

Teacher Version of Succession Worksheet

This document complements the “Student Worksheet on Succession” with more background for the teacher. The student worksheet by itself should contain enough information to allow students to complete the tasks (if necessary with some additional help from web resources).

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I. Pedagogical Goals

The main purposes of the exercises in the student worksheet accompanying this document are:

- to provide a “substitute” for real data from field experiments which could not be carried out or completed for any reason,
- to be used as preparation for a project, where students can practise the visualization and analysis of the data they will generate in their own project,
- to train students in the interpretation of results from graphs and tables,
- to practice making an argument based on real data, and
- to make students aware of potential pitfalls when planning their own projects.

(The importance of the last point should be stressed because students frequently underestimate the need for careful analysis and documentation during an experiment. In the data presented here, there will be ambiguities and outright errors by the teams that recorded them which will likely pose problems in the analysis.)

For this exercise some background knowledge in **biological succession**, **competition** and **life in aquatic environments** may be required from the students.

As explained in the worksheet, all data presented are real data: they are the results of a class project that had to fit into the time frame available within the school's schedule. Consequently, scientific requirements in term of e.g. blind tests, redundancies or error estimates had to be reduced to fit the time table. (As an example: a second set of discs was also deployed as replicates or replacement in case samples were damaged or lost. However, the second set was not analysed due to insufficient time.) Thus, these data are a good representation of the kind of result one can expect at the end of a class project.

The worksheet provides a number of tasks from which the teacher should chose those that are appropriate for the capabilities of the students and the goal of a course. At the same time, the data are open for interpretation and thus there are not necessarily correct or incorrect answers. The task for the students is to analyse the available data, interpret them and make a plausible argument for their results. For the assessment of the results, examples of possible outcomes of an analysis will be given below.

The central tasks for the students are:

- visualization of measured data,
- description of the results,
- interpretation and discussion of their findings.

For the visualization, data files are provided in LibreOffice and OpenOffice format (.odt-files) and in Excel format (.xlsx). If the students are not familiar with charting in spreadsheet software, numerous tutorials (or video tutorials) for the different software packages are available on the web.

II. Background

II.1 Experimental Framework

The data used here are from a student project that took place during 10 consecutive weeks in the spring (March 26 to May 28) of 2018. The location was a pier outside the GEOMAR Helmholtz Centre for Ocean Research in Kiel, Germany. Construction of the discs as well as retrieval and analysis were done by the students.

The time frame for the experiment was chosen to fit into the time table of that particular school, but also with a view to environmental conditions: at higher latitudes, winter deployments are usually not advisable as no or very few organisms will settle on the discs. Summer deployments are more interesting and projects during this time can be shorter. Depending on the native species found in the area, most organisms spawn in late spring or early summer, i.e. as soon as the required temperature is reached and there is enough food available for the feeding larvae.

The experimental procedure described in the student worksheet is a variation of the VIRTUE-s approach (see <https://virtue-s.eu/> for details).

It should again be noted that the design of this experiment was adapted to the school's schedule and the time the students were able to dedicate to the project. The straightforward way to examine succession on discs would have been to either

- put one (better: several) discs into the water in early spring, then examine them every week for a period of 10 weeks to see what additional growth has occurred in the preceding week, and put them back into the water until the next week. (Note, however, that this is quite difficult without damaging the growth on the discs.) Or:
- deploy 10 discs at the same time in early spring and retrieve and examine one of them every week.

Since it was much easier for the school to have the whole class examine all of the discs together on one single day, a “reverse approach” was chosen, where one disk was deployed every week and after 10 weeks all disks were retrieved and analysed at the same time.

This, however, has implications for the interpretation of the results, which we will address below.

II.2 Topics

Depending on the setup, a range of different ecological topics can be addressed with this type of method. (Aspects considered in this worksheet are emphasized in **bold print**.)

1. Differences and similarities of ecological succession between terrestrial and aquatic environments.
2. Comparison of spring, summer and autumn recruitment.
3. **Effects of light availability on community structure.**
4. **Development of species diversity with time.**
5. **Changes in community structure with time.**
6. Comparison of succession in two different aquatic locations.
7. Phenology vs. Succession.
8. Others...

II.3 Contents of this set of documents

The student worksheet includes:

- Information on materials and methods used in the experiment.
- A description of the analysis methods for the discs after retrieval.
- Data obtained by the school class.
- Data files for use with spreadsheet software.
- Tasks for the students and hints to help with the interpretation of their results.

The teacher version assumes that the teacher is familiar with the contents of the student worksheet. It adds:

- Examples for visualising the results in diagrams.

- Examples for the interpretation of the results (including facts and further information about the recruitment of the most common fouling organisms).

III. Data Analysis

The tables with the data and the tasks for visualization are given in the student worksheet. Here, we will proceed directly to the resulting graphs. (All figures shown in this document were created with LibreOffice using the spreadsheet data provided with this exercise.)

In this section we will show plot examples that display the expected outcomes of the corresponding tasks in the student worksheet, before we continue to the discussion and analysis in the next section.

III.1 Environmental factors

Sea surface temperature and salinity were recorded at weekly intervals during the experiment (Figure 1).

