Respiratory metabolism in the marine macroalga, *Ulva* spp.: Exploratory studies with the respiratory electron transport system (ETS)

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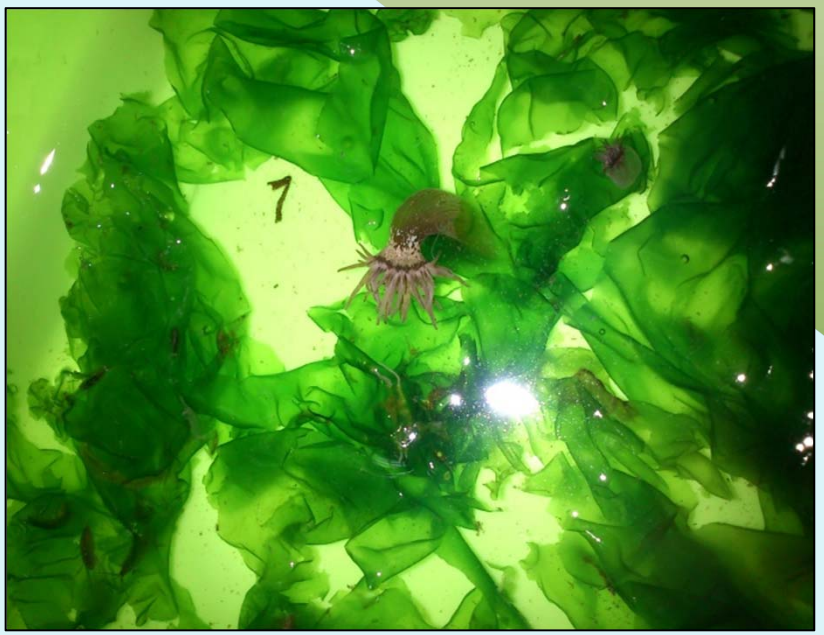
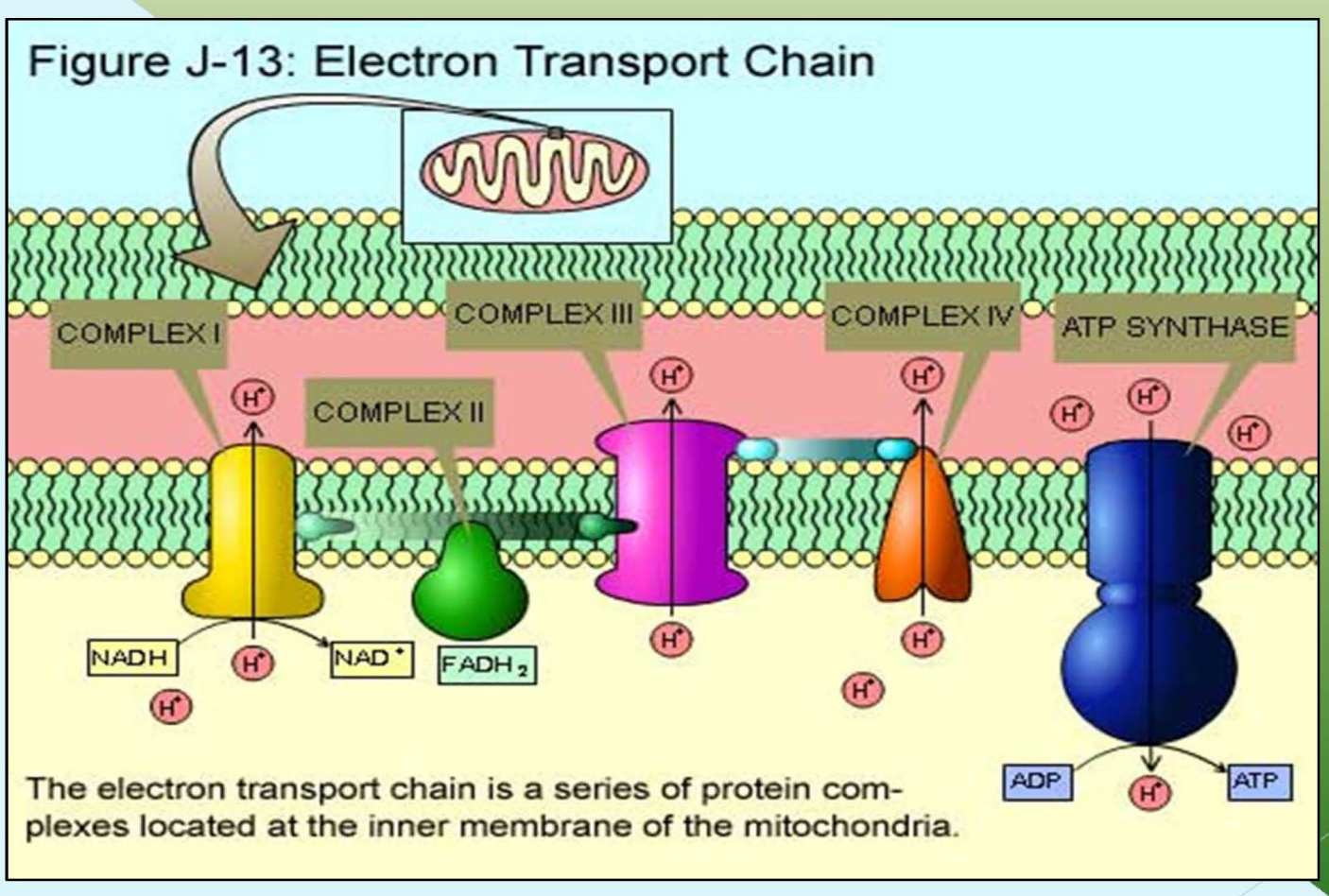
Ulva specie



Ulva rigida **C.Agardh**

Ulva rotundata **Bliding**

ETS (Electron Transport System) (Packard et al., 1971)



http://thebiochemsynapse.wordpress.com/tag/electron-transport-chain/

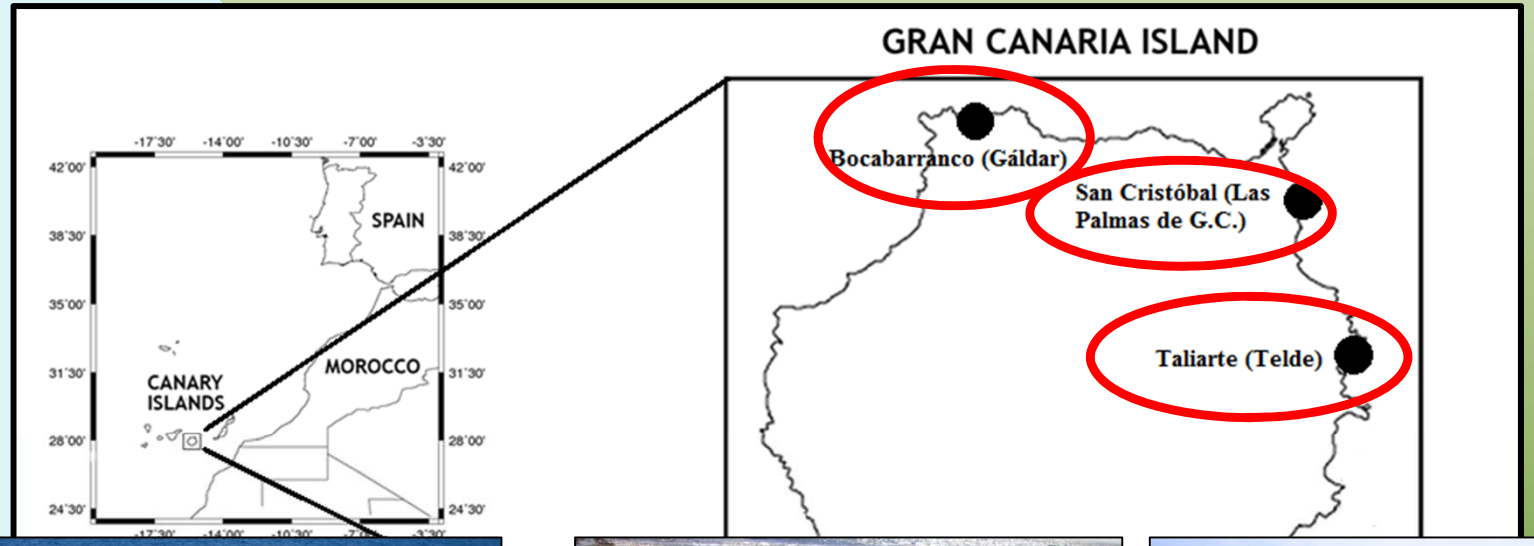
Objetives

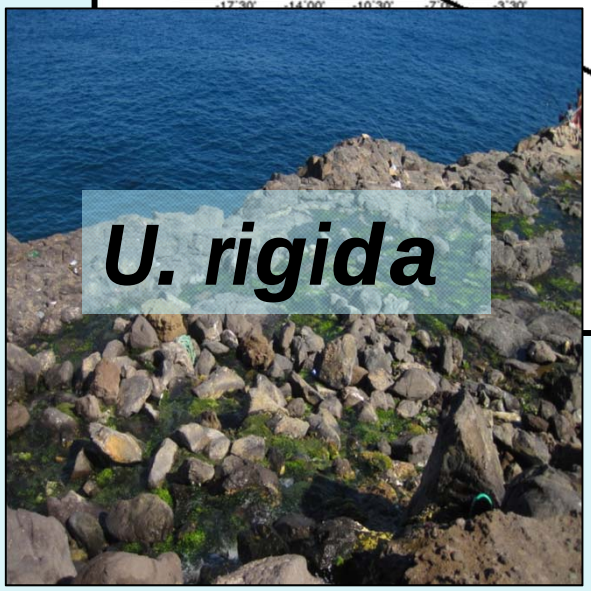
- Optimize the homogenization method to determine the activity of the electron transport system (ETS).
- Study of the relative importance of the three enzymes, NADH-dh, NADPH-dh and Succinate-dh in determine ETS activity.
- Determine the relationship between:
  - a) Dry mass and chlorophyll.
  - b) Dry mass and potential respiration.
  - c) Potential respiration and chlorophyll.

