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Assessing the relationship between R and ETS in an ocean acidification experiment

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Abstract

The ETS (electron transport system) method has been widely applied in aquatic ecosystems to estimate planktonic respiratory activity. As with other enzymatic methods, the ETS represents a proxy of activity, which needs to be transformed to actual respiration rates (R) by means of R/ETS ratios. Paradoxically, few studies have focused on the variability of R/ETS, and most of these were performed with single organisms under culture conditions. Here, we explore, by the first time, the variability of R, ETS and R/ETS of natural microplanktonic communities enclosed inside mesocosms, treated with different CO_2 additions. The experiment lasted 30 days, during which the ecosystem underwent abrupt changes in community structure through a transition from oligotrophy to highly eutrophic conditions after nutrient-induced fertilization. R and ETS increased significantly during the phytoplankton bloom period at the two mesocosms with higher CO_2 concentrations, coinciding with a rise in diatoms and heterotrophic bacteria cell numbers. However, we did not find any clear relationship between R/ETS and community structure or biomass. R and ETS were weakly, but significantly, correlated considering all the mesocosms. Nevertheless, the R/ETS ratio was highly variable, ranging more than 3 fold in magnitude during the experiment. Average R/ETS (both before and after fertilization) were >1, which is an unexpected result, assuming that ETS represents potential respiration rates. Together with the meagre published literature on the topic, our results suggest that R/ETS may be too variable as to apply constant ratios to different marine communities and environmental conditions. Thus, until we understand the factors driving the R/ETS changes and are able to constrain the range of variability of the ratio we should be cautious when deriving actual respiration from ETS, unless we measure the two variables at the same time during fieldwork experiments and cruises.

Introduction

Respiration is a key factor in organic carbon utilization and energy flow in oceanic ecosystems. It is regarded to be an ubiquitous process, occurring at all depths and in all regions, so it can be used as an indicator of metabolism in terms of individual, population and community for marine ecology studies (Bangqin et al. 2005). In addition, the significant role of the oceanic biological pump in controlling atmospheric CO_2 levels has recently been emphasized. The equilibrium between pCO_2 in the atmosphere and in the ocean surface is largely controlled by the photosynthetic fixation and the subsequent reoxidation in both euphotic and deep layers. Thus, it is essential to make reasonable estimates of the rates of oxygen consumption (respiration and oxidation) and primary production within the communities to fully understand the carbon cycle and evaluate the role of the oceanic areas as sources or sinks of CO_2.

The rates of oxygen consumption are generally low and consequently difficult to measure in the ocean, particularly in the deep cold waters. Hence, various attempts have been made to overcome the problems associated with the estimates of plankton respiration (see review in Arístegui et al. 2005). In 1971, Packard developed an

enzymatic method, the electron transport system (ETS), as an index of potential respiration for planktonic organisms. The determination of ETS activity in plankton is thought to estimate, under saturation of substrates, the maximum overall capacity of the enzymes associated with the respiratory electron transport system (i.e., potential respiration) in both eukaryotic and prokaryotic organisms (Packard 1985). As Williams (1984) mentioned, there are several potential advantages in using the ETS assay to infer respiration. For example, the high sensitivity of the assay, the capability of obtaining fast and fine details of the respiratory metabolism distribution, and the avoidance of in vitro effects, especially when working in oligotrophic or deep waters, where the more direct techniques of measuring actual oxygen consumption are not sensitive enough. However, the main caveat of the method, as with any other enzymatic method, is that it only represents a proxy of activity, which needs to be transformed to actual rates, providing a ratio of conversion that can be constrained.

Since the introduction of the ETS method, several improvements were made to the assay (e.g. Kenner & Ahmed 1975) and different authors used the ETS approach to estimate the respiratory activity of specific components of marine plankton (King & Packard 1975; Kenner & Ahmed 1975; Christensen et al. 1980; Bidigare et al. 1982; Finlay et al. 1983; Packard et al. 1983; Packard 1985; Hernández-león 1988; Schalk 1988; Martinez 1991). Paradoxically, few studies focused on the most relevant aspect: looking at the relationship between actual respiration (R) and ETS, and hence at the variability of the R/ETS ratio, with the aim to elucidate whether ETS could be universally used to derive actual respiration in freshwater and marine ecosystems (Packard & Williams 1981; Vosjan et al. 1987; Mimura et al. 1988; Vosjan et al. 1990; Arístegui & Montero 1995; Arístegui et al. 2005; Reinthaler et al. 2009). It has been found that empirical R/ETS ratios for bacteria, phyto- and zooplankton are very different and show a wide range of values and large standard deviation (SD) around each ratio (**Table 1**). This suggests that the relationship between ETS activity and R is not constant and may vary with the type of plankton community. In spite of this, R has been inferred in the past from ETS using different R/ETS ratios derived from natural communities (e.g. Packard & Williams 1981; Arístegui & Montero 1995), sometimes without any reasoning behind the selection of one or the other conversion ratio (e.g. Bangqin et al. 2005; Ramírez et al. 2006).

Indeed, it is necessary to constrain the variability of the R/ETS ratio in different surface waters and deep ocean natural communities, as well as to understand the main processes affecting the variability of this index. The scarce available data on ETS studies are difficult to compare, due to the specificity of the method that might vary between labs. Unfortunately, it has never been carried out a standardization exercise of the ETS assay

among the different users of the method. Moreover, the meagre database on R/ETS is insufficient to draw any clear conclusions on its variability. Consequently, every researcher should relate their ETS analyses with actual rates of oxygen consumption in order to obtain their own R/ETS for each study, until we have more robust information about the variability of the ratio. Unless this is done, respiration estimated from ETS and R/ETS ratios obtained from the literature won't be reliable and credible.

Table 1. Published empirical actual respiration to ETS ratios (R/ETS) and standard deviations (SD) for various groups of marine organisms and for freshwater and marine plankton communities.

Organisms	**R/ETS** (Average ±SD)	**Comments**	**References**
Bacteria	1.1±0.24	Cultures	Christensen et al. 1980
Bacteria	0.75±0.16	Cultures	Packard 1985
Diatoms	0.17±0.01	Cultures	Kenner and Ahmed 1975
Chlorophytes	0.17±0.04	Cultures	
Coccolithophorids	0.16±0.07	Cultures	
Dinophyceae	0.18±0.02	Cultures	
Isochrysis1	0.53	Cultures	Martínez 1992
Isochrysis1/	0.72	Cultures	
Isochrysis2	0.29	Cultures	
Isochrysis2/	0.56	Cultures	
Thalassiosira1	0.53	Cultures	
Thalassiosira2	0.64	Cultures	
Thalassiosira1/	0.92	Cultures	
Chaetoceros1	0.99	Cultures	
Chaetoceros2	0.98	Cultures	
Tetraselmis1	0.67	Cultures	
Tetraselmis2	0.99	Cultures	
Protozoa	0.25±0.02	Cultures	Finlay et al. 1983
Microplankton	0.16	NO Africa upwelling	Packard et al. 1974
Microplankton	0.32	-	Owens & King 1975
Microplankton	0.18	Eutrophic lakes	Jones & Simon 1979
Microplankton	0.34	Gulf of Maine	Packard & Williams 1981
Microplankton	0.33	-	Packard 1985a
Microplankton	3.01±3.75	NO Mediterranean	Mimura et al. 1988
Microplankton	0.32	Sea if Banda	Vosjan & Nieuwland 1987
Microplankton	0.13	Eutrophic lakes	Span 1988
Microplankton	0.23±0.71	North Sea	Vosjan et al. 1990
Microplankton	0.72±0.06	Oligotrophic lakes	Toth & Drits 1991
Microplankton	0.32±0.16	Oligotrophic lakes	Del Giorgio 1992
Microplankton	0.89±0.4	Oceanic regions (superf. waters)	Arístegui & Montero 1995
Microplankton	1.14±0.22	NA Central Ocean (superf. waters)	Hernández et al. 1999
Microplankton	0.68±0.11	Mesopelagic NE Atlantic	Arístegui et al. 2005
Bacterioplankton	5.3	NADW (4000m)	Reinthaler et al. 2006

With the aim of contributing to disentangle the causes and magnitude of variability of the R/ETS, and its relationship with community structure, we estimated R and ETS on natural marine communities enclosed inside mesocosms, along an ocean-acidification experiment. The objective of our work was twofold. First, investigate how the variability in the structure and biomass of the autotrophic and heterotrophic communities, under different trophic conditions (oligotrophic vs eutrophic environments), may affect the R/ETS index. We hypothesized that the R/ETS ratio would not be constant, but rather vary with changes in the contribution of different groups to the planktonic community, as well as in the ratio of autotrophic to heterotrophic organisms. Second, examine the effects of acidification on R, ETS and R/ETS, simulating future pCO_2 scenarios due to Climate Change forced by anthropogenic activities.

Methods

Experiment design and sampling

The experiment was carried out during the KOSMOS16 project at the Pier of Taliarte, Canary Islands, running from 2 March to 5 April 2016. Eight 1100 m^3 mesocosms tanks were installed and supplied with seawater pumped directly from the adjacent offshore by the GEOMAR team. Randomly chosen, different pCO_2 concentrations were applied to each tank: the gradient was from 400 μatm (daytime concentration at the atmosphere and sea surface) up to 1450 μatm (simulating a future scenario of an acidified surface ocean). We chose the following mesocosms for our study: MK5, MK6, MK7 and MK8 with 400, 1450, 700 and 1150 μatm respectively.

The sampling procedure was designed in order to collect the entire water column of the mesocosms at every extraction. Water was collected every two days from the beginning of the experiment until the depletion of nutrients (t17). After seventeen days (t18), nutrient enriched water was added to all the mesocosms, and the sampling changed to a daily basis in order to accurately follow the development of the bloom (t19-t25). Over the last days (t25-t29), the sampling was again shift to every two days. Meanwhile, complementary experiments were run within the mesocosms to study the effects of the acidification on a large number of variables.

