



# Zooplankton biomass and electron transport system activity around the Balearic Islands (western Mediterranean)



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## ABSTRACT

Measuring electron transport system (ETS) activity in zooplankton provides an index of respiration, theoretically, the potential respiration rate. We apply the ETS technique to estimate potential respiration and carbon demand from the zooplankton community in the upper 200 m of the water column near the Balearic Islands. The investigation was focused on two areas with different oceanographic conditions: the Balearic and Algerian subbasins. It compared the biomass, potential respiration and specific potential respiration of different size fractions (53–200, 200–500, >500  $\mu\text{m}$ ) in both areas. In these regions the largest contribution to respiration was found in the larger sizes. The specific respiration (per unit biomass) was greater in smaller fractions, indicating that they have a more active metabolism. Both biomass and potential respiration increased in the Algerian subbasin and for both regions biomass and potential respiration were greater in shallow waters over the continental shelf (<200 m). Using Kleiber's law as a tool to investigate the relationships between these two variables, we found that the exponential relation coefficient ( $b$ ) was less than 0.75, indicating that the respiration was depressed (shifted down). In cultures and in eutrophic ocean waters (upwelling areas)  $b$  normally is greater than 0.75, consequently we intuit that the low value of  $b$  over the Balearic and Algerian subbasins indicates that the zooplankton is not well fed and that they are living under oligotrophic stress.

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## 1. Introduction

Zooplankton plays an important role in carbon transfer and vertical flux in the oceans. They are part of the “biological pump” because they transfer carbon fixed by phytoplankton, through the food web to deeper levels of the ocean (Longhurst & Harrison, 1989). For this reason zooplankton respiration has been widely studied in recent decades by the scientific community (Bämsted, 1980; Childress, 1968; Conover, 1960; Del Giorgio & Williams, 2005; Gómez et al., 1996; Hernández-León & Gómez, 1996; Hernández-León & Ikeda, 2005; Ikeda, 1970; King & Packard, 1975; Mayzaud et al., 2005; Minutoli & Guglielmo, 2009, 2012; Packard & Gómez, 2013; Packard et al., 1974). However, *in situ* measurements of zooplankton respiration remain difficult to carry out in practice. Capturing a natural zooplankton community and isolating it in good physiological condition in a simulated *in situ* environment long enough to make a physiological and ecologically meaningful respiration measurement, challenges the possible. Hence, the drive to develop biochemical indices of respiration. The activity of the respiratory electron transport system (ETS), theoretically measures potential respiration ( $\phi$ ) and since the early work of King & Packard, 1975 and Finlay et al., 1983 its relationship with respiration has been shown to be better

than the Kleiber relationship between respiration and biomass (Packard & Gómez, 2008). Accordingly, we use ETS activity to calculate respiration thereby reducing the problems associated with incubation of zooplankton in a controlled environment (Gómez, 1991).

Oxygen is the final electron acceptor of the aerobic respiratory electron transport system (Gnaiger, 2009). The ETS method (Packard et al., 1971, 1974) saturates the mitochondrial ETS with NADH and succinate, and ETS microsomal with NADPH. It then uses a tetrazolium salt 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT), often called tetrazolium violet, as the electron acceptor. The reaction generates the reduced tetrazolium which is, in the parlance of organic chemistry, a formazan. In this process 1 mol of  $\text{O}_2$  consumed is equivalent, stoichiometrically, to 2 mol of INT reduced and 2 mol of formazan produced. The formazan is measured spectrophotometrically at 490 nm. This is a measurement of the ability of mitochondria and microsomes to transfer, physiologically, electrons from substrates (NADH, NADPH and succinate) to a final electron acceptor. It is the respiratory capacity (or potential respiration) and in the parlance of enzymology, the maximum velocity of the ETS, its  $V_{\text{max}}$ .

Respiratory  $\text{O}_2$  consumption is affected by several factors that do not affect the  $V_{\text{max}}$  of the respiratory ETS. Among these short-term factors are nutritional level, activity level, and behavioral shifts. They can change respiration at the physiological level, but they do not change

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the potential respiration at the biochemical level. The potential respiration is determined by the concentration of the enzyme complex NADH dehydrogenase-lipoprotein in the inner membrane of mitochondria (Nelson & Cox, 2005). This enzyme complex is constitutive and therefore part of the mitochondrial machinery. This  $V_{max}$  does not change rapidly with changing external nutritional conditions, behavior, or the amount of substrate metabolized. Indeed, it is a permanent characteristic of the cell and as such varies along with variations in carbon, nitrogen, protein and total biomass of the cell. However because this  $V_{max}$  is a property of organic catalysts it will vary with the temperature, pressure, pH, and ionic strength of the surrounding chemical and physical fields. Furthermore, because this  $V_{max}$  is the respiratory potential, it and the respiration rate will move in parallel with these four factors.

Respiration can be expressed as a function of biomass according to the power function:  $R = aW^b$ , and for years it has been accepted that the coefficient  $b$ , equaled 0.75 for all organisms. This is known as “3/4 power law” or “Kleiber’s law”. Ikeda, 1970, 1985 investigated the Kleiber relationship in zooplanktonic organisms. Here, we investigated the ETS-biomass relationship in both studied areas because the exponent of the power function,  $b$ , seems to indicate the physiological or nutritional state. Other authors have found that the ETS-biomass exponential relation coefficient ( $b$ ) is close to or above 0.75 in organisms that are well fed, while it is lower in organisms found in oligotrophic conditions (Gómez et al., 2008; Herrera et al., 2011; Martínez, 2007; Packard & Gómez, 2008).

