Respiratory metabolism in macroalgae: Using the respiratory electron transport system (ETS) to detect stress and different physiological states.

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Introduction

Metabolism study of green algae (*Ulva*) communities, inhabiting intertidal pools of Gran Canaria. As an index of metabolic status and stress we used the electron transport system (ETS) to differentiate between different growing conditions in the natural environment. This technique has been successfully used to study many different marine planktonic organisms including bacteria, phytoplankton and zooplankton, but it has not been used to study marine macroalgae.

In this first phase of our research we have developed the methodology for homogenizing *Ulva* and have used a standard spectrophotometric-based kinetic enzyme assay to describe the impact of nutrient limitation on the metabolic capacity in samples collected in the wild and maintained in controlled culture.

Figure 1: Map of Gran Canaria. We collected samples at San Cristobal, Bocabarranco and Taliarte

Material and methods

Two homogenization methods

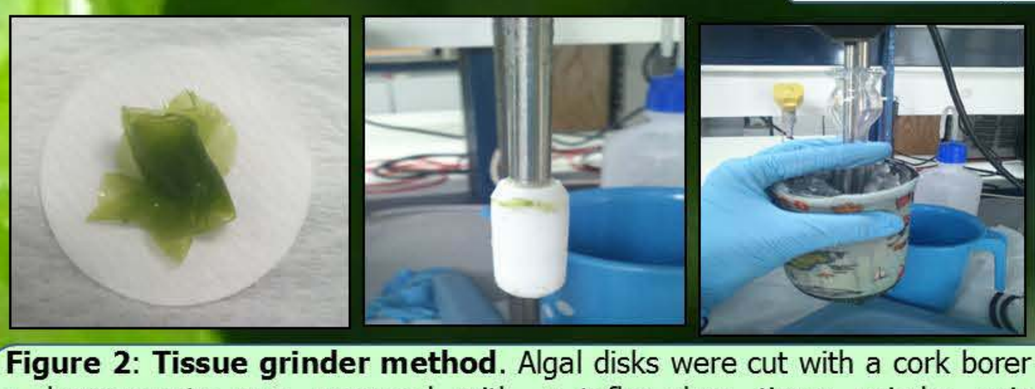


Figure 2: **Tissue grinder method**. Algal disks were cut with a cork borer and a homogenate was prepared with a teflon-glass tissue grinder using a disintegrated glass microfibre filter (GF/C Circle, 25mm) as the abrasive. We followed the Kenner and Ahmed (1975) ETS methodthe modifications of Gómez et al., 1996.

Figure 3: **Liquid nitrogen method**. Algal disks were cut as before, put into eppendorf tubes with the disks submersed in Liquid nitrogen, and homogenized with with a plastic pestle. The chlorophyll was measured according to (Mitchell and Kiefer, 1984).

Choice of the homogenizion method: The difference in the Specific ETS from the two methods was significant, in addition to its, we decided to use the tissue-grinding method because it was less expensive and easier.