Community oxygen respiration

Water samples were collected in 10L plastic containers every sampling morning, and transported to the laboratory. Once in the room, variable volumes of seawater were transferred to smaller bottles for different measurements. Around 5L seawater from

each mesocosms was tempered from in situ temperature to 21ºC for the dissolved oxygen analysis.

Rates of oxygen consumption were measured directly as the difference in the dissolved oxygen between 125 mL borosilicate bottles incubated in the dark during 12-24 h, and controls (zero time). For each water sample, 4 replicates for initial oxygen determination and 4 replicates for the final determination (dark incubation) were filled using a silicone tube; appropriate homogenization and dispensation of the sample was required in order to avoid turbulence or bubble formation. Fixation of the initial replicates with the Winkler reagents, were carried out immediately before the filling of the bottles. For the final replicates, the fixation was performed 24 hours later. Incubations were accomplished inside small containers filled with enough water to cover the bottles once closed, in a dark and acclimatized room (21ºC).

The entire content of the initial and final bottles were titrated in about 3 min. The final end point of the titration was controlled by means of an automated precise oxygen titration system, with colorimetric end point detection (Williams & Jenkinson 1982).

Oxygen concentration was also measured using membrane-inlet mass spectrometry (MIMS) (Kana et al. 1994). Samples were taken from the same container as for the BOD bottles by inserting the outflow plastic tube into the bottom of a glass tube and allowing the water to overflow. Five replicate tubes for initial and final oxygen determination were filled this way for every sampling time. Each tube was capped with a plastic stopper. The tubes for initial oxygen concentration were analysed immediately after being filled, whereas, the final tubes were incubated during 12-24 hours inside thermostatic baths at 18ºC. Briefly, the method is based on the spectrometric determination of the ratio of argon to oxygen in the sample. The other gases in the sample can diffuse through a permeable membrane and be collected in a stream of helium. The oxygen concentration is then derived from this ratio by determining the solubility of argon corrected for salinity and temperature. The rates of oxygen consumption were calculated as a difference between initial and final dissolved oxygen. Furthermore, in order to test the linearity of oxygen consumption during the incubation time, we carried out time series measurements for some cases using MIMS (**Fig. 1**).

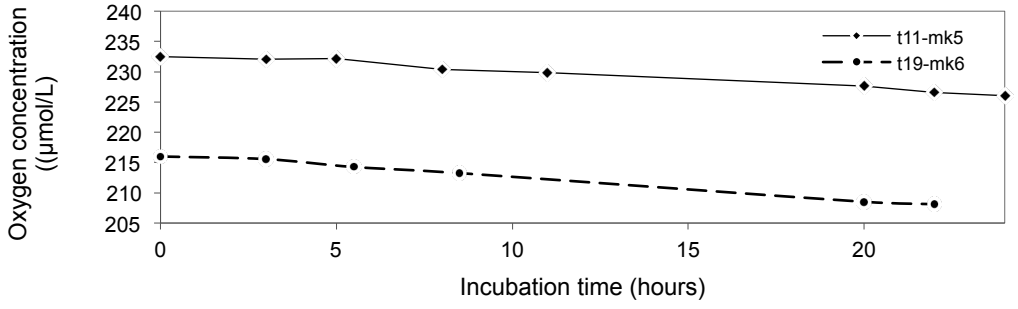


Figure 1. Oxygen consumption, measured with membrane-inlet masses spectrometry (MIMS), along the incubation time of water samples of MK5 (t11) and MK6 (t19).

ETS activity measurements

ETS activity was measured by means of the tetrazolium reduction technique according to Arístegui and Montero (1995). The assay was first proposed by Packard (1971) and modified by Kenner & Ahmed (1975). It is based on the oxidoreductase activity of tetrazolium when interacts with the coenzyme Q-cytochrome B complex of the ETS and the consequent INT-formazan production. The amount of INT-formazan produced can be measured from its absorption at 490 nm and be converted to the equivalent oxygen utilization.

During the first sampling times of the experiment, 3L of each sample were filtered through a Whatman GF/F filters (47 mm diameter) applying a gentle vacuum. Due to the high concentration of particulate material in all the mesocosms after several days, the filtration procedure was adjusted to 1.5 hours in order to avoid long filtration times and damage the sample. Filters were folded into cryovials, flash-frozen in liquid nitrogen and stored at -80ºC until assayed. Back in the laboratory, filters were homogenized in 2.8 mL of M phosphate buffer at (0-4ºC) for 1 min in a Teflon glass tissue grinder. The filter homogenate was transferred to a graduated glass tube to record the total volume. For each sample, 3 cryovials were prepared and kept at (0-4ºC): two replicates with 0.5 mL substrate solution and 0.35 mL 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT) solution, and a third one with 0.5 mL blank solution and 0.35 mL water. A volume of 0.9 mL of the filter homogenate was added to the 3 cryovials and immediately they were incubated for 15 min at 18ºC in a thermostatic bath. After the incubation, the reaction was quenched with 0.25 mL of a solution of phosphoric acid and concentrated formalin. The quenched reaction mixture was centrifuged for 15 min at 18ºC at 1400 RPM and stored on ice. The volume was recorded and the supernatant transferred to a new tube for a second centrifugation during 10 min. The absorbances at 490 nm and 760 nm were read within 2 hours at room temperature on a spectrophotometer. The 490 nm peak is proportional to INT-formazan production and the 760 nm peak measures the turbidity of the sample. In addition, filter and reagent blanks were run daily. The first one by filtering Milli-Q water and assaying the homogenate as the other samples, and the second by assaying just the reactants.

ETS activity was calculated by the following equation:

$$ETS_{ASSAY}(\mu l\ O_2 l^{-1} h^{-1}) = 60 \times H \times S \times (corr\ OD)/(1.42 \times V \times f \times t)$$

where 60 converts minutes to hours, H is the homogenate volume (in mL), S is the volume of the quenched reaction mixture (in mL), corr OD is the absorbance of the sample at 490 nm corrected for blank absorbance, V is the volume of seawater filtered

(in L), f is the volume of the homogenate used in the assay (in mL), t is the incubation time (in min), and 1.42 converts the INT-formazan formed to oxygen units (in µL).

$$ETS_{SITU} = ETS_{ASSAY} \times e^{\left(Ea/R \times \left(1/T_{ass} - 1/T_{is}\right)\right)}$$

Where *Ea* is the Arrhenius activation energy (in kcal mol^{-1}), R is the gas constant, T_{ass} and T_{is} are the assay and *in situ* temperatures (in Kelvin degrees).

Size fractionation of chlorophyll a

Size fractionation was prepared for chlorophyll a determination by filtering 100-250 mL of the different seawater samples (MK5- MK8) through a filtration tower mounted with different pore-size polycarbonate membrane filters (0.2, 2 and 20 µm). Filtration for total chlorophyll a was performed in the same way as explained above, through 0.2 µm pore-size polycarbonate membrane filters.

Chlorophyll a was measured fluorometrically following the recommendations of Yentsch & Menzel (1963) and Holm-Hansen et al. (1965), as described in Strickland & Parsons (1960). The bench fluorometer (Turner Design) required a previously calibration with pure chlorophyll a (Sigma Co.). Pigments were extracted from the filters in cold acetone (90%) during 24h and the acetone extracts were further acidified in order to subtract the pheopigment values from the original values representing total pigments.

Prokaryotic and eukaryotic abundances determined by flow cytometry

Water samples (2mL) for bacteria determination were collected, fixed with 1% paraformaldehyde (final concentration) and stored at -80ºC until analysis (Kamiya et al. 2007). Phytoplankton groups were analysed on fresh samples (4ml). Because of the autofluorescence of phytoplankton, fixation of the samples previous to the cytometric analysis was not necessary. Samples were analysed by flow cytometry (FACSCalibur, Becton Dickinson), with a 15 mW laser set to excite at 488 nm. Subsamples (400 µL) for determination of heterotrophic bacteria were stained with the fluorochrome SYBR Green I (4 µL) at room temperature for 20 minutes (Marie et al. 1997). Different flows were applied depending on each group: for bacteria we used a flow rate of 16 µL min^{-1} during 1.5 minutes and for Synechococcus and picoeukaryotes, a flow rate of 60 µL min^{-1} during 2.5 minutes. Nanoeukaryotes were counted on fresh samples with a Cytobuoy cytometer, provided with flow-image, using a flow rate of 300 µL min^{-1} during 7 minutes.

Measurements of the side scatter and fluorescence emission at 530 nm permitted the enumeration of the total bacterial population. The rest of cells in unstained samples emitted autofluorescence, which was measured with orange (585 nm) and red (>650 nm) detectors. These autofluorescence properties (FL2 and FL3) and side scatter differences were used to distinguish and enumerate three groups of phytoplankton: Synechococcus spp., picoeukaryotes and nanoeukaryotes (Olson et al. 1993; Zubkov et al. 1998;). The calibration of the flow rate was made every 10 samples. Data acquisition and analysis were done with Cell-Quest software (Becton Dickinson).
Biomass of Synechococcus, picoeukaryotes and heterotrophic bacteria were estimated by multiplying the abundance data obtained by flow cytometry, by the average cell carbon content calculated from each group of organism biovolume, using the conversion factors obtained by Montero et al. (unpublished) from samples collected in coastal waters of Gran Canaria (**Table 2**). Nanoeukaryotes abundances were converted into biomass using the corresponding size average biovolumes obtained by Montero et al. (unpublished) from oceanic waters near Cape Guir (NW Arica) (**Table 2**) and applying the conversion factor of 220 fgC μm^{-3} proposed by Borsheim & Bratbak (1987).

Table 2. Cell carbon content and cell biovolume averages used for biomass determination.

