Ocean Acidification Effects on Phytoplankton Community Structure in Gran Canaria Island during a Mesocosm Experiment

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Index

1. Abstract ........................................................................................................................................ 4

2. Introduction .................................................................................................................................. 5

3. Methods
   3.1 Set-up of mesocosm experiment and nutrient fertilization .......... 7
   3.2 Samples analyses ....................................................................................................................... 8
   3.3 Data analyses and statistics ........................................................................................................ 9

4. Results
   4.1 Carbonate chemistry and nutrient evolution .......................................................... 9
   4.2 Evolution of phytoplankton abundances along the experiment ............. 12
   4.3 Effect of $pCO_2$ on different phytoplankton growth rates before
       and after fertilization ................................................................................................................. 15
   4.4 Overall community structure trends ..................................................................................... 16

5. Discussion
   5.1 Effects of $pCO_2$ on different taxonomic groups and
       community structure ................................................................................................................ 19
   5.2 Potential consequences for carbon cycling ................................................................. 23

6. Conclusions .................................................................................................................................. 24

7. Annex ......................................................................................................................................... 24

8. Acknowledgements ..................................................................................................................... 24

9. References .................................................................................................................................... 27

10. TFT Report .................................................................................................................................. 34
1. Abstract

The regulation of marine carbon cycling and ocean-atmosphere CO₂ exchange is closely related to phytoplankton dynamics. The large increase of atmospheric CO₂ levels since the industrial revolution has caused important changes in surface ocean pH and carbonate chemistry. Such changes have been shown to slow down calcification in corals and coralline macroalgae and change competition between phytoplankton groups. Here we investigated whether plankton community structure and net growth rates of phytoplankton groups are affected by pH changes along a pCO₂ gradient simulating oligotrophic or eutrophic ocean conditions. The present experiment was performed over 3 March to 3 April 2016 in the eastern coast of Gran Canary island (27°59’22.8” N, -15°22’0.8” Wº) on seven different mesocosms. Our results show how on oligotrophic conditions nanoeukaryotes and dinoflagellates had a positive response to pCO₂ increases, while no significant responses were observed on the other phytoplankton groups. Conversely, on high nutrient concentrations, heterotrophic bacteria and diatoms show a positive response on their net growth rates as pCO₂ increased, while picoeukaryotes and flagellates presented a negative response. The main changes in the structure of the phytoplankton community occurred at a CO₂ threshold of 700-850 ppm. Our data suggest that the combination of an increase in atmospheric CO₂ concentrations together with nutrient fertilization would favour the presence of large diatoms and hence the vertical carbon flux in the ocean.
2. Introduction

About half of the cumulative anthropogenic CO\textsubscript{2} emissions between 1750 and 2011 have occurred in the last 40 years (IPCC, 2014) and if CO\textsubscript{2} emissions continue rising at current rates, atmospheric concentrations could reach between 730 and 1020 ppm levels according to climate-carbon cycle models (IPCC, 2007). Serving as a buffer for global climate change, the ocean has absorbed around 30\% of the anthropogenic CO\textsubscript{2} since the beginning of the industrial revolution (Sabine et al., 2004) inducing critical changes in the chemistry of seawater, especially in the carbonate system. Projections indicate that the pH will drop 0.3 units from its present value (Caldeira & Witckett, 2003) leading to a 150\% increase in H\textsuperscript{+} and 50\% decrease in CO\textsubscript{3}\textsuperscript{2–} concentrations (Orr et al., 2005). It has also been suggested that this drop will promote a drastic lowering of calcium carbonate saturation states, with different impacts on shell-forming marine organisms (Doney et al., 2009) as well as a rise in CO\textsubscript{2}, presumably increasing photosynthetic efficiency at different levels between autotrophic groups (Riebesell 2004) which are responsible of about half of the global primary production on Earth (Field et al., 1998).

Indeed, photosynthesis directly depends on the concentration of CO\textsubscript{2} at the site of carbon fixation and, as such, it could promote significant implications for marine primary productivity (Rost et al., 2008). Present-day CO\textsubscript{2} levels are not saturating the carboxylase reaction of algal RUBISCO, and this potentially sets a limit to algal photosynthesis and growth (Beardall & Raven, 2004). Nearly all marine phytoplankton have evolved concentrating mechanisms (CCMs) to overcome the low CO\textsubscript{2} affinity in marine environments, although there are clear evidences that CCMs vary within and among dominant groups and taxa of prokaryotic cyanobacteria and eukaryotic marine phytoplankton (Burkhardt et al., 2001; Rost et al., 2003; Giordano et al., 2005). The different energetic and nutrient costs of CCMs may modulate how CO\textsubscript{2} affects primary production, elemental composition, and phytoplankton community structure in the ocean.

The fate of phytoplankton community composition and diversity is often difficult to predict, as complex community interactions also depend on the original community composition as well as the intensity and frequency of disturbances. A meta-analysis of published experimental data about phytoplankton growth rates have found that ocean acidification (OA) may impact competition both within and between groups, and appeared to be a stronger driver of functional diversity changes than temperature (Dutkiewicz et al., 2015). This impacts may promote changes on carbon cycle of phytoplankton food web and consequently, it could change how is kidnaped in the seafloor along years. For example, in some mesocosms studies, it has been reported that bacterial abundance may benefit directly and indirectly from decreasing seawater pH (Endres et al., 2014; Thingstad et al., 2008) probably due to the enhancement of production and exudation of carbon-rich components under OA conditions (Engel et al., 2014). At the same time, it is important to emphasise that the responses observed are not always in agreement, probably due to different methodologies and environmental conditions used in studies. For example, Fu et al. (2007) reported that doubling pCO\textsubscript{2}
alone had no significant effect on the growth of either *Synechococcus* or *Prochlorococcus* in culture experiments while another study had reported lower abundance of *Synechococcus* at high pCO$_2$ in an enclosed experiment with nutrient addition (Paulino et al., 2008). These responses seem to be controlled by other variables, such as nutrient availability (Lomas et al., 2012). Hence, it seems necessary to study the response of phytoplankton communities to OA under (natural or simulated) oligotrophic (low nutrient) and eutrophic (high nutrient) conditions. Other recent studies have reported that picoeukaryotic cells will benefit from nutrients’ addition at high CO$_2$ (Paulino et al., 2008; Brussard et al 2013; Schulz et al., 2013; Schaum et al., 2012), although at the same time the impact of viral attacks may be intensified (Chen et al., 2015). Diatoms appeared to have neutral or slightly positive responses to increasing CO2 concentrations (Rost et al., 2003; Dutkiewicz et al., 2015), but it has been showed that it could depend on specific-species composition (Kim et al., 2006). On the contrary, coccolithophores are negatively affected due to dissolution of their carbonate shells under high OA (Berry et al, 2002; Rost et al., 2003). At higher trophic levels, there is little information on OA impacts, but Aberle et al. (2013) found almost no direct effects of OA on microzooplankton composition and diversity.