The Balearic archipelago is located in the boundary area between the northern, more saline and colder waters, originated in the nearby Gulf of Lion, and the southern, less saline and warmer oligotrophic waters of the Algerian basin, where the Balearic channels control the meridional mass transport and fluxes of the water masses (Pinot et al., 2002). Those authors indicate that through the Mallorca channel, severe winters are associated with an increased inflow of northern waters, whereas milder winters result in a higher northward flow of recent Atlantic water and can be related with the zooplankton biomass and its biodiversity (Fernández de Puelles et al., 2007, 2009). In relation to that, other biological studies in the same area showed, that whether seasonal or interannual variability was investigated, the distribution of the zooplankton communities was strongly influenced by the inflow/outflow of those waters (Fernández de Puelles et al., 2003, 2004). Temporal studies during a decade (1994–2003) found that hydrographic changes were reflected in the planktonic distribution and linked to large atmospheric factors (Fernández de Puelles et al., 2004). Accordingly, due to the proximity of the Atlantic, the Mallorca channel could be considered as a suitable place for long-term studies to observe zooplankton changes in relation to large-scale climatic fluctuations (Fernández de Puelles & Molinero, 2007, 2008). This study focuses on the zooplankton  $\phi$  in three size fractions in the upper 200 m of the water column in these two water masses.

In order to elucidate the role of epipelagic zooplankton in the upper 200 m of the ocean water column we calculate  $\phi$ , biomass, and average annual carbon demand for this community near the Balearic Islands. Also we investigated the relationship,  $\phi$ -biomass, as a possible indicator of zooplankton physiological state and the availability of food in the area. This study was part of the IDEADOS program to understand the relationships between environmental conditions and nectobenthonic communities in the oligotrophic Balearic and Algerian subbasins of the western Mediterranean.

## 2. Material and methods

### 2.1. Study area and sampling methods

Samples were collected during the oceanographic cruise IDEADOS 0710 (11 to 29 of July 2010) in the western Mediterranean Sea off the northwest and southwest of Mallorca, the largest of the Balearic Islands.

The sample sites were on the continental shelf and slope at the edge of the Balearic subbasin (Sóller studied zone) and the Algerian subbasin (Cabrera zone) respectively over a range of depths from 200 to 900 m (Fig. 1). The zooplankton collection was performed by vertical net hauls being raised at a speed of  $1 \text{ m s}^{-1}$ . The microzooplankton ( $53\text{--}200 \mu\text{m}$ ) was sampled with a Calvet net of  $53 \mu\text{m}$  mesh size. The mesozooplankton was captured with WP2 net  $200 \mu\text{m}$  mesh after which the haul was divided into fractions of  $200\text{--}500 \mu\text{m}$  and  $>500 \mu\text{m}$ . The samples were taken at different times of day: morning between 7:00 and 9:00 h, midday between 12:00 and 14:00 h, afternoon between 17:00 and 19:00 h and midnight between 24:00 and 02:00 h (GMT) at each station. However, because of technical problems on board it was not possible to obtain samples from station C200 during the afternoon. The 1 L samples of each station were split into 2 separate aliquots of 0.5 L. These 0.5 L subsamples were filtered and immediately frozen in liquid nitrogen at  $-196 \text{ }^\circ\text{C}$  and stored at  $-80 \text{ }^\circ\text{C}$  until ETS activity and protein analyses could be performed.

### 2.2. Hydrographic data

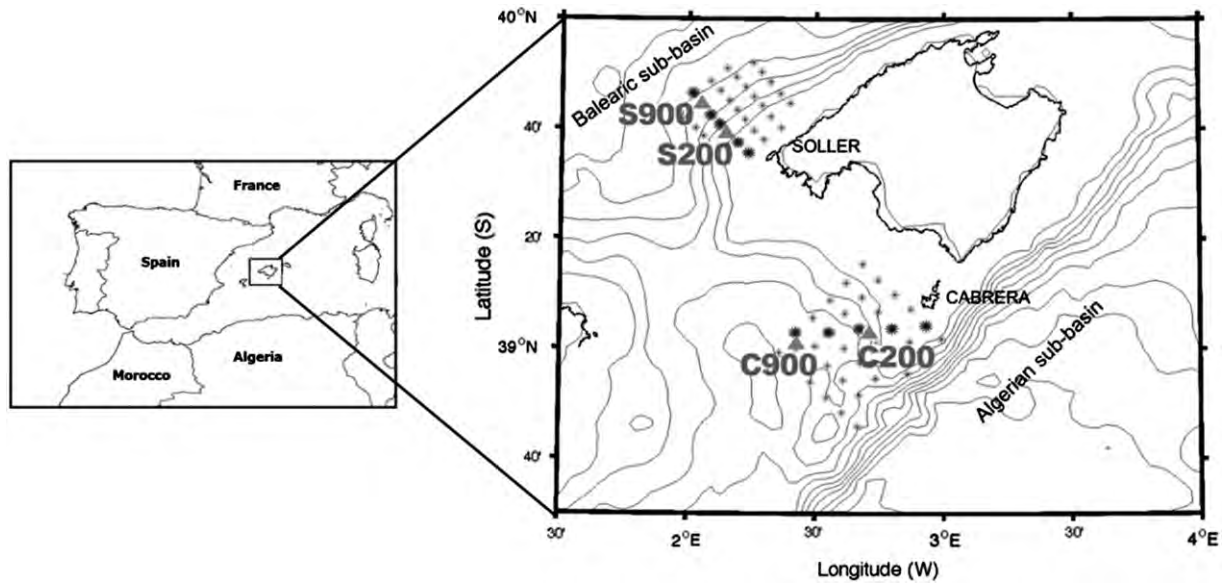
Hydrographic data were obtained with a SBE-911 CTD recorder operating at a sampling rate of 24 Hz, deployed at an average speed of less than  $1 \text{ m s}^{-1}$ . The CTD transects were separated by 4.5 nmi, with stations placed 2.8 nmi apart at the Sóller ground, in the north, and 5 nmi apart at the Cabrera ground, in order to reach the 1000 depth isobath in each case. Hydrographic parameters (salinity, potential temperature, dissolved oxygen, turbidity, fluorescence and Photosynthetically Available Radiation (PAR)) were processed using the Sea Bird Electronic Data Processing routines and according to the standard protocols developed for IEO data analysis (López-Jurado et al., 1995). Salinity and oxygen were calibrated on board using the standard salinity bottles and the winkler method, respectively. Temperature and salinity were considered accurate within  $0.005 \text{ }^\circ\text{C}$  and 0.003 units respectively. Temperature data ( $^\circ\text{C}$ ) were processed with ODV software to obtain vertical profiles. Chlorophyll-a concentrations were determined using the fluorometric technique (Holm-Hansen et al., 1965) after filtration of 1.5 L of seawater on Whatman GF/C Filters. The samples were frozen at  $-20 \text{ }^\circ\text{C}$  for further laboratory analysis. Chlorophyll-a was extracted with 90% acetone, and measured using a PerkinElmer 204 spectrofluorometer.