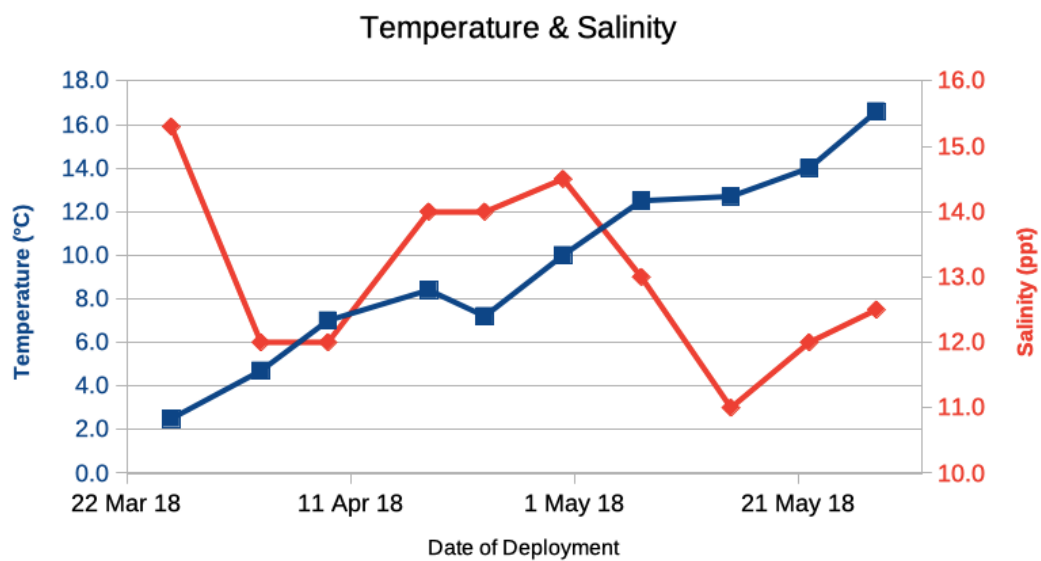


Figure 1: Changes of temperature and salinity with time during the 10-week project

Note that - different from the following plots - Figure 1 gives the date of deployment on the x-axis. This will be a point to keep in mind later, when “weeks in the water” are used as the time variable.

III.2 Biomass

The data for biomass on the upward and the downward facing part of the Petri dish (see Fig. 1 in the student worksheet) are shown in Figure 2.

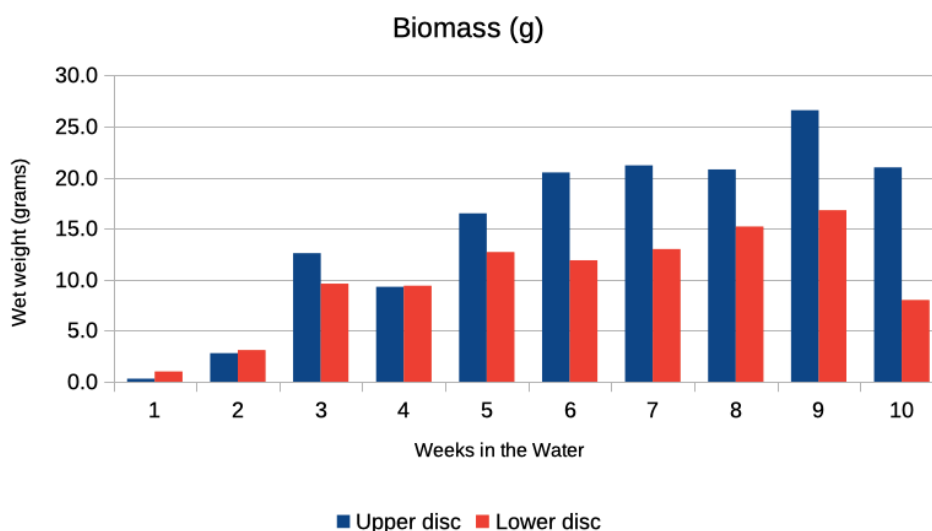


Figure 2: Final biomass (wet weight) after different lengths of time in the water during the 10-week project.

From now on, “weeks in the water” is used to denote time. Note, that while it is tempting to use phrasing such as “the disc from week 3” this may lead to ambiguities because it does not refer to week 3 of the experiment: The disc that has been **in the water** for 3 weeks was actually deployed in week 8 of the experiment, i.e. on May 15. In this document, the word *week* will always refer to *weeks in the water* to denote the different lengths of exposure.

III.3 Percentage Cover

The visual estimation of percentage cover that was given as a task to the students is a fairly subjective method with an accuracy of maybe 10%. The advantage of this method, however, is that it is fast and even younger pupils can do it by themselves. From the photos provided with the student worksheet, the following results were obtained by visual estimation:

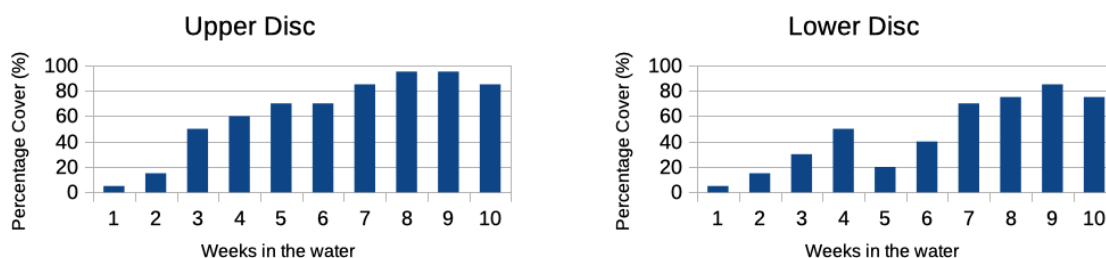


Figure 3: Time series of Percentage Cover (%) of upper and lower discs

If the students worked in teams and did individual estimations, their results will probably differ by 5 to 10%. This will give them a sense for the accuracy and reliability of this method.

