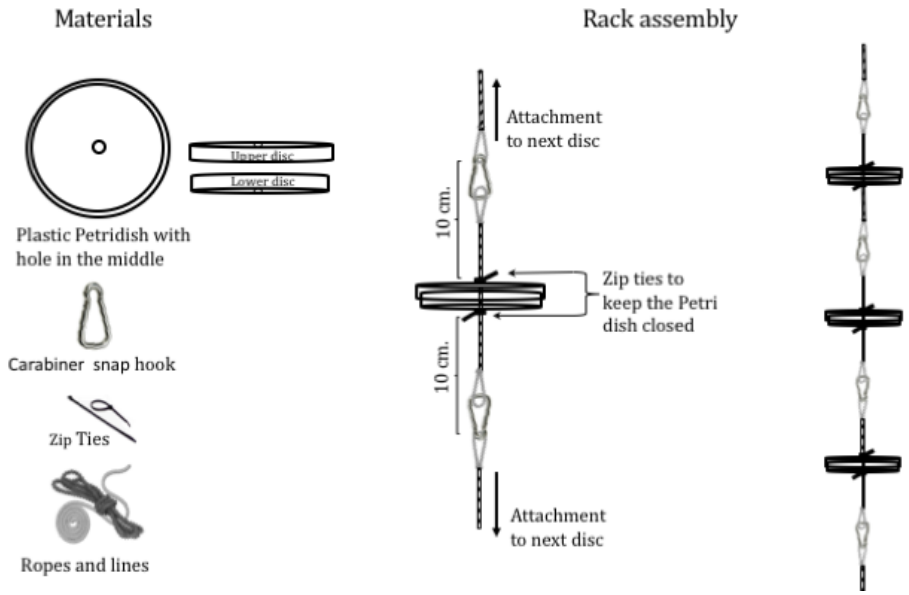


Figure 1: Rack construction using plastic petri dishes

The discs and racks used for the experiment were new. They were made of plastic (polystyrol) and the the same materials were used during the entire investigation. The construction is shown in Figure 1. Petri dishes with holes in the middle were used as discs and attached to short plastic lines. These were connected to each other using stainless steel carabiner snap hooks. A weight was placed at the end of the rack so the construction would stay underwater. A maximum of three disc pairs was suspended from every line.

With this configuration it was possible to add successive pairs of discs to an existing line without disturbing the older discs. One disc-pair (upper and lower part of the petri dish, see Figure 1) was prepared for each date of deployment.

For the labelling of the discs, two methods were used to ensure proper identification later:

- Labelled fabric tape (using a permanent marker) was attached to the inner side of the discs.
- Colour-coded zip ties were fastened to the lines above the discs.

II.3 Rack deployment

Before the project was started it was decided that discs would be deployed successively during 10 consecutive weeks in the spring (March 26 to May 28). The location was a pier outside the GEOMAR Helmholtz Centre for Ocean Research in Kiel, Germany, in a protected harbour with very little ship activity.

- A disc was deployed at each deployment date by adding it to a new or existing line.
- The discs were placed about 0.5 – 1.5 meter from the sea surface to ensure that the rack was underwater at all times.
- The position of the discs was switched every now and then so that every disc had changing random positions along the line (top, middle or bottom) during the duration of the project. This was done to eliminate light and depth effects.

II.4 Measurement of environmental parameters

To record the changes in temperature and salinity of the surface water, these parameters were measured every week when a new disc was deployed. Seawater was collected with a bucket at the site and temperature was immediately measured using a thermometer. Salinity was determined using a refractometer.

II.5 Retrieval of racks and quantitative analysis of discs

- The retrieval of the rack (all discs at the same time) took place one week after the deployment of the last disc. (Thus, the first discs had been in the water for the full 10 weeks from March 26 to June 4, while the last (newest) discs had only been in the sea for 1 week, i.e. from May 28 to June 4.)
- The racks were transported to the laboratory in buckets filled with seawater. There, the racks were dismantled and the upper and lower discs were separated.
- The students paired up in teams. Each team was responsible for 1 set of discs (upper and lower).
- The discs (upper and lower) were placed individually in labelled deep dishes and submerged in seawater.
- The percentage of the disc covered with fouling organisms was estimated using the guide for Visual Estimation of Percentage Cover[2].
- An initial identification of the organisms was made.
- Biomass was estimated:
  - The discs were allowed to drip dry.
  - Each disc was weighed separately on the kitchen scale.
  - Results were recorded in a protocol sheet.
  - The weight of a dry reference disc was subtracted to give the weight of the (wet) biomass.
- Photos of the discs were taken for documentation and later estimation of percentage cover, either visually (as control) or using an image processing program.