### 2.3. ETS analysis

Potential respiration was estimated according to the ETS method (Packard et al., 1971, 1974) with modifications (Gómez et al., 1996; Owens & King, 1975; Packard & Christensen, 2004). Subsamples were sonified with an ultrasonic processor (Sonic Vibra-Cell, Model VCX130, USA) for 45 s in 1.5 mL of Milli-Q double-distilled water, then centrifuged for 10 min at 4000 rpm at  $0 \text{ }^\circ\text{C}$ . A 0.5 mL aliquot of the supernatant was added to a 1.5 mL of solution containing the substrates 0.2% (v/v) Triton X-100. Samples were incubated at  $18 \text{ }^\circ\text{C}$  for 20 min after which the reaction was stopped with a quench solution consisting of 50% phosphoric acid (0.1 M) and 50% of formaldehyde (36%). The absorbance reading was performed in a spectrophotometer (Beckman DU 650, USA) at 490 nm and 750 nm to correct for turbidity. ETS activity was calculated for *in situ* temperature using the Arrhenius equation and activation energy of  $15 \text{ kcal mol}^{-1}$  (Packard et al., 1975). Potential respiration ( $\phi$ ) was calculated from ETS activity according to Packard & Christensen, 2004. The rate of potential oxygen consumption ( $\phi$ ) was converted to carbon demand rate assuming a respiratory quotient (RQ) of 0.85 (King et al., 1978).

$$CD(\mu\text{gCh}^{-1}\text{m}^{-3}) = \phi(\mu\text{LO}_2\text{h}^{-1}\text{m}^{-3}) \cdot 0.85 \cdot 12/22.4 \quad (1)$$

where CD is the carbon demand, 0.85 is the RQ, 12 is the weight of 1 mol of carbon and 22.4 is the volume in  $\mu\text{L}$  of  $1 \mu\text{mol}$  of  $\text{O}_2$ . Biomass was



**Fig. 1.** Map of study area. Hydrographic stations are marked with asterisks (in bold transects used to obtain vertical temperature profiles shown in Fig. 2). Zooplankton sampling stations are marked with triangles: S200 and S900 located in Balearic subbasin (Sóller) at 200 and 900 m depth respectively and C200 and C900 in Algerian subbasin (Cabrera) at 200 and 900 m respectively.

estimated in mg of protein by the method of Lowry et al., 1951, as amended by Rutter, 1967. We studied the relationship between  $\phi$  ( $\mu\text{mol O}_2 \text{ h}^{-1} \text{ m}^{-3}$ ) and biomass ( $\text{mg protein m}^{-3}$ ) in both studied areas, and in the different size fractions. This relationship is represented by the equation:

$$\phi = aW^b \quad (2)$$

or expressed in logarithmic terms

$$\log \phi = b \log W + \log a. \quad (3)$$

#### 2.4. Statistical analysis

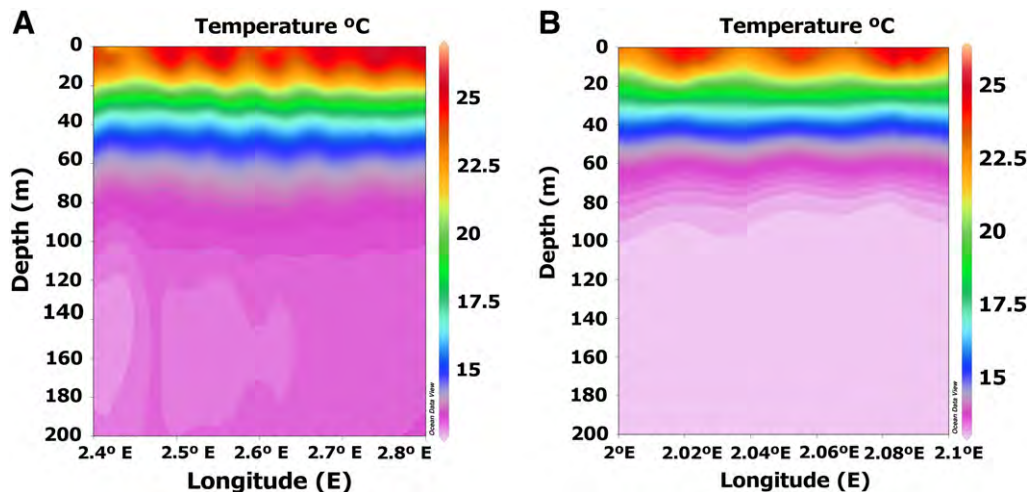
The data were analyzed using the program R Development Core Team 2010 (R Foundation for Statistical Computing, Vienna, Austria). To confirm normality,  $\phi$ , biomass and specific  $\phi$  data were analyzed by the Shapiro Wilk test and the homoscedasticity of the residuals was

assessed graphically,  $\phi$ -biomass and specific  $\phi$  data were not normal and statistical differences between stations, times of day and fractions were tested using Kruskal–Wallis test; and between regions of Cabrera and Sóller were assessed using Wilcoxon Mann–Whitney test. To study the correlation between  $\phi$ -biomass we obtain the regression equations, using a confidence limits of 95% and the Pearson correlation coefficient.

### 3. Results

#### 3.1. Hydrographic data

In both sampling sites the water column was stratified with a thermocline between 20 and 40 m in which the temperature dropped from 26 °C to 14 °C (Fig. 2). Surface chlorophyll-*a* values ranged from 0.06 to 0.15  $\text{mg m}^{-3}$  in Sóller and from 0.03 to 0.10  $\text{mg m}^{-3}$  in Cabrera, the deep chlorophyll maximum (DCM) was at 50–70 m depth with values between 0.20 and 0.50  $\text{mg m}^{-3}$  in Sóller, and in



**Fig. 2.** Temperature profiles showing the thermocline in A: Cabrera and B: Sóller. Transects used to obtain vertical profiles are shown in Fig. 1 (asterisks in bold).