III.4 Scatter plot of biomass versus percentage cover

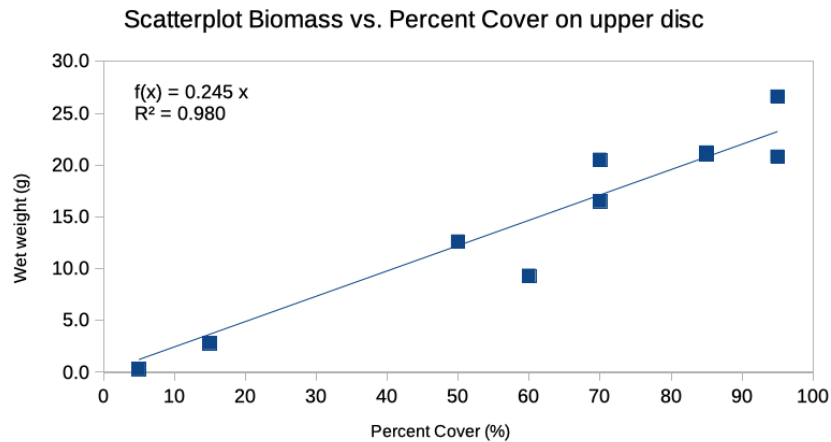
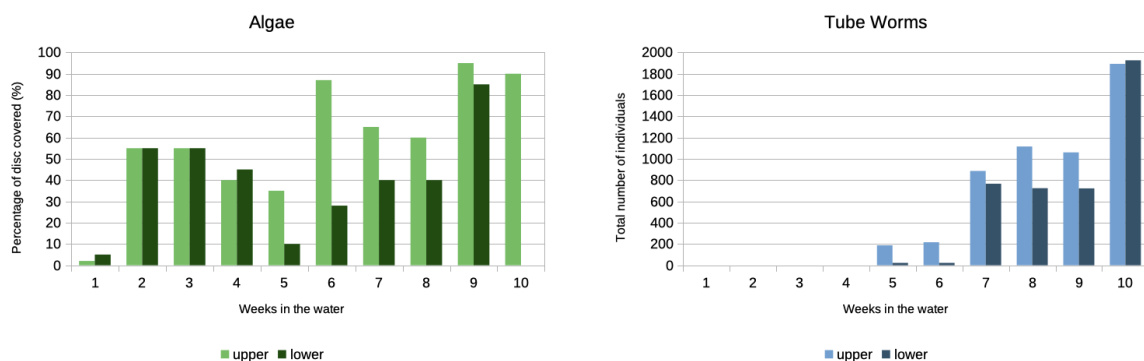


Figure 4: Scatterplot and regression line for biomass (in grams) vs. percent cover (%) for the upper discs.

LibreOffice computes a correlation coefficient of 0.96 for the two variables. (The relationship for the lower disc - not shown - is less pronounced.) Although this correlation is high (and statistically significant with a probability value $p < 0.001$) one should not forget that we are looking at only 10 measurements.

III.5 Species identification and counting

The results given in Table 3 of the Student Worksheet can be presented in different ways. The number of organisms can be plotted in column graphs individually for each species (Figure 5):



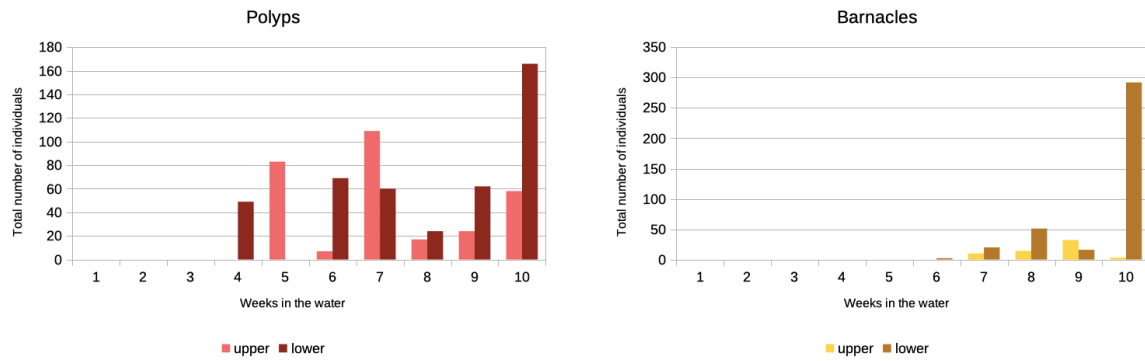


Figure 5: Development of the species composition on a disc over time. The percentage cover (Algae) and number of individuals (Tube Worms, Polyps, Barnacles) are shown for the upper and the lower discs.

