

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.

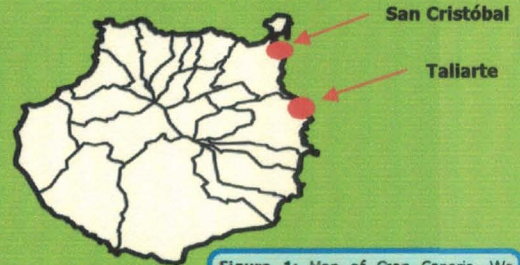


Figure 1: Map of Gran Canaria. We collected samples at San Cristobal and Taliarte (Teide).

Material and methods

Two homogenization methods

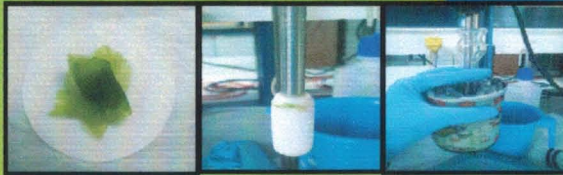


Figure 2: Tissue grinder method. Algal disks were cut with a cork borer and a homogenate was prepared with a teflon-glass tissue grinder using a disintegrated glass microfibre filter (GF/C Circle, 25mm) as the abrasive. We followed the Kenner and Ahmed (1975) ETS method.



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

Results

1. **Choice of the homogenization method:** The difference between both methods was significant, in addition, we decided to use the tissue-grinding method because it was less expensive and easier.

2. **Correlation between different biomass proxies.** There is a good correlation between dry weight, optical density and chlorophyll a & b (Figs 5, 6, & 7).

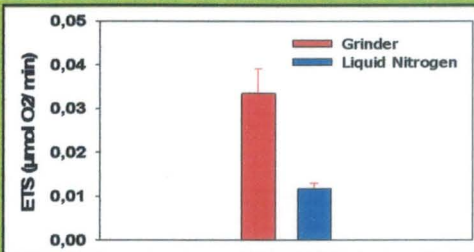


Figure 4: Comparison between ETS total in both homogenization methods.

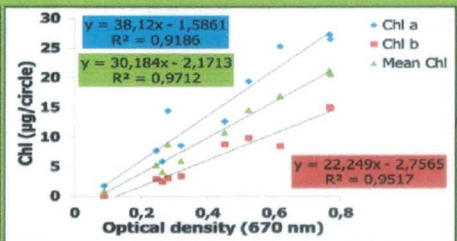
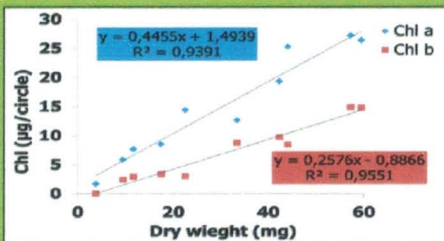
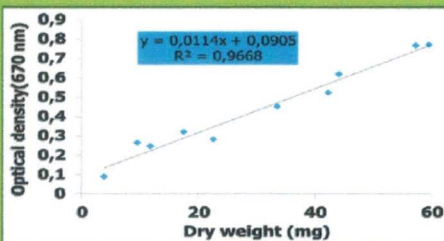


Figure 5, 6 & 7: Correlation between Dry weight, Optical density and Chlorophyll a & b.

3. Evolution of ETS with time

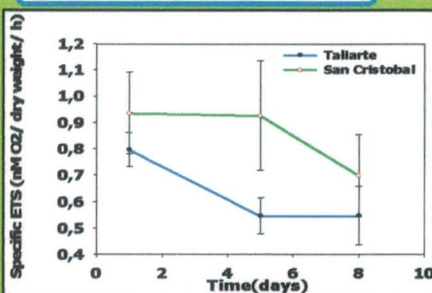


Figure 8: ETS time-course in *Ulva* from two different locations.

We collected the algae (day 1) and stored them at 20°C for 8 days without nutrients with a 10-14 photoperiod. In spite of the apparent differences between the two areas they were not statistically different because of the large standard deviation in the San Cristobal data after 5th day. (We used PERMANOVA v.1.6 software (M.J. Anderson, 2005).

4. Relation between Biomass and Respiratory activity (Kleiber)

	Taliarte	San Cristóbal
T ₁	y = 0,5211x - 2,3324 R ² = 0,5664	y = 0,3544x - 2,1173 R ² = 0,419
T ₅	y = 0,6153x - 2,602 R ² = 0,7828	y = 0,1582x - 2,0325 R ² = 0,1316
T ₈	y = -0,2676x - 1,0757 R ² = 0,0391	y = 0,1688x - 2,0487 R ² = 0,0593

Table 1: The Kleiber coefficient decreases from the first to the last day due to starvation.

Conclusions

- 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.
- There is a good correlation between dry weight, optical density and Chlorophyll so we used the optical density at 670 nm as a reference to measure biomass.
- The differences in the 8-day ETS time courses for the two areas were not statistically different.
- Due the starved conditions we could see a decrease in the Kleiber coefficient with the time.

References

- Kenner and Ahmed, 1975. Measurements of Electron Transport Activities in marine Phytoplankton. *Marine Biology* 33, 119-127.
- Mitchell and Kiefer, 1984. Determination of absorption and fluorescence excitation spectra for phytoplankton. *Lecture notes on coastal and estuarine studies*, VOL. 8, pp. 157-169.
- Anderson, M.J. 2005. PERMANOVA: a FORTRAN computer program for permutational multivariate analysis of variance. Department of Statistics, University of Auckland, Auckland, Nueva Zelanda.