**Table 1**Biomass, potential respiration ( $\phi$ ) and carbon demand for the zooplankton 53–100  $\mu\text{m}$  size.

[0.3 mm] Station	Sampling time	$\phi$	Biomass	sp. $\phi$	Carbon demand
		( $\mu\text{mol O}_2 \text{ h}^{-1} \text{ m}^{-3}$ )	( $\text{mg prot m}^{-3}$ )	( $\mu\text{mol O}_2 \text{ h}^{-1} \text{ mg prot}^{-1}$ )	( $\mu\text{mol C h}^{-1} \text{ mg prot}^{-1}$ )
S200	Midday	0.12	0.15	0.82	0.70
S200	Midnight	0.14	0.10	1.38	1.17
S200	Morning	0.11	0.09	1.14	0.97
S200	Midday	0.10	0.12	0.84	0.71
S200	Afternoon	0.11	0.04	2.78	2.37
S200	Midnight	0.07	0.08	0.97	0.82
S900	Morning	0.07	0.20	0.36	0.31
S900	Midday	0.09	0.09	1.01	0.86
S900	Midnight	0.14	0.17	0.84	0.71
S900	Afternoon	0.10	0.09	1.15	0.98
C200	Midday	0.28	0.16	1.77	1.51
C200	Midnight	0.20	0.13	1.52	1.29
C200	Midday	0.10	0.14	0.67	0.57
C200	Midnight	0.30	0.60	0.51	0.43
C200	Morning	0.11	0.04	2.92	2.48
C200	Midday	0.17	0.30	0.58	0.49
C900	Midnight	0.25	0.14	1.79	1.53
C900	Midday	0.10	0.07	1.48	1.25
C900	Afternoon	0.26	0.18	1.44	1.23
C900	Morning	0.25	0.54	0.46	0.39
C900	Midday	0.19	0.76	0.25	0.22

Cabrera the DCM was between 60 and 80 m with values between 0.21 and 0.27  $\text{mg m}^{-3}$ .

### 3.2. Zooplankton biomass, potential respiration ( $\phi$ ) and carbon demand

$\phi$ , biomass and carbon demand per volume of sea water were estimated for the different size fractions (53–200, 200–500, >500  $\mu\text{m}$ ) at different times of day (morning, midday, afternoon and midnight) at each station as shown in Tables 1, 2 and 3. Based on estimations of carbon demand (Table 5), and assuming an error associated with a seasonally varying uncertainty, we can estimate the average annual carbon demand. Zooplankton carbon demand due to respiration in the upper 200 m, was  $12.14 \pm 1.79 \text{ g C yr}^{-1} \text{ m}^{-2}$  in Cabrera, while in Sólter was  $7.17 \pm 1.32 \text{ g C yr}^{-1} \text{ m}^{-2}$ .

When we analyze data of each station and depth we found no significant differences between different times of day in  $\phi$  ( $p > 0.05$ ) and biomass ( $p > 0.05$ ). As a result we pooled all the biomass data from all sampling times. Significant differences between Cabrera and Sólter in  $\mu\text{mol O}_2 \text{ h}^{-1} \text{ m}^{-3}$  ( $p < 0.01$ ) for all sizes of zooplankton as shown in Fig. 3A and in total biomass ( $\text{mg protein m}^{-3}$ ), ( $p < 0.01$ ) as shown in Fig. 3B being higher in the area of Cabrera. Both variables are higher in the Cabrera region. Furthermore in Cabrera,  $\phi$  is greater over the continental shelf, (Fig. 4,  $p < 0.05$ ). The biomass is also greater over the shelf, but the difference is not significant (Fig. 4,  $p > 0.05$ ). In the Sólter region, no significant differences were observed in  $\phi$  ( $p > 0.05$ ) and in the biomass ( $p > 0.05$ ) between the shelf and continental slope stations as shown in Fig. 4.

In the Sólter region the contribution of  $\phi$  appears greater in the larger sizes, but this difference is not significant ( $p > 0.05$ ). However, if we

**Table 2**Biomass, potential respiration ( $\phi$ ) and carbon demand for the zooplankton 200–500  $\mu\text{m}$  size.

Station	Sampling time	$\phi$	Biomass	sp. $\phi$	Carbon demand
		( $\mu\text{mol O}_2 \text{ h}^{-1} \text{ m}^{-3}$ )	( $\text{mg prot m}^{-3}$ )	( $\mu\text{mol O}_2 \text{ h}^{-1} \text{ mg prot}^{-1}$ )	( $\mu\text{mol C h}^{-1} \text{ mg prot}^{-1}$ )
S200	Midday	0.09	0.20	0.44	0.38
S200	Midnight	0.23	0.27	0.87	0.74
S200	Morning	0.15	0.19	0.78	0.66
S200	Midday	0.14	0.15	0.93	0.79
S200	Afternoon	0.22	0.28	0.80	0.68
S200	Midnight	0.09	0.14	0.65	0.56
S900	Morning	0.06	0.02	2.59	2.20
S900	Midday	0.08	0.19	0.45	0.39
S900	Midnight	0.21	0.27	0.75	0.64
S900	Midday	0.17	0.22	0.79	0.67
S900	Afternoon	0.17	0.29	0.59	0.50
C200	Midday	0.32	0.39	0.81	0.69
C200	Midnight	0.47	0.58	0.81	0.69
C200	Midday	0.24	0.51	0.47	0.40
C200	Midnight	0.50	1.09	0.46	0.39
C200	Morning	0.24	0.50	0.49	0.42
C200	Midday	0.44	0.83	0.54	0.46
C900	Midnight	0.16	0.29	0.56	0.48
C900	Midday	0.11	0.28	0.41	0.34
C900	Afternoon	0.29	0.47	0.63	0.53
C900	Morning	0.17	0.37	0.46	0.39
C900	Midday	0.21	0.22	0.96	0.81