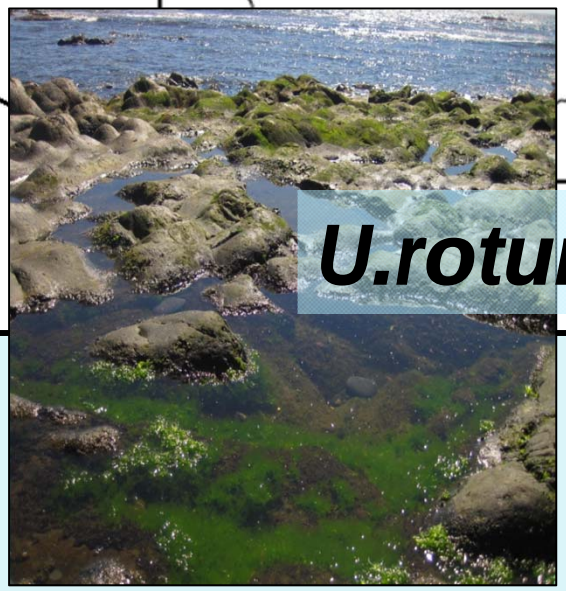
Objetives

- Study the variability of the potential respiration, dry mass and chlorophyll in three different locations around Gran Canaria.
- Determine the time-course of metabolism in aquaria with filtered sea water over a week.
- Determine the relation between biomass and respiratory activity in the three different locations.

Sampling locations





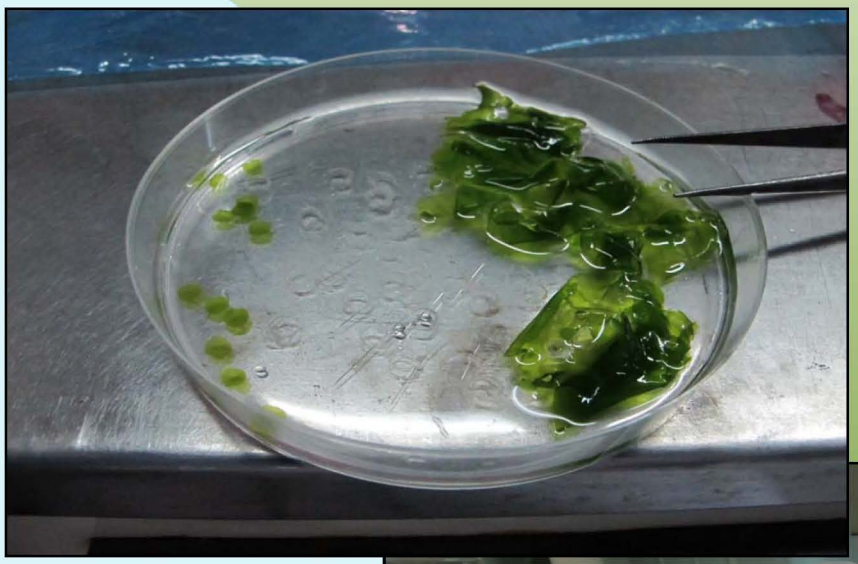


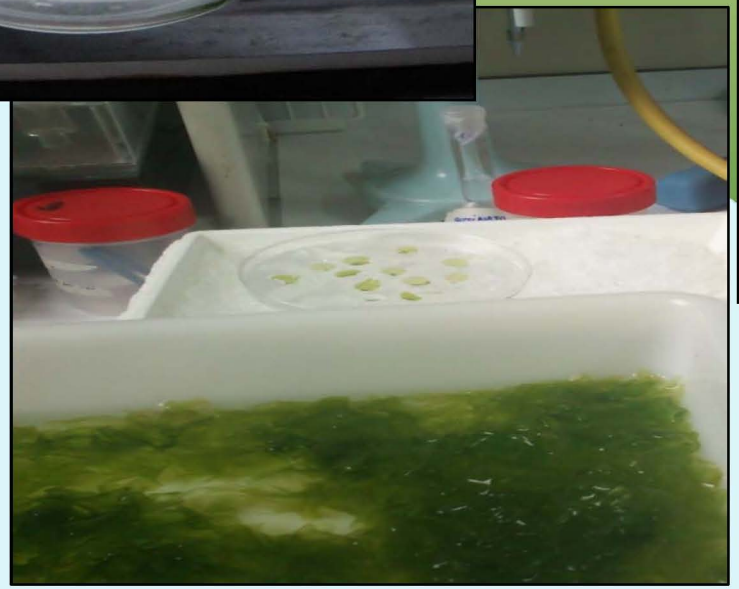
Preparation of cell-free extracts. Optimization for activity measures of the respiratory electron transport system (ETS)

Homogenization methods

Material and methods

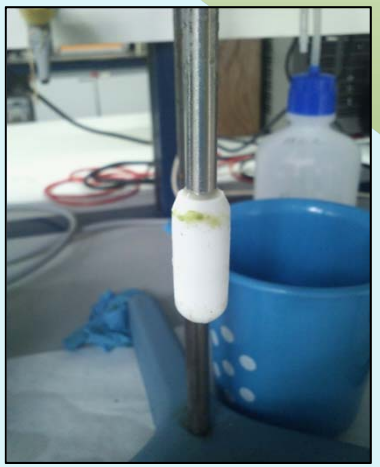
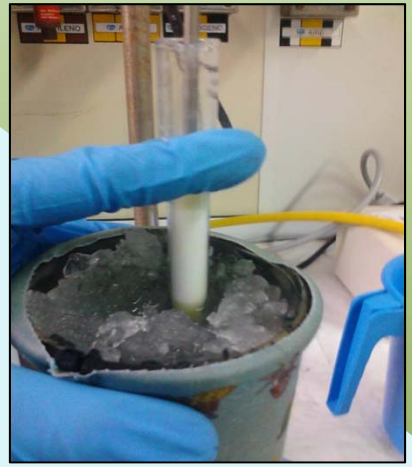
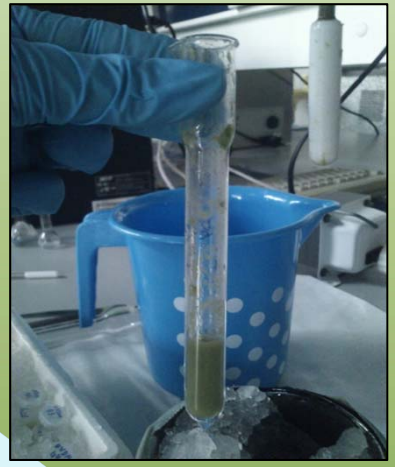
Ten replicated samples - 20 identical algal circles (0, 9 cm diameter)





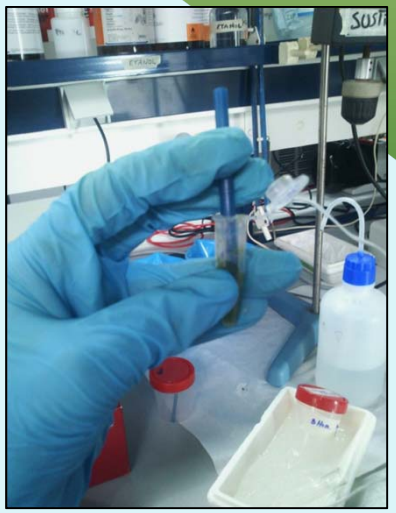
Material and methods

- **Grinder method:** 2 ml of TRIS solution (100mM, pH 8, 5) during 4 minutes.

