

Introduction

Protists are eukaryotic, predominantly unicellular organisms, diverse from plants, animals and fungi. Protists come in many different shapes and forms, they vary greatly in cellular organisation and differ in motion patterns. These organisms are distributed worldwide, and can be found not only in any water, but also in the sands, muds, marshes and on some plants. Many species of protists remain undiscovered, or sequenced, but not yet identified or described.

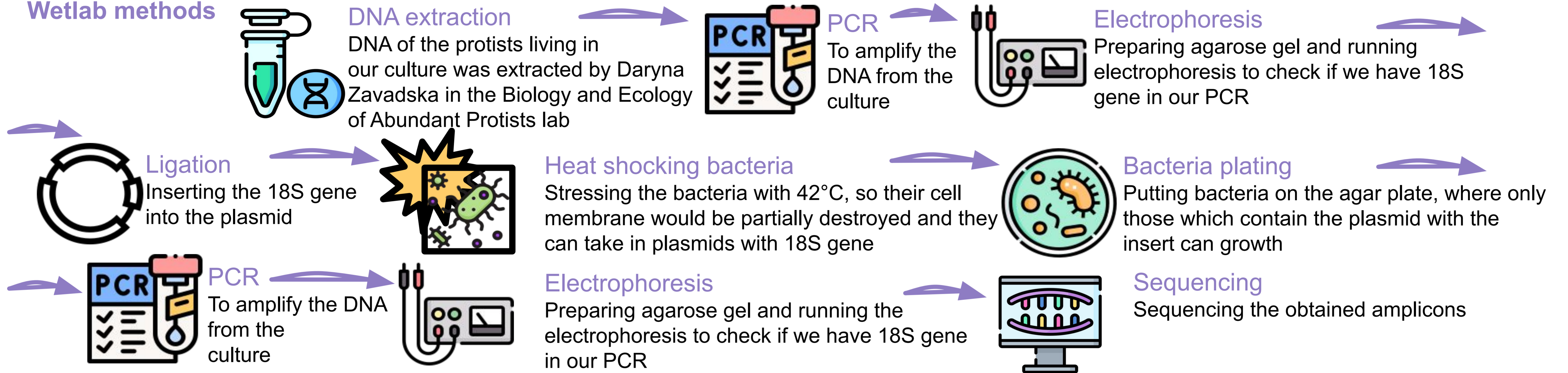
Project goal

We assigned ourselves two tasks. First one was sequencing and describing the morphology of the protists species from the particular culture, taken from the Solway Coast, Scotland. The second goal was to find the most abundant protist in the culture and define its ecology.

Project overview

Our work was divided into the wetlab part and the microscopy part. In the wetlab part, we multiplied the 18S ribosomal RNA regions of the DNA found in our culture, and inserted the 18S gene into plasmids, which were then propagated in bacteria. We have grown bacterial colonies, and picked ones that contained the inserted 18S sequences. Then we amplified the DNA once again and sequenced it. In the second part of the project, we observed our culture under the microscope to get more information about the protist species living in the culture and to be able to compare the microscopy images and sequence results.