**Table 3**  
Biomass, potential respiration ( $\phi$ ) and carbon demand for the zooplankton >500  $\mu\text{m}$  size.

Station	Sampling time	$\phi$	Biomass	sp. $\phi$	Carbon demand
		( $\mu\text{mol O}_2 \text{ h}^{-1} \text{ m}^{-3}$ )	( $\text{mg prot m}^{-3}$ )	( $\mu\text{mol O}_2 \text{ h}^{-1} \text{ mg prot}^{-1}$ )	( $\mu\text{mol C h}^{-1} \text{ mg prot}^{-1}$ )
S200	Midday	0.20	0.50	0.40	0.34
S200	Midnight	0.27	0.81	0.33	0.28
S200	Morning	0.30	0.59	0.51	0.43
S200	Midday	0.13	0.19	0.68	0.57
S200	Afternoon	0.10	0.52	0.20	0.17
S200	Midnight	0.05	0.21	0.26	0.22
S200	Morning	0.17	0.12	1.36	1.16
S900	Morning	0.09	0.16	0.55	0.47
S900	Midday	0.04	0.14	0.30	0.26
S900	Midday	0.17	0.48	0.36	0.30
S900	Afternoon	0.19	0.76	0.25	0.21
C200	Midday	0.28	0.65	0.43	0.37
C200	Midnight	0.31	0.86	0.36	0.30
C200	Midday	0.30	1.09	0.28	0.24
C200	Midnight	0.14	0.60	0.24	0.20
C200	Morning	0.26	0.79	0.32	0.27
C200	Midday	0.17	0.54	0.32	0.27
C900	Midnight	0.26	0.96	0.27	0.23
C900	Midday	0.10	0.35	0.29	0.25
C900	Afternoon	0.15	0.43	0.35	0.30
C900	Morning	0.10	0.11	0.91	0.78

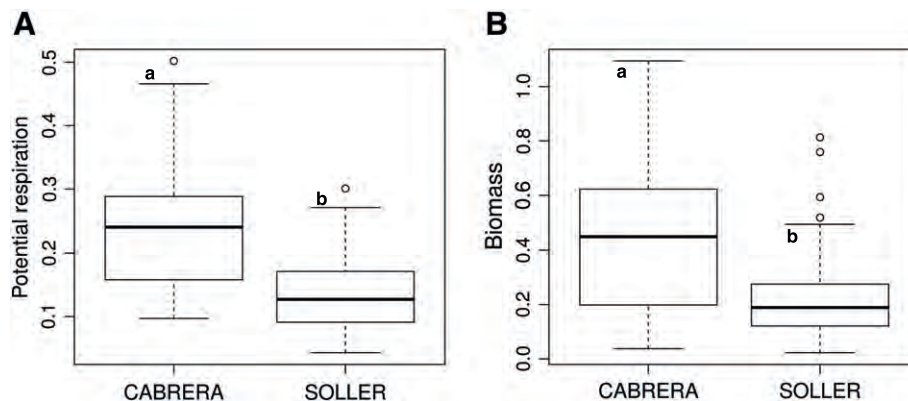
analyze the potential respiration per unit of biomass (specific  $\phi$ ) in smaller fractions it is significantly greater ( $p < 0.01$ ), (Fig. 5A and B). In Cabrera the greatest contribution to the  $\phi$  occurs in the fraction 200–500  $\mu\text{m}$  but it is not significantly different from the other fractions ( $p > 0.05$ ). On the other hand, specific was higher in the smaller size fractions ( $p > 0.01$ ), (Fig. 6A and B).

### 3.3. Relationship between potential respiration ( $\phi$ ) and biomass

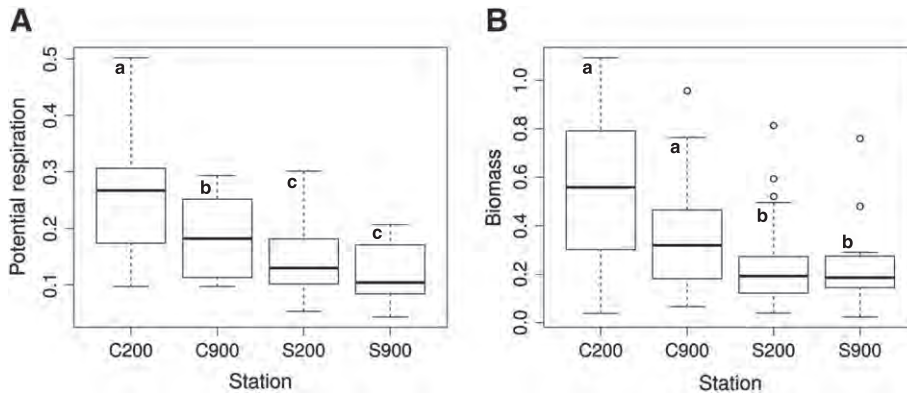
Fig. 7A shows the regression lines for Cabrera and Sóller and Fig. 7B shows them for different size fractions (53–200, 200–500, >500  $\mu\text{m}$ ). Table 4 shows the coefficients of regression and determination coefficients of each equation, and the 95% confidence intervals (CI) of the b coefficient. As shown in Table 4 the coefficient b was lower than 0.75 in both, Sóller ( $0.36 \pm 0.09$ ) and Cabrera ( $0.35 \pm 0.08$ ). In the pooled data the slope of the regression equation in the smaller size (53–200  $\mu\text{m}$ ) was lower than the other sizes, and had a lower correlation between  $\phi$  respiration and biomass (Table 4, Fig. 7B).