Synechococcus	**Picoeukaryotes**	**Heterotrophic bacteria**		**Nanoeukaryotes**	
		LNA	HNA	Small size	Large size
100 fgC $cell^{-1}$	444 fgC $cell^{-1}$	18 fgC $cell^{-1}$	46 fgC $cell^{-1}$	25 μm^{-3} /cell	100 μm^{-3} /cell

Results

Community respiration and ETS activity

The rates of respiration along the whole experiment ranged from 3.31 to 13.54 μmol O_2 d^{-1} L^{-1} in all the mesocosms. Despite the different treatments of pCO_2 within the mesocosms, a general trend was observed (**Fig. 2**). The community showed variable respiration, ranging about two fold, during the first days of the experiment (Period 1: from t-1 to t19). A more pronounced increase in oxygen consumption was noted after the fertilization (Period 2: from t19 to t29) in all the tanks (MK5, MK6 and MK8), with the exception of MK7, where the bloom showed a delay of 2-3 days with respect to the other treatments since the nutrient addition was performed later. Indeed, no significant differences were observed in average R among the treatments (ANOVA, $P > 0.05$), in spite that the plankton community in MK6 consumed 29% more dissolved oxygen than in MK7 (t-test, $P < 0.05$) (**Fig. 3A**). Furthermore, comparing the development of respiration and ETS, we found that during the first period, R was significantly higher

than ETS in all the mesocosms (Student's t-test, $P < 0.01$). However, during the second period R and ETS were statistically similar in all the treatments, except in MK7 (Student's t-test, $P < 0.01$). ETS activity presented a similar range of variability than R along the experiment, and the highest values after the bloom were also observed in MK6 and MK8 (**Fig. 3B**).

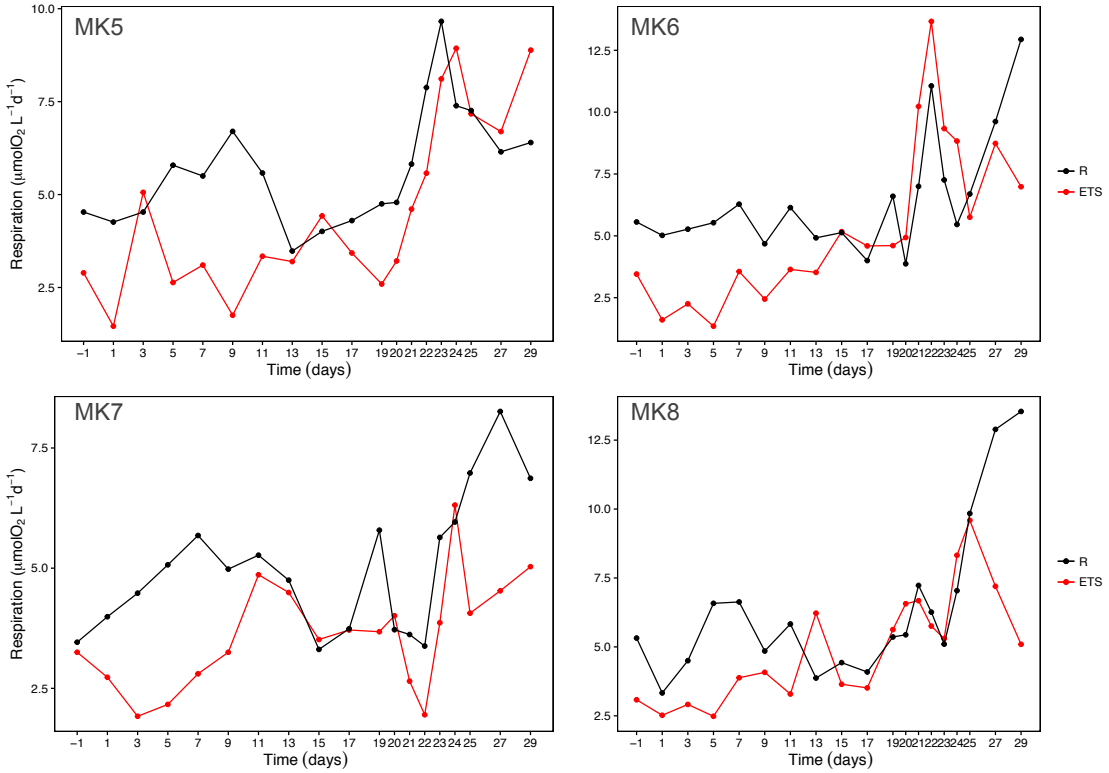


Figure 2. Temporal evolution of oxygen consumption (respiration rates, R) and ETS activity at each of the studied mesocosms.

Table 3. Average (±SD) ratios of R/ETS, ETS/Chla and A/H (Autotrophic to heterotrophic biomass of picoplankton and nanoplankton) from the two periods of the experiment: before (Period 1: t1-t19) and after (Period 2: t20-t29) fertilization.

		Period 1	Period 2
R/ETS	Mk5	1.81 ±0.94	1.19 ±0.36
	Mk6	1.98 ±0.99	1.02 ±0.41
	Mk7	1.48 ±0.56	1.43 ±0.33
	Mk8	1.49 ±0.54	1.25 ±0.60
ETS/Chl	Mk5	6.42 ±3.72	6.89 ±3.02
	Mk6	6.56 ±3.98	6.26 ±2.22
	Mk7	7.18 ±4.31	5.76 ±5.24
	Mk8	6.16 ±3.27	4.74 ±2.07
A/H	Mk5	1.33 ±0.56	1.27 ±0.19
	Mk6	1.46 ±0.59	0.99 ±0.26
	Mk7	1.27 ±0.43	1.54 ±0.54
	Mk8	1.49 ±0.71	0.97 ±0.17

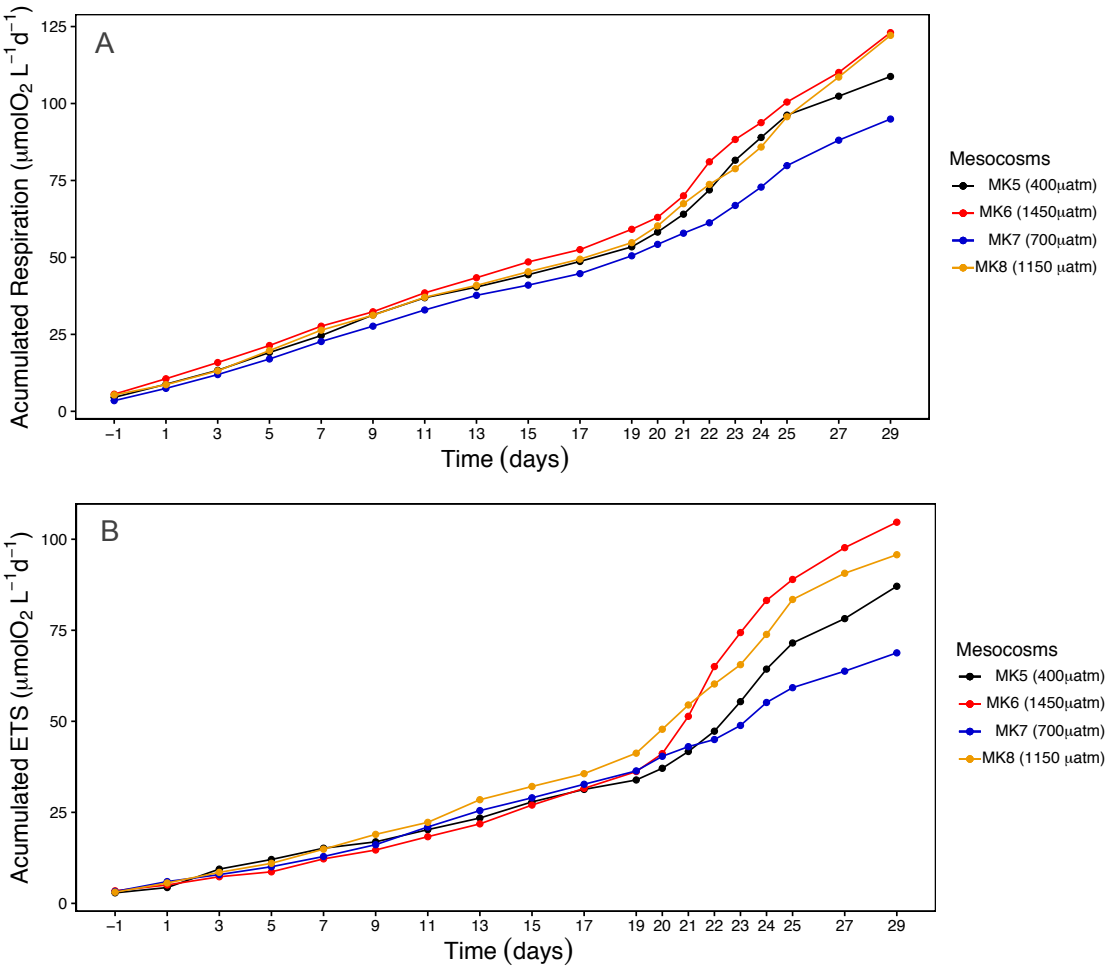


Figure 3. Accumulated respiration (A) and ETS activity (B) along the experiment.