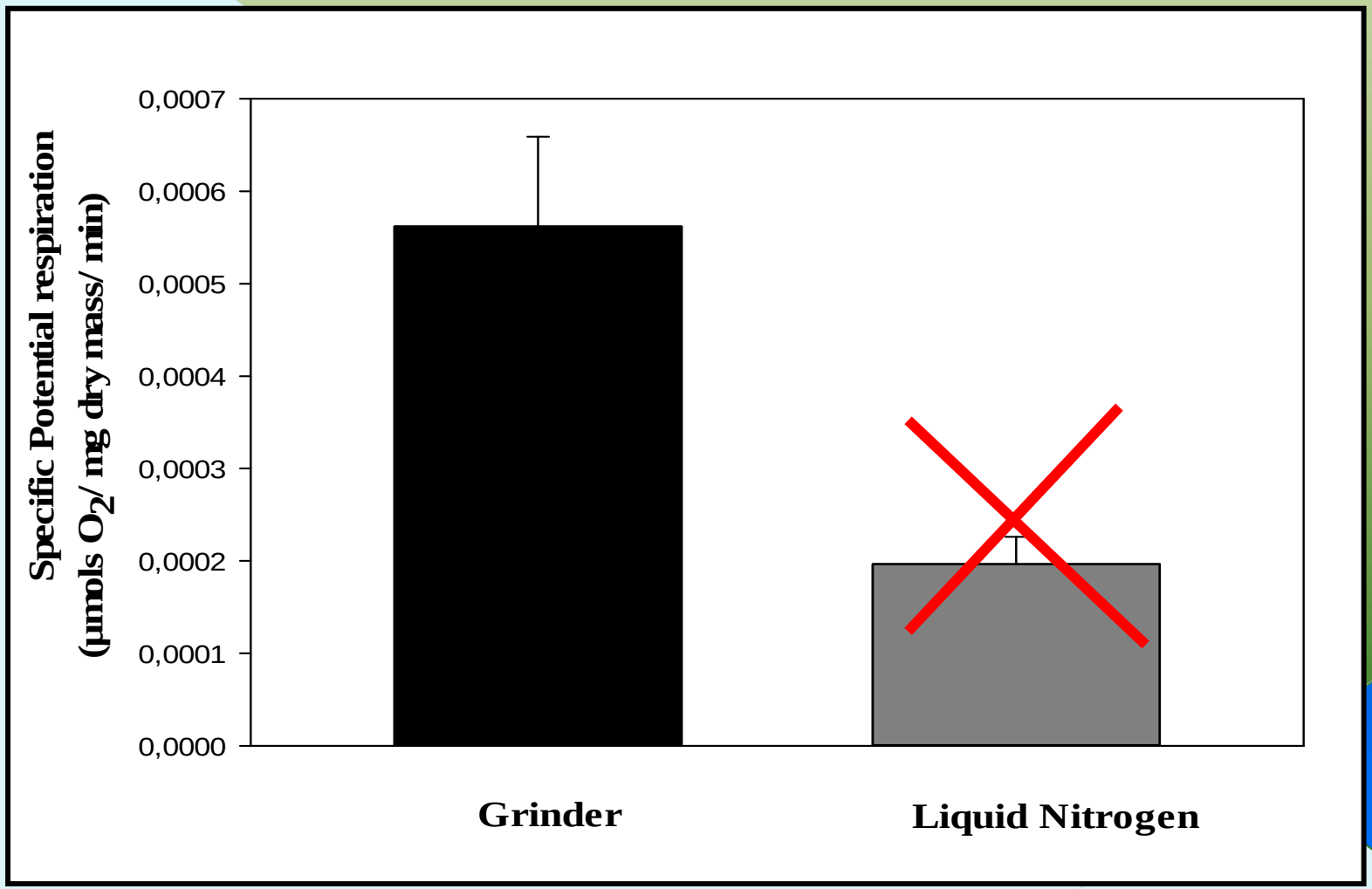

- **Liquid nitrogen method:**





The homogenate was centrifuged for 8 minutes at 2500 rpm and maintain in an ice-bath at 0–4 °C to preserve the enzymatic activity (Gómez et al., 1996).

Comparison between specific potential respirations in the two different homogenization methods



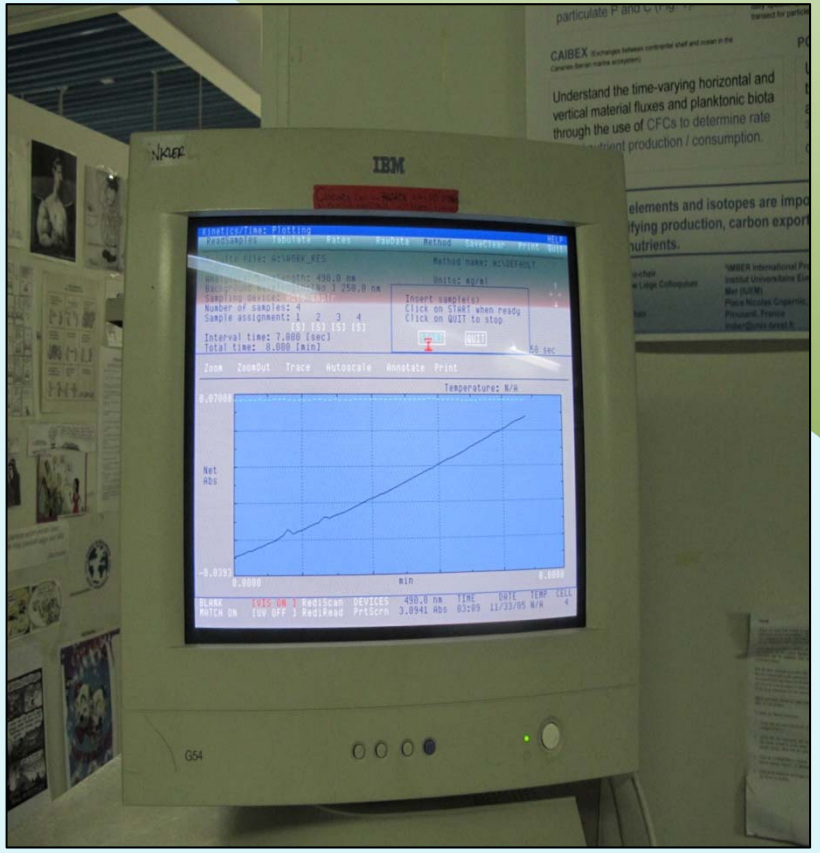
Material and methods

Maintenance: incubation chamber inside the aquaria with filtered sea water (0,45 µm) at 20ºC with 10-14h photoperiod during one week

<u>Analysis: day 1, 5 and 8</u>

Material and methods

Beckman DU® 650 spectrophotometer at 490 nm and 750 nm (turbidity) at 20±1°C for 8 min in kinetic mode.

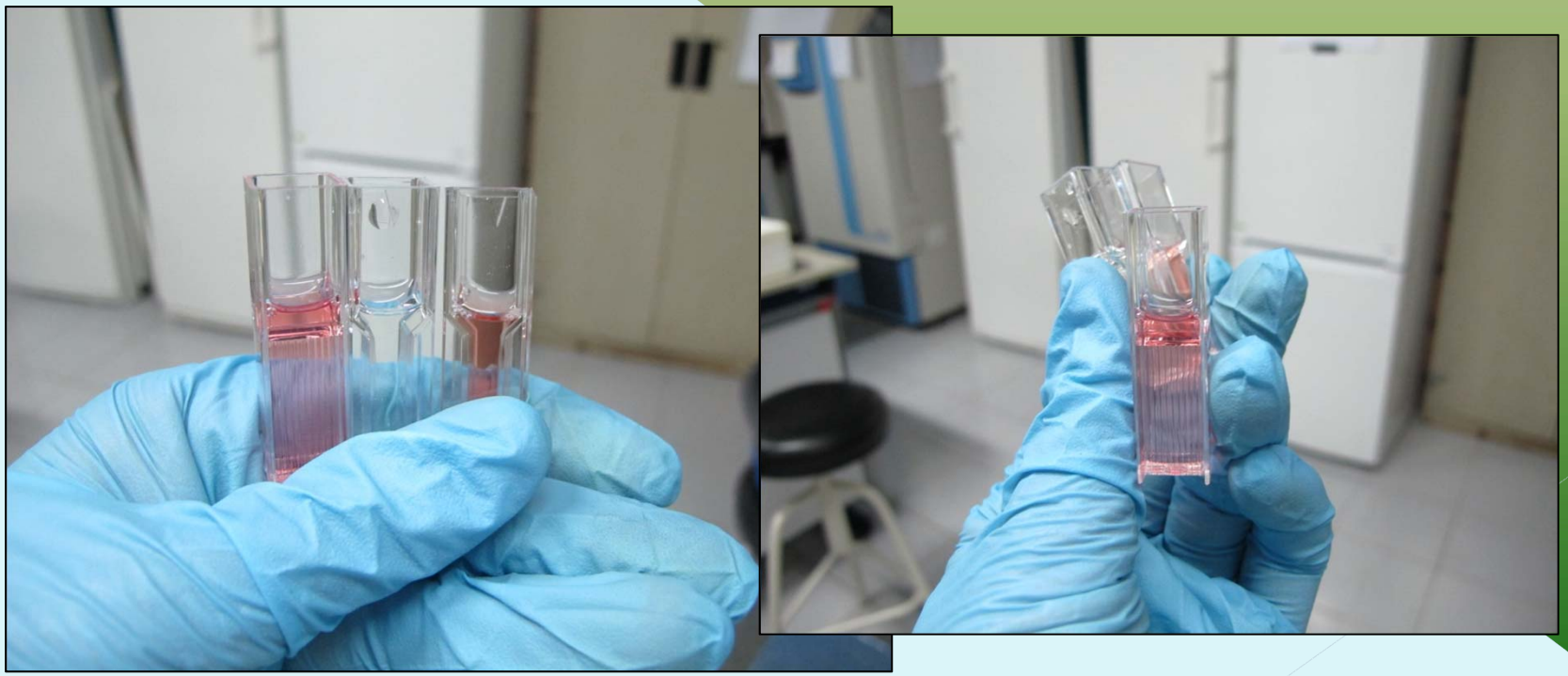


Material and methods

Addiction of sustrates: nicotine adenine dinucleotide (NADH), nicotine adenine dinucleotide phosphate (NADPH) y succinate.