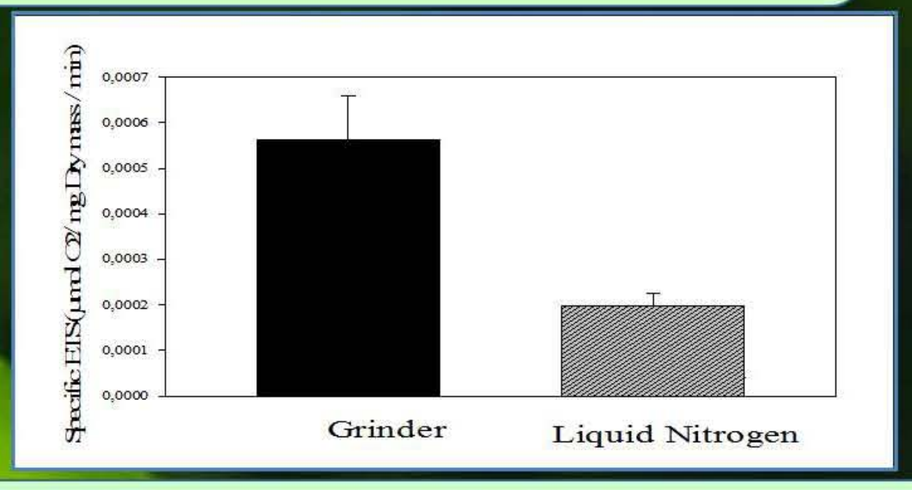
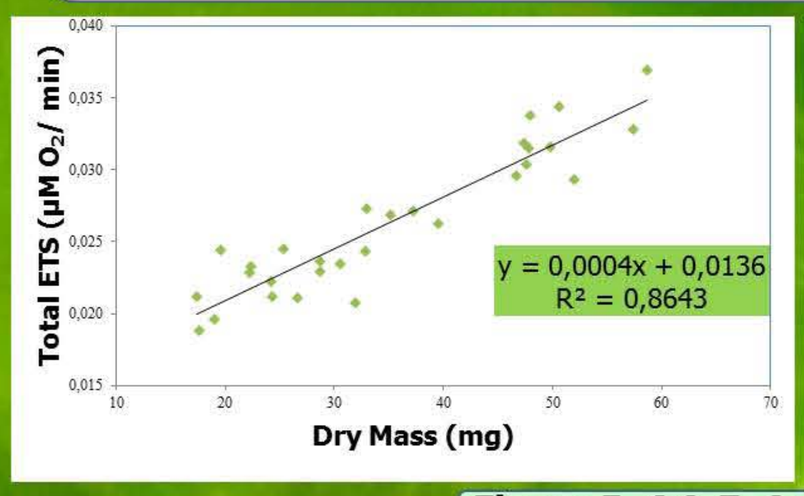
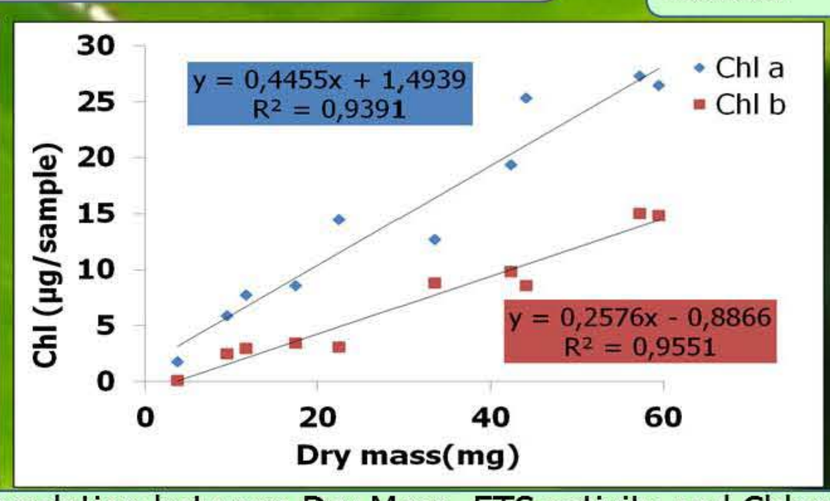


Figure 4: Comparison between ETS total in both homogenization methods.

Results

1. Correlation between different biomass proxies. There is a good correlation between Dry Mass, ETS activity and chlorophyll (Fig. 5, 6, & 7)