Relationship between R and ETS

In order to compare different variables with the respiration data set, our data were log transformed to achieve normality. First, we looked for a common relationship between R and ETS for the whole experiment (**Fig. 4**). The two variables showed a significant, although relatively weak, positive correlation ($r^2 = 0.3$, $P < 0.001$). The regression equation obtained is:

$$\log R = 0.359 \log ETS + 0.526 \quad (1)$$

n=76 r^2=0.31 p-value < 0.001

Being *R* and *ETS* expressed in µmol O_2 L^{-1} d^{-1}

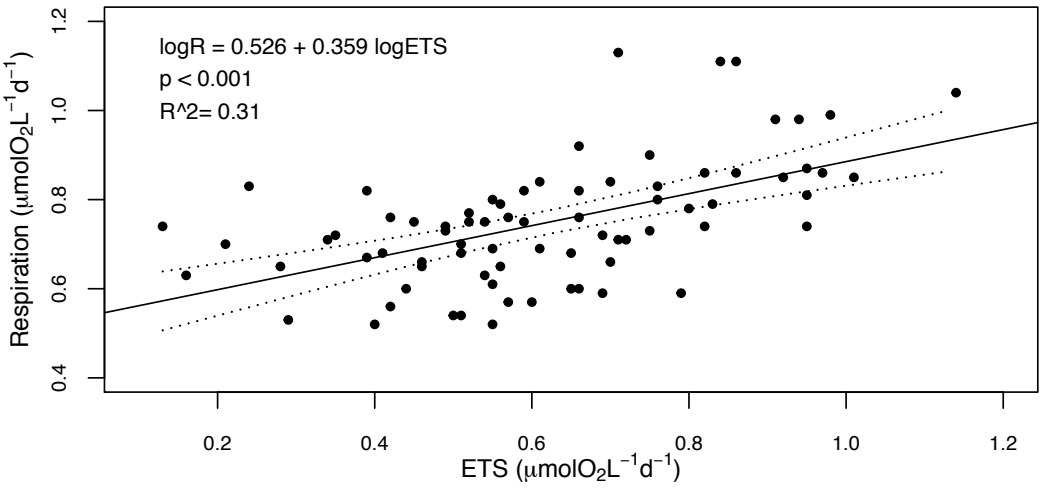
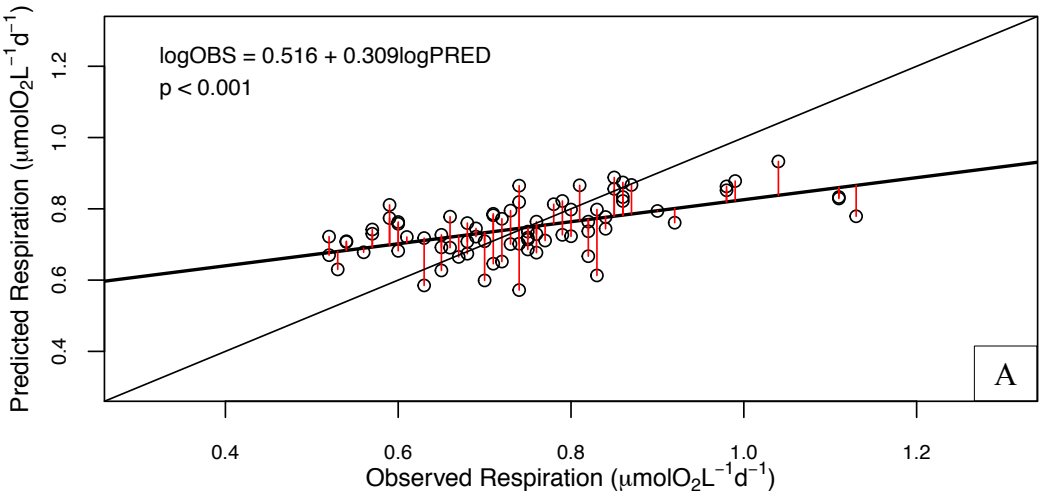


Figure 4. Relationship between community respiration (R) and ETS activity for the whole data set. Data are log transformed for the regression analysis. The line of best fit (straight line) and the confidence intervals (dashed lines) are shown.

The performance of this equation can be assessed as a plot of predicted versus observed values (**Fig. 5A**). Moreover, we have tested the mean value of R/ETS of all the studied mesocosms (1.47 ±0.68, n=76) to predict respiration rates (**Fig. 5B**). Despite the slope of the regression equation obtained in the second prediction is not significantly different from unity (P<0.001), the use of a constant ratio more than doubles the residual standard error obtained in equation 1: from 6.4% to 17.9%. The R/ETS ratios for all the mesocosms were generally higher during the first period (**Fig. 6**). The lower ratio values were found between t15 and t17, just before fertilization, when nutrients were completely depleted. After nutrient addition, the ratios fluctuated from 0.5 to 1.5 (**Fig. 6**). We did not find any significant correlation between R/ETS and pCO2, chlorophyll a or biomass (P > 0.05).



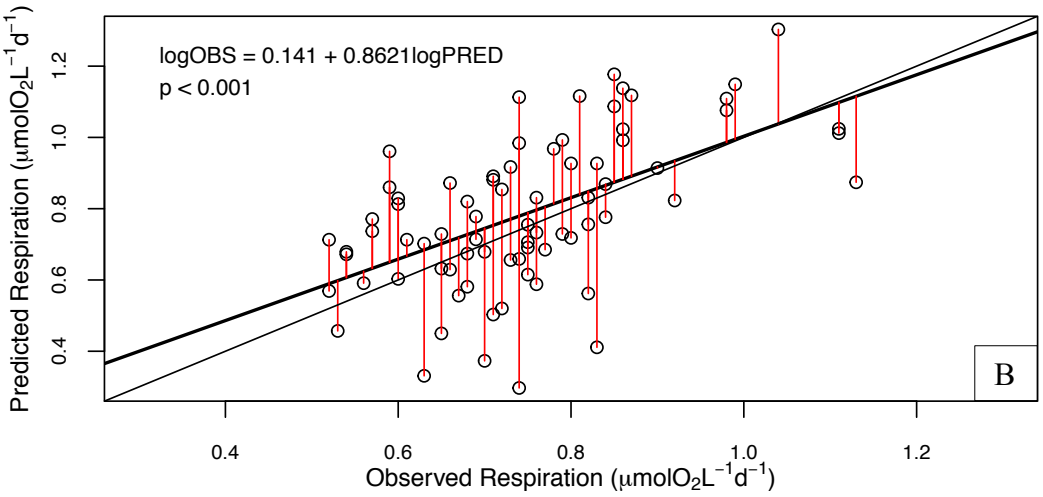


Figure 5. Observed plankton respiration as a function of the predicted respiration calculated from equation 1 (**A**). Observed plankton respiration as a function of predicted respiration using the mean respiration to ETS ratio of all the experiments R/ETS = 1.47; *n* = 76 (**B**). Data was log-transformed for the two analyses.

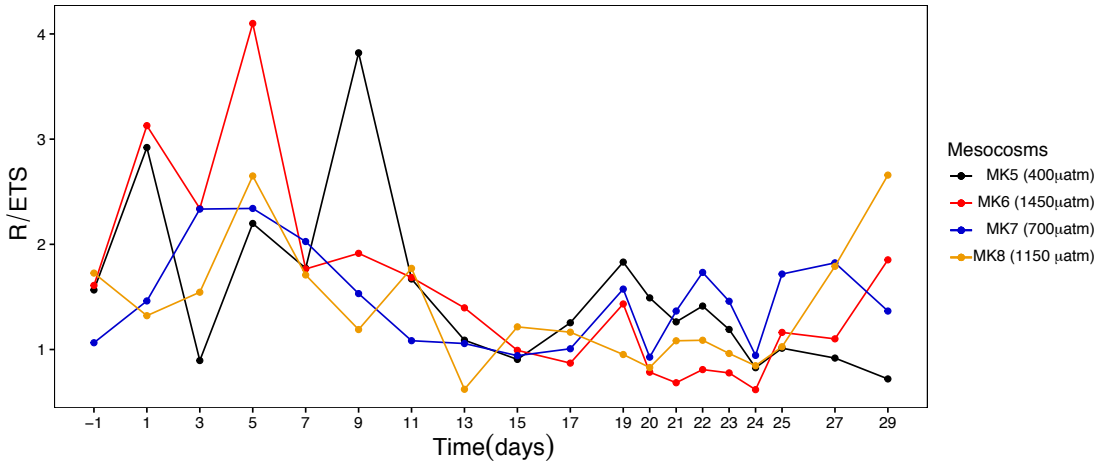


Figure 6. Evolution of the respiration to ETS ratio (R/ETS) along the experiment.

Plankton community structure: biomass and chlorophyll a

We estimated the biomass of certain groups of picoplankton (heterotrophic bacteria, *Synechococcus* and picoeukaryotes) and nanoplankton (nanoeukaryotes) to look at the evolution of the A/H ratio (Autotrophic/Heterotrophic pico- and nanoplankton) (**Table 3**). The pico- and nanoplanktonic community was dominated by the autotrophic biomass even before fertilization (≈ 62 % of total biomass), in spite that in terms of abundance, the heterotrophic prokaryotes (namely bacteria) were the dominant group (96.7 % of the total cell mL^{-1}). Total bacteria presented similar average values in all the mesocosms until the addition of nutrients (ANOVA, P > 0.05) (**Fig. 7**). After fertilization, average bacterial biomass varied between treatments (ANOVA, P<0.01). We applied a Tukey post hoc test in order to see whether the mean differences in

bacterial biomass between each pair of mesocosms was or not statistically significant (**Table 4**). The two mesocosms with higher CO_2 concentration (MK6 and MK8) supported a similar high prokaryotic abundance in comparison with MK5 and MK7 (**Fig. 7**).

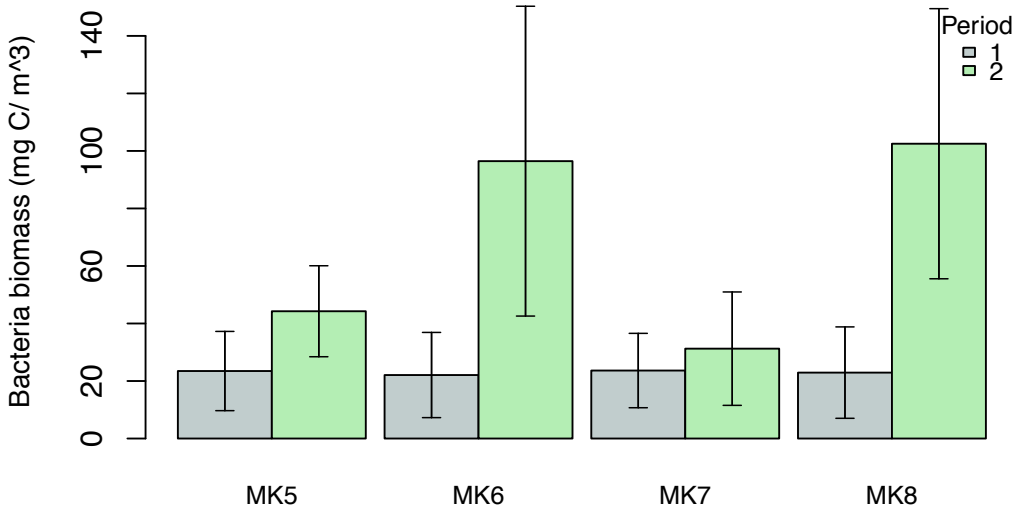


Figure 7. Average bacterial biomass (mgC m^{-3}) for period 1 (before fertilization) and period 2 (after fertilization) measured in the different mesocosms. Error bars show standard deviation.