To detect and measure ETS in any biological community → water-soluble tetrazolium-salt, INT.

The reduction of 2 mols of INT (Formazan) = natural reduction of 2 atoms of oxygen (or 1 molecule of O_2).



Material and methods

Dry mass (Lovegrove, 1966): 60ºC 24 h

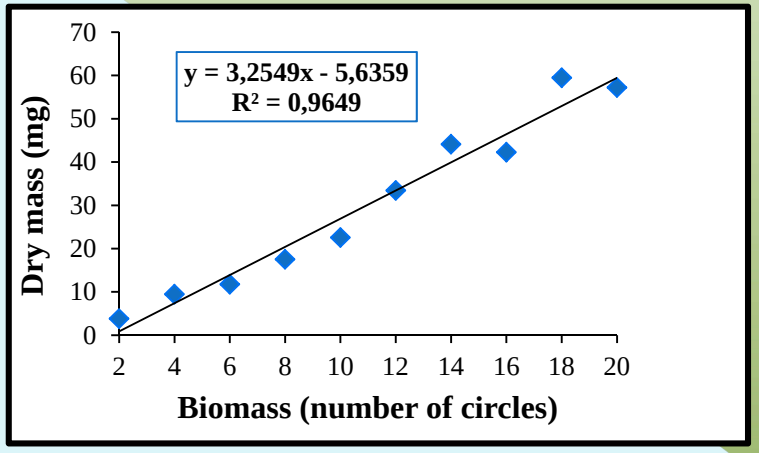
The extraction of chlorophyll was according with Mantoura and Llewellyn (1983).

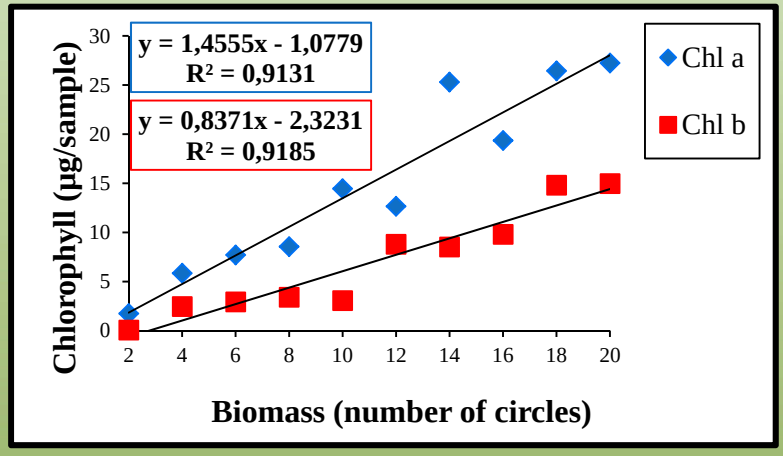
Chlorophyll Determinations (Mitchell and Kiefer ,1984)

- **Chlorophyll *a* = 11.93 E664 – 1.93 E647**
- **Chlorophyll *b* = 20.36 E647 – 5.50 E664**

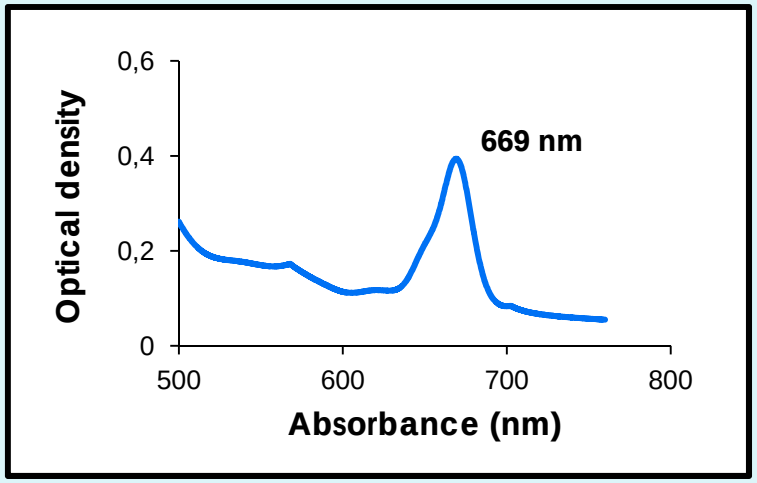
Nº circle/sample	Dry mass (mg/ sample)	Optical density at 664 nm	Optical density at 647 nm	Chl *a* (µg/sample)	Chl *b* (µg/sample)
2	3,80	0,0015	0,0004	1,74	0,06
4	9,49	0,0052	0,0026	5,86	2,46
6	11,76	0,0067	0,0033	7,71	2,93
8	17,52	0,0077	0,0037	8,56	3,40
10	22,56	0,0127	0,0049	14,45	3,07
12	33,44	0,0116	0,0074	12,67	8,79
14	44,11	0,0224	0,0102	25,30	8,53
16	42,29	0,0174	0,0094	19,36	9,81
18	59,49	0,0239	0,0136	26,45	14,82
20	57,22	0,0246	0,0139	27,25	14,98

Material and methods

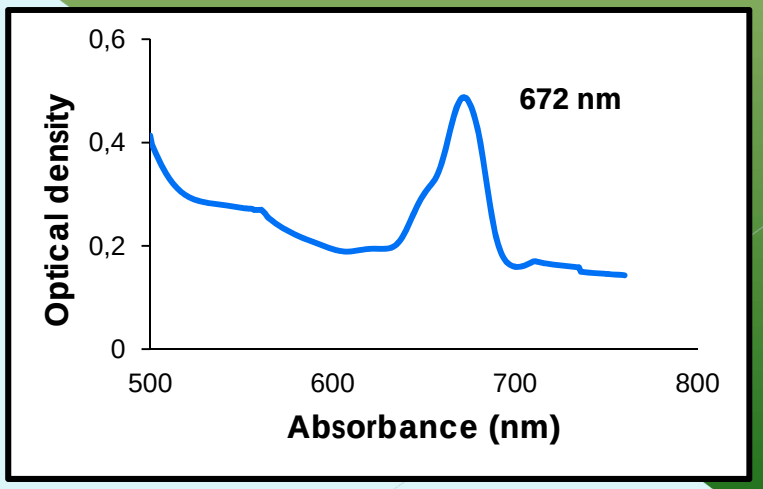




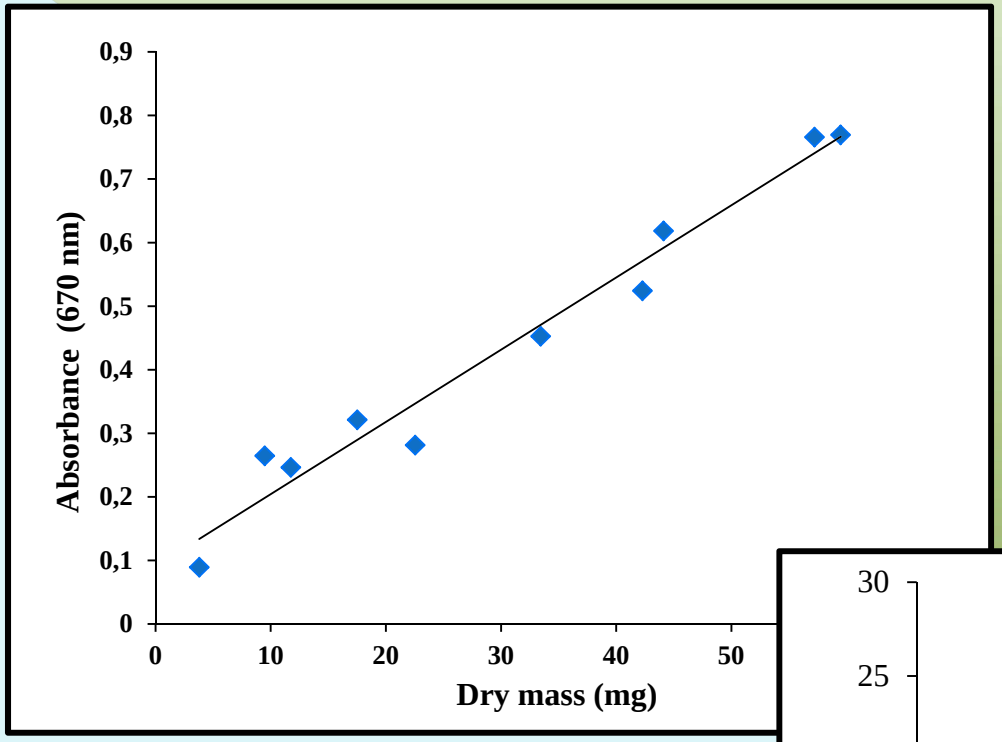
Chlorophyll maximun peak (with filter and without filter)