All these OA effects at different trophic levels and taxonomic groups of the planktonic community could lead to changes in the pools and fluxes of organic carbon mediated by the nature and size structure of the community, with profound implications on carbon sedimentation and sequestration in the deep ocean. It has been reported that high levels of pCO$_2$ could lead into a counterintuitive cycling of carbon by bacteria, causing a substantial decline of net community production despite higher primary production of organic carbon (Engel et al., 2013; Thingstad et al., 2008). This increase of bacterial activities would exacerbate the competition between phyto- and bacterioplankton for inorganic nutrients inducing a decline of autotrophic productivity and the corresponding carbon flux to the deep ocean.

With the aim of contributing to disentangling the influence of OA in shaping plankton community structure, we ran a series of experiments inside enclosed mesocosms under an acidification gradient. We wanted to test the hypothesis that OA influences the composition and abundances of autotrophic and heterotrophic communities (from bacteria to microplankton) and that nutrient fertilization would exacerbate these differences.

Seven mesocosms with different pCO$_2$ levels (from 368 to 1287 ppm) were installed at the pier side of Taliarte harbour, in Gran Canary Island. The experiment lasted 29 days during early spring 2016. Mesocosms’ experiments are an ideal platform to assess potential effects of changing chemistry on plankton community as they allow for species interaction and competition in quasi natural environment. Most of the previous mesocosms studies published in the literature were carried out in cold or temperate seas (Aberle et al., 2013; Sommer et al., 2015; Brussard et al., 2013; Thoisen et al., 2015; Rossoll et al., 2013). On the contrary, our study took place in subtropical warm coastal
waters, with low nutrient concentrations similar to open ocean values (González-Dávila et al., 2003) and a phytoplankton community dominated by cyanobacteria and small eukaryotes (Arístegui et al., 2001; Arístegui & Montero, 2005; Baltar et al., 2009).

3. Methods

3.1 Set-up of a mesocosm experiment and nutrient addition

The mesocosm study was conducted at the pier side of Taliarte’s harbour, in the eastern coast of Gran Canary island (27°59’22.8” N, -15°22’0.8” W), from 3 March to 3 Abril 2016. No specific permission was required to collect water from this area, and the study did not involve endangered or protected species. Eight floating, pelagic mesocosms (volume ~ 1100 m$^3$), made of thermoplastic polyurethane (TPU), were situated on a floating platform. The filling of the mesocosms started after all mesocosms were moored in position with adjacent harbour seawater. There was no risk of differences between enclosed water bodies in terms of seawater chemistry and plankton community abundance and composition, because the mesocosms were all filled simultaneously.

CO$_2$ enrichment was carried out gradually over 3 days ($t$-3, $t$-2, $t$-1) by adding CO$_2$-enriched water aerated with pure CO$_2$ (99.995%) for a minimum of 24h. To reach an even distribution of CO$_2$-enrichment water throughout the mesocosm, a dispensing device (termed “spider”) was slowly moved up and down during the injection. By using this technique total alkalinity remained constant while dissolved inorganic carbon (DIC) increased, mimicking on-going ocean acidification (Dickson et al., 2007). A CO$_2$ gradient was established along mesocosms, with target $p$CO$_2$ concentrations ranging from 368 to 1287 µatm. This approach involves the use of regression statistics for assessment of possible CO$_2$ effects and increases the chance of detecting a threshold level for any CO$_2$ sensitive processes. Samples were collected by means of a PVC tub with a closure system at the two extremes. The tub’s volume was 5L and its content was gently mixed to ensure a homogeneous depth-integrated water sample.

In order to simulate the upwelling of deeper, nutrient-rich waters, dissolved inorganic nutrients were added on $t$18 using the same dispersion device as described above, to yield concentrations of 5 µM NO$_3$, 0.32 µM PO$_4$, and 2.5 µM Si. The amount of inorganic nutrients added to each mesocosm was calculated on the basis of volume determinations, depending on mesocosm volume. Water samples from each mesocosm were collected in the morning between 9:00 and 11:00, either every two days ($t$-3 to $t$17) or daily ($t$19 to $t$25) in order to obtain greater temporal resolution during the fertilization period.
### 3.2 Samples analyses

CO₂ concentrations were calculated from measured temperature, dissolved inorganic carbon (DIC) and total alkalinity (TA) by members of the GEOMAR group. The samples were pressure-filtered to exclude calcareous particles and particulate organic material because they can influence the precision of the carbonate chemistry measurements. DIC was calculated as the mean of the best three out of four measurements of 2 mL sample volume. According to the open-cell method described in Dickson et al. (2003), TA was determined by potentiometric titration. Nitrate, nitrite, phosphate and silicate were determined from water samples following the methods based on spectrophotometric techniques developed by Murphy and Riley (1962) and Hansen and Grasshoff (1983). Ammonium concentrations were determined fluorometrically following Holmes et al. (1999). The analysis was performed two hours after sample collection by the GEOMAR group.

Heterotrophic bacteria (HB), small photosynthetic eukaryotic cells (picoeukaryotes), and *Prochlorococcus* and *Synechococcus* type cyanobacteria, were counted by means of a FACScalibur (Becton and Dickinson) cytometer. Picoeukaryotes, *Prochlorococcus* and *Synechococcus* samples (4 mL) were analysed *in vivo* after 30-60min of collecting samples. Heterotrophic bacteria were fixed with 2% final concentration of formaldehyde, incubated for 30 min at 4°C and then stored frozen in liquid nitrogen until analysed. To count HB, 400 µl was stained with DMS-diluted SYTO-13 (Molecular Probes Inc.) stock (10:1) at 2.4µM final concentration. Bacteria were identified by their signatures in a plot of side scatter (SSC) versus green fluorescence (FL1). High DNA (H-DNA) bacteria and low DNA (L-DNA) bacteria were separated in the scatter plot. Small phytoplankton groups were identified by an interactive analysis of multiple bivariate scatter plots of side scatter, red fluorescence and orange fluorescence. Samples were run at low speed for HB (16 µL min⁻¹) and medium speed for the other photosynthetic cells (60 µL min⁻¹). A suspension of yellow–green 1 µm latex beads (≈10⁶ beads ml⁻¹) was added as an internal standard (Polysciences, Inc.).