The diagrams may also be combined for all organisms (Figure 6). (Note that now the number of organisms is shown on a logarithmic scale.)

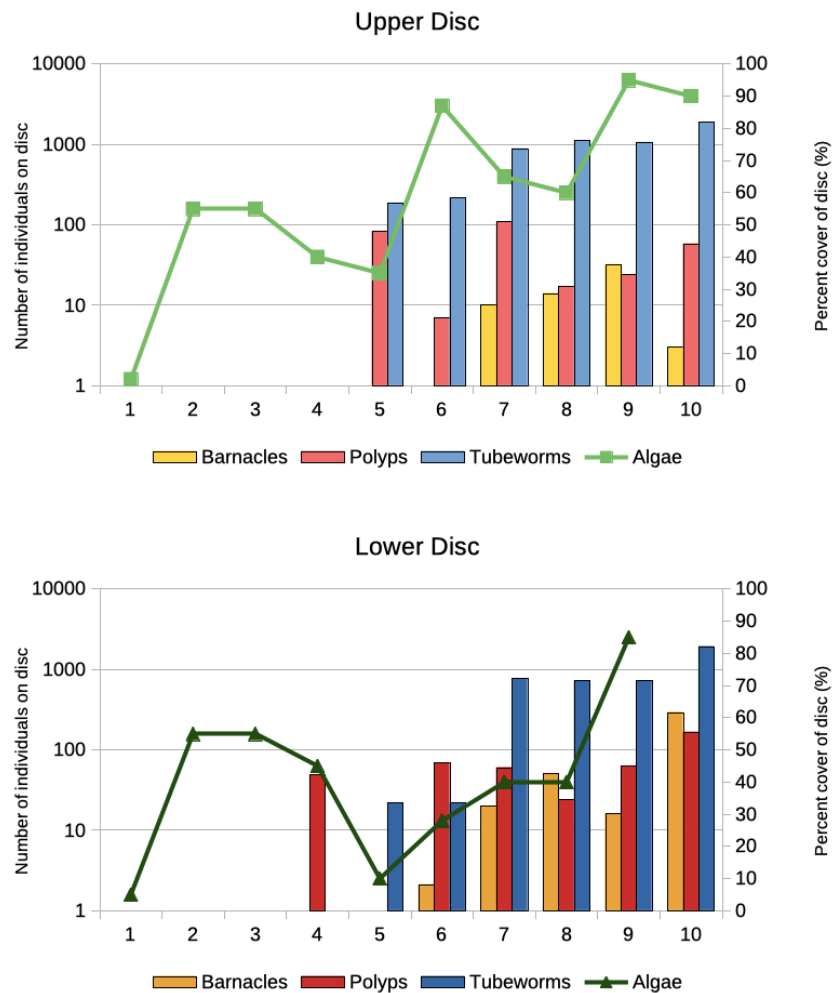


Figure 6: Succession of fouling organisms on the upper and lower disc. The line shows the growth of algae (right Y-axis) and the columns the total number of invertebrates (left Y-axis; note that the scale is logarithmic).

III.6 Species richness

By simply counting how many different species are present on the discs for each week, a diagram was constructed showing the change in species richness with time (Figure 7).

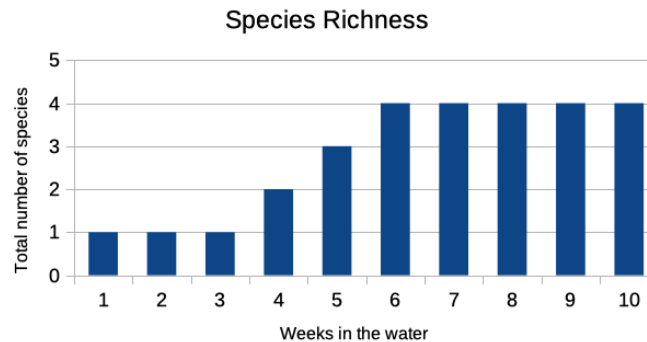


Figure 7: Change of species richness on the discs with time

Species richness on the discs increases with time. By week 6, it has reached its maximum (in terms of the number of species considered in this study) and does not decrease anymore after that.

III.7 Biodiversity

The calculation of biodiversity indices is described elsewhere in the set of VIRTUE-s documents (see: <https://virtue-s.eu/biodiversity-calculations>).

Here, we use Simpson's Index of Diversity D (see student worksheet, section IV.7) to compare the biodiversity of the oldest upper and lower discs at the end of the experiment (week 10):

Upper disc	n	Σ	N	D
Tube Worms	1893			
Polyps	58	3584868	1954	0.06
Barnacles	3			
Lower disc				
Tube Worms	1926			
Polyps	166	3819912	2384	0.32
Barnacles	292			

Table 1: Components of the computation of Simpson's Index.

IV. Interpretation

Using the diagrams above, the students can now interpret the development of each single species as well as compare the growth of the individual species on the upper disc and the lower disc. If they analyse the data carefully, they will also find some contradictions in the data which we will address below.

Again, the numbering used here corresponds to the numbers of the tasks in the student worksheet:

Tasks 1 - 3 are mostly about describing the features found in the diagrams and giving very simple interpretations. Tasks 4 - 8 go deeper into the analysis of the biological data and their interpretations.