[2] available for download here:
https://virtue-s.eu/english-content/visual-estimation-percentage-cover

- Counting the organisms:
  - The discs (still immersed in water) were placed under the stereo microscope.
  - A counting grid was placed on top of the disc.
  - Discs were first examined under the lowest magnification of the stereo microscope to identify the major species.
  - Then, a magnification was used that allowed to see an entire quadrat on the grid while still recognising the organisms.
  - Organisms in several random quadrats were counted manually.
  - Results were recorded in the protocol sheet.
  - From the numbers obtained for the random quadrats, data were extrapolated to the total area of the disc.

Only organisms that could clearly be assigned to a particular disc were counted. ("Visiting" species that might have moved from one disc to another during transport were not considered.) In this experiment, the organisms for analysis were barnacles, tube worms and polyps. In addition, the percentage of the disc covered by filamentous macroalgae, which are difficult to count individually, was estimated visually.

III. Data recorded

III.1 Environmental factors

Table 1 shows the data for temperature and salinity at each date of deployment:

Date of deployment	Temperature (°C)	Salinity (ppt)
26. Mar.	2.5	15.3
3. Apr.	4.7	12.0
9. Apr.	7.0	12.0
18. Apr.	8.4	14.0
23. Apr.	7.2	14.0
30. Apr.	10.0	14.5
7. May	12.5	13.0
15. May	12.7	11.0
22. May	14.0	12.0
28. May	16.6	12.5

Table 1: Data for temperature and salinity

(Data available in files Table1.ods and Table1.xlsx)

Note that the unit "ppt" for Salinity refers to "parts per thousand". (Strictly speaking, salinity is expressed as a mass fraction of gram dissolved salt per kilogram of sea water.)

III.2 Biomass

Table 2 gives the biomass data (in grams) on the discs at the time of analysis.

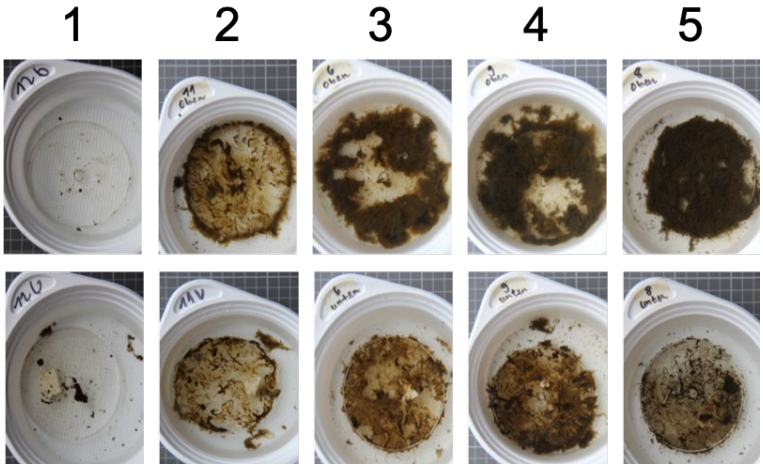
	Biomass (g)	
Weeks in the water	Upper disc	Lower disc
1	0.3	1.0
2	2.8	3.1
3	12.6	9.6
4	9.3	9.4
5	16.5	12.7
6	20.5	11.9
7	21.2	13.0
8	20.8	15.2
9	26.6	16.8
10	21.0	8.0

Table 2: Data for biomass (wet weight)

(Data available in files Table2.ods and Table2.xlsx)

III.3 Percentage cover

Here, we show photographs of the discs placed in the water for the 10 weeks from mid-April to end of May (Figure 2).



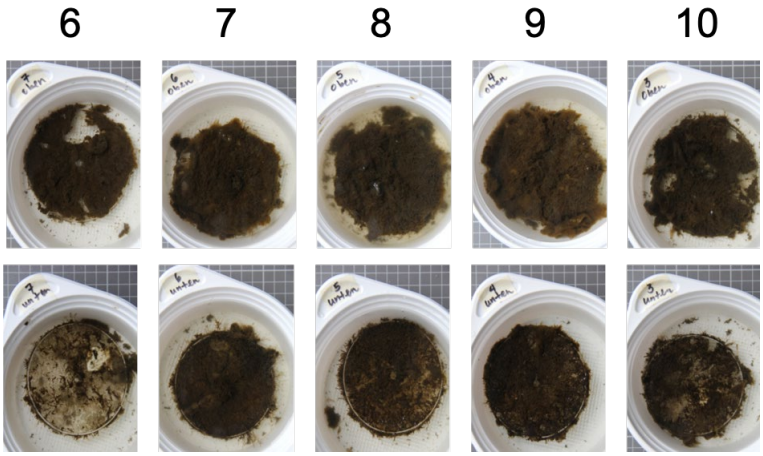


Figure 2: Photographs of upper and lower discs (in upper and lower row of each pair). Numbers indicate weeks in the water (top pairs weeks 1-5, bottom pairs weeks 6-10).