A CytoBuoy cytometer (Dubelaar and Gerritzen, 2000) was used to count nanoeukaryotic cells (2-20 µm). The light scattered from each passing particle is measured at two angles - forward scatter (FWS) and sideward scatter (SWS)- to provide information on size and shape of the particles. The fluorescence emitted by photosynthetic pigments in algal cells is detected at three different wavelengths and these fluorescence signatures of Chl-a, phycocerythrin and phycocyanin, displayed as red, orange and yellow, respectively, assist in determining the algal type of each particle. Samples (about 15-10 mL) were analysed *in vivo* during 7 minutes at a flow rate of 300 µL min⁻¹).

Samples (100 mL) for microphytoplankton (>20 µm) were fixed with alkaline Lugol’s iodine (1 % final concentration), sedimented in Utermöhl chambers and counted by means of an inverted microscope following the Utermöhl method by the GEOMAR group.
3.3 Data analyses and statistics

To determine potential $pCO_2$ effects on phytoplankton abundances, the daily deviation ($AD_i$) of each mesocosm was calculated by subtracting observations ($X_i$) from the mean of all mesocosms ($\bar{X}$) on the specific sampling day $AD_i = X_i - \bar{X}$. These daily deviations were averaged over time, according to $\frac{1}{N} \sum_{t=1}^{N} (AD_i)$ with $N$ being the number of sampling days, in order to obtain the mean deviations ($MD$) of each mesocosm with respect to a particular parameter. Calculated mean deviations were tested against real average $pCO_2$ of the different mesocosms by linear regression. Days -1, 1, 2 were excluded from the analysis because $pCO_2$ levels had not yet reached the correct concentrations. Linear regression analyses were undertaken using R software (http://www.r-project.org/). In order to visualize the level of similarity of all phytoplankton species biomass and check whether there was a change in the community structure, a MDS (Multidimensional Scaling) plot was performed with the software PRIMER v6. Bray-Curtis similarities were computed following the square-root transformation of biomass in order to diminish the importance of the most abundant species and to take the less abundant species into consideration.

4. Results

4.1 Carbonate chemistry and nutrient evolution

After 3 days of $pCO_2$ adjustment, all mesocosms reached $pCO_2$ target levels and the average $pCO_2$ concentrations were calculated for each mesocosm during the two periods (Tab.1).

<table>
<thead>
<tr>
<th>Mesocosm</th>
<th>M5</th>
<th>M7</th>
<th>M3</th>
<th>M4</th>
<th>M8</th>
<th>M2</th>
<th>M6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target $pCO_2$ ($\mu$atm)</td>
<td>400</td>
<td>700</td>
<td>850</td>
<td>1000</td>
<td>1150</td>
<td>1300</td>
<td>1450</td>
</tr>
<tr>
<td>Average $pCO_2$ ($\mu$atm)</td>
<td>403</td>
<td>663</td>
<td>810</td>
<td>899</td>
<td>1021</td>
<td>1115</td>
<td>1287</td>
</tr>
<tr>
<td>(BF)</td>
<td>403</td>
<td>663</td>
<td>810</td>
<td>899</td>
<td>1021</td>
<td>1115</td>
<td>1287</td>
</tr>
<tr>
<td>Average $pCO_2$ ($\mu$atm)</td>
<td>368</td>
<td>604</td>
<td>690</td>
<td>768</td>
<td>829</td>
<td>974</td>
<td>1021</td>
</tr>
<tr>
<td>(AF)</td>
<td>368</td>
<td>604</td>
<td>690</td>
<td>768</td>
<td>829</td>
<td>974</td>
<td>1021</td>
</tr>
</tbody>
</table>

Table 1. Average $pCO_2$ ($\mu$atm) concentrations for the two periods, “BF” = before fertilization and “AF” = after fertilization. Symbols and colours indicated here are used in all following figures.
The $pCO_2$ concentrations were allowed to vary naturally and treatments remained fairly separate over the entire experiment (Fig. 1). The variability in the highest $pCO_2$ mesocosms was greater than in the others, mostly due to the fact that differences in $pCO_2$ concentrations between atmosphere and seawater were greatest during outgassing processes. The decrease in $pCO_2$ from $t20$ to $t25$ was driven by biological uptake, as productive biomass increases after nutrient fertilization (Kim et al., 2006). The $pCO_2$ levels were adjusted to expected values after fertilization.

**Figure 1.** Time evolution of $pCO_2$ ($\mu$atm) throughout the whole experiment. Dashed line indicates fertilization day, which distinguishes between the period before fertilization “BF”, and after fertilization “AF”. The colours indicate the $pCO_2$ concentrations: high (red), intermediate (grey) and low (blue) $pCO_2$ levels (legend on Tab.1).

Nutrient concentrations decreased substantially to low levels during the first period (Fig.2). There was low inorganic nitrogen (~1,46 $\mu$mol L$^{-1}$ NO$_3$ (NO$_2$+NO$_3$) and 0,47 $\mu$mol L$^{-1}$ NH$_4$) relative to phosphate (~ 0,17 $\mu$mol L$^{-1}$) in all mesocosms at the start of the study period, compared to the canonical Redfield nutrient stoichiometry (C: N: P = 106:16:1; Redfield, 1958). These concentrations are within the natural range data obtained from European Station for Time Series in the Ocean at the Canary Islands (ESTOC) located in the northeast Atlantic subtropical gyre (González-Dávila et al., 2003) and temporal dynamics between phosphate and nitrates showed decoupling. Nitrate and nitrite concentrations decreased from 1,46 $\mu$mol L$^{-1}$ to 0,011 $\mu$mol L$^{-1}$ during the first three days (-2,4 d$^{-1}$), whereas phosphate concentrations progressively diminished at an average rate of 0,16 d$^{-1}$ throughout the nutrient depleted period (BF: Before fertilization).
After fertilization (AF period) NO\textsubscript{x} and PO\textsubscript{4} increased their concentrations (3.43 µmol L\textsuperscript{-1}; 0.15 µmol L\textsuperscript{-1} respectively) and NO\textsubscript{x} decreased faster than phosphates (Fig.2). An abnormal pattern was observed on M7 treatment, with slower decrease of NO\textsubscript{x} and PO\textsubscript{4}, and a delayed peak of NH\textsubscript{4}. The slower decrease of the silicates indicates that the diatoms are using it in a more progressive way, instead of using it quickly. Moreover, ammonium decreased very quickly in the first three days (-1.2 d\textsuperscript{-1}) and barely reached zero on the BF period (low nutrient concentrations).

*Figure 2.* Temporal development of NO\textsubscript{x}, PO\textsubscript{4}, NH\textsubscript{4} and SiO\textsubscript{2} concentrations (µmol L\textsuperscript{-1}) in the high (red), intermediate (grey) and low (blue) pCO\textsubscript{2} treatments during the course of the experiment.