Although not all of the diagrams are required for each task, the students should be aware of all diagrams and able to use them for their arguments. (In the following discussion, the figures that were already shown above will be repeated to facilitate reading.)

It is important to keep in mind that these data were produced without replicates and every number resulted from the analysis of one single disc. Consequently, explanations have to remain speculative, whereas in a real scientific study, replicates would strengthen the reliability of the data and their interpretation. Nevertheless, this will be a good exercise for students to examine causalities and make educated data-based deductions.

IV.1 Change of temperature and salinity

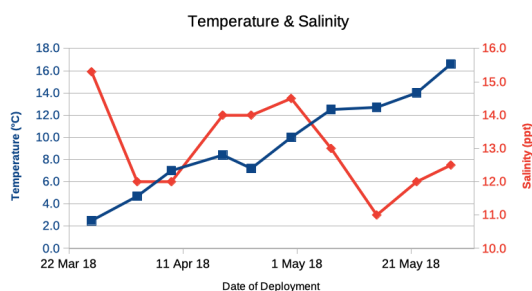


Figure 8: Changes of temperature and salinity with time during the 10-week project

Temperature showed a steady increase in spring from a cold winter temperature of 2.5 °C in late March to 16.6 °C in late May. This temperature increase clearly reflects the seasonal warming of the sea surface, with a slight interruption in the week of April 23 (i.e. corresponding to the 5th deployment).

Salinity varied between 11.0 and 15.3 ppt. Salinities between 14 and 20 ppt are normal in this part of the Baltic Sea, but slightly higher or lower salinities are not uncommon. Particularly in the first two weeks of May, however, there was a marked decrease of salinity to its minimum value before it started to increase again. The reason for the changes in salinity is not obvious. There may have been rain or freshwater input from a river. (If the students enter the coordinates of the sampling site into Google maps or similar software and zoom out, they may see the small river Schwentine to the east of the sampling site. From here, lower salinities (freshwater mixed with surrounding water) can reach the position of the rack.) All of these interpretations are plausible.

IV.2 Increase in biomass with time

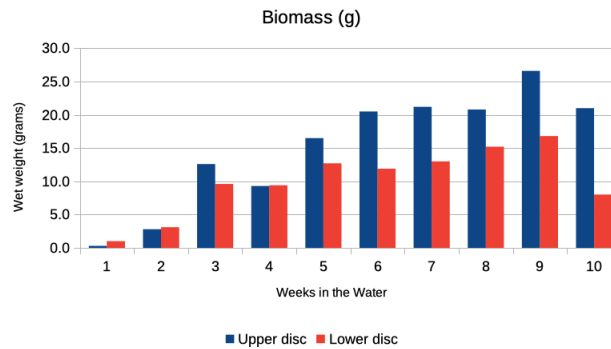


Figure 9: Final biomass (wet weight) after different periods of time in the water during the 10-week project.

Generally, the biomass on the lower discs is less than on the upper ones. This indicates that either there is less growth on the discs facing downward, or the species growing there have less biomass.

Biomass shows an almost continuous increase with increasing duration of deployment. There are some slight variations which may be due to different initial conditions when the discs were deployed, but these are minor. However, a pronounced decrease in biomass appears in the oldest discs: both the upper and lower disc that have been in the water for 10 weeks have significantly less biomass than the discs that were deployed one week later.

Although not necessarily always true, it is reasonable to assume that the longer the discs have been in the water the more organisms should be settling or growing on them (at least up to some point). However, there may also be a (continuous/periodic?) loss of biomass due to grazing and predation by organisms that “visit” the discs, such as fish or snails. One could also speculate that loss of biomass on the discs may happen by mechanical stress (wave action during storms?) or careless handling during recovery or analysis. Each of these interpretations is valid, and we have no way of determining which one is correct.

IV.3 Percentage cover and biomass

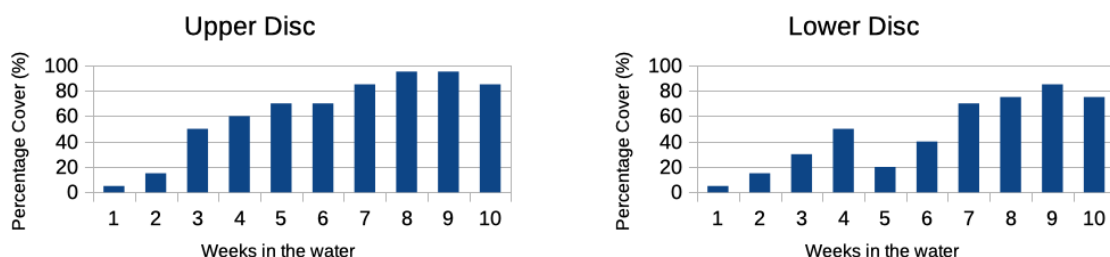


Figure 10: Time series of Percentage Cover (%) of upper and lower discs

Although the tendencies in percentage cover are very similar to those of biomass, this does not hold for every detail of the time series. While on the upper discs there is an almost steady increase in percentage cover with time of exposure, the lower discs show a marked decrease in percentage cover between 4 and 5 weeks in the water. Both percentage cover and biomass show a decrease between week 9 and 10, but this is much more pronounced in biomass than in percentage cover.