## 4. Discussion

The average biomass is higher in Cabrera than Sóller and decreases over the continental slope. Other authors have studied the abundance of zooplankton in the area, although the diversity of methods and nets used makes comparisons difficult. The study conducted by Fernández de Puelles et al., 2003 on seasonal and interannual variability at southern of Mallorca, during 1994–1999 in 3 stations at 75, 100 and 200 m depth using a Bongo net of 250  $\mu\text{m}$ , showed that the zooplankton decreases from the coast to the oceanic stations. During these 6 years the authors argue that the biomass of zooplankton was low, similar to that in other western Mediterranean areas (Fernández de Puelles et al., 2003). There were also peaks in late spring in neritic and coastal areas, and peaks in winter in most oceanic areas. Other authors also show the importance of winter mixing that enables the entry of nutrients from deep zones to the surface waters, this fertilization of the euphotic zone increases the primary and secondary production. In previous studies in the area between 2003 and 2004, although it was difficult to identify regularities in the temporal dynamics of zooplankton, biomass maxima occurred in late winter and summer (February



**Fig. 3.** A: Zooplankton potential respiration ( $\mu\text{mol O}_2 \text{ h}^{-1} \text{ m}^{-3}$ ), (average for all samples) and B: Biomass ( $\text{mg protein m}^{-3}$ ), (average for all samples); in Cabrera and Sóller. Different letters above boxes denote significant differences ( $p < 0.05$ ).



**Fig. 4.** A: Zooplankton potential respiration ( $\phi$ ) ( $\mu\text{mol O}_2 \text{ h}^{-1} \text{ m}^{-3}$ ) and B: Biomass ( $\text{mg protein m}^{-3}$ ); in each station at different depths. Different letters above boxes denote significant differences ( $p < 0.05$ ).

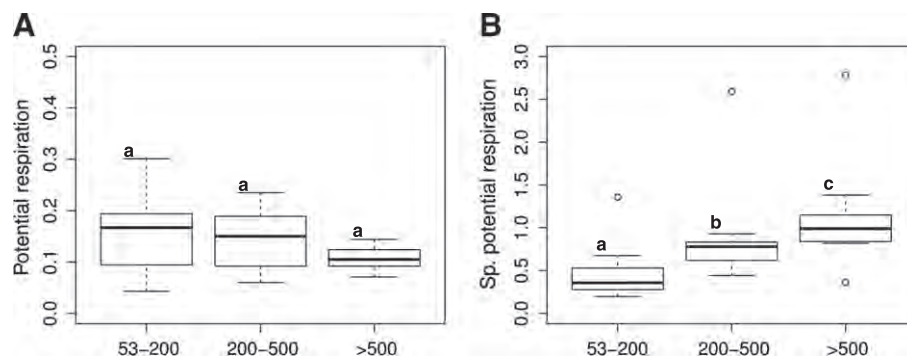
and June), (Cartes et al., 2008). We have carried out the sampling in the summer when there is a marked stratification and nutrients have not yet upwelled from deeper waters.

$\Phi$  is higher in the Cabrera region. It is related to biomass and since it is controlled by constituent mitochondrial proteins, it is a good living biomass predictor. However, it should be noted that although there is a good relationship between respiration and biomass, it is not a causal one, that is why higher zooplankton biomass in Cabrera is reflected in higher  $\phi$ . The biomass simply packages the enzyme complexes that constitute the ETS. This is seen clearly in Fig. 1 of West et al., 2002 and is evident from the measurements of Weibel & Hoppeler, 2005. It is the enzyme kinetics of these enzymes that is the causal basis of the ETS activity (Packard & Gómez, 2008). Note that here, as we speak of “ETS activity”, we are referring to the *in vitro* ETS activity, the activity measured by our enzyme assay for ETS activity. This contrasts with the *in vivo* ETS activity which is the time-varying ETS activity that changes with food availability, stress, and swimming activity. It is the *in vivo* ETS activity of the whole organism that should be the respiration rate.

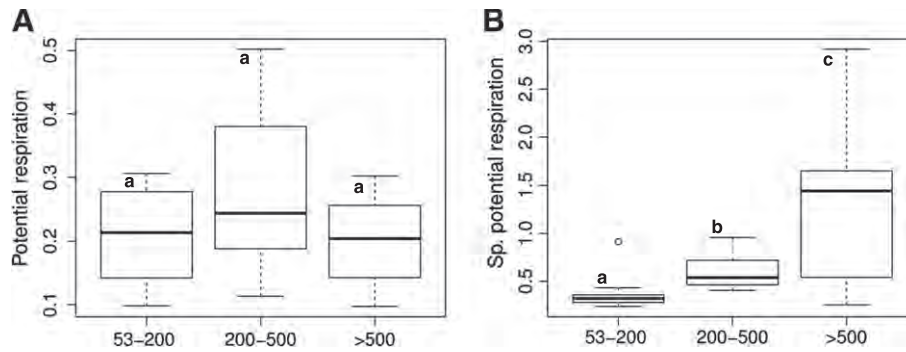
With regard to respiration and biomass, the relationship is also not causal and is even less coupled than the  $\phi$ -biomass relationship because of the influence of variables such as food availability, hormonal levels, and swimming activity (Weibel & Hoppeler, 2005). Herrera et al., 2011 has shown that food availability in mysids impacts respiration rate but not  $\phi$ . The relationship between biomass and  $\phi$  can be a good indicator of the physiological state of an organism. For many years the paradigm of “Kleiber’s law” (Kleiber, 1961)

has been accepted dogma in animal physiology. This “law” is based on an exponential relationship between biomass and respiration with a coefficient close to 0.75 or 3/4. Recently, the metabolic theory of ecology (MTE) was developed on the basis of Kleiber’s law (Brown et al., 2004) and has been touted by Whitfield, 2006 as biology’s new unifying theory. However, even more recently, many authors have found variations in Kleiber’s coefficient (b) in different taxonomic groups (Atanasov, 2010; Glazier, 2005, 2006). These findings shed doubt on the reliability of this exponent and argue that it is not a constant for all species and sizes of organisms as claimed (Dodds et al., 2001; Riisgård, 1998).

