Micro- and mesozooplankton respiratory metabolism: comparison of simultaneous incubation experiments and ETS activity.

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1. Introduction

The contribution of the different plankton components to the Ocean respiratory carbon losses is of prime importance for the improvement of models dealing with global climate changes and plankton production.
However, the obtention of plankton respiratory losses for the separate groups by classical incubation methods is extremely time- and labour consuming.
Enzymatic methods like the activity of the respiratory electron transport system (ETS) overcome most of the experimental inconveniences, but gives potential, not actual respiration rates.
In order to use ETS as valid estimator of respiration rates, it is necessary to determine wheter there exist robust relationships between ETS activity and directly measured O_2 consumption.

2. Aims

1.- The obtention of robust relationships between directly measured O_2 consumption rates and ETS activity for different zooplankton components.

2.- To ascertain whether the relationships between respiration rates and ETS for different zooplankton size-fractions are also different and can be used as a proxy for respiration rates.

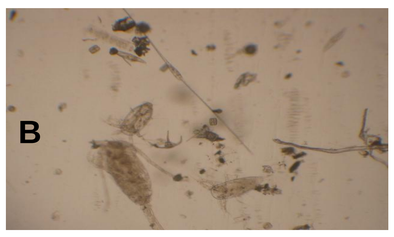
3. Methods

The study was based in the comparison of O_2 consumption rates obtained by classical incubation methods with those obtained from ETS activity in the incubated organisms.

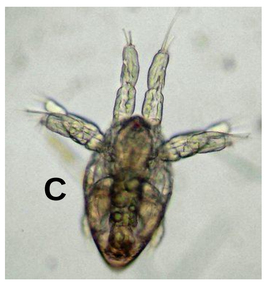
Experimental organisms

The study included three groups of experimental zooplankton organisms (Fig. 1):

- **>200 µm mixed zooplankton** (mesoplankton) from 3 stations in the NW Mediterranean in March 1999 (Alcaraz et al. (2007). Obtained by 100 – 0 m depth vertical net hauls made with a 200 µm-mesh WP-2.



- **<200 >50 µm mixed zooplankton** (metazoan microzooplankton) from a station 1 nM offshore Barcelona. Samples obtained from november 2003 to january 2004. 40 – 0 m depth vertical hauls made with a 50 µm-mesh net..



- **Copepod nauplii**. (nauplii II-IV) obtained from a laboratory culture of the cyclopoid *Oithona davisae* maintained by the Zooplankton Ecology Group in the Institute of Marine Sciences.

Fig. 1.- The three experrimental zooplankton groups: **A**: Mixed mesozooplankton; **B**: Mixed microzooplankton; **C** *Oithona davisae* nauplii.

Direct measurements of respiration rates:
O_2 consumption rates were measured by 24 h-duration incubation experiments at "in situ" temperature as described in Alcaraz et al. (1998). In most of the experiments, semi-continuous analysis of dissolved O_2 concentration were obtained with pulsed polarographic O_2 electrodes (ENDECO®, Fig. 2). Time - O_2 concentration relationships were fitted by linear regression (Fig. 3). In few cases, the incubation was made in BOD bottles and the O_2 concentration measured by Winkler titration (Fig. 4).

ETS activity:
After the incubations, the experimental organisms were transferred by filtration into GF/A glass fibre filters, immediately frozen in liquid N_2 and maintained at -80 ºC until the analysis of the ETS activity. These were performed according to Packard et al. (1996).

Fig. 2.- Incubation flasks showing the electrode and the pressure control

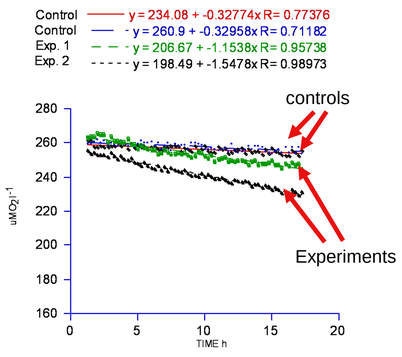
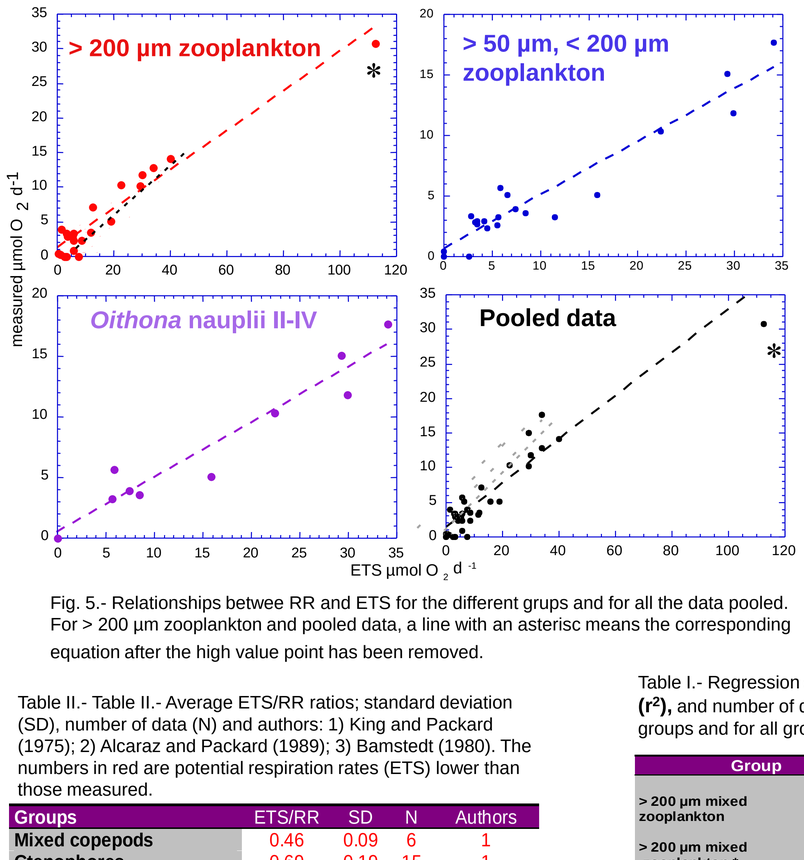