Biomass as well as percentage cover give higher total numbers for the upper than for the lower discs, indicating that growth is more vigorous on the discs facing upward.

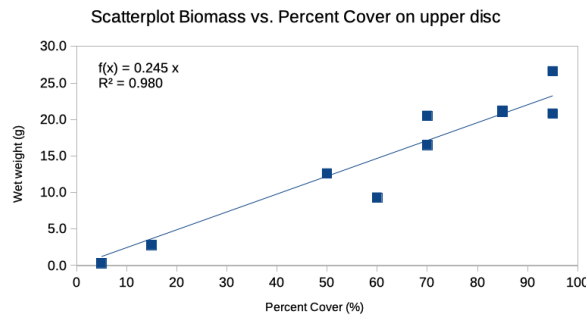


Figure 11: Scatterplot and regression line for biomass (g) vs. percent cover (%) for the upper discs.

At least for the upper discs, there is a clear linear correlation between percentage cover and biomass. The high and statistically significant correlation coefficient of 0.96 seems to confirm this. (For the lower discs, the correlation coefficient is lower with 0.72, but still significant.)

Clearly, one would expect biomass to initially correlate with percentage cover, at least up to the point where the disc is completely overgrown (100% cover) and biomass growth continues in layers on top of each other. The relation does not have to be linear because different organisms add different amounts of biomass per unit area. The fact that we see a stronger decline of biomass than percentage cover between weeks 9 and 10 could be due to the large percentage cover already present: in week 9 the upper discs were almost completely overgrown and the lower discs were also covered to more than 80%. One could suspect that on the disc that was in the water for 10 weeks, growth in several layers had already taken place and that somehow (maybe during strong wave action) the top layers of the overgrowth were lost (taking biomass - presumably mostly algae - with them), while the bottom layers remained and still mostly covered the same area, but now with considerably less biomass. This hypothesis is supported by the photos of the discs after 9 and 10 weeks. (However, as already discussed in the last section, careless handling during transport may also be the cause.)

IV.4 Development of species on the upper and lower discs

IV.4.1 Algae

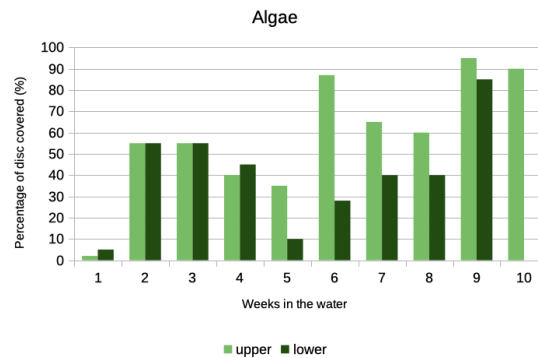


Figure 12: Percentage cover (%) of algae

Apparently, algae on the upper and the lower side grow equally fast on disks that have been in the water for less than 4 weeks. They seem to “explode” from week 1 to week 2, from less than 5% to more than 50% cover. However, diligent students will probably notice that some of the other data, i.e. the photos of week 2 (Figure 2 in the student worksheet), the estimates of total cover (Figure 3 in this document) and the development of biomass (Figure 2 this document), are in complete contradiction to the numbers reported for percent cover of algae in week 2. If simply for the fact that an estimate of algae cover alone cannot possibly be significantly larger than an estimate of the total cover, we have to conclude that the numbers in week 2 have not been reported correctly by the team of students responsible for those discs.

So, ignoring the apparent fast initial growth of algae, what else can we say? Since we already see some algae after week 1, it is still plausible to argue that algae are the first to populate the discs, particularly at the time of year of the late deployments (from about discs 6 to 1, i.e. mid-April to early June) where there would definitely be sufficient sunlight.

Data from week 3 and 4 indicate that initially algal growth happens at similar rates on the upper and lower discs. On older discs (week 5 and above), however, there are notably less algae on the lower discs. This may well be due to shading. When the algae (and other organisms) on the upper discs cover a good portion of the surface, this can reduce the light reaching the lower disc making it harder for the algae there to grow to their full potential.

The reduced growth of algae on the upper discs of weeks 4 and 5 relative to week 3 again seems questionable in the light of the photos. Although the exact type of growth cannot be determined from the photos, in week 4 no other species have been counted on the upper disc and in week 5 only a comparatively small number of tube worms. Thus, we have to assume that the photos show mostly algae, and the cover seems to be as high or higher than in week 3.

For the oldest disc (week 10), we do not have any numbers for algae on the lower disc, but as discussed above (section IV.3) we have to assume that a large part of the algae was lost on that disk.