The abundances of the larger phytoplankton size groups (dinoflagellates and diatoms) are shown for some of the sampling days in **Figure 8**. Biomasses could not be inferred since biovolumes where not estimated. At t1, diatoms presented more than an order of magnitude higher cell numbers than dinoflagellates. In general the two groups increased after the fertilization, particularly in the two mesocosms with higher pCO_2 levels. Diatoms were abundant from the start of the experiment (**Fig. 8B**), and dropped after silicates were depleted in t5 (data not shown). Nevertheless, after the addition of new nutrients, diatoms started to grow again and achieved the maximal abundance (more than 1000 cells /mL) at t27 in MK8. In the low pCO_2 mesocosms, diatoms peaked at t25, declining from t27 onwards.

Table 4. Tukey post hoc test's results for the bacteria biomass observed during the second period. "Diff." corresponds to the mean difference between each pair of mesocosms.

	Diff	P
MK6-MK5	52.19	0.0306
MK7-MK5	-13.00	0.8852
MK8-MK5	58.26	0.0133
MK7-MK6	-65.19	0.0048
MK8-MK6	6.06	0.9863
MK8-MK7	71.26	0.0019

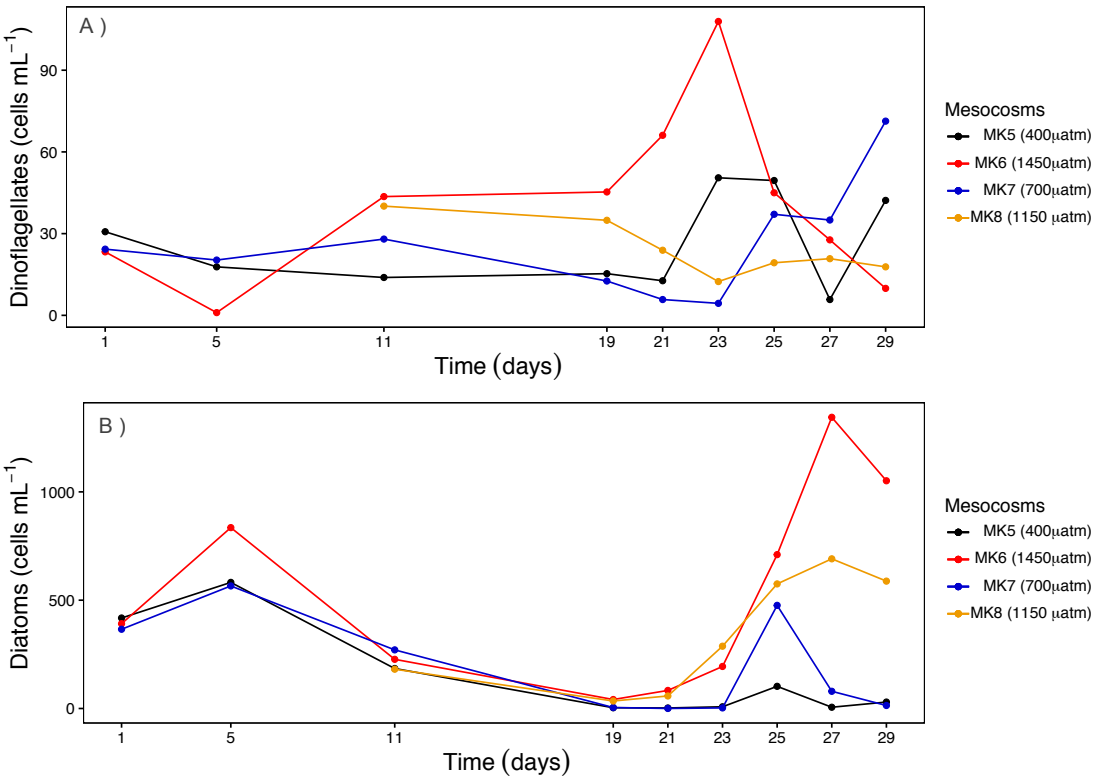


Figure 8. Temporal evolution of the cell concentration (cells mL^{-1}) of dinoflagellates (A) and diatoms (B) along the experiment.

Chlorophyll a concentration ranged from 0.28 to 4.5µg L^{-1} along the experiment. It was statistically higher during period 2, (ANOVA, P<0.01), but no significant differences between mesocosms were observed at any period. The daily measured respiration rates (R) presented similar low (although significant) correlations with both chlorophyll and biomass (**Table 5**). ETS activity showed a rather similar relationship with biomass (r^2=0.39, P<0.001) than with chlorophyll a (r^2=0.31, P<0.001) (**Table 5**).

Table 5. Pearson correlation coefficients between community respiration data (R) and ETS activity (ETS) with other variables.

	R			ETS		
	R^2	F-statistic	P-value	R^2	F-statistic	P-value
Chlorophyll a	0.30	31.76	0.00***	0.31	33.36	0.00***
Total picoplankton biomass	0.31	34.54	0.00***	**0.39**	47.63	0.00***
Autotroph picoplankton biomass	0.07	5.957	0.02*	0.06	5.155	0.03*
Bacteria biomass	0.17	15.14	0.00	0.26	26.42	0.00***

In order to look at changes in community structure and see whether these are reflected in respiratory metabolism, we compared the averages of the autotrophic/heterotrophic biomass (A/H) ratio of pico- and nanoplankton from the different mesocosms,

considering separately the two periods of the experiment. During the first period, the A/H ratios were not significantly different (P> 0.05) (**Fig. 9A**). Conversely, we found significant differences in the averages during period 2 (ANOVA, P <0.001) (**Fig. 9B**). In any case the A/H ratio shows a significant relationship with either with both R and ETS (P>0.05) (**Table 5**).

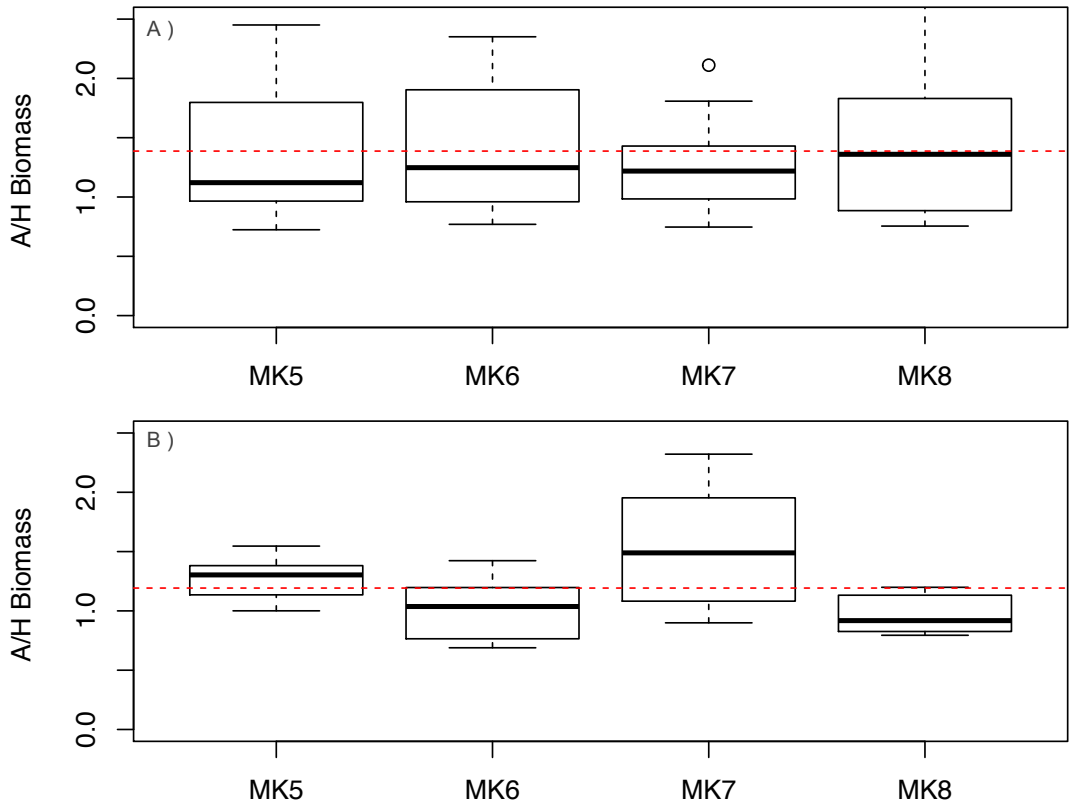


Figure 9. Boxplots of the average values of the autotrophic/heterotrophic biomass ratio (A/H) during periods 1 (**A**) and 2 (**B**). The red line represents the mean value of the averages. Only picoplankton and nanoplankton size fractions are considered.