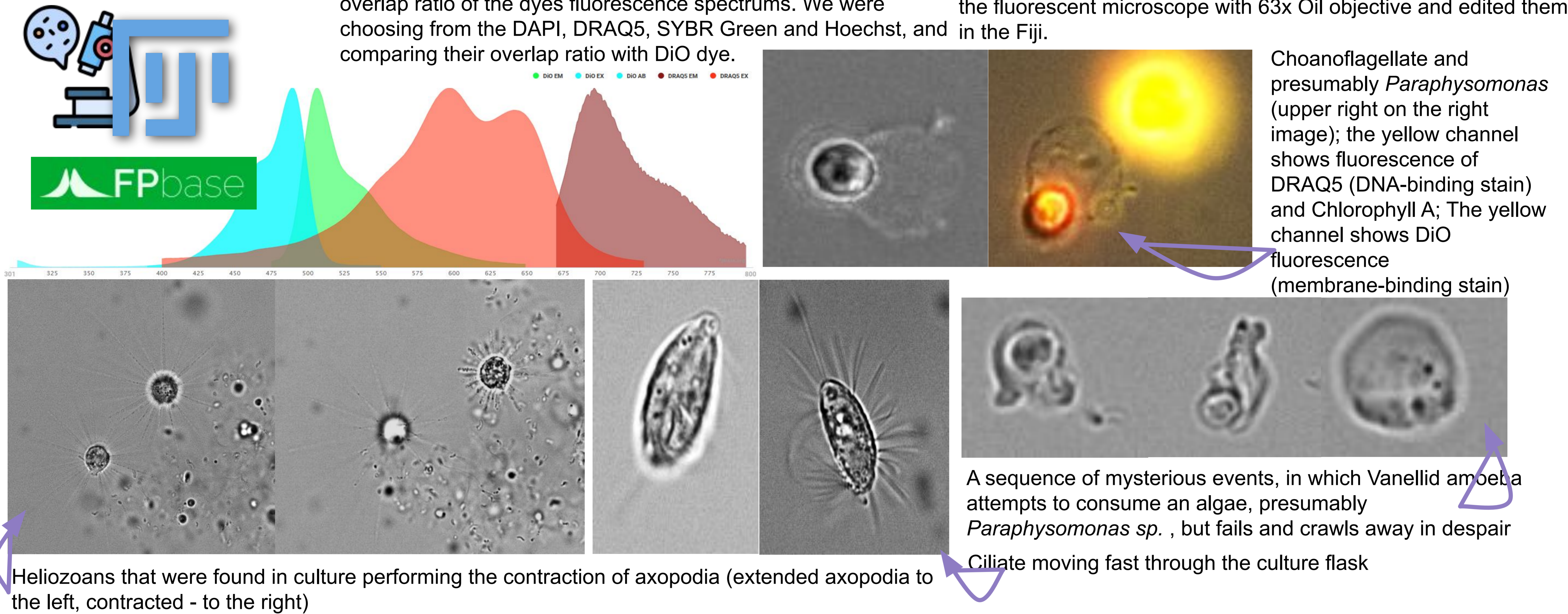
Wetlab methods



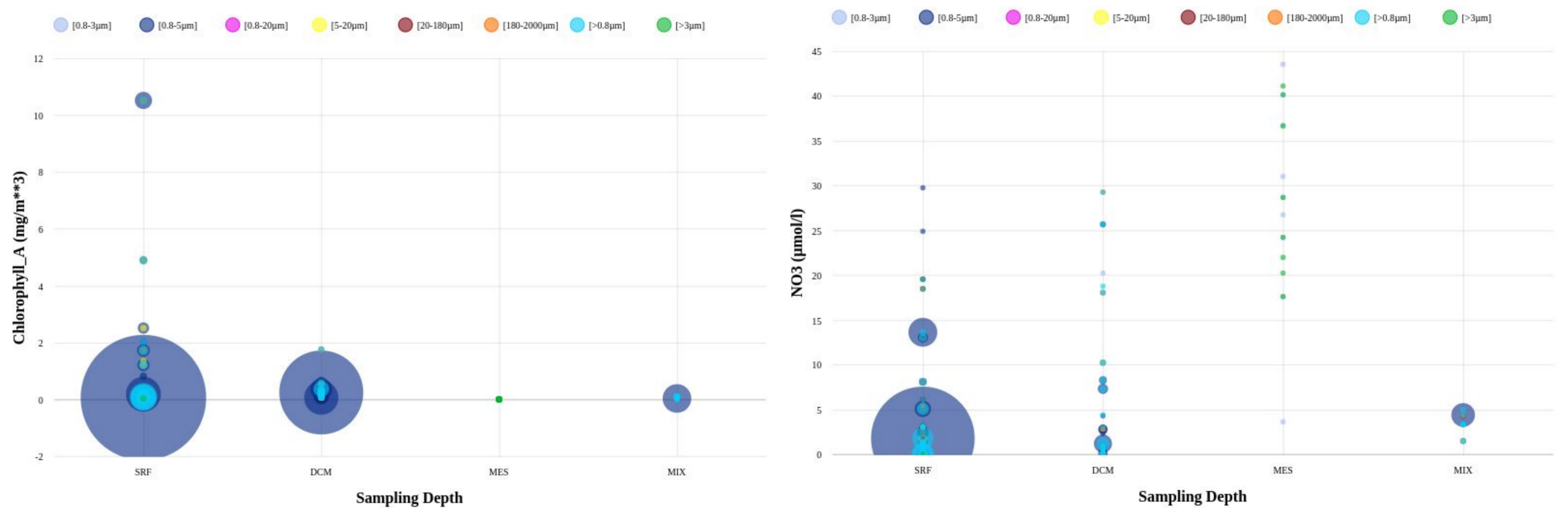
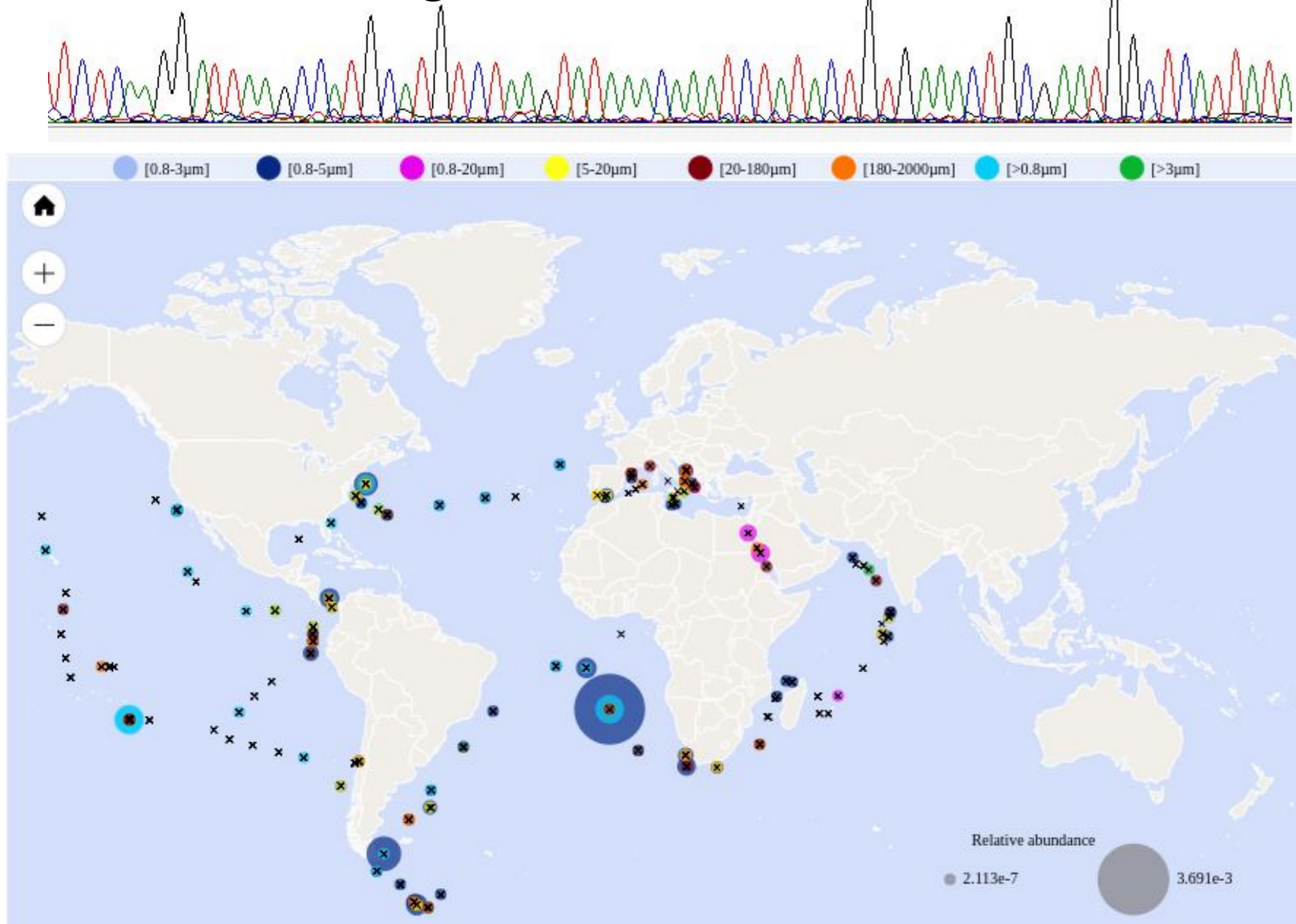
Drylab methods

We used the FPbase (<https://www.fpbase.org/>) to calculate the overlap ratio of the dyes fluorescence spectrums. We were choosing from the DAPI, DRAQ5, SYBR Green and Hoechst, and comparing their overlap ratio with DiO dye.

We took pictures of the protists in the culture we investigated with the fluorescent microscope with 63x Oil objective and edited them in the Fiji.



We used BioEdit to check quality and process our sequences, and BLAST to identify species according to the sequencing results, and Tara Oceans V9 metabarcoding database (<https://oba.mio.osupytheas.fr/ocean-atlas/>), to investigate geographical distribution and ecological factors that influence distribution of *Paraphysomonas sp.* in the global Ocean.



The global distribution of *Paraphysomonas sp.* suggests a strong dependence on the Chlorophyll A and NO₃ concentrations; *Paraphysomonas sp.* occurs worldwide.

Conclusion

With the help of BLAST we processed the results of our sequences, and got the photosynthetic chrysophycean algae *Paraphysomonas*, which we could see everywhere in our culture microscopy as well. *Paraphysomonas* is globally distributed and inhabits the low chlorophyll and low nitrate waters, and it can tell us a lot about the water quality it lives in, in particular that the water is quite clean. This fact can help a lot in the ecological research. Extensive microscopy revealed the presence of many more protists that co-exist with *Paraphysomonas* in the culture; they were identified by morphology, and more sequencing will be done on the culture in the future.