IV.4.2 Tube Worms

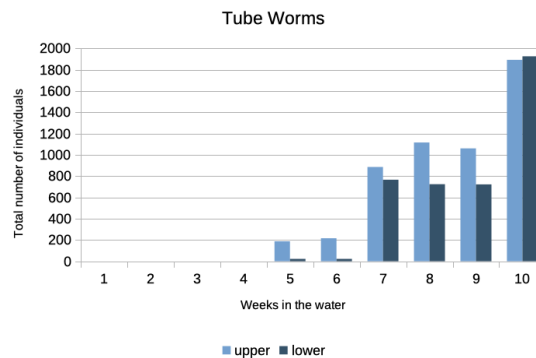


Figure 13: Number of individuals of tube worms

Tube worms are the most abundant species on the discs. (Note that their total number reaches almost 2000 individuals on the oldest disc, where barnacles only number close to 300.) They do not appear on the youngest discs at all, and on the upper discs only on those that had been deployed more than 5 weeks (i.e. before late April). On the lower discs substantial increase in individual numbers is seen only after 7 weeks in the water. There seem to be abrupt changes in growth on both sides of the discs between weeks 6 and 7 and 9 and 10.

Tube worms grow preferentially on the upper disc (weeks 5 to 9). (However, on the oldest discs (week 10) growth on the lower side is reported as slightly stronger than on the upper side.)

As stated in the hints for the students, *Polydora sp.*, the tube worm species found on these discs, build their tubes from sediment that settles on the discs. This can explain the preferential recruitment on the upper disc: currents are sufficiently weak to allow sediments to accumulate on the discs within 4 weeks. For the lower discs the situation is less clear: although sediment would not “rain down” from above as on the upper discs, it could still be carried there by water movement and then adhere to the surface. It seems likely, however, that this sediment would be made up of lighter particles and take longer to accumulate, which would explain the lower numbers of tubeworms on the lower discs.

The unexpectedly large number of tubeworms on the lower disc in week 10 is difficult to explain. There might be a connection to the loss of biomass on that disc (see section IV.7 below) or it may also be a simple counting error.

IV.4.3 Polyps

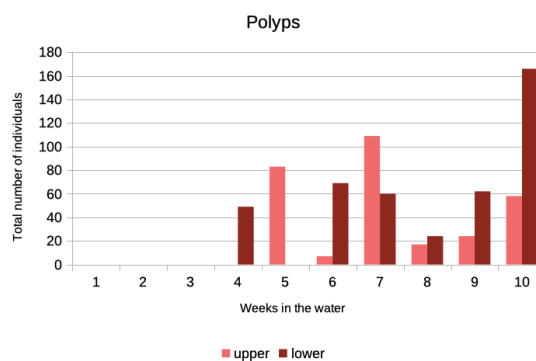


Figure 14: Number of individuals of polyps

The first polyps appear on the (lower) disc that had been deployed for 4 weeks, but their further appearance is somewhat erratic: in the discs of week 5, they are only on the upper disc, in week 6 mainly on the lower disc. The population seems to be very low on the discs from week 8 while higher numbers are found on the oldest discs.

Initially, polyps in this experiment do not show any clear preference for upper or lower disc, but for the 3 oldest discs a preference for the lower disc seems to establish itself.

The planula larvae of polyps, specifically of *Obelia sp.*, are positively phototactic while they are still drifting in the water as plankton, but they become negatively phototactic when it is time to settle. This would lead us to expect more polyps in the “shady places”, i.e. on the lower discs.

IV.4.4 Barnacles

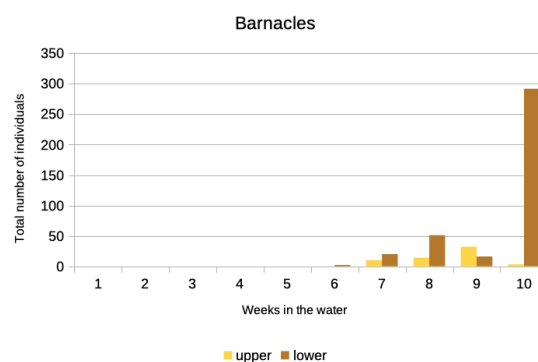


Figure 15: Number of individuals of barnacles

No barnacles were found on discs that had been in the water for less than 6 weeks (i.e. deployed after April 23). This could either mean that the settlement of barnacles was finished by mid-April, or that later arrivals did not yet settle because they require the presence of something else on the discs before they are able to populate it.

Except for the disc of week 9, barnacles settle preferably on the lower discs, with very high numbers on the lower side of the oldest disk.

As stated in the hints for students, 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, which in this location usually occurs between March and mid-April. After spawning, the barnacle larvae are planktonic and positively phototactic. This is an advantage since they feed on phytoplankton which is abundant in the upper water column. The last larval stage, the cypris larva, is the stage which finally settles on a substrate after some days to weeks. Thus, if the barnacles spawned in March to mid-April, we would indeed expect to see them settling on the discs by late April or May. Nevertheless, due to the uncertainty in timing (we do not have any data on the presence or absence of cypris larvae in the water), the second of our initial hypotheses cannot be ruled out from these data: it is conceivable that barnacles may not settle on a “blank” surface before at least a thin biofilm has established itself on it.

Because the cypris stage is negatively phototactic, settlement on the lower disc would be preferred. Furthermore, barnacles are filter feeders and obtain their food by “sieving” food particles from the water column. Consequently, for those settling on the upper discs, sediment raining onto the discs may clog their filtering apparatus and put them at a disadvantage.

IV.5 Order of appearance of the organisms

Strictly speaking, from this experiment it is not possible to determine the order of appearance of the species. Instead of following the time history of one single disc, the growth on discs that were deployed at different times and for increasingly briefer periods was examined simultaneously at the end of the experiment.

