The effect of global change factors as stressors in the molecular ecophysiology of marine plankton.

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1. INTRODUCTION

We all know that Planet Earth has undergone important changes throughout geological history, but we also know that antropogenic factors are decisive in current changes.

The biggest factor of global change on the planet is carbon dioxide (CO_2) which absorbs the solar infrared radiation and causes the greenhouse effect, with all its side effects; for example , the partial melting of poles will produce a mixture of polar freshwater and marine water which will change marine currents.

Ultraviolet radiation (UVR) is another global change factor that suposes a threat to organisms, which are also suceptible to it because it reaches the earth surface through the holes created in the ozone layer.
In order to then see if the radiation can cause any damage, we are especially interested in Rubisco accumulation, which is an enzyme which uses atmospheric $C0_2$ during the dark phase of photosynthesis, sequestring it from the atmosphere. Also, D1 protein which is fundamental in the electron transport chain during photosynthesis, and PCNA (proliferating cellular nuclear antigen) which is an auxiliary protein of the DNA polymerase, is fundamental in replicating DNA, hence in cell division and growth.

2. HYPOTHESIS

1st Hypothesis

UVR radiation is going to damage the DNA and the proteins, which will cause mutations and metabolic unbalances and cells will .

2nd Hypothesis

Alternatively, if cells have repair mechanisms, they will be able to overcome UVR affects and they will continue growing and accumulating proteins related to photosyntheis and growth.

3. MATERIAL

Biological material aim of study are oceanic microscopic algae:

Dunaliella tertiolecta

Phaeodactylum tricornutum

Dunaliella tertiolecta is a biflagellated microalgae that has no cell walls and has a marginal chloroplast, a central nucleus and a membrane which allows it to change its volume according to the changes in osmotic pressure. Some Dunaliella species are rich in carotenoids which are a source of Vitamin A , antioxidants, and so the extracts of Dunaliella are used a lot in cosmetics and health.

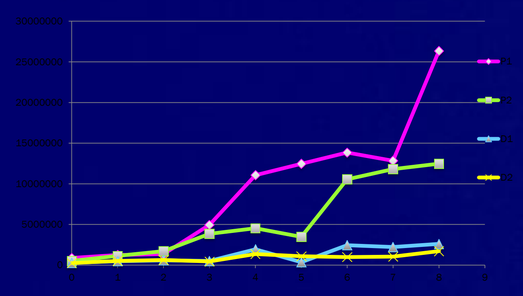
Phaeodactylum tricornutum is a diatom, therefore dependent on silicate for its growth. This species can change its shape according to environmental conditions. It can even grow without silicon.

4. METHODOLOGY

1st session (2nd December 2014)

We have worked with two of *Dunaliella* and two of *Phaeodactylum cultures*. We grew them by bubbling them with air and exposure to light , for photosynthesis to create the sugars, needed for growth. Cultures were grown at 16ºC because they are oceanic species. The day the experiment was started was called 'Time 0' or 'control day', because the cells were not exposed to any treatment yet. We submitted the cells to 1,5 hours of high ultraviolet B radiation (UVB) and we counted the cells every day to get a measure of the cellular density. This is one of the most important variables in oceanography. Afterwards, cells were centrifuged and frozen at -80ºC.

growth rates logistic model				
Time	P1	P2	D1	D2
0	888.000	504.000	288.000	256.000
1	1.248.000	1.144.000	488.000	512.000
2	1.360.000	1.720.000	590.000	624.000
3	4.952.000	3.856.000	504.000	448.000
4	11.064.000	4.536.000	1.944.000	1.360.000
5	12.456.000	3.480.000	368.000	1.088.000
6	1.384.000	1.056.000	2.448.000	992.000
7	1.284.000	1.180.000	2.224.000	1.041.000
8	26.344.000	1.248.000	2.616.000	1.720.000
r(d-1				



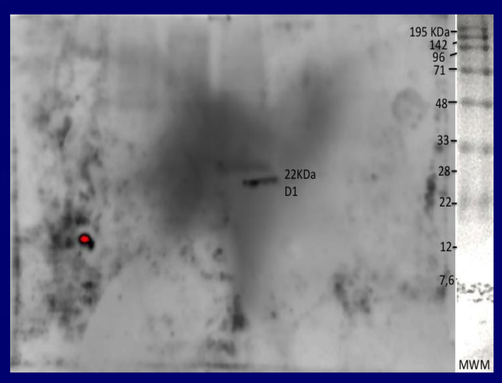
2nd Session (27th January 2015)

We studied the accumulation of Rubisco, D1 and PCNA as markers of stress. We did PAGE (Polyacrylamide Gel Electrophoresis). We extraceted the total proteins from the pellets according to extraction protocols. PAGE consists of separating the proteins from the total protein pool because they contain electric charge and different molecular weights. Proteins were denatured , and then an electrical field was applied. Then the proteins migrated according to their molecular weights.

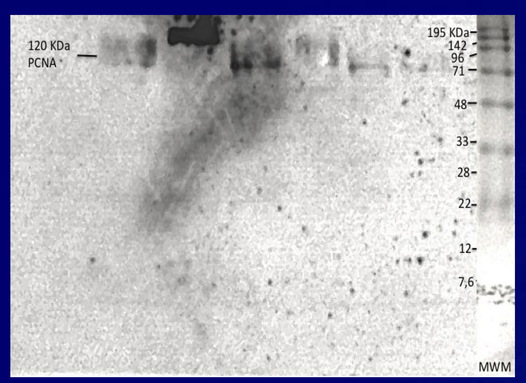
Rubisco was detected by staining the gels with Comassie Blue (specific dye which binds to proteins).

3rd Session (13th April 2015)

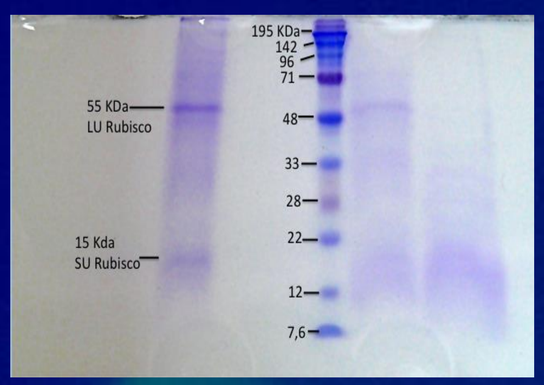
PCNA and D1 were detected by western blots. For this purpose the proteins were transferred/blotted (by applyimg an electric field) from the gels to a different support called membrane made of a synthetic polymer called PVDF. The membrane were incubated with specific antibodies recognising PCNA and D1 and the bands were visualized by using an image analyser.



Western blots: D1



PCNA



Rubisco

4. CONCLUSIONS

1.The results of cellular counts that we gathered over 9 days support hypothesis 2. It does not seem like there is any damage to DNA. Therefore we had to try to see if the UVB irradiation had produced an accumulation of certain proteins indicating estress or not.

2. Rubisco weighs 55Kda and it was detected by staining with comassie, indicatinmg that it was still abundant. PCNA is a large protein bout 100 Kda, and D1 is smaller (22 Kda) since they are less abundant they were detected by using antibodies.

3. The presence of Rubisco, PCNA and D1 indicates that Dunaliella and Phaeodactylum were able to repair any damage caused by UVR. This allowed cells to continue growingn and scaped from death.

Greatfulness: We want to thanks to the researchers who have worked with us learning and supporting us, and our classmates that helped us in our project.