The ETS/Chl ratio yields a proxy of the relative contribution of autotrophs to total microplankton respiration (**Table 3**). We observe higher values of this ratio during period 2 than in period 1 in MK5. However, the rest of mesocosms, MK5 presented lower ETS/Chl ratios for period 2 (**Table 3**). Like other mesocosms, MK5 presented high values of ETS for the second period but, interestingly, chlorophyll as well as diatoms sharply decreased (**Fig. 8B)**. In MK6, the ETS activity more than doubled from period 1 to period 2 (**Fig. 2**) and consequently the R/ETS ratio was lower and close to 1. The ETS/Chl ratio, however, remained very similar between periods in comparison with MK5. MK8 behaved similarly to MK6, supporting high prokaryotes biomass, leading to a low A/H ratio (**Table 3**). During period 1, diatoms in MK8 were not measured, but we expected to have similar abundances as the rest of the treatments (**Fig. 8B**). Chlorophyll

a instead, seemed to reach the highest mean value (2.29 μg L^{-1}) for the second period. This is reflected in the ETS to Chl ratio, showing the lowest value at period 2 among all mesocosms (**Table 3**). MK7 did not behave as the other mesocosms. All the groups started to grow two days later, due to the delay in the fertilization of the tank, hampering the comparison of this mesocosms with the rest (**Fig. 2** and **Fig. 3**).

Discussion

One of the goals of this study was to look at the effects of CO_2 concentrations on the respiratory metabolism. Linear regression analyses detected no significant relationship (F-test, $P > 0.05$) between community respiration and pCO_2 in any of the mesocosms, agreeing with previous observations by other authors (Egge et al., 2009; Motegi et al. 2013; Tanaka et al., 2013). However, when plotting the accumulated R and ETS (Fig 3), we may observe significant enhancements of both actual and potential respiratory rates at high pCO_2 after nutrient fertilization. These enhancements matched higher cell growth of diatoms and bacteria under high CO_2 treatments (Figs. 7, 8), which might be responsible of the elevated respiratory activity. The potential implications at the ecosystem level could be notorious. Indeed, Piontek et al (2013), in another mesocosms study in an Arctic fjord, observed that the increase of both heterotrophic use of carbon and phytoplankton production at elevated CO_2, stimulated by nutrient enrichment, resulted in an increase of exportable POC to depth.

Past studies on plankton respiration have reported significant correlations between ETS and chlorophyll, with ETS peaks at the chlorophyll maximum (e.g. Packard et al. 1983; Martinez 1991; Del Giorgio 1992; Estrada et al. 1992; Packard & Christensen 2004), suggesting that when seawater chlorophyll is high, respiratory activity is mainly due to phytoplankton (Packard & Christensen 2004). By contrast, in this study, in spite of the phytoplankton dominance during the two periods, ETS correlates much better with community biomass than with Chla. This could result from the presence of a complex and variable in time ecosystem with large amounts of organic matter and bacteria, where phytoplankton is dominated by mixotrophic forms with low chlorophyll content. Harrison (1986) and Martínez (1991) reported a mean ETS/Chl ratio of 1.7 and 1.9 $mgO_2\,mgChl^{-1}\,d^{-1}$ for the Canadian Arctic and the Barent Sea, respectively, with values as low as 0.7 at the chlorophyll maximum layer , suggesting a high contribution of autotrophs to the total microplankton respiration. Our results, however, show values around 6 $mgO_2\,mgChl^{-1}\,d^{-1}$, which are more in accordance with those (5 $mgO_2\,mgChl^{-1}\,d^{-1}$) reported by Martínez (1990) for the oligotrophic western Mediterranean Sea. In general (except MK5), we obtain higher values of the ETS/Chl ratio for the first period (nutrient depleted), although in the second period (nutrient replete) the values are still

high. The low abundances of diatoms during the second period in MK5 could be the reason of the higher ETS/Chl ratio found in this mesocosms.

The R/ETS ratio is believed to represent the fraction of the maximum respiratory capacity that an organism or community is currently using (Martínez 1992). According to this, the R/ETS should be lower than 1. However, the mean R/ETS value for all our mesocosms was 1.47, showing MK6 the highest mean R/ETS value (1.98) for the period 1 and the lowest (1.02) value for the second period of all the data set. Several authors have obtained variable R/ETS ratios in natural communities ranging several fold in magnitude, although in most cases the ratio was lower than 1 (e.g. Packard & Williams 1981; Arístegui & Montero 1995). Albeit R and ETS are usually significantly correlated, (like in our study) the R/ETS ratio is known to vary depending on the planktonic community structure and seasonality (Hobbie et al. 1972). Another cause for the observed global variability may be due to the methodological differences between the published studies. The ETS assay is a measure of the respiratory potential of the community that reflects the recent past prior to the sampling, whereas the physiological measurement of the respiration may reflect changes in the organisms occurring during the incubation period (Del Giorgio 1992). Indeed, it has been shown that the R/ETS ratio is directly related to growth rates in bacteria and phytoplankton cultures (Christensen et al., 1980; Martínez 1992). For that reason, it is reasonable to think that R/ETS ratios obtained at certain times and locations cannot be extrapolated to every region because the communities sampled may not be comparable (e.g. Packard and Williams, 1981).

The general, but not fully contrasted, believe is that autotrophic organisms present lower R/ETS ratios than heterotrophs (Kenner & Ahmed 1975; Span 1988; Del Giorgio 1992;). Photosynthesis, like respiration, involves an electron transport system. Therefore, when autotrophs dominate the plankton community, the ETS assay would measure the potential activity of both processes, yielding higher ETS estimates, and hence lower R/ETS, than measured taking into account only the respiratory process (Packard 1985). Supporting this hypothesis, Martínez (1991) reported the highest values of ETS at the chlorophyll maximum layer for all the sampled stations during a cruise in the Barents Sea. On the contrary, it is thought that R/ETS ratios are higher with increased bacteria contribution to overall respiration, since bacteria are potentially more metabolically active than other organisms (Williams 1984; Azam & Fuhrman 1984; Harrison 1986). In our experiment, the R/ETS ratios were lower during the second period, after nutrient fertilization, coinciding with the phytoplankton bloom and the rise of diatoms. However, in the two periods average R/ETS were higher than 1, like in some other studies where heterotrophic organisms dominated (e.g. Mimura et al., 1988; Reinthaler et al., 2009). Abiotic processes (e.g. nitrification or peroxide formation)

could not explain the high oxygen consumption with respect to ETS. In fact there was not evidence of nitrification and, although peroxide increased over ambient levels, this took place during the second half of the experiment, and the effect at best would only explain 5-10% of the oxygen consumption (Mark Hopwood pers. com.).

The application of a regression equation has been found to be a more reliable approach than the use of a mean R/ETS to transform ETS activity to actual respiratory rates but unfortunately there are very few published studies to compare with (Packard & Williams 1981; Del Giorgio 1992; Arístegui & Montero 1995). Yet, the knowledge and comparison of R/ETS ratios from different studies is very useful to explore the variability of the ratio in different organisms and natural communities and the causes affecting this variability. Our mean R/ETS ratio is in general high compared with those found in cultures and some natural communities (see table 1). Moreover, the ratio shows a large variability along the experiment (ranging from <1 to >3). This is however not surprising when looking at the published R/ETS ratios that span more than an order of magnitude, showing no clear pattern or relationship with community structure at the taxonomic or functional levels.

Conclusions

This work represents one of the few studies where actual respiration rates (R) and ETS activity are measured simultaneously in natural marine communities. Moreover, it is the single one where R and ETS are measured under controlled conditions (inside mesocosms) and during a relatively long period of time (30 days), where the ecosystem undergoes drastic changes in nutrients' concentrations, CO_2, and community structure.

Our results indicate that R and ETS are weakly, but significantly, correlated along the experiment in all the mesocosms with different CO_2 treatments, which shifted from oligotrophic to highly eutrophic conditions. Nevertheless, the R/ETS ratio is highly variable, ranging more than 3 fold in magnitude during the experiment. The average R/ETS is somewhat higher under nutrient depleted conditions, compared to the bloom period after nutrient fertilization. In all cases, however, the average ratio is >1, which is an unexpected result assuming that ETS represents a proxy of potential respiration. Abiotic oxygen production (consumption) rates or any other methodological issues cannot explain the high (low) R (ETS) values leading to the observed high R/ETS ratios. We therefore suggest that either the ETS assay does not account for all the potential respiratory activity, or there is a temporal mismatch between R and ETS that is reflected in the ratio.

We did not find any clear pattern of change at the plankton community level that might explain the variability of the R/ETS ratio, with the exception of a somewhat lower R/ETS during the nutrient replete period, when diatoms and heterotrophic bacteria bloomed. In fact, cumulative R and ETS clearly increased in the high CO_2 treatments, after nutrient fertilization, matching a rise in diatoms and bacteria in these mesocosms compared with the low CO_2 treatments.

All in all, our results (together with the published literature) indicate that R/ETS may be too variable as to apply constant ratios in different marine regions and trophic conditions. More studies with natural communities (either on cultures or controlled field experiments) are necessary to understand the factors driving the variability in R, ETS and their ratio. Not until we explore, understand and constrain this variability we will be sure that ETS can be used to quantify respiration in surface and deep-water marine communities. Meanwhile, we must be cautious to interpret respiration rates derived from ETS activity or, better, we should estimate simultaneously R and ETS during fieldwork experiments and cruises.

Acknowledgements

I want to thank the GEOMAR group for letting us participate in the KOSMOS16 experiment and for helping us with the logistics and complementary measurements of nutrients, CO2, chlorophyll and the large size phytoplankton data. Also I am grateful for the cytometry samples provided by Adriana Rodriguez and for the contribution of all the GOB members during the experiment**.** Finally, I would like to make a special mention to Isabel Baños, for the advice and help that has provided us with, not to forget the respiration measurements and the ETS assay.

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Descripción detallada de las actividades desarrolladas durante la realización del TFG

El desarrollo de las actividades se ha realizado progresivamente y de acuerdo al tipo de análisis requerido a lo largo del experimento. La colaboración con el equipo GEOMAR ha sido una gran oportunidad ya que se han podido realizar una gran diversidad de actividades que han permitido obtener los datos para la realización del TFG. Todas ellas se llevaron a cabo en el "Grupo de Oceanografía Biológica" (GOB), bajo la supervisión de mis Tutores, Javier Arístegui y María Fernanda Montero y con la colaboración de Isabel Baños, una estudiante de doctorado del grupo.