(Original photos available as files Photos_of_Discs_Week1-5.png and Photos_of_Discs_Week6-10.png.)

III.4 Species identification and counts

Using species identification plates[3], organisms were identified and counted.

Except for algae, for which percent cover was estimated, organisms on the discs were counted manually through the stereo microscope. Values given in Table 3 are the extrapolated total number of individuals on a disc. For the cell shaded in grey no results were handed in by the students.

Weeks in the water	Algae		Barnacles		Polyps		Tube Worms	
	(%)		(number of individuals)		(number of individuals)		(number of individuals)	
	upper disk	lower disk	upper disk	lower disk	upper disk	lower disk	upper disk	lower disk
1	2	5	0	0	0	0	0	0
2	55	55	0	0	0	0	0	0
3	55	55	0	0	0	0	0	0
4	45	40	0	0	0	49	0	0
5	35	10	0	0	83	0	187	22
6	87	28	0	2	7	69	216	22
7	65	40	10	20	109	60	886	766
8	60	40	14	51	17	24	1116	724
9	95	85	32	16	24	62	1061	722
10	90		3	292	58	166	1893	1926

Table 3: Abundance of different species: Algae in percent cover, other organisms in total number on the disc.

(Data available in files Table3.ods and Table3.xlsx)

[3] these can be downloaded from here: https://virtue-s.eu/english-content/species-identification-plates

IV. Analysis and Visualisation of the Data

Your task now is to analyse the data obtained from this project. To this purpose, the data have been made available in spreadsheet format for LibreOffice, OpenOffice and Excel (see files cited above). All of the following tasks can be solved by using any of these programs. If in doubt, consult a tutorial for your software of choice. (YouTube has a wide selection of visual guides, and there is plenty of advice in forums.) All of the graphing tasks can also be done on paper with a pencil and ruler.

1. Create a time series plot (bar chart or line graph) for temperature and salinity.

2. Create a bar chart (column graph) of the time series of the values for biomass on the upper and lower discs with the time information as "weeks in the water" on the x-axis.

3. Estimate the percentage cover of the discs shown in the photos above (Figure 2, provided in files Photos_of_Discs_Week1-5.png and Photos_of_Discs_Week6-10.png). For this visual estimation use the guide below (Figure 3, provided in the file Visual_Estimation_of_Percentage_Cover). Create a new table for percentage cover similar to Table 2. Plot bar graphs of the data in your table.

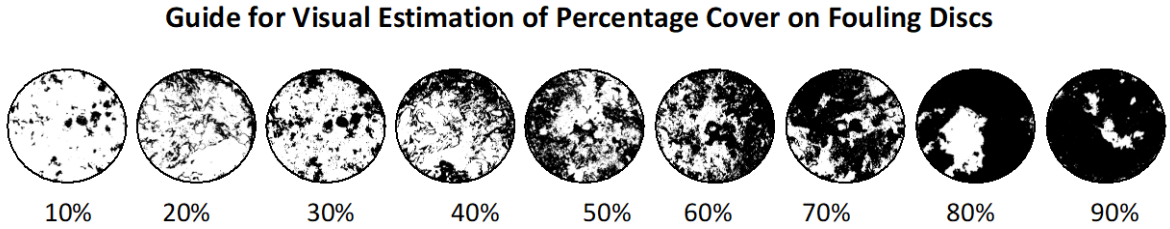


Figure 3: Tool for visual estimation of percentage cover

If you are working in teams, have each team member do their own estimation of percentage cover and compare the results afterwards. Calculate the differences between the team's estimates and from these estimate the average error margin of this method.

4. Create a scatter plot of biomass on the y-axis versus percentage cover on the x-axis for the data of the upper discs. (Optionally, have the spreadsheet software add the regression line and let it calculate the correlation coefficient.[4])

5. Plot the results in Table 3 in different ways:
   - Plot the percentage cover of algae and the number of organisms of each species as a function of time in bar graphs individually for each species. Differentiate between upper and lower discs.
   - Optional challenge: Combine the diagrams for all organisms in a plot for the upper and lower disc respectively. Use a logarithmic scale for the number of organisms and a second linear y-axis for the percentage cover of algae.

6. Count how many different species are present on the discs each week (this time, you do not need to differentiate between upper and lower disc) and construct a diagram showing the change in species richness (number of species) with time.