Fig. 3.- Electrode graphs of O_2 concentration inIncubation experiments

Fig. 4.- BOD bottles for respiratory incubation experiments

4. Results and Discussion



The relationships between ETS values and respiration rates (RR) for the different groups were statistically significant (Fig. 5 and Table I).
The regression coefficients for > 200 µm zooplankton were not statistically different from those of other groups ($p < 0.05$), even after a single point from the mesozooplankton (*) was removed.
For the pooled data the differences with respect the remaining groups were also non significant ($p < 0.05$).

Fig. 5.- Relationships betwee RR and ETS for the different grups and for all the data pooled. For > 200 µm zooplankton and pooled data, a line with an asterisc means the corresponding equation after the high value point has been removed.

Table II.- Table II.- Average ETS/RR ratios; standard deviation (SD), number of data (N) and authors: 1) King and Packard (1975); 2) Alcaraz and Packard (1989); 3) Bamstedt (1980). The numbers in red are potential respiration rates (ETS) lower than those measured.

Groups	ETS/RR	SD	N	Authors
Mixed copepods	0.46	0.09	6	1
Ctenophores	0.69	0.19	15	1
Medusae	1.19	1.13	36	1
Mixed zooplankton	0.65	0.25	7	1
Mixed zooplankton	0.39	0.11	6	2
Acartia tonsa	1.25	0.42	5	3
Acartia tonsa	1.75	1.45	5	3
Acartia tonsa*	2.27	0.23	5	3
Mixed zooplankton	**2.46**	**1.35**	**18**	**This study**
Mixed microzooplankton	**1.75**	**0.66**	**17**	**This study**
Oithona Nauplii II-IV	**2.07**	**0.59**	**10**	**This study**
Total (pooled data)	**2.09**	**1.75**	**45**	**This study**

* Winter values

Table I.- Regression eaquations, determination coefficients **(r^2),** and number of data (**n**) for the different zooplankton groups and for all grouped.

Group	Equation	R^2	n
> 200 µm mixed zooplankton	RR = 0.28 ETS + 1.05	0.93	18
> 200 µm mixed zooplankton *	RR = 0.36 ETS + 0.29	0.88	17
< 200, > 50 µm mixed zooplankton	RR = o.441 ETS + 0.65	0.91	17
Nauplius II-IV Oithona davisae	RR = 0.45 ETS + 0.44	0.92	10
Pooled data	RR = 0.31 ETS + 1.46	0.87	45
Pooled data *	RR = 0.41 ETS + 0.51	0.88	44

* Same as the above with the largest value point removed

ETS/RR ratios (Table II) were similar to those obtained by Bamstedt (1980) for *Acartia tonsa.*
The values ranged from **1.75** to **2.46**, with an average value for pooled data of **2.09.** These values contrast with previous ones in which **ETS** gave potential respiration rates **lower** than those directly measured (seeTable II). The reason could be the improvement of the analysis of ETS activity by the two substrates method (Packard et al. 1996).
The relationship between **calculated** and **measured** respiration rates, using the **regression equation** for the pooled data, and the average **ETS/RR ratio** explain the same percentage of the variance, **88 %** in both cases (Fig. 6).

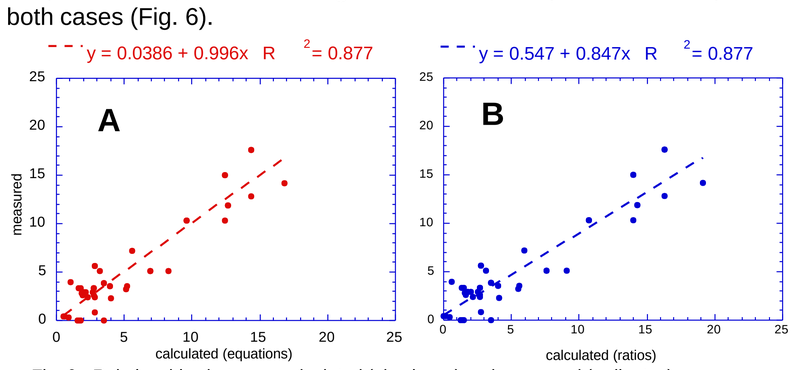


Fig. 6.- Relationships between calculated (abscissae) and measured (ordinates) respiration rates for the threegroups of zooplankton (pooled data). A: Equations for pooled data as in Table I. B: ETS/RR ratios (Table II).

This relatively good agreement obtained between observed and calculated respiration rates (Fig. 6), both using the **regression equation for pooled data (A)** and the **average ETS/RR** ratio **(B)**, could be due to the relatively homogeneous environmental conditions (mainly temperature and food) experienced by all the zooplankton groups before catching them and during the incubation experiments.

5. Conclusions

•The ETS-RR regression equations and ETS/RR ratios for the different zooplankton components appear to be relatively constant.

• At least when the experimental conditions (mainly temperature and food) are similar, both regression equations and ETS/RR ratios are robust predictors of actual respiration rates (RR). The variance explained is in both cases up to 88 %.

• The factors affecting the variability of the relationships between ETS-derived and directly measured RR must be ascertained in order to efficiently use ETS activity as a proxy of respiration rates.

6. References

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