Silicate concentration was significantly lower under elevated pCO\textsubscript{2} (p<0.05, Fig.3) after fertilization, mainly due to biological uptake of diatoms (p<0.05, Fig. 4) (Bellerby et al., 2008).
Evolution of phytoplankton abundances along the experiment

The abundances of planktonic groups during the course of the experiment are presented in Fig. 5. Heterotrophic bacteria abundance showed a small increase on day 3, which coincides with a little increase in *Synechococcus* abundances (Fig. 5a). Interestingly, five days before nutrient addition, heterotrophic bacterial abundances increased in all mesocosms until t18 with an average concentration of $2 \times 10^6 \pm 3 \times 10^5$ cells mL$^{-1}$ (Fig. 5a). The average highest abundances of $3.8 \times 10^6$ cells mL$^{-1}$ during the phase “AF” were observed for the intermediate and high pCO$_2$ treatments.
We differentiated two subpopulations: High DNA bacteria (high fluorescence ones) and Low DNA bacteria (low fluorescence ones) (Gasol et al., 1999). Our data show how throughout almost the whole experiment LNA bacteria abundance overcame HNA abundances, but not in a significant way (see Annex.a).

*Synechococcus* abundances remained very low \((1.9 \times 10^4 \text{ cells mL}^{-1})\) during the first period in all mesocosms, and peaked at \(t24\) with maximum abundance of \(5.3 \times 10^5 \text{ cells mL}^{-1}\) and \(5.1 \times 10^5 \text{ cells mL}^{-1}\) in M2 (1300 µatm) and M4 (1000 µatm) respectively (Fig. 5b). *Prochlorococcus*, one of the most abundant cyanobacteria in the oceans, was absent during all experiment (data not shown). Owing to the fact that this group has been frequently observed in the Canary Islands seawaters (Aristegui & Montero, 2005), we postulate that the enclosure inside the mesocosms is responsible for that disappearance.

Picoeukaryotes showed a sharp drop during first five days in all mesocosms, and only the two treatments with the least \(pCO_2\) increased their abundance some days after fertilization \((t24)\) (Fig. 5c).

Autotrophic nanoeukaryotes diminished in all mesocosms during the first days in the same way as picoeukaryotes. We differentiated two groups amongst this cluster which showed different behaviours in the two periods. Nanoeukaryotes I, smaller and with less FL3, where the only that predominated during the first period (Fig. 5d), while nanoeukaryotes II, larger and with more FL3, increased in the second period, together with nanoeukaryotes I (Fig. 5e). The delayed bloom observed on M7 indicates, that this mesocosm behaved differently compared with the others but it has not been excluded from the statistical analysis because it did not cause significant differences in the results obtained.

The last group analysed was the microphytoplankton, formed of diatoms, large flagellates and dinoflagellates. Throughout the entire experiment, diatoms were the most abundant group of microphytoplankton (~87% on BF period, ~72% on AF period) and showed a little bloom at \(t5\), followed by a decrease until nutrient addition (Fig. 5g). Diatoms also showed a pronounced increase on high \(pCO_2\) treatments during the AF period. Dinoflagellates and flagellates remained practically constant during the BF period and increased on \(t23\), five days after nutrient addition (Fig. 5f, h).
Figure 5. Temporal development of total heterotrophic bacteria (a), *Synechococcus* (b), picoeukaryotes c), autotrophic nanoeukaryotes I (d), autotrophic nanoeukaryotes II (e), large flagellates (f), diatoms (g) and dinoflagellates (h) abundances (cell mL$^{-1}$ or Log cell mL$^{-1}$) in the high (red), intermediate (grey) and low (blue) $p$CO$_2$ treatments during the course of the experiment.
4.3 Effect of $pCO_2$ on different phytoplankton growth rates before and after fertilization

To compare $pCO_2$ effects, we analysed the data of the two phases separately: a) before nutrient addition (BF) (days 3-17) and b) after nutrient addition (AF) (days 19-29). As phytoplankton abundance may also be regulated by grazing and viral lysis, we refer to changes in phytoplankton abundances over time as net phytoplankton growth.

Before nutrient addition, nanoeukaryotes I and dinoflagellates had a positive growth under high $pCO_2$ concentration ($p<0.05$; Tab.2). The significantly positive response of nanoeukaryotes I+II is due to the contribution of nanoeukaryotes I, which is more abundant in this period. After nutrient addition, differences in heterotrophic bacteria and microphytoplankton abundances between treatments were significant ($p<0.05$) reporting positive responses on high $pCO_2$ treatments (Tab.2). The microphytoplankton response is mainly due to diatoms response, because they involved a ~72% microphytoplankton abundance in this period. Conversely, picoeukaryotes and flagellates reported negative significant responses on high $pCO_2$ treatments ($p<0.05$; Tab.2).

<table>
<thead>
<tr>
<th>Period</th>
<th>BF</th>
<th>AF</th>
<th>r²</th>
<th>p</th>
<th>Slope</th>
<th>r²</th>
<th>p</th>
<th>Slope</th>
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<tbody>
<tr>
<td>Group</td>
<td>r²</td>
<td>p</td>
<td></td>
<td>Slope</td>
<td></td>
<td>r²</td>
<td>p</td>
<td>Slope</td>
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<tr>
<td>Heterotrophic bacteria</td>
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<td>0.63</td>
<td>-</td>
<td></td>
<td>0.49</td>
<td>0.08*</td>
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<tr>
<td>LNA Bacteria</td>
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<td>0.5</td>
<td>-</td>
<td></td>
<td>0.55</td>
<td>0.05*</td>
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<tr>
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<td>0.25</td>
<td>0.15</td>
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<tr>
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<td>-</td>
<td></td>
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<td>0.28</td>
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<tr>
<td>Synechococcus</td>
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<td></td>
<td>0.14</td>
<td>0.39</td>
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<td>0.59</td>
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<td>0.03**</td>
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<tr>
<td>Nanoeukaryotes I+II</td>
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<td><strong>0.002</strong>*</td>
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<td>0.26</td>
<td>0.24</td>
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<td><strong>0.005</strong>*</td>
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<tr>
<td>Dinoflagellates</td>
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<td><strong>0.04</strong></td>
<td>Pos.</td>
<td></td>
<td>0.26</td>
<td>0.24</td>
<td>-</td>
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<tr>
<td>Flagellates</td>
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<td>-</td>
<td></td>
<td>0.73</td>
<td><strong>0.01</strong></td>
<td>Neg.</td>
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</tr>
<tr>
<td>Diatoms</td>
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<td>0.18</td>
<td>-</td>
<td></td>
<td></td>
<td>0.74</td>
<td><strong>0.01</strong></td>
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Significant codes:  
* 0.05  
**0.01  
***0.001

Table 2. Changes in phytoplankton abundances during the two periods (“before fertilization”, BF; “after fertilization”, AF) determined by the slope direction of the linear regression. “Pos.” means significantly increasing abundance with increasing $pCO_2$ and “Neg.” means significantly decreasing abundance with increasing $pCO_2$. 