Packard & Gómez, 2008 argue that the MTE and Kleiber’s law describe respiration over a large size range but not short periods of time or different physiological states; and propose the EKM based on potential respiration and availability of substrates, not in biomass. Aguiar-González et al., 2012 show this to be the case in experiments with marine bacteria. Field studies of zooplankton show different ratios close to or greater than 0.75 in optimal feeding conditions, and below 0.75 when the organisms are found in oligotrophic areas or poor feeding (Gómez et al., 2008; Herrera et al., 2011; Martínez, 2007; Packard & Gómez, 2008). In our results the coefficient b is lower than 0.75 in Cabrera and Sóller (Table 4) which suggests that the zooplankton is nutrient limited and that both areas are oligotrophic, this is consistent with the hydrographic data. However, the slope of the regression equation in the smaller sizes (53–200  $\mu\text{m}$ ) is lower than the other sizes, and has a lower correlation between  $\phi$  and biomass (Table 4). One reason may



**Fig. 5.** Sóller data. A: Potential respiration ( $\phi$ ) ( $\mu\text{mol O}_2 \text{ h}^{-1} \text{ m}^{-3}$ ) for the different size fractions (53–200, 200–500, >500  $\mu\text{m}$ ); B: Specific potential respiration (sp.) ( $\mu\text{mol O}_2 \text{ h}^{-1} \text{ mg prot}^{-1}$ ) for the different size fractions (53–200, 200–500, >500  $\mu\text{m}$ ). Different letters above boxes denote significant differences ( $p < 0.05$ ).



**Fig. 6.** Cabrera data. A: Potential respiration ( $\phi$ ) ( $\mu\text{mol O}_2 \text{ h}^{-1} \text{ m}^{-3}$ ) for the different size fractions (53–200, 200–500, >500  $\mu\text{m}$ ); B: Specific potential respiration (sp.) ( $\mu\text{mol O}_2 \text{ h}^{-1} \text{ mg prot}^{-1}$ ) for the different size fractions (53–200, 200–500, >500  $\mu\text{m}$ ). Different letters above boxes denote significant differences ( $p < 0.05$ ).

be that this fraction had greater diversity of organisms including autotrophs, herbivores and omnivores. Conover, 1960 showed that carnivorous zooplankton has higher respiratory rate than herbivorous zooplankton did. Furthermore, because we know from the work of Herrera et al., 2011 that the nutritional state of the zooplankton is a factor, the low values of b in the small zooplankton could indicate that they were undernourished. As expected  $\phi$  generally increased, while specific  $\phi$  decreased with increasing body weight, this indicates that the small size fractions are metabolically more active, Ikeda, 1970 found similar correlations in studies in different groups of planktonic animals.

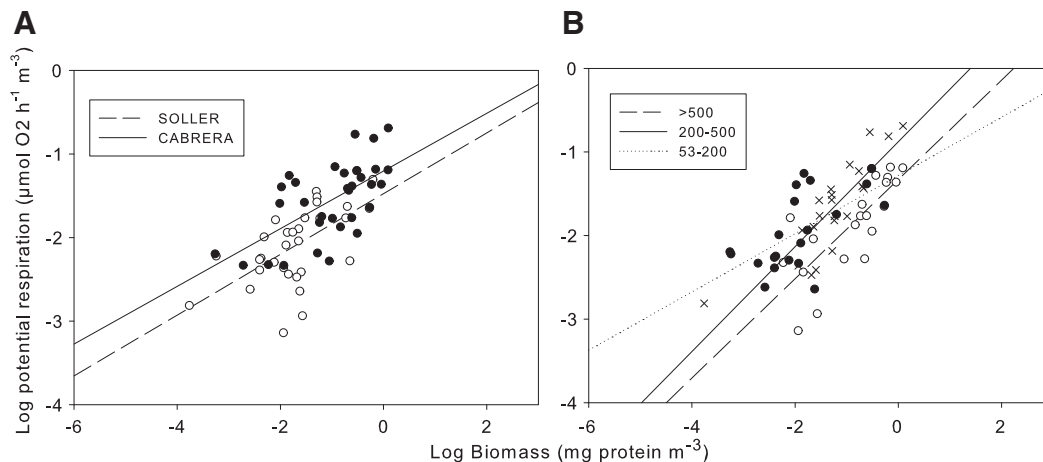
Comparing our biomass and  $\phi$  data with those published by other authors, the biomass values obtained are lower than in the tropical eastern North Pacific (King et al., 1978) and in areas of upwelling (Packard, 1979) which can be two orders of magnitude greater than those obtained in the Sóller stations. In contrast, compared with the values obtained by Minutoli & Guglielmo, 2009 in different Mediterranean areas, the biomass is similar to some of our stations, but  $\phi$  values are lower, resulting in a lower specific  $\phi$  and lower carbon demand per cubic meter in these areas of the Mediterranean Sea (Table 5). Considering carbon demands presented in Table 5, it seems clear that Sóller and Cabrera are oligotrophic areas, however compared to the values presented by Minutoli & Guglielmo, 2009 it seems that within the Mediterranean, the Balearic Sea is an area richer in nutrients and supports, via the food chain, a zooplankton energy demand that is up to 10 times higher in this area than outside.

Frontal mesoscale events between Mediterranean waters and waters of Atlantic origin, and input of cold northern water into the channels (Fernández de Puelles et al., 2004; Pinot et al., 1995), could act as fertilization mechanisms that increase productivity off the Balearic Islands.

Here, we estimated the average annual carbon demand from epipelagic zooplankton respiration. According to data of primary production (PP) obtained by Bosc et al., 2004 from the 4-year SeaWiFS time series over the 1998–2001 period, our values represent a 19.7% of PP in Cabrera and 12% in Sóller. Assuming that the study area is a moderately productive (PP between 250 and 1000  $\text{mg C d}^{-1} \text{ m}^{-2}$ ), carbon consumption by metazooplankton (>200  $\mu\text{m}$ ) is 22.2% of the PP according to Calbet, 2001. Of this carbon, an average of 25% is loss by respiration, which means that 5.5% of the PP is consumed due to respiration (Calbet, 2001). These estimates are consistent with the data obtained in this study using the ETS technique, if we consider that our carbon demand estimate is the “potential” carbon demand, and in this estimate is included the microzooplankton (53–200  $\mu\text{m}$ ) demand.

## 5. Conclusions

1. Potential respiration and biomass per cubic meter in epipelagic western Mediterranean zooplankton were higher over the Algerian sub-basin than over the Balearic subbasin.