670 nm



Material and methods



Peso seco

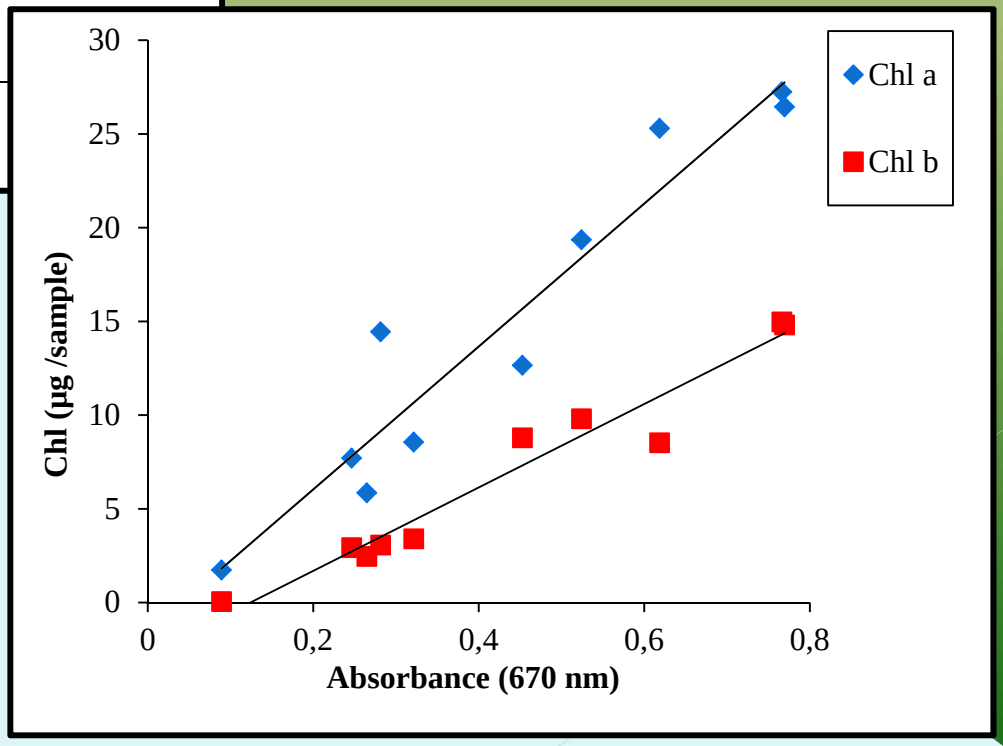
y= 0,0114x + 0,0905
R^2= 0,9668

Chl *a*

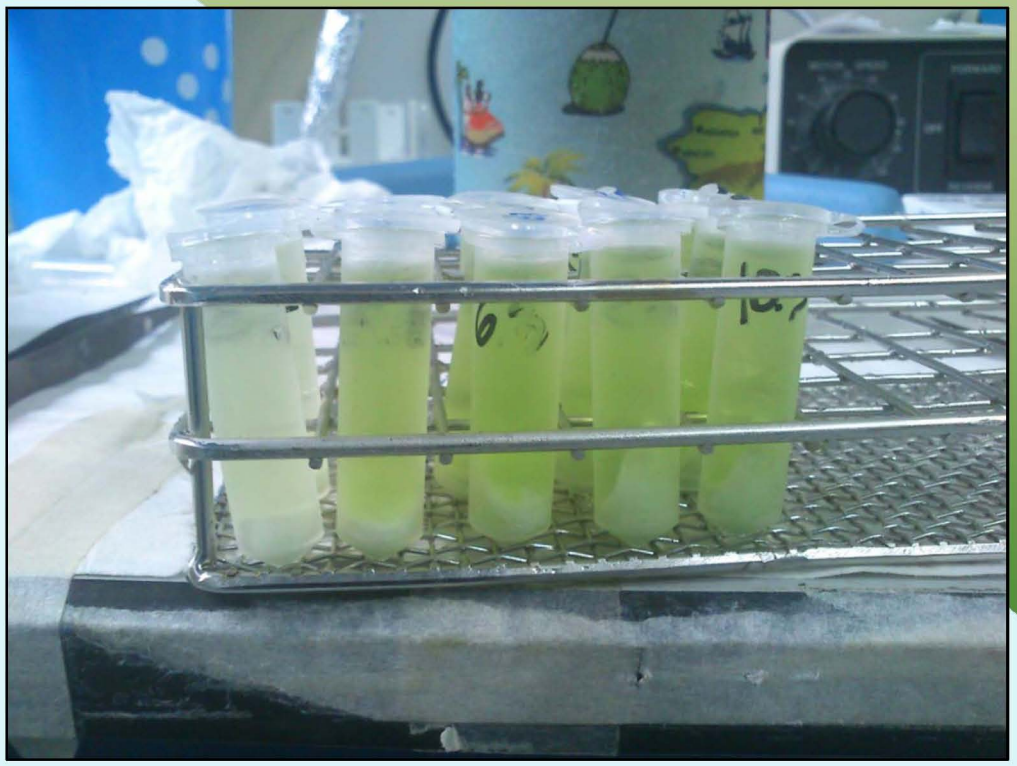
y= 38,12x – 1, 5861
R^2= 0,9186

Chl *b*

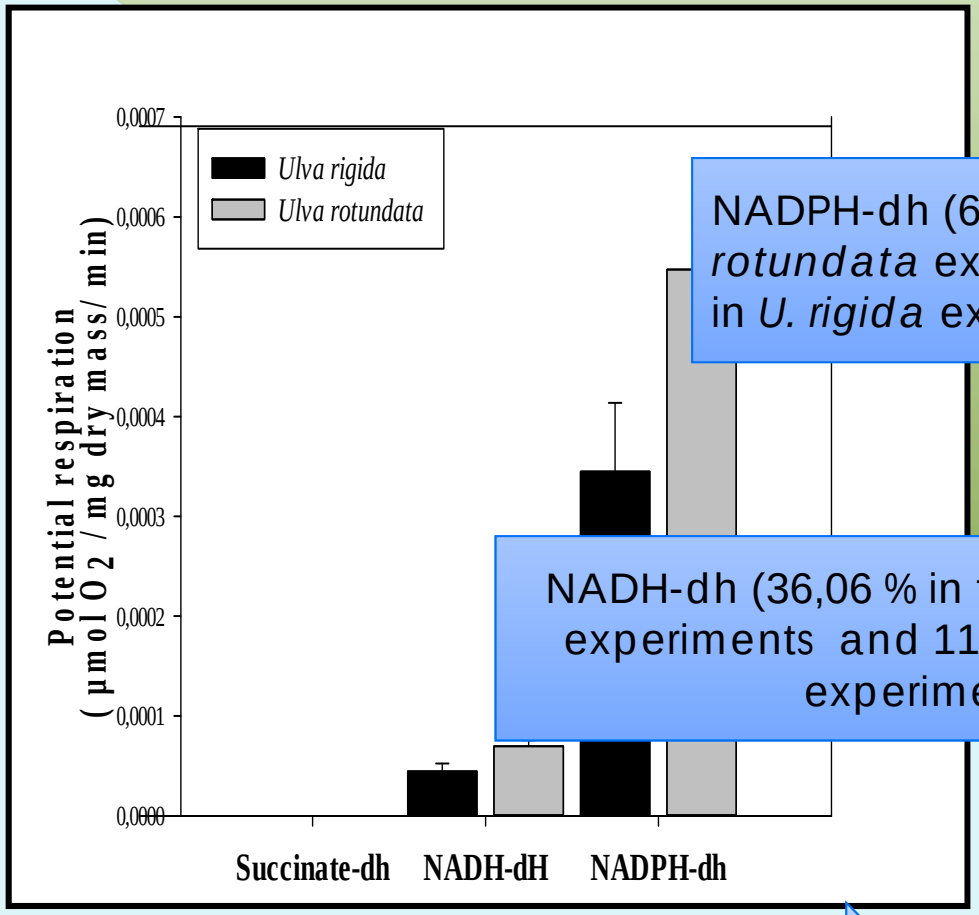
y= 22, 249x – 2,7565
R^2= 0,9517



The relative importance of the three enzymes, NADH-dh, NADPH-dh and Succinate-dh in determine ETS activity.