4.4 Overall community structure trends

Abundance data of each group and species were transformed to biomass (see Annex b to see the conversion factors used). During the first period, the total biomass in all treatments remained relatively constant (Fig.6). Moreover, after three days of adding inorganic nutrients, the biomass of high $p$CO$_2$ treatments increased more than the others (Fig.6), although not in a statistically significant way (Fig.7, $p>0.05$).

![Figure 6. Temporal development of total biomass in the high (red), intermediate (grey) and low (blue) pCO$_2$ treatments during the course of the experiment.](image)

![Figure 7. Total biomass (mg C m$^{-3}$) versus pCO$_2$ for the seven treatments on the two periods. BF “before fertilization” and “AF” after fertilization. Symbols indicate the mean total abundances in the high (red), intermediate (grey) and low (blue) pCO$_2$ treatments](image)
In the second period (AF), some phytoplankton abundances on treatments with less $p$CO$_2$ (400µatm and 700µatm) were more interrelated (more proximity between dots means more similarity) than the other treatments (Fig 8b, surrounded by a black circle). The availability of a range of $p$CO$_2$ levels enable us to see the threshold at which the effects of $p$CO$_2$ begin to be strong enough to affect community structure. In this case, the tipping point was observed between 700 ppm and 850 ppm treatments.

![Bray-Curtis non-parametric MDS plots showing similarities between community structure using days as replicates at (a) before fertilization period (BF) between treatments, (b) after fertilization period (AF) between treatments.](image)

Figure 8. Bray-Curtis non-parametric MDS plots showing similarities between community structure using days as replicates at (a) before fertilization period (BF) between treatments, (b) after fertilization period (AF) between treatments.

Interestingly this difference was not observed during the first period (Fig 8a), when nutrients, mainly silicates, were practically exhausted and probably limiting the growth of some phytoplankton species.
The contribution in percentage terms of diatoms’ biomass was the greatest in all treatments during the first period, while after fertilization only in high $p$CO$_2$ treatments (Fig.9). Moreover, the contribution of nanoplankton’ biomass seems to be more important in low $p$CO$_2$ treatments after the fertilization period (Fig.9).
5. Discussion

5.1 Effects of $p$CO$_2$ on different taxonomic groups and community structure.

Predicting the ocean’s role in the global carbon cycle requires an understanding of the coupling between carbon and growth-limiting elements in biogeochemical processes. Therefore, understanding OA effects at ecosystem level is important, as complex communities could either dampen or aggravate the CO$_2$ effects experienced at the single species level. Past studies on ocean acidification have reported significant changes in competitive fitness between phytoplankton types to significantly alter community structure (Dutkiewicz et al., 2015; Bérmudez et al., 2016; Hare et al., 2007). In the present study, shifts in the community structure can be seen in Fig.9, showing a transition from nanoeukaryotes’ to diatoms’ biomass predominance as $p$CO$_2$ increased during and after the bloom. This shift was related with positive net growth rates on diatoms on high $p$CO$_2$ treatments (Tab.2), so an apparent CO$_2$ limitation was affecting them or giving them an advantage over other phytoplankton species. More than half of the organic carbon flux to the deep ocean is responsible for diatoms (Nelson et al., 1995, Dugdale & Wilkerson 1998), so the impact of $p$CO$_2$ on this group has to be carefully studied. Riebesell et al. (1993) reported how the CO$_2$ supply can limit growth rate but not the total biomass attained by a diatom bloom after exhaustion of nitrate and phosphate. The non-significant response observed for the first period suggests that nutrients may be playing an important role on the success of diatoms. During the two periods, nutrient concentrations were depleted rapidly, but the silicate concentrations were much scarcer in the first period than in the second. The strong correlation between silicates and diatoms (Fig.4) suggests that when silicates were limiting diatom’s growth, no apparent effects of $p$CO$_2$ could be seen in their net growth rates or biomass. In fact, Egge & Aksnes, (1992) confirmed that diatoms tend to predominate in the phytoplankton community except when silicate is scarce, although other studies have reported the opposite result (Hare et al., 2007). Therefore, it is not easy to establish whether this positive $p$CO$_2$-response is due to changes in the use of nutrient ratios, carbon limitation or both simultaneously. Tortell et al. (2002) associated the CO$_2$-dependent shift to diatoms predominance with a significant change in the use of nutrients, with higher ratios of nitrate:silicate (N:Si) and nitrate:phosphate (N:P) consumption by phytoplankton in the low CO$_2$ treatment. In the present study, the slower decrease of nitrates and silicates on low $p$CO$_2$ treatments in the bloom period (Fig.2) could support that hypothesis. Field experiments have demonstrated that they are capable of indirect or direct HCO$_3^-$ uptake (Tortell & Morel, 2002; Cassar et al., 2004; Martin & Tortell 2006) and for that reason, in general, their growth is not limited by carbon (Tortell et al. 1997, Goldman 1999). However, high-efficiency concentrating mechanisms have been reported (CCMs) on some diatom species (Reinfelder, 2011). The inorganic carbon acquisition strategies are likely to vary among diatom species (Kim et al., 2006; Tortell et al., 2008), and for that reason, the initial community composition could have a significantly greater impact than high CO$_2$ on phytoplankton biomass (Egger et al., 2014). In the first period, the most important diatom genus in terms of biomass and
abundance was *Dactyliosolen*, while in the second *Ginardia* was the most important genus on high \(p\text{CO}_2\) treatments. These two genus have been reported in the Canary waters (Moro et al., 2003), and it seems that *Guinardia* benefited from high \(p\text{CO}_2\) concentrations during bloom and immediately thereafter. In future studies, *Guinardia*’s CCMs should be studied to clarify the results obtained in the present study.