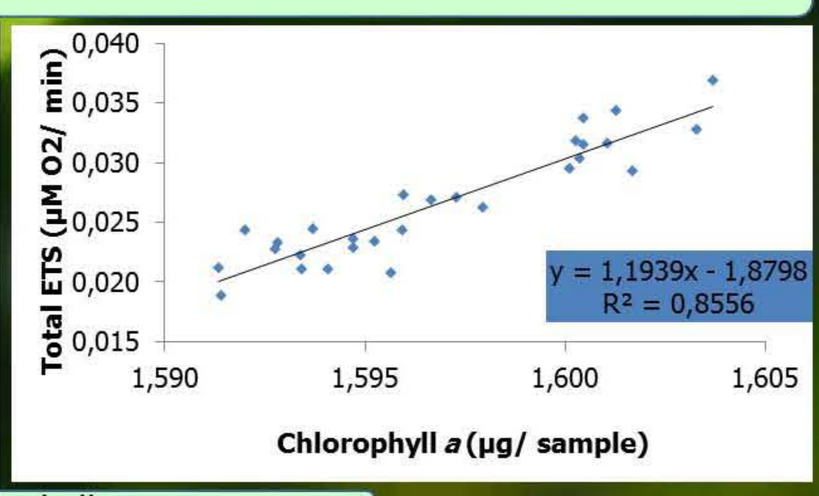


Figure 5, 6 & 7. Correlation between Dry Mass, ETS activity and Chlorophyll *a*

2. Relative importance of NADH, NADPH and Succinate in determine ETS activity.

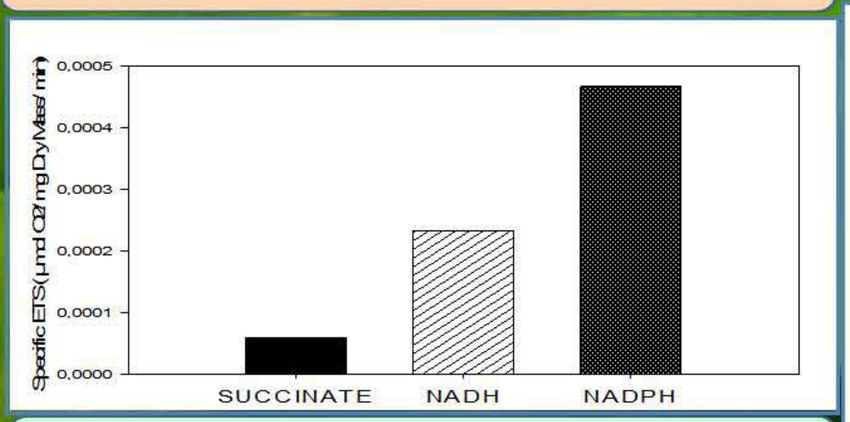


Figure 9. Reactive contribution in the ETS activity with different samples of algae.

3. Time-course of metabolism in aquaria with filter sea water over a week.

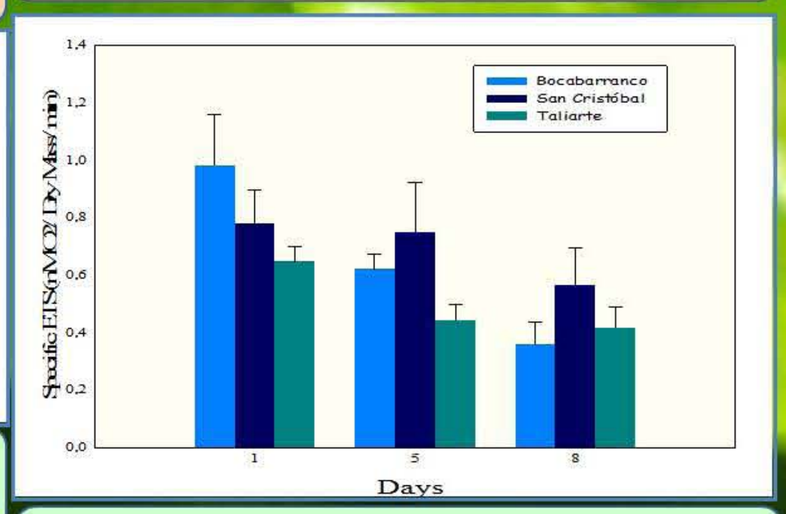


Figure 8. Specific ETS time-course in Ulva from three different locations.

4. Variability of the ETS activity, Dry Mass , nutrients and Chlorophyll in three different locations around Gran Canaria.

	Bocabarranco	Taliarte	San Cristóbal
Specific ETS (nM O_2/ dry mass/ min)	0,9844	0,6481	0,7805
Dry Mass (mg)	23,4402	40,4123	32,3344
Chlorophyll (µg/ sample)	1,5931	1,6009	1,5958
Ammonium (mg/l)	2,78	1,23	1,58
Nitrate(mg/l)	2,4	1,2	2,7

Table 1:Comparation of the Specific ETS, Dry Mass and Chlorophyll measures at the collected day in the three locations.

Conclusions

1. Comparison between ETS means in both homogenization methods demonstrated a significant difference. We used the tissue-grinding method because it was less expensive and easier.
2. There is a good correlation between Dry Mass, ETS activity and Chlorophyll using the optical density at 670 nm as a reference to measure biomass.
3. The contribution from the Succinate, NADH, and NADPH in the ETS activity is 7,8%, 30,7 % and 61,5 % respectly.
4. The differences in the 8-day ETS time courses for the two areas were statistically different.
5. Bocabarranco has the highest ETS activity and agree with the high levels of nutrients and Taliarte has the smalls level of nutrients coinciding with the smallest ETS activity.
6. Taliarte has the highest Dry Mass, probably due its a different Ulva sp. than the other two places.

References

Kenner, R., Ahmed, S., 1975. Measurements of electron transport activities in marine phytoplankton. Mar. Biol. 33, 119–127. the modifications of Gómez et al., 1996

Mitchell, G and Kiefer, D. 1984. Determination of absorption and fluorescence excitation spectra for phytoplankton. Lecture notes on costal and estuarines studies, VOL. 8, PP. 157-169.