Thus, we do not know when the first macroalgae actually settled on the disc that had been in the water for 10 weeks. However, in the Baltic algae spores are present all year round and in March there is sufficient light to support algal growth and development. Besides, algae were found on all discs in all weeks of the experiment. Therefore, it seems safe to assume from the data that the macroalgae were first to settle on every disc.

For tube worms, we argued that sediment has to have accumulated on the hitherto empty disc before successful settling could be achieved. For polyps, there is no clear argument for any particular point in time. The appearance of barnacles on the discs coincides well with the phenology/seasonality of recruitment. In fact, for all animal species spawning cycles would probably play a role, which in turn could depend on water temperature.

Thus, based on the data available, the order of appearance of the organisms on the discs in this experiment is:

Macroalgae first, tube worms and polyps second, barnacles last.

IV.6 Effects of temperature or salinity

The most common algae, *Ectocarpus*, has 2 stages in its life cycle, a haploid gametophyte, which is less tolerant to salinity changes and a more tolerant diploid sporophyte. For the low algal cover on the week-5 disc (Figure 5 and Figure 6) it can be speculated that this may have been caused by the sudden and persistent decrease in salinity that happened just after deployment in late April (Figure 1). Young algae that settled during this time may have been stressed with the very low salinities, which may have influenced their growth and development in the succeeding weeks. Algae that settled earlier on discs that had been in the water a few weeks longer may have reached a more tolerant stage in their life cycle and thus were less severely affected.

In terms of temperature, there may well be an influence of water temperature on spawning of larvae, but this is not visible from the data.

IV.7 Competition

In terms of a potential competition for space, obviously the first to arrive would be at an advantage, unless the late arrivals manage to displace them or profit from external influences that affect a competitor.

As pointed out above, at this time of the year the first to arrive seem to be algae, with tube worms and polyps after that. There is no indication for a competition for space between tube worms and algae. Tube worms could well build their tubes underneath the algae: they do not need light, and the algae would not be affected by the tubes. At least at the later stages of growth on the discs, polyps and algae as well as polyps and tube worms do not seem to compete for space because polyps are mainly found on the lower while algae and tube worms populate the upper side. Theoretically, there could be competition for space between the settling larvae of polyps and barnacles: they both avoid light and settle preferably on the lower disc. However, when the barnacles had finally settled, there was still more than 20% free space on the lower discs (Figure 3). Besides, both species can co-exist with each other because they do not compete for food: barnacles are filter-feeders (eating phytoplankton and detritus) and polyps are

predators preying on small zooplankton. On the other hand, barnacles that may have tried to settle on the upper side of the discs found very little space remaining there. Even if they did settle on the upper side of the discs (Figure 15) they probably get smothered eventually by algae, tube worms and sediment.

An interesting argument could be made for a competition on the lower side of the discs between algae and the three animal species: the image of the oldest disc indicates that most of its algal growth may have fallen off at some point thus opening up space for the other species to reclaim. This could explain the comparatively high total number of each of the other three on the lower side of the disc of week 10.

It should be noted, though, that there is no clear separation between the species. Although there are intermediate periods during the initial settlement stages where some species may only be found on the upper or the lower disc but not on both (weeks 4 to 6), in the more “mature” settlement stage every species that is present on one side of the discs is also present on the other.

IV.8 Biodiversity

For the disc that had been in the water for 10 weeks, Simpson’s index is 0.06 for the upper and 0.32 for the lower disc (cf. Table 1). Thus, the chance to pick two individuals at random that are not from the same species is only 6% for the upper disc while it increases to 32% for the lower. Although species richness is higher on the lower disc, the absolute value is still fairly low.

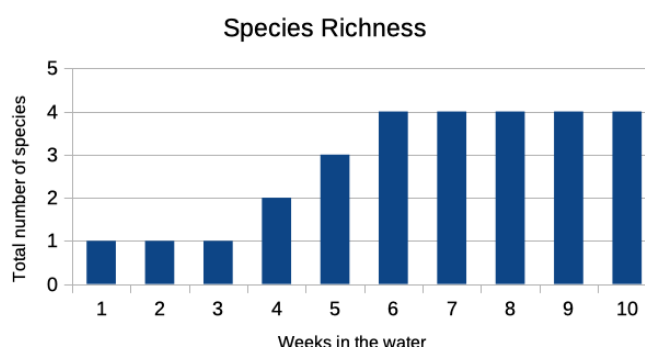


Figure 16: Change of species richness on the discs with time

This can be attributed to the disproportionately high numbers of tube worms. In terms of individuals, the upper disc is almost completely dominated by them. On the lower disc, they still dominate by far, but at least barnacles contribute significantly to the mix.

It should be kept in mind however that only the 3 animal species were used in the analysis of Simpson’s Index. The algae cannot be included in the calculation because individual counts could not be made. Species richness (another index for biodiversity which considers all 4 species) peaked after the 6th week of deployment. This does not mean that the community has already reached its climax stage, but it is due to the low number of species present in this experiment and the fact that juvenile stages of other benthic organisms will only settle on the discs later in the year. (For example, at this particular location mussels will start to grow in the summer, and by autumn they will have completely overgrown everything else.)

V. Further Reading

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