The other group which also reported positive net growth by increasing \(p\text{CO}_2\) on rich-nutrient period was heterotrophic bacteria (LNA+HNA) (Tab. 2, \(p<0.05\)). Some studies have attributed this response to accumulation of gel particles (TEPs), such as substrate, to attach to and accelerate nutrient availability on rich \(p\text{CO}_2\) treatments (Endres et al., 2014; Engel et al., 2014). Grossart et al. (2006) also reported an increase in bacterial abundances of particle-attached bacteria at high \(p\text{CO}_2\) during a bloom decline when the release of algal-derived organic matter was high. Bacterial abundance and bacterial growth would be very different, depending on whether bacterial growth is controlled by the system’s production of labile DOC, or by the parameters determining bacterial success in algal–bacterial competition for nutrients (Thingstad et al., 2008). Endres et al., (2014) showed increased bacteria abundances associated with increased extracellular release of organic compounds by autotrophs under excess availability of \(\text{CO}_2\) during the nutrient limitation period. In our case, the non-significant correlation during the nutrient depleted period (Tab. 2, \(p<0.05\)) does not correlate with the study mentioned above, although we do not have organic compounds data to determine whether the extracellular release of organic compounds was high or not. Another explanation for the bacteria’s behaviour observed could be related with silicates. Thingstad et al. (2008) showed how silicate would seem to have a large potential for changing the ratio between availability of organic carbon and nitrogen to the heterotrophic prokaryotic community. In treatments with silicates and low carbon concentrations where diatoms predominated, a high proportion of the material produced autochthonously was unavailable to bacteria possibly due to chemical form, or because of physical protection (for example, inside diatoms). In the present study, diatoms biomass predominated equally in all treatments during the first period (Fig.4), so the immobilization of autochthonous material could be the responsible for the lack of a positive increase in bacterial abundance. However, in order to test these hypotheses, the different forms of carbon should be collected in future studies of ocean acidification.

For bacteria, direct uptake of substrate from seawater is restricted to simple molecules such as mono- and disaccharides and amino acids (LMW-DOM), which are present in very low concentrations in seawater. In contrast, the high- molecular weight (HMW-DOM) fraction needs to be hydrolysed prior to microbial uptake. The increase observed on day 3 (Fig. 5a) was probably due to the initial consumption of LMW-DOM fraction, and the second increase, before fertilization (day 15), could be associated with the remineralization of more refractory organic material fraction. In oligotrophic environments, LNA bacteria may represent the most active and predominating bacterioplankton community (Zubkov et al., 2001; Jochem et al., 2004; Longnecker et al., 2006), although in the present study the two heterotrophic bacteria groups showed
similar behaviours on different treatments during the course of the experiment (See Annex.a).

*Synechococcus* is ubiquitously distributed throughout the world and recognized as one of the main components of picophytoplankton in the ocean (Johnson & Sieburth, 1979; Waterbury et al., 1986). As observed in other studies, no significant correlation on net growth rates between $pCO_2$ treatments were observed before or after fertilization (Fu et al., 2007; Lomas et al., 2012). Similar results have been reported under nutrient-depleted conditions on Maugendre et al. (2014), suggesting that ocean acidification has a very limited impact on this plankton community. Conversely, there are studies reporting negative responses in growth rates on high $pCO_2$ levels (Traving et al., 2014; Paulino et al., 2008) and positive responses (Dutkiewicz et al., 2015, Bermúdez et al., 2016). These differing results highlight the need to perform ocean acidification studies as close as possible to real natural conditions, since the results can vary if they are carried out in isolated cultures or in mesocosm studies. Suffrian et al. (2008) showed that the effects of elevated CO$_2$ on single plankton species observed in laboratory studies are not comparable to those obtained in experiments simulating close to natural conditions, since such complex systems seem to have a higher capacity to buffer changes in $pCO_2$. The apparent photosynthetic affinity for inorganic carbon of *Synechococcus* ranges between a low-affinity and high-affinity (Kaplan and Reinhold, 1999; Price et al., 2002; Badger and Price, 2003), and unresolved oxygen-depend process could affect the functioning of CCMs (Woodger et al., 2005). The lack of response observed in this group between treatments in the two periods could be associated with the lack of a direct effect of $pCO_2$ on this group or with indirect effects, such as a possible dependency of growth upon viral lysis of heterotrophic bacteria (Weinbauer et al., 2011) or high grazing rates observed in the two periods (Armengol et al., in progress).

*Prochlorococcus* disappeared since the beginning of the experiment in all mesocosms, so no possible effect of $pCO_2$ could be tested (data not shown). There is evidence to suggest that *Prochlorococcus* was virtually unaffected by elevated $pCO_2$ (Fu et al., 2007), probably due to the lack of differences in CCM physiology or gene expression observed under different $pCO_2$ conditions (Hopkinson et al., 2014).

Recent studies on ocean acidification are consistent with small-sized phytoplankton benefits from high $pCO_2$ levels in terms of growth rate and biomass accumulation (Bermúdez et al., 2016; Newbold et al., 2012; Brussaard et al., 2013). Some authors suggest that the picoplankton success on high $pCO_2$ environments could be associated with a decrease in microzooplankton predation or in viral production (Larsen et al., 2008; Meakin et al., 2011). In contrast, during our study, picoeukaryotes’ net growth rates decreased in high $pCO_2$ treatments during the bloom period (Tab.2, $p < 0.05$), while in the nutrient-depleted period, no apparent effects were observed, as pointed out by Newbold et al. (2012). It has been suggested that phytoplankton that lack an efficient carbon concentrating mechanism (CCM) might be favoured in a high CO$_2$ environment (Riebesell 2004). The operation of CCMs is energetically expensive and, because cell
membranes are freely permeable to CO₂, additional metabolic costs are incurred in limiting the efflux of CO₂ from the cell (Giordano et al., 2005). It may be that the energetic savings made by downregulating CCMs are significant under elevated pCO₂ in at least some picoplankton species (Meakin et al., 2011), although these benefits may be offset to some degree by the increased costs of other metabolic processes (Raven and Johnston, 1991). The high-efficiency evidences CCMs reported in this group (Iglesias-Rodríguez et al., 1998) could incur an expense in terms of additional energy on high pCO₂ treatments, although as we have seen previously, CCMs can vary between species, making it difficult to compare results between studies with different initial phytoplankton communities. Therefore, future work should examine the increase of pCO₂ on grazing dynamics, as well as focusing on the CCMs of different picoeukaryotes groups and their sensitivities to pCO₂ and nutrient availability.

The positive growth rates observed during the nutrient-poor period in the nanoeukaryotes I group as pCO₂ increased (Tab.2, p<0.05), tally with the findings of another study conducted in Bering Sea, where community composition shifted from diatoms and towards nanophytoplankton on high pCO₂ (Hare et al., 2007). Before fertilization, the predominant population of nanoeukaryotes was nanoeukaryotes I, smaller and with less FL3, whereas during the second period it was nanoeukaryotes II, larger and with more FL3. It is known that the size of cells is important for a variety of processes in seawater, such as diffusion-limited uptake of substrates, resource allocation, predator-prey interactions, and gravitational settling. The relative surface area can be of greater importance than cell diameter, mass or volume with respect to processes such as nutrient uptake, because it is the surface that interferes with the outer medium containing the substrate reservoir. The relative surface area of a phytoplankton cell increases with decreasing size, but also with increasing eccentricity of the cells. Consequently, the small nanoeukaryotes I should be better competitors for resources during the nutrient-poor period (Grover, 1989) but the negative significant correlation of their size as pCO₂ increased (see Annex.e) is not still resolved. Reinfelder (2011) put forward a theoretical relationship between cell size, bulk seawater concentration of CO₂aq, and cell carbon-specific reaction-diffusion CO₂ supply rate. For cells with radii smaller than approximately 10 μm, CO₂ reaction-diffusion could support high specific carbon fixation rates (>1 d⁻¹) at bulk seawater CO₂aq concentrations >10 μM. At CO₂aq concentrations from 3 to 20 μM, reaction-diffusion-supported specific carbon fixation rates drop sharply as cell radius increases from 10 μm to 20 μm. The size of the nanophytoplankton is normally between 2 and 20 μm, but in this study the exact size of the nanoeukaryotes is not available. If the size of nanoeukaryotes I had been smaller than 10 μm, our results might be in agreement with the above-mentioned study. In any case, the relationship between size and pCO₂ is still not well known, and therefore further studies should explore this area further.