Results



NADPH-dh (63,89% in the *U. rotundata* experiments and 88,59 % in *U. rigida* experiments)

NADH-dh (36,06 % in the *U. rotundata* experiments and 11,41 % in *U. rigida* experiments)

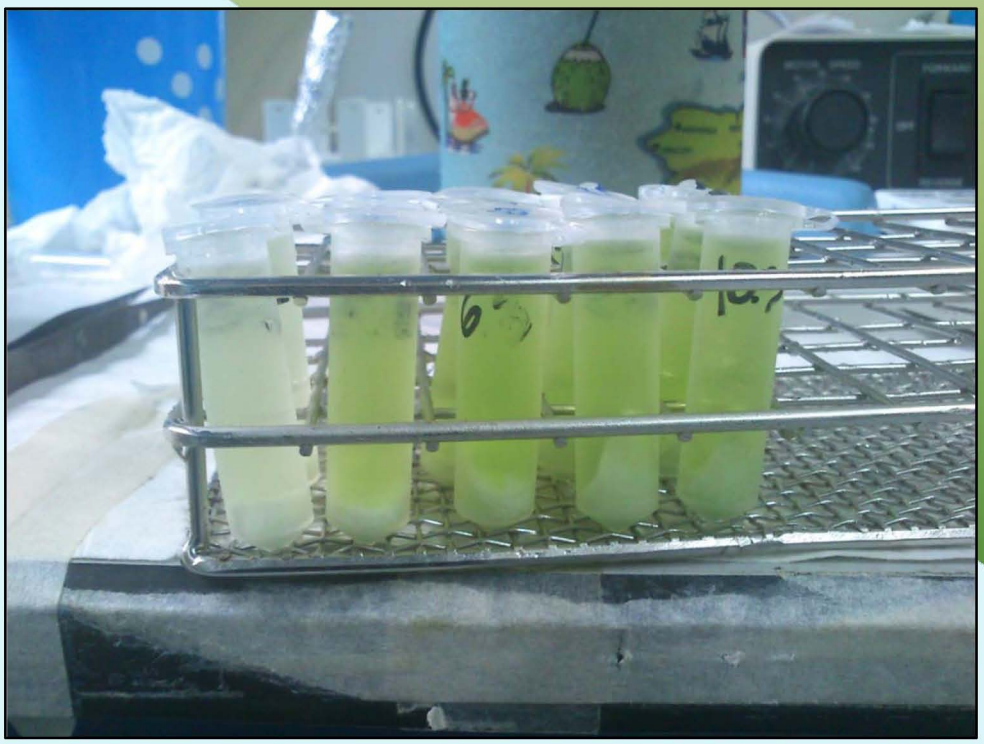
Succinate-dh could not be measured because was below the limit of detection.

Determine the relationship between:

a) Dry mass and chlorophyll.

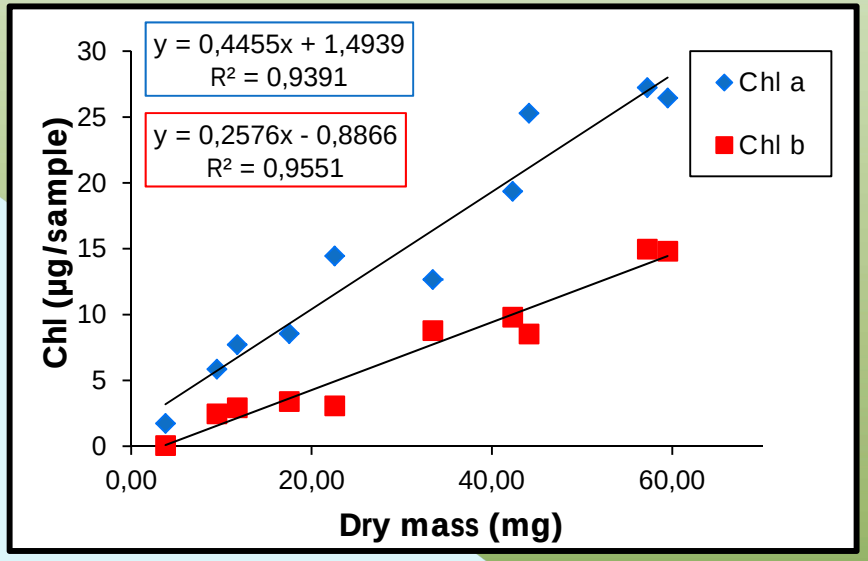
b) Dry mass and potential respiration.

c) Potential respiration and chlorophyll.