Reunión inicial. Planificación y temporización

En esta primera reunión los dos tutores de empresa me presentaron a todos los miembros del GOB y explicaron el funcionamiento del grupo. Entre todos repasamos los aspectos básicos de lo que iba a tratar el experimento y nos pusimos de acuerdo para repartirnos las tareas requeridas: poner en marcha todo el equipamiento, adaptar los laboratorios según los análisis que íbamos a realizar y revisar si el material del que disponíamos era el necesario, a fin de tener todo preparado para cuando el experimento empezara. La planificación debía de ser muy precisa y acorde a las fechas del muestreo. Además, se establecieron características más personales como cuáles iban a ser mis tareas, cómo íbamos a organizar las próximas reuniones, qué tipo de control se iba a seguir de las distintas actividades realizadas y cuáles iban a ser los objetivos de mi TFG. Por último, especificamos los conocimientos que debería repasar y adquirir para poder formar parte del experimento y poder desarrollar un buen trabajo. Al tratarse de un experimento con muchas otras personas era necesario mostrar una gran responsabilidad e implicación.

Revisión bibliográfica

Inicialmente se me recomendó leer los primeros artículos publicados con respeto al ensayo del ETS: inicio del método, mejoras del mismo y su relación con la respiración, ya que parte de mi trabajo iba a estar enfocado a ese tema:

Kenner, R. A. and Ahmed, S. I. 1975. Measurements of electron transport activities in marine phytoplankton. Mar. Biol., 33, 119–127.

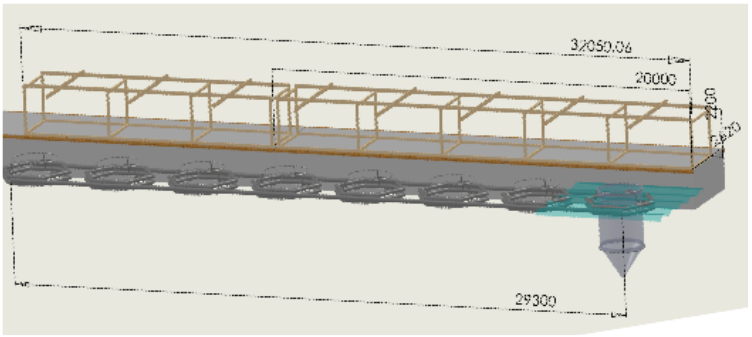
Packard, T. T. 1985. Measurement of electron transport activity of microplankton. Adv. Aquat. Microbiol., 3, 207–261.

Packard, T. T., and P. J. L. B. Williams. 1981. Rates of respiratory oxygen consumption and electron transport in surface seawater from the northwest atlantic. Oceanol. Acta 4: 351–358.

Arístegui , J. and M. F. Montero. 1995. The relationship between community respiration and ETS activity in the ocean. J. Plankton Res. 17: 1563–1571.

Para tener un visión más general, mi supervisora me recomendó un libro de la biblioteca de ciencias básicas de la ULPGC: "Del Giorgio, P. A & Williams, P J. L. B. (Eds.) 2005. Respiration in aquatic ecosystems. Oxford University Press, USA". Este libro me ha sido de gran ayuda ya que se trata de una revisión de los diferentes estudios sobre la respiración en los principales sistemas acuáticos de la biosfera. Los capítulos que más me han interesado son aquellos haciendo referencia a los principales métodos utilizados para la medición del la respiración en el océano y aquellos describiendo información existente sobre diferentes estudios realizados.
Más adelante por mi cuenta fui buscando otros artículos más actuales, según mi necesidad de entender cierto método o de buscar resultados similares o distintos a los míos, con el fin de empezar a tener una idea global de lo que se ha realizado a lo largo de la historia sobre este tema.

Muestreo en los mesocosmos



Dentro del muelle de Taliarte, el equipo de GEOMAR instaló 8 mesocosmos de 1100m^3 a lo largo del pantalán de PLOCAN. El agua para rellenarlos fue bombeada desde el exterior del muelle. A continuación se inyectaron diferentes concentraciones de pCO_2 a cada tanque, obteniendo un gradiente que variaba desde 400 matm (concentración actual en la atmósfera y superficie del agua) hasta 1450 matm (simulando un escenario futuro de un océano superficial acidificado). El procedimiento de muestreo fue diseñado con el fin de integrar toda la columna de agua de los mesocosmos cada vez que se extraía agua. Durante los primeros tiempos del experimento (prebloom) se muestreó cada dos días; más adelante, una vez se añadieron los nutrientes (bloom), diariamente y por último, después del bloom, se llevaron a cabo otros dos tiempos de muestreo, completando finalmente los 20 días (desde el t-3 hasta el t29).

Se organizaron turnos de muestreo a fin de repartirse el trabajo, ya que las mismas que les tocaba coger las muestras de agua se encargaban de transportar el material de muestreo al pantalán y realizar la recogida de todo el volumen necesario para los diferentes grupos de investigación; todo con un cuidado extremo cuidado para evitar cualquier contaminación o deterioro de las muestras. Cada mesocosmos se muestreaba de 6 a 7 veces ya que los tubos empleados tenían solo capacidad de unos 5 litros. Se necesitaba rellenar dos garrafas de 10 litros (4 tubos), mientras los tubos restantes eran empleados para las mediciones de gases disueltos.

Cálculo del consumo de oxigeno por las comunidades fitoplanctónicas

La respiración en el océano siempre ha sido un proceso muy difícil de medir, principalmente porque los gases implicados son muy sensibles tanto a cambios físicos y químicos, como biológicos. Consecuentemente, en la mayor parte de los estudios nacionales e internacionales biogeoquímicos las mediciones de respiración aún no son rutinarias. Las tasas de respiración pueden ser derivadas de varias maneras, desde la comparación entre la producción y el consumo de un producto o reactivo, por estimación de las actividades enzimáticas involucradas en la respiración, predicciones a partir de la biomasa, o por modelos inversos de la composición o actividad de la comunidad. En este trabajo hemos medido la respiración mediante el consumo de oxígeno utilizando un sistema microWinkler, el ensayo enzimático de la cadena transportadora de electrones (actividad ETS) introducido por Packard, según las modificaciones descritas en Arístegui y Montero (1995), y por último midiendo el consumo de oxígeno mediante espectrometría de masas (Membrane Inlet Mass Spectrometer; MIMIS), aunque éste ultimo no lo he considerado en mi trabajo.

Método Winkler

Este método se basa en la diferencia de oxigeno entre las botellas finales, incubadas entre 12-24 h en oscuridad, y las iniciales (tiempo cero). Los resultados obtenidos representan el consumo neto de oxigeno producido dentro de la botella, que puede deberse tanto a procesos bióticos como abióticos; aunque al ser la oxidación química principalmente impulsada por la luz, se considera que el consumo de oxigeno es debido solamente a respiración de organismos.

Para desarrollar este método es necesaria la preparación de 4 tipos de soluciones. La solución 1 de sulfato manganoso y la solución 2, que se trata de una solución alcalina formada por NaOH y NaI, son las necesarias para la fijación de las botellas en el momento cero y el momento final después de la incubación en oscuridad. Las otras dos soluciones son una de ácido sulfúrico y la otra de tiosulfato; éstas en cambio se emplean en el momento de la titulación de las botellas, cuando se realiza la valoración.

La transferencia del agua de las diferentes muestras en las botellas de boro-silicato (BOD) requiere de gran precisión. Se debe evitar en todo momento la generación de turbulencia y/o burbujas, ya que éstas podrían interferir en la concentración de oxigeno. Las botellas están calibradas y específicamente diseñadas con una tapa cónica para ayudar a eliminar burbujas y, una vez éstas estén cerradas, permitir su estanqueidad. La adición de los diferentes reactivos también debe realizarse con sumo cuidado.
Para la determinación del oxigeno disuelto del tiempo cero y final de cada muestra se utilizaron botellas por cuadruplicado. La titulación de las botellas se realizó mediante un sistema automatizado microWinkler, donde la cantidad total de yodo fue determinada espectrofotométricamente a 456nm por el cambio de color producido durante la valoración con tiosulfato. Todas las mediciones fueron realizadas en un laboratorio atemperado a 21ºC. La conversión de la cantidad de yodo a concentración de oxigeno se basa en una relación estequiométrica que depende de todos los reactivos utilizados durante el análisis y de varios parámetros como son el volumen exacto de las botellas, la cantidad de tiosulfato adicionado durante la titulación, y las temperaturas de fijación e *in situ*.

Espectrometría de Masas (MIMS)

Aunque los datos obtenidos mediante esta técnica no son tratados en mi trabajo, durante las prácticas aprendí el funcionamiento básico del instrumentos y en varias ocasiones ayudé a la persona encargada a pasar las muestras. El MIMS presenta varias ventajas sobre el Winkler como son la simplicidad a la hora de medir las muestras, el hecho de que se puede emplear para medir gran cantidad de replicados en tiempo real con ninguna o poca preparación de las muestras y por último que los volúmenes pueden ser bastante pequeños.
La muestra se introduce dentro de la cámara de vacío de espectrómetro de masas a través de una membrana semipermeable permitiendo su medición exacta. En nuestro caso lo utilizamos con la intención de medir la respiración; es decir, la diferencia de oxigeno disuelto en el agua entre un momento inicial y otro final después de la incubación de la misma muestra. Para ello trabajamos con la relación oxigeno/argón. El argón es un gas que en el océano solo se ve afectado por procesos físicos, a diferencia del oxigeno o el nitrógeno que se ven influenciados tanto por procesos físicos como biológicos. Estos gases son los que encontramos en mayores concentraciones en aguas aeróbicas. Conociendo la cantidad de argón que hay en la muestra y la contribución de los procesos físicos a su concentración, podemos conocer la cantidad de oxigeno que ha sido consumido biológicamente. La interpretación de los datos es un poco más complicada ya que depende de muchas variables y al ser tan sensible es fácil que dé

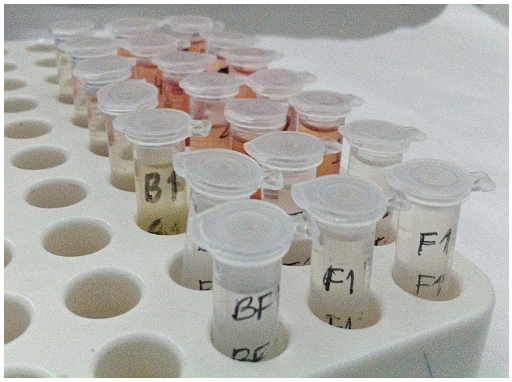
errores, por esta razón realizamos 5 réplicas por muestra y esperamos a que la señal se estabilice antes de cambiar de réplica.