7. Calculate the Simpson's Index of Diversity for the oldest upper and lower discs. Basically, Simpson's Index is a measure of the probability that two randomly selected individuals from a

[4] If you are not familiar with these terms, see https://milnepublishing.geneseo.edu/natural-resources-biometrics/chapter/chapter-7-correlation-and-simple-linear-regression/ for a good introduction. For a video tutorial of how to apply this in a spreadsheet, see e.g. https://www.youtube.com/watch?v=f4_GwWdUNqI .

sample will not be from the same species. It ranges from 0.0 (no probability because all individuals are from the same species) to 1.0 (100% probability). To compute the index, use the definition:

$$D = 1 - \frac{\sum_i^I n_i(n_i - 1)}{N(N-1)}$$

where

D = Simpsons Diversity Index
n_i = Number of individuals of species i
I = total number of species
N = Total number of individuals of all species.

With this, you can compare the biodiversity of the upper and the lower disc at the end of the experiment (week 10) using the data from the project (use tube worms, polyps and barnacles only).

V. Interpretation of the Results

Using the diagrams you created above, you are now ready to interpret the results of the project. In section V.2 we give some hints that will help you in this analysis and provide some key words for additional information on the different organisms that you can find on the internet.

Important: the data presented here are the original data from the student project. They have not been altered to make them look nicer or tampered with to reflect idealized textbook situations. They contain measurement uncertainties, counting errors and also data gaps. Thus, they reflect the real situation of the student project where this is what you have to work with. As a consequence, in many interpretations there will not be a clear-cut "wrong" or "right". What we are looking for is rather an "educated maybe": use the data, try to see patterns and trends, but also discuss potential sources of error or uncertainties. Explain where the results fit your expectations and biological theory and where they differ. Make the best of the data you have!

V.1 Tasks

Discuss the following aspects:

1. How do temperature and salinity change over the duration of the experiment?
2. Compare the change in biomass with time on the upper and the lower discs. Which features are worth noting? Interpret your findings.
3. Do you see any relation between biomass and percentage cover of the discs? Explain. (Optionally: How well are biomass and percentage cover of the discs correlated? What does this mean?)
4. Describe and discuss the growth of the four species: what development is there as a function of "time in the water" and in relation to upper and lower discs?
    a. Algae
    b. Tube Worms
    c. Polyps
    d. Barnacles
5. Explain the apparent order of appearance of the organisms on the discs. What could be the reasons for this?
6. Is it plausible that water temperature or salinity may have affected settlement and growth on the discs? If so, when and in which way?
7. Which species could be competing with each other for the same type of resources? What resources are those? Do you see evidence for this in the data?

8. Compare the diversity indices of the upper and lower disc at the end of the experiment. How do they differ? Explain the difference.

V.2 Hints

To help you interpret the data, here are some hints you can use. (You may not be familiar with some of the words used here, but they are very easy to look up on the web.) Although most of this information is relevant for this experiment, you will have to decide for yourself which parts are important for your interpretation of the data.

- The data presented here were collected by students in a class project. Thus, the results are "real" and not idealised. For example, the students may have accidentally scraped off some of the organisms while handling the discs. Consequently, some of the data may not be unconditionally reliable.
- The sampling site was on the west shore of the Kiel Fjord at the geographical coordinates 54°19'47.6"N 10°09'00.2"E.
- There may be a shading effect when upper discs are sufficiently overgrown to reduce the amount of light available for the lower disc.
- The most common algae were *Ectocarpus*, a brown filamentous alga. It has two stages in its life cycle, first a haploid gametophyte, which is less tolerant to salinity changes, and later a more tolerant diploid sporophyte.
- *Polydora sp.*, the most common tube worm species found on the discs, rely on sediments (which gradually accumulate on the discs from the water column) to build their tubes. The currents in the Kiel Fjord are fairly weak.
- The release of barnacle larvae by adult animals is determined by the concentration of phytoplankton and by turbidity. It mostly coincides with the phytoplankton bloom in spring. Barnacle larvae are planktonic. The earlier larvae stages are positively phototactic (which is an advantage since they feed on phytoplankton). The last larval stage, the cypris larva, is the stage which after some days or weeks settles on a substrate. This stage does not feed and is negatively phototactic.
- The planula larva of polyps, specifically of *Obelia sp.,* are part of the zooplankton drifting in the water. At this stage, they are positively phototactic but they become negatively phototactic when it is time to settle on a solid surface.
- The organisms may be competing for space on the discs or for the same type of food.
- Depending on the native species found in the area, most organisms spawn in late spring or early summer, i.e. as soon as the right temperature is reached and there is enough food available for the feeding larvae.

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