Another possible explanation for the increase of nanoeukaryotes I during the first period could be that their small size gives them an advantage in decreasing the risk of being depredated by other organisms. In fact, Hansen et al. (1997) reported that heterotrophic
dinoflagellates preferably ingest prey near to their own size rather than smaller. Aberle et al., (2013) reported a positive CO$_2$ effect on dinoflagellates, as in the present study, during the nutrient-depleted period (Tab.2, p <0.05). The dinoglagellates’s form II RubisCO has the lowest carboxylation: oxygenation specificity factor among eukaryotic phytoplankton (Badger et al. 1998, Whitney & Andrews 1998), giving them a disadvantage with regard to carbon fixation in the high-O$_2$, low-CO$_2$ modern ocean. As a consequence, dinoflagellates are likely to require a CCM to sustain even their relatively slow rates of growth and photosynthesis, and, consequently, it could give them an advantage in high pCO$_2$ media, as observed in our study. Be that as it may, direct experimental evidence for CCMs in marine dinoflagellates is limited, and results vary with species and experimental conditions (Reinfielder et al., 2011).

5.2 Potential consequences for carbon cycling

An apparent shift in phytoplankton community structure was found between 700-850 ppm (Fig.8) and this may explain why we did not observe significant differences in total biomass between the CO$_2$ treatments (Fig.7). This result could be important because this threshold is within the atmospheric pCO$_2$ levels that are predicted in the future ocean models (IPCC, 2007). If the future ocean reaches these pCO$_2$ levels the structure of the phytoplankton community could change with important consequences on the flow of the organic carbon moved by the marine trophic chain. Similar results were observed in another study, showing how CO$_2$-dependent taxonomic shifts could occur in the absence of a detectable difference in total primary productivity or biomass (Tortell et al., 2002). In another mesocosm experiment, Engel et al., (2013) observed that addition of CO$_2$ led to an increase in the competition between bacterioplankton and phytoplankton, thus causing a decrease in autotrophic phytoplankton biomass. These findings were not observed in our study, where it seems that some autotrophic organisms, like diatoms, benefited in treatments with higher pCO$_2$. Thingstad et al., (2008) proposed the “counterintuitive carbon cycle”, particularly pronounced in diatom-dominated systems, where the carbon/mineral nutrient ratio in phytoplankton production is high. When bacterial growth rate is limited by mineral nutrients, excess organic carbon is accumulated in the system, while when bacteria is limited by organic carbon, the addition of labile dissolved organic carbon reduced phytoplankton biomass and activity and also the rate at which total organic carbon accumulates. In our study we did not observe a reduction of phytoplankton biomass, although this could be due to enhanced growth caused by nutrient fertilization. Most of the studies carried out suggest that the export of sinking organic carbon may be lower under future warming scenarios (Laws et al., 2000, Bopp et al. 2001) but more studies are needed to test it.
6. Conclusions

Despite the apparent changes in taxonomic composition and community structure observed along the experiment, total biomass did not differ significantly between the CO$_2$ treatments. The main changes in community structure were observed over 700-850 ppm of pCO$_2$ after nutrient fertilization. Future studies should consider this range as an important transition threshold in order to perform ocean acidification experiments. In our simulated-bloom situation, the net growth rates of heterotrophic bacteria and diatoms were higher in high pCO$_2$ treatments, while picoeukaryotes and flagellates responded oppositely. The assessment of the impact on specific groups needs to be undertaken with extreme caution, since the complexity of marine ecosystems and different composition communities respond differently to pCO$_2$ perturbations.

7. Acknowledgements

I wish to thank Javier Arístegui for enabling me to form part of this project, supervising my work and contributing to the phytoplankton analyses. Also, I am very grateful to GEOMAR for setting up the experiment and analysing nutrients, pCO$_2$ and microphytoplankton samples. I would also like to thank Nauzet Hernández for his patience and encouragement and - last but not least - Isabel Baños, Alba Filella, Acorayda and Minerva Espino for being such a very welcoming team and helped me in everything I needed.

8. Annex

a) Temporal development of total heterotrophic bacteria (a), LNA/HNA ratio abundances (cell mL$^{-1}$) in the high (red), intermediate (grey) and low (blue) pCO$_2$ treatments during the course of the experiment
b) Conversion factors used to pass abundances to biomass

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<td>HNA bacteria</td>
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Change of units (mg C/m³) = (cel/ml) * (FgC/cel) * (10⁻¹² mg/1Fg) * (1mL/10⁻⁶ m³)

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A. Rodríguez-Santos

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*Chaetoceros.mag*10 272  
*Chaetoceros.min*10 272  
*Chaetoceros.decipiens* 432  
*Chaetoceros.Didymus* 209  
*Leptocylindrus* 68  
*Asterionellopsis.glacialis* 108  
*Ceratulina* 723  
*Corethron* 1322  
*Thalassiosira.nitzschioides* 1057  
*Cylindrotheca* 64  
*Bacteriastrum.hyalinum* 140

c) Regression lines between SSC and $pCO_2$ concentrations
9. References


A. Rodríguez-Santos


A. Rodríguez-Santos


10. TFT Report

Descripción detallada de las actividades realizadas en el TFT

Inicialmente, éste trabajo empezó con el procesamiento de unas muestras diferentes a las utilizadas. Durante los primeros 4 meses analicé las muestras de un experimento de acidificación océánica que se realizó en las instalaciones del BEA (Banco Español de Algas) durante 15 días. Estas muestras formaban parte de un estudio para comprobar si una alga nociva encontrada en un proyecto de KOSMOS proliferaba en medios con más $pCO_2$. Al no obtener los resultados deseados estas muestras fueron redirigidas a mí para poder realizar mi TFT. En ese momento, me enseñaron a utilizar el citómetro de flujo para procesar tanto muestras de bacterias como de picoeucariotas, tal y como se explica en el apartado de “Methods”. También me mostraron cómo se realiza el lavado de material de laboratorio y cómo se preparan varios compuestos necesarios para los análisis.