**Fig. 7.** Relationship between potential respiration ( $\phi$ ) and biomass for A: Cabrera and Sóller and B: different size fractions (53–200, 200–500, >500  $\mu\text{m}$ ) around the Balearic Island.

**Table 4**

Coefficients of regression equation  $\log \phi = b \log W + \log a$  ( $\pm 95\%$  CI) for different areas and size fractions.  $\phi$  = potential respiration ( $\mu\text{mol O}_2 \text{ h}^{-1} \text{ m}^{-3}$ ),  $W$  = biomass ( $\text{mg prot m}^{-3}$ ).

Data	a	b	r <sup>2</sup>	n	p-value
Sóller	-1.47 ± 0.16	0.36 ± 0.09	0.37	32	<0.01
Cabrera	-1.20 ± 0.10	0.35 ± 0.08	0.39	32	<0.001
53–200 $\mu\text{m}$	-1.29 ± 0.22	0.35 ± 0.11	0.37	21	<0.01
200–500 $\mu\text{m}$	-0.88 ± 0.13	0.63 ± 0.09	0.73	22	<0.001
>500 $\mu\text{m}$	-1.32 ± 0.13	0.59 ± 0.12	0.58	21	<0.001

2. The Kleiber coefficient for the zooplankton in these two regions near the Balearic Islands was less than the so-called standard value of 0.75. We argue that this low value indicates that the zooplankton was under nourished.

3. Carbon demand estimated from epipelagic zooplankton ETS activity represented 19.7% of primary production in Algerian subbasin and 12% in Balearic subbasin.

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**Table 5**

Review of published data of biomass and potential respiration ( $\phi$ ) calculated from ETS activity. data were expressed as  $\mu\text{L O}_2 \text{ h}^{-1} \text{ m}^{-3}$ , protein data were converted to dry weight using the dry weight–protein ratio of 5.2 (Postel et al., 2000).

Reference	Size ( $\mu\text{m}$ )	Region	st	Deep	Biomass (mg dry wt.)	$\phi$ ( $\mu\text{L O}_2 \text{ h}^{-1} \text{ m}^{-3}$ )	sp. $\phi$ ( $\mu\text{L O}_2 \text{ h}^{-1} \text{ DW}^{-1}$ )	C demand ( $\mu\text{g C h}^{-1} \text{ m}^{-3}$ )	
Present work	53–200	Western	S200	0–200	0.50	2.42	5.69	1.10	
	53–200	Mediterranean	S900	0–200	0.72	2.30	3.62	1.05	
	53–200		C200	0–200	1.19	4.38	5.72	1.99	
	53–200		C900	0–200	1.76	4.71	4.68	2.15	
	200–500		S200	0–200	1.08	3.48	3.21	1.59	
	200–500		S900	0–200	1.03	3.11	4.46	1.41	
	200–500		C200	0–200	3.37	8.25	2.57	3.76	
	200–500		C900	0–200	1.68	4.23	2.59	1.93	
	>500		S200	0–200	2.19	3.91	2.29	1.78	
	>500		S900	0–200	2.00	2.76	1.58	1.26	
	>500		C200	0–200	3.92	5.44	1.40	2.48	
	>500		C900	0–200	2.40	3.42	1.97	1.56	
	King et al. (1978)	>212	Eastern tropical	2	0–83	2.86	15.54	5.44	7.08
		>212	North Pacific	6	0–79	4.97	37.34	7.51	17.00
>212			10	0–44	4.75	27.73	5.84	12.63	
>212			14	0–78	4.18	26.15	6.26	11.91	
>212			18	0–79	8.34	64.05	7.68	29.17	
>212			20	0–68	30.74	257.35	8.37	117.19	
>212			25	0–70	14.43	126.71	8.78	57.70	
>212			29	0–65	31.08	238.46	7.67	108.59	
>212			33	0–65	20.46	152.92	7.47	69.63	
>212			34	0–65	27.38	108.00	3.94	49.18	
>212			37	0–65	22.15	139.08	6.28	63.33	
>212			40	0–78	10.10	34.74	3.44	15.82	
>212			44	0–78	7.95	44.10	5.55	20.08	
>212			46	0–81	9.25	60.62	6.56	27.60	
Packard (1979)	>102	Northwest Africa	30	0–200	26.00	80.08	3.08	36.47	
	>102	Upwelling	31	0–200	59.00	150.45	2.55	68.51	
	>102		36	0–200	32.00	213.44	6.67	97.19	
	>102		37	0–200	19.50	197.93	10.15	90.13	
	>102		62	0–200	14.50	136.01	9.38	61.93	
	>102		70	0–200	13.00	147.55	11.35	67.19	
	>102		78	0–200	20.00	269.60	13.48	122.76	
	>102		85	0–200	11.50	156.06	13.57	71.06	
	>102		89	0–200	14.50	184.01	12.69	83.79	
	>102		97	0–200	19.50	95.55	4.90	43.51	
	>102		99	0–200	5.00	133.00	26.60	60.56	
	>102		104	0–200	11.00	95.48	8.68	43.48	
	>102		105	0–200	9.50	87.02	9.16	39.63	
	>102		119	0–200	13.00	62.53	4.81	28.47	
>102		122	0–200	18.00	165.06	9.17	75.16		
Minutoli and Gugliemo (2009)	>335	Western	VA	0–200	0.79	0.18	0.23	0.08	
	>335	Mediterranean	V4B	0–200	1.22	0.42	0.34	0.19	
	>335		V3B	0–200	0.64	0.23	0.35	0.10	
	>335		V1A	0–200	0.90	0.20	0.23	0.09	
	>335		V2	0–200	0.55	0.14	0.25	0.06	
	>335	Eastern	V6	0–200	0.39	0.12	0.31	0.05	
	>335	Mediterranean	V7	0–200	0.50	0.14	0.31	0.06	
	>335		V8	0–200	0.26	0.08	0.32	0.04	
	>335		VIERA	0–200	0.40	0.12	0.33	0.06	
	>335		V10	0–200	1.01	0.35	0.36	0.16	



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