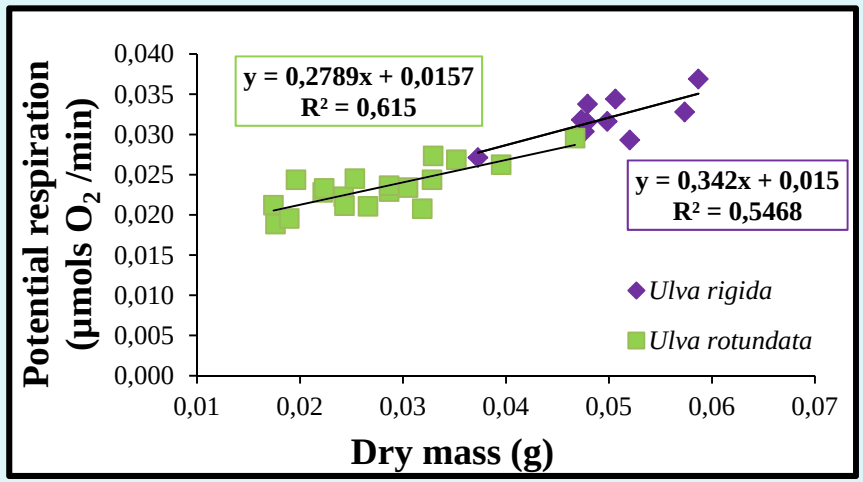


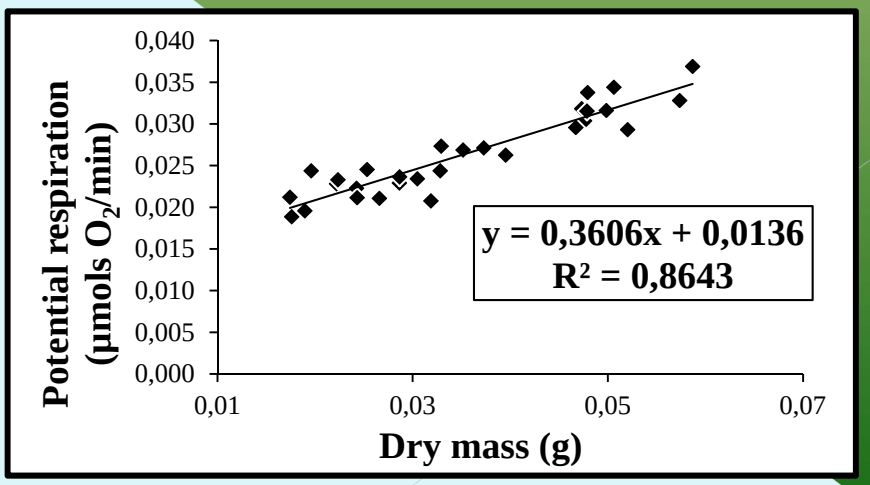
Results

- **a) Dry mass and chlorophyll**

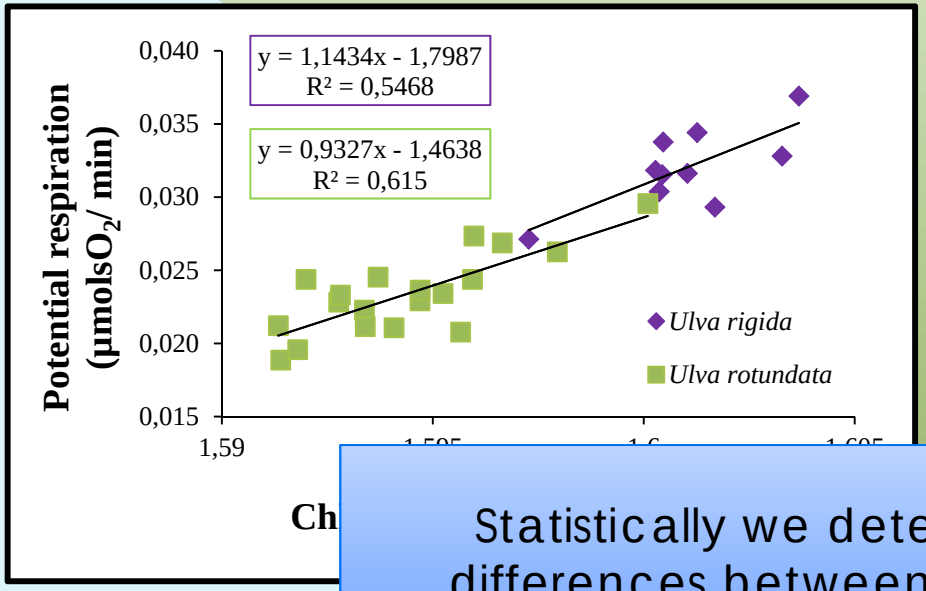


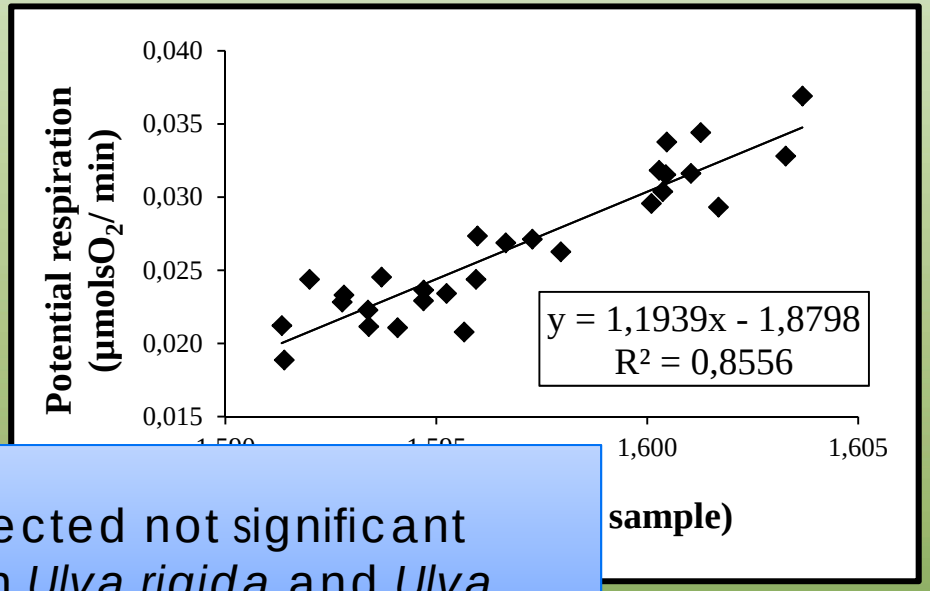
- **b) Dry mass and potential respiration**



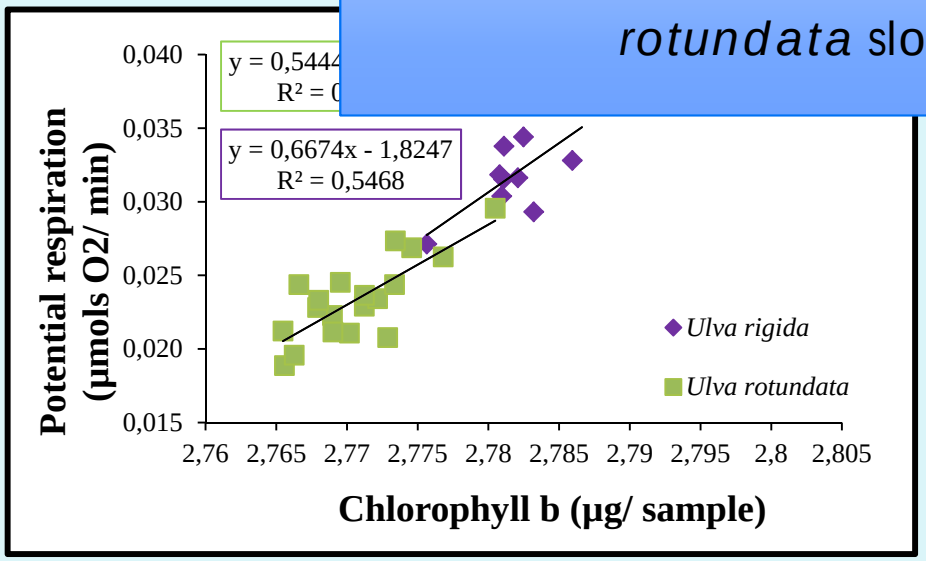


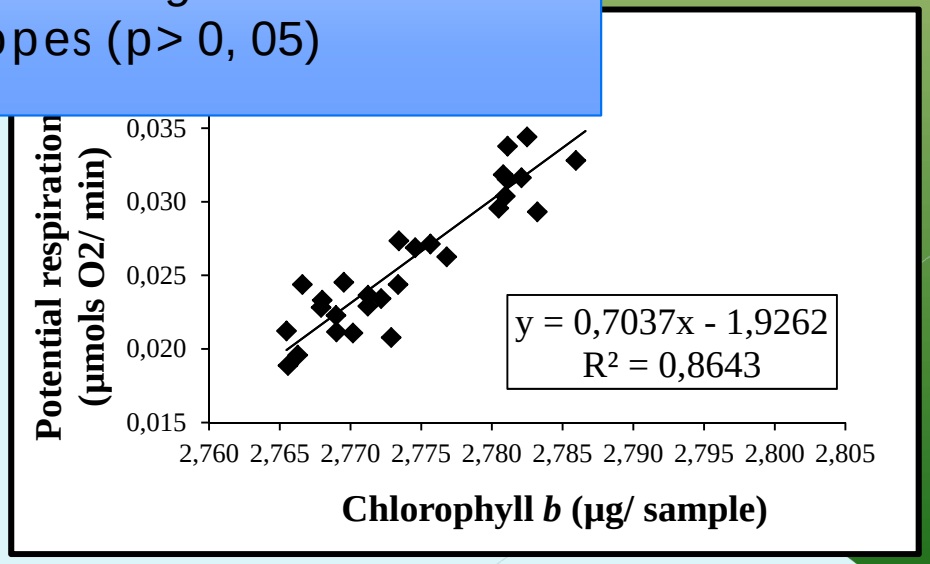
- c) potential respiration and chlorophyll.





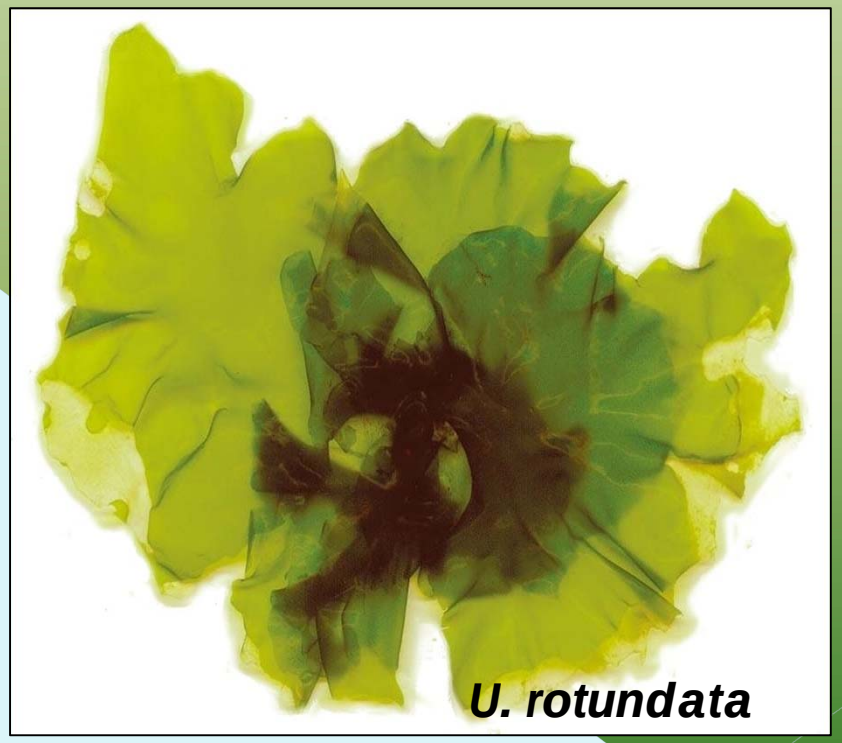
Statistically we detected not significant differences between *Ulva rigida* and *Ulva rotundata* slopes (p> 0, 05)





Study the variability of the potential respiration, dry mass and chlorophyll in three different locations around Gran Canaria.

http://www.seaweed.ie/descriptions/ulva_rigida.php



http://projet-tpe-algues-vertes.e-monsite.com/pages/qu-est-ce-qu-une-maree-verte.html