A continuación pasé varias semanas filtrando y procesando las muestras de nanofitoplancton con un microscopio invertido de auto-fluorescencia (estas muestras fueron sin duda, las más difíciles de leer). Encontramos resultados que no se ajustaban a lo esperado y buscamos una posible explicación. Concluimos que la forma en que se añadió el CO$_2$ a las bolsas no fue la adecuada, al observar cómo la mayoría de poblaciones de fitoplancton morían poco después de iniciar el experimento. Estas muestras se desecharon y me permitió encargarme de algunas muestras de otro experimento, KOSMOS16. El hecho de no poder utilizar los datos que tanto esfuerzo me había costado obtener me enseñó una gran lección sobre el mundo de la ciencia. A veces mucho del esfuerzo que inviertes en algo no acaba llegando a buen puerto, pero hay que aprender de los errores cometidos a lo largo del camino, para no volver a cometerlos cuando lo intentas otra vez. Esos meses de trabajo me sirvieron para dominar el citómetro de flujo, varias técnicas y adquirir soltura a la hora de procesar una gran cantidad de datos.

El equipo de GEOMAR, supervisado por el doctor Ulf Riebesell y el “Grupo de Oceanografía Biológica” (GOB) nos reunimos para organizar el muestreo y revisar el diseño del experimento del proyecto KOSMOS16. Gracias a GEOMAR se dispuso del material necesario para montar los mesocosmos y todo su equipo hizo un gran trabajo. Durante prácticamente un mes entero nos dirigíamos cada mañana al muelle del puerto de Taliarte para muestrear los 8 mesocosmos que había instalado el grupo GEOMAR. Aproximadamente se tardaban unas 3 horas en recoger todas las muestras y nos íbamos turnando los días de muestreo. Se muestreaba de 6 a 7 veces cada mesocosmo, ya que había que rellenar dos garrafas de 10 litros con tubos que tenían una capacidad de 5 litros. Los tubos restantes se empleaban para medir gases disueltos y metales. Todo este procedimiento se realizaba con un traje especial para evitar la contaminación y los tubos eran cuidadosamente agitados para garantizar la homogeneidad de la columna de agua. Los equipos de muestreo estaban formados por una mezcla de estudiantes de diferentes lugares del mundo, permitiéndonos conocer otros alumnos que estudiaban lo mismo que nosotros, practicar inglés y entablar nuevas amistades. Una vez que cada grupo tenía sus
pertinentes garrafas de aguas las trasladábamos a nuestros laboratorios y empezábamos los diferentes análisis, siempre manteniendo estrictos protocolos para no contaminar las muestras.

Aproximadamente mis análisis terminaban hacia las 18:30h, aunque algunos días terminaba mucho más tarde. La citometría se pasaba “in vivo”, de modo que las muestras no tenían que ser manipuladas de ninguna forma. Al finalizar el experimento pasé varias semanas pasando las muestras de bacterias, que habían sido almacenadas a -80°C y tenían que ser debidamente teñidas son SYTO13. Debido a algunos resultados no congruentes, todas las muestras se analizaron dos veces. También me encargué de analizar unas muestras de citometría de un experimento con peróxido de hidrógeno que propuso uno de los miembros de GEOMAR a mitad del estudio.

Una vez con los datos en bruto empecé a realizar gráficos y análisis estadísticos. Seguidamente escribí la memoria, complementándola con la lectura de la bibliografía que hablaba sobre el mismo tema.

**Entrenamiento recibido**

Tanto el grupo de GEOMAR como el “grupo de oceanografía biológica” me enseñaron pacientemente a realizar los muestreos matutinos. La metodología usada para la citometría de flujo con el FACSscalibur y el CytoSense me la enseñó mi tutor Javier Aristegui, mostrándome pacientemente como identificar las diferentes poblaciones en los diagramas X-Y. Para entender estos diagramas tuve que aprender qué tipo de pigmentos son específicos de cada especie del fitoplancton y si se trataban de organismos autótrofos o heterótrofos. Acorayda y Minerva, las técnicas de laboratorio de nuestro grupo, me ayudaron a entender el funcionamiento del laboratorio, dónde se guardaba el material y pequeños trucos cuando el citómetro no funcionaba correctamente.

Los datos se trabajaron con Excel y después con R-Studio. Este último programa no lo dominaba en absoluto pero gracias a Nauzet Hernández y tutoriales de YouTube logré aprender satisfactoriamente. Después aprendí a usar el programa estadístico PRIMER con el fin de realizar un análisis PERMANOVA, aunque al final me quedé en un MDS debido a la naturaleza de los datos de los que disponía.

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

La relación con todos los científicos, técnicos, personal de mantenimiento y de seguridad que trabajan en el IOCAG ha sido muy buena. En todo momento me sentí integrada en el equipo y siempre me ayudaron en todo lo necesario. Intenté ayudar al equipo en todo lo posible y aprendí que cada uno tiene una función indispensable cuando se trabaja en equipo.
Aspectos positivos y negativos más significativos relacionados con el desarrollo del TFG

En general, he disfrutado mucho estudiando un tema que me apasiona. Tener la suerte de estudiar el cambio climático y sus repercusiones en la oceanografía biológica ha sido muy enriquecedor. Poder colaborar con el proyecto KOSMOS y el grupo de GEOMAR también ha sido muy satisfactorio. Conocer otros estudiantes internacionales siempre te hace enriquecer tanto como científica como persona. Otro aspecto positivo ha sido aprender a trabajar sin descanso durante muchos días. Teniendo en cuenta el cansancio acumulado durante el muestreo, la alegría con la que muchos de nosotros afrontábamos el día a día me pareció un aspecto muy importante a la hora de realizar nuestro trabajo.

El grado de organización que requiere una gran cantidad de datos también me ha sorprendido. Si no hubiera sido por la meticulosa nomenclatura y la profesionalidad a la hora de almacenarla, me hubiera sido imposible realizar los diferentes análisis.

Como aspectos negativos, creo que no he aprendido muy bien a administrarme el tiempo. Para otro trabajo, es un aspecto que debo mejorar con mucho ahínco.

Valoración personal del aprendizaje conseguido a lo largo del TFG

La realización de este trabajo me ha permitido ver en primera persona cómo funciona el mundo de la investigación científica. He aprendido a buscar respuestas a problemas que iban presentándose y a no conformarme con la primera solución que encontraba, mejorando mi visión crítica. El apoyo por parte de todo el equipo me hizo trabajar en un ambiente donde realmente me sentía muy a gusto, provocando que las horas se fueran volando y con ganas de volver al día siguiente.
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