Electron transport system (ETS) activity. Ensayo enzimático

Cada día de muestreo se filtraron entre 1 y 3 l de agua de mar de cada mesocosmos, a través de filtros de fibra de vidrio (Whatman GF/F) con un tamaño de poro de 0.45 micras, utilizando una bombas de vacío. Los filtros se doblaron y guardaron dentro de crioviales que fueron introducidos en nitrógeno liquido con el objetivo de producir la congelación inmediata de las células para evitar la pérdida de actividad enzimática; posteriormente se almacenaron a -80ºC hasta el análisis.

Una semana antes del ensayo habilitamos una zona del laboratorio con todo el equipamiento necesario y justo dos días antes de empezar con los análisis, preparamos todos los reactivos y soluciones: sustrato, blanco, INT, tampón de homogeneización y "quench". Al tratarse de un ensayo enzimático la preparación de los reactivos ha de ser muy precisa. Posteriormente, se dispensaron en distintos recipientes y almacenaron a -20ºC para su conservación. Cada día antes de empezar el experimento, tuvimos que comprobar que los baños termostáticos estaban a 18ºC, ya que la temperatura es un factor muy determinante en el funcionamiento de las enzimas, e ir a buscar hielo para mantener todos los reactivos y soluciones a una temperatura de 0-4ºC. La cantidad de coenzimas (NADH y NADPH) necesarias se pesaron y añadieron a diario a la solución sustrato.

Durante una semana mi supervisora y yo conseguimos hacer el ensayo de todas las muestras del experimento, realizando 19 por día. La coordinación entre ambas fue esencial con tal de conseguir un buen resultado, ya que esta técnica requiere de muchos pasos para la obtención final de los datos.



Los filtros junto con el material recogido fueron homogeneizados en 2,8 ml de solución tampón con una varilla de teflón, a 0-4ºC durante 1 minuto. Mientras tanto, se prepararon tres replicados por muestra: dos con la cantidad exacta de solución sustrato e INT, y un tercero con solución blanco y agua Mili-Q. Además, se realizó un replicado de blanco de reactivo utilizando filtros limpios (sin muestra). Una cantidad de 0.9 ml del crudo homogenizado fue transferido a los tres crioviales e inmediatamente después, se pusieron a incubar a 18ºC durante 15 minutos en un baño termostático. Para parar la reacción se empleó la reacción "quench" de ácido clorhídrico y formol. La mezcla de reacción inactivada se centrifugó una primera vez

durante 20 minutos a 1400 rpm. El sobrenadante fue transferido a crioviales limpios y se aplicó una segunda centrifugación durante 15 minutos. Finalmente se midió espectrofotométricamente la absorbancia del sobrenadante a 490 nm y 760 nm, una vez las muestras se ajustaron a la temperatura ambiente del laboratorio. El pico de 490nm es proporcional a la producción de INT-formazan y el de 760nm mide la turbidez de la muestra. La actividad del ETS fue calculada mediante la ecuación de Packard and Williams (1981):

$$ETS_{ASSAY}(\mu l\ O_2 l^{-1} h^{-1}) = 60 \times H \times S \times (corr\ OD)/(1.42 \times V \times f \times t)$$

y corregida a temperatura *in situ* mediante la ecuación de Arrhenius:

$$ETS_{SITU} = ETS_{ASSAY} \times e^{\left(Ea/R \times \left(1/T_{ass} - 1/T_{is}\right)\right)}$$

Formación recibida

Durante el desarrollo del TFG he adquirido formación en cuanto a la metodología de muchas técnicas de análisis, incluyendo la interpretación de los resultados obtenidos, el entendimiento de los principios en los que se basa cada una y el funcionamiento de los instrumentos empleados (espectrofotómetro, centrífugas, baños termostáticos, microWinkler, MIMS y citometría de flujo).

Para la obtención de la concentración de oxígeno mediante el microWinkler, tuve que aprender unas nociones básicas en la programación con MSDOS ya que éste funciona con este lenguaje. Asimismo, entender en general los fundamentos bioquímicos relacionados con el consumo de oxigeno y comprender las reacciones químicas que se producen durante el análisis a causa de los reactivos añadidos a las muestras. En el caso del ETS, asimilar la base y metodología de la técnica resultó más complicado, ya que ésta requiere gran cantidad de pasos y más reactivos, siendo éstos bastante sensibles. Además, la obtención final de los datos impone aplicar diferentes formulas que dependen de muchos parámetros.

Los datos de respiración de ambos métodos se trabajaron primeramente en Excel para visualizar las tendencias generales y comprobar si eran coherentes a lo esperado. Más adelante, una vez adquirido todo el conjunto de resultados, éstos se trataron con el programa estadístico R-Studio, ya que permite realizar muchos más análisis y test de una manera más organizada y estética; además lo conocía de antemano debido a su utilización en la asignatura de Estadística.

Nivel de integración e implicación dentro del departamento y relaciones con el personal

Recién llegada del Erasmus en Alemania, me incorporé al grupo de investigación de Oceanografía Biológica en el IOCAG. La relación con todos los técnicos, científicos, personal de mantenimiento y de seguridad que trabajan en la empresa ha sido en todo momento excelente. Desde un primer momento me he sentido muy integrada dentro del centro. Las personas del grupo siempre se han ofrecido a ayudarme en lo posible ante cualquier situación, como por ejemplo sobre las técnicas utilizadas durante las prácticas, sobre el funcionamiento de algunos equipos a la hora de analizar las muestras o cualquier otra cuestión relacionada que me haya surgido.

En general la dinámica en el laboratorio ha permitido una buena integración por parte de todos los miembros. Además, al estar en relación con todos ellos he podido aprender mucho más de lo previsto. Cada uno de los miembros tiene mucha más experiencia que yo y están especializados en ciertos métodos o técnicas, lo que me conlleva a ampliar mis conocimientos y a ver otros puntos de vista. Asimismo, he intentado ofrecer mi ayuda en todo momento cuando se ha requerido y no perturbar el trabajo de los otros. Desde limpiar o transportar material, sustituir a la persona que le tocaba muestrear o ayudar en algunos análisis cuando la persona encargada estaba ocupada en hacer otra cosa.

Aspectos positivos y negativos más significativos relacionados con el desarrollo del TFG

Como visión general, la experiencia ha sido muy positiva. El grado de colaboración e integración del departamento y la utilización de muchas técnicas e instrumentos. Abordar un experimento en el laboratorio de forma independiente requiere cierta responsabilidad, lo cual considero que es una experiencia muy enriquecedora. Por otro lado, haber participado en la campaña KOSMOS16 y haber estado en contacto con otros científicos y estudiantes internacionales es también un aspecto positivo.
Durante los días de muestreo estábamos trabajando desde las 9 de la mañana hasta las 5-6 de la tarde sin prácticamente interrupciones. A pesar del cansancio acumulado que esto implica, el conocimiento y la metodología de trabajo adquiridos son de gran utilidad, ya que suponen un buen entrenamiento para afrontar experimentos de mayor escala en un futuro y mejorar la manera de trabajar eficientemente y en grupo.
Otra cuestión es que al estar formando parte de un experimento del que dependen más personas y de larga duración, no es posible disponer de todo el conjunto de datos hasta al cabo de dos meses de haber empezado. Esto se traduce en que al final solo dispones

de alrededor un mes y medio para analizar, comentar y redactar las partes más importantes del trabajo. Es por esta razón que durante el desarrollo de las practicas he incrementado y mejorado mi capacidad de organización, aspecto que en mi opinión es imprescindible adquirir.

Valoración personal del aprendizaje conseguido a lo largo del TFG

Mis conocimientos y mi experiencia en el laboratorio y en general en el campo de la investigación de la oceanografía biológica han aumentado enormemente, al verme incluida, desde un primer momento, en el experimento KOSMOS16. La formación recibida conforme a los tipos de análisis y técnicas que íbamos a utilizar ha sido excelente, consiguiendo su máxima comprensión. El gran apoyo por parte de todos los miembros del grupo ha sido de gran ayuda en el desarrollo de las prácticas y del TFG. No dudaron en ayudarme bajo ninguna circunstancia, ya fuera a comprender mejor las actividades a realizar, buscar información relacionada con mi trabajo, enseñarme la dinámica del laboratorio o a explicarme nuevas técnicas también relacionadas, que aunque no las haya tratado mucho durante el experimento, puede que sí las utilice en un futuro.

Son unas prácticas muy completas, se ajustan a lo esperado y además proporcionan una gran variedad de oportunidades para aprender, entender y ampliar los conocimientos sobre la oceanografía biológica y el cambio global en un ámbito científico.

Por último destacar la labor ejercida por mis dos tutores Javier Arístegui y María Fernanda Montero, mi supervisora/colaboradora, Isabel Baños, y mi Tutor Académico, Antonio Juan González, que han prestado mucha atención a mi desarrollo del trabajo, y han ofrecido su tiempo ante cualquier duda o cuestión. Sin ellos no habría obtenido un resultado tan satisfactorio durante la elaboración del TFG.

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