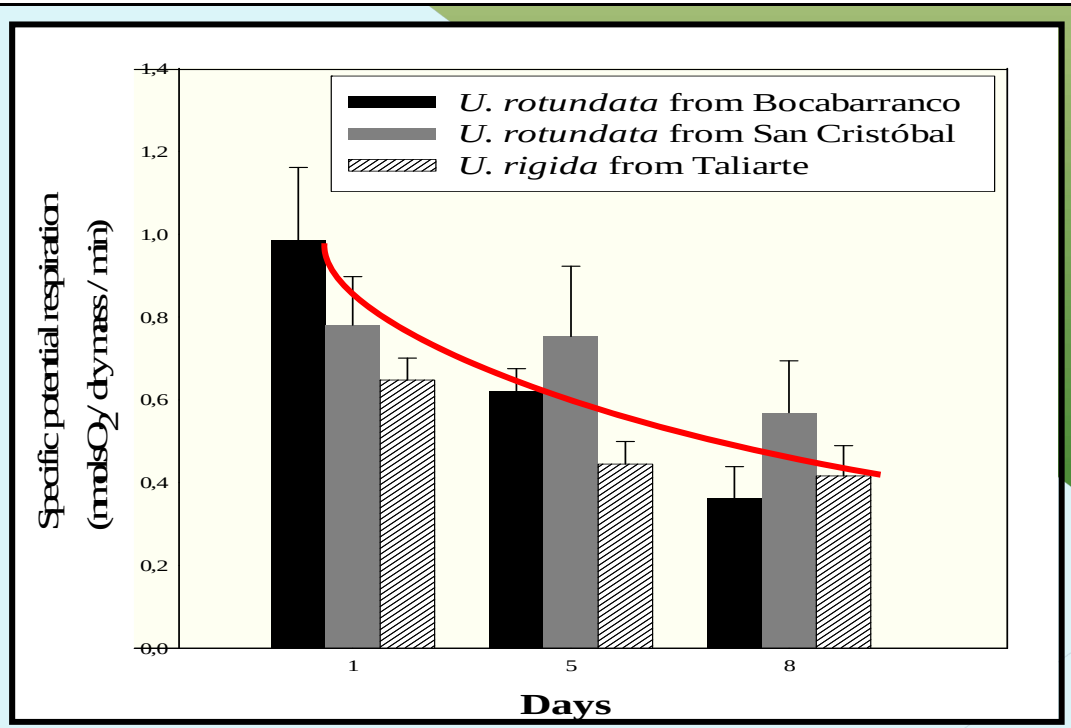
Results

	Ulva rigida (Taliarte)	*Ulva rotundata* (Bocabarranco)	*Ulva rotundata* (San Cristóbal)
Specific potential respiration (nmols O_2/ dry mass/ min)	0,65 ± 0,05	0,98 ± 0,18	0,78 ± 0,12
Dry Mass (mg/sample)	49,65 ± 5,93	23,44 ± 5,51	32,33 ± 7,31
Chlorophyll *a* (µg/ sample)	1,60 ± 0,0018	1,59 ± 0,0016	1,59 ± 0,0022
Ammonium (mg/l)	1,23	2,78	1,58
Nitrate (mg/l)	1,2	2,4	2,7

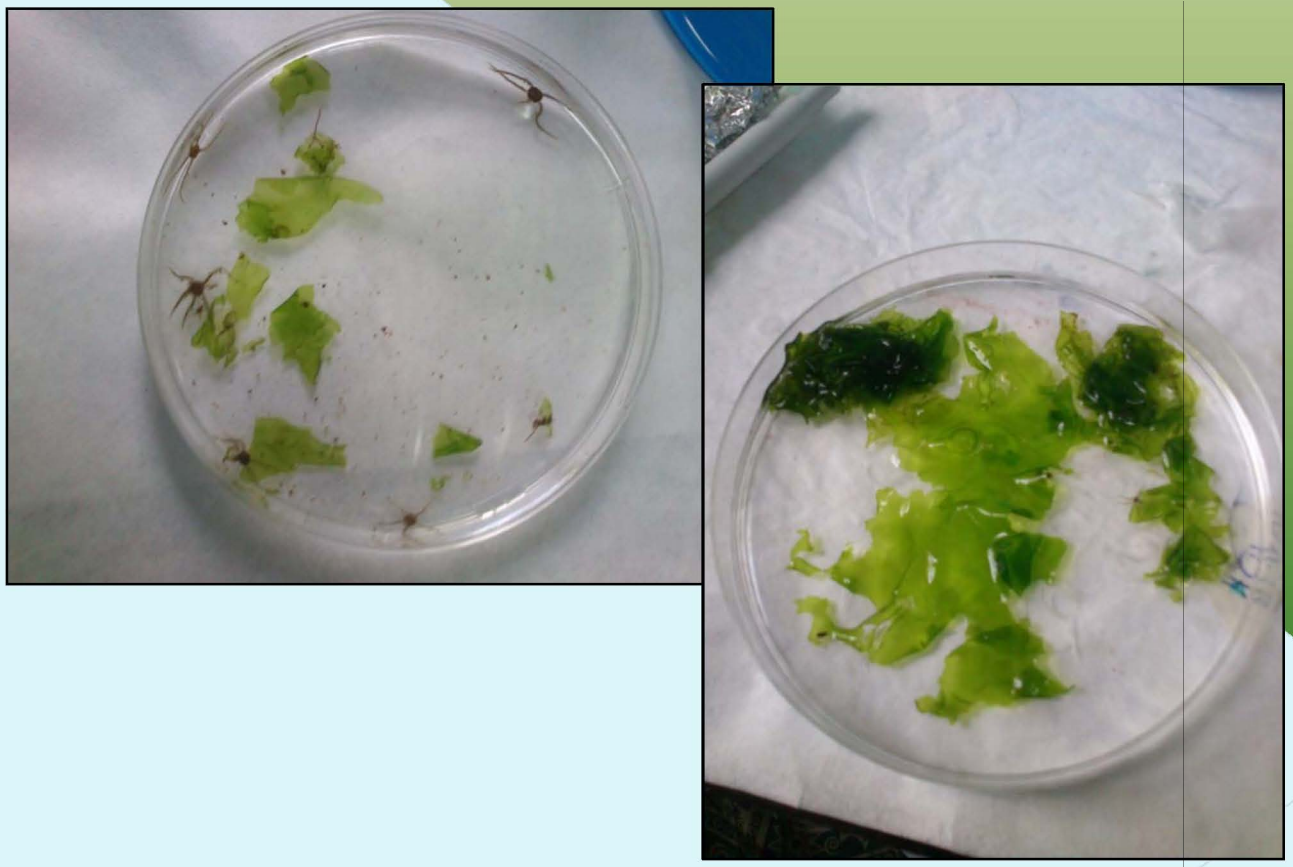
Time-course experiments of metabolism in aquaria with filtered sea water over a week.

Results

Time (days)	*Ulva rigida* (Taliarte)	*Ulva rotundata* (Bocabarranco)	*Ulva rotundata* (San Cristobal)
1	0,6481 ± 0,0526	0,9844 ± 0,1765	0,7805 ± 0,1171
5	0,4447 ± 0,0543	0,6220 ± 0,0533	0,7532±0,1692
8	0,4159 ± 0,0735	0,3612 ± 0,0771	0,5688 ± 0,1256



Relation between Biomass and Respiratory activity (Kleiber) in the three different locations



Kleiber coefficient

Time (days)	*Ulva rigida* (Taliarte)	*Ulva rotundata* (Bocabarranco)	*Ulva rotundata* (San Cristóbal)
1	y = 0,5211x - 2,3324 r^2 = 0,5664	y = 0,2605x - 2,0078 r^2 = 0,4303	y = 0,4255x-2,25 r^2 = 0,547
5	y = 0,6156x - 2,658 r^2 = 0,7828	y = 1,0329x - 3,2564 r^2 = 0,8131	y = 0,1584x- 2,0469 r^2 = 0,1316
8	y = -0,1945x - 1,192 r^2 = 0,0077	y = -0,1421x - 1,7225 r^2 = 0,0186	y = 0,169x -2,064 r^2 = 0,0593

Conclusions

- Comparison between potential respiration in both homogenization methods demonstrated a significant difference. We used the tissue-grinding method because it was less expensive and easier.
- The most important contribution to the ETS activity of *Ulva* spp. was NADPH, followed by NADH and succinate.
- There was good correlation between the biomass parameters, dry mass, chlorophyll, and optical density at 670 nm. As a result, we used this optical density as a measure of biomass.

Conclusions

- *Ulva rotundata* from Bocabarranco had the highest potential respiration consistent with the high levels of nutrients and *Ulva rigida* from Taliarte has the lowest potential respiration coinciding with the lowest level of nutrients in the area. However *Ulva rigida* from Taliarte has the highest dry mass. The differences in the 8-day potential respiration time courses for the three areas were statistically different. There was a loss between 25 to 60% of its initial potential respiration over this time period.

- There was a decrease in the Kleiber coefficient from the first day to the last day, suggesting a shift in the nutritional state.

ACKNOWLEDGEMENTS

- A mis tutores, May, Susi y Ted, por ayudarme a empezar en este mundo tan dificil y aguantar mi inexperiencia.

- A Federico Maldonado, no solo por su ayuda profesional en el laboratorio, si no por la gran paciencia que ha tenido conmigo en este año, por sus consejos, sugerencias y sobre todo por su gran amistad y cariño incondicional.

- A mis grandes amigos Ali, Gara, Elian, Yeray y Carlos que dia a dia me han apoyado en todo.

- A todos mis compañeros del laboratorio por mostrarte el funcionamiento del mismo.

- Este trabajo está soportado por el poyecto BIOMBA (CTM 2012-32729/MAR).