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Microzooplankton grazing upon picoplankton: The effect of future carbon dioxide levels in the ocean

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Microzooplankton grazing upon picoplankton: The effect of future carbon dioxide levels in the ocean

Este trabajo se presenta por el alumno Kai García Neefjes para la obtención del título de Graduado en Ciencias del Mar por la Universidad de Las Palmas de Gran Canaria, y es dirigido por el Dr D. Santiago Hernández León del Instituto de Oceanografía y Cambio Global (IOCAG).

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Abstract

Impacts of ocean acidification on marine biota were observed in a wide range of marine systems. However, there is no abundant literature about the effect of a future high pCO_2 scenario on microzooplankton grazing in the ocean. Here, we used two different mesocosms under the Bioacid/Kosmos Project (GEOMAR) to study the response of high pCO_2 concentrations upon phytoplankton growth and microzooplankton grazing. Mesoscosms were installed inside the Taliarte harbor (east coast of Gran Canaria), and two pCO_2 levels were assayed, one simulating present conditions (400 µatm) and another at future scenarios of increased pCO_2 (1450 µatm). Microzooplankton grazing experiments were developed using the 2-point method, which is a modification of the classical dilution method. Our results did not show the high pCO2 treatment affecting directly the picoplankton communities (picoeukaryote and *Synechococcus* organisms), but we found some significant correlations between grazing and growth rates in both treatments.

Introduction

Ocean acidification due to the CO_2 increment in the ocean is one of the main problems faced in the context of global change. The excess of carbon dioxide released to the atmosphere is taken up by the ocean and therefore dissolved, generating carbonic acid (H_2CO_3). The increase of hydrogen ions promotes a decrease of the seawater pH (Newbold et al., 2012). Oceans served as one of the largest sinks for anthropogenic CO_2, absorbing ca. 30 % of anthropogenic carbon dioxide (Sabine et al., 2004). Thus, an important impact of acidification is expected.

Acidification changes seawater chemical speciation and biogeochemical cycles which also impacts directly in the biota (Fabry et al., 2008; Guinotte and Fabry, 2008; Doney et al., 2009; Kerr, 2010; Sabine and Tanhua, 2010). Prokaryote and eukaryote organisms in the range of 0.2–2.0 µm in cell diameter (Groisillier et al., 2006; Zubkov and Tarran, 2008; Zubkov, 2009) showed different responses in relation to acidification (Newbold et al., 2012). Recent studies have shown a 4-fold increase in *Synechococcus* photosynthesis when incubated at high CO_2, while *Prochlorococcus* did not show significant changes (Fu et al. 2007). Other studies have reported that picoeukaryotic cells will benefit from nutrient addition at high CO_2 concentrations (Paulino et al., 2008; Brussaard et al 2013; Shulz et al., 2013; Schaum et al., 2012).

Microzooplankton organisms are mostly grazers (Calbet and Landry, 2004; Sherr and Sherr, 2007) feeding on autotrophic and mixotrophic organisms. These organisms are in the range between 20 and 200 µm, and include heterotrophic organisms such as protozoa and metazoa. They play an important role in the flux of energy and matter through the marine food web, acting as a link between low and higher throphic levels (Beers and Stewart, 1967; Parsons and LeBrasseur, 1970; Berk et al., 1977). Furthermore, recent studies show that effects of ocean acidification on microzooplankton composition and diversity (e.g., Aberle et al., 2013). Microzooplankton grazing is of paramount importance in order to better understanding the biological pump and so, the fate of organic carbon in the water column (Schmoker et al., 2013). So, therefore, it is of importance to confirm the plasticity of these organisms to acidification and how they adapt to future ocean changes.

The aim of this work was to study the effect of high pCO_2 concentrations upon phytoplankton growth and microzooplankton grazing. Two treatments, one at present pCO_2 conditions and another simulating future pCO_2, were assayed. In order to assess both processes the 2-point method was employed, which is a modification of the dilution technique proposed by Landry et al. (1984). This method provides the grazing rates of the experiments and determine growth rates of phytoplankton.

Material and Methods

We performed a total of 10 acidification experiments during March 2016 under the Kosmos/Bioacid Project (GEOMAR, Germany), whose goal was to analyze the outcome of possible future oceanic scenarios due to acidification. Phytoplankton growth and microzooplankton grazing were measured using the 2-point method (Strom and Fredrickson, 2008), which is a modification of the dilution method proposed by Landry and Hasset (1982). This method has three main assumptions: (1) phytoplankton growth is not affected by the presence or absence of other phytoplankton organisms; (2) the probability of a phytoplankton cell being consumed is a direct function of the rate of encounter of consumer with prey cells; and (3) change in the density of phytoplankton over time can be represented by an exponential equation. The method is based in the incubation of two treatments by triplicate: one treatment with 100 % whole seawater (WSW), and the other treatment with a dilution of 5% WSW. The 100% WSW treatment contained all organisms smaller than 200 µm and provided phytoplankton growth rates in the presence of microzooplankton grazers (k=net growth). The 5% WSW treatment was sufficiently diluted to assume that encounters between grazers and prey were practically null. In this treatment, net growth rate was assumed to be equivalent to the phytoplankton intrinsic growth rate (µ). Net growth rates in each bottle (k, d^{-1}) were calculated from initial and final picoplankton readings in each dilution treatment:

$$k = \frac{1}{t} \ln\left(\frac{P_t}{P_0}\right)$$

where P_t = final picoplankton. concentration, P_0 = initial picoplankton concentration, and t = incubation time in days. Using these two treatments, microzooplankton grazing rates (g, d^{-1}) were calculated as:

$$g = \mu - k$$

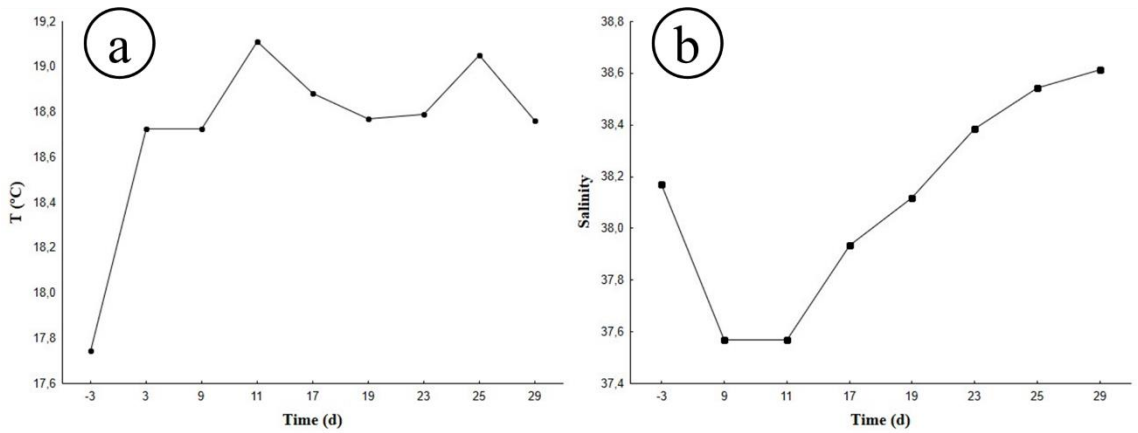
Grazing was negative in some experiments but they did not differ significantly from zero.

Water for dilution experiments was collected from mesocosms with 400 µatm and 1450 µatm using a 5 L PVC pipe, and gently transferred into two 25 L polycarbonate carboys. Treatment at 100% WSW was filtered through a 200 µm mesh attached at the end of the tube, and treatment at 5% WSW was gravity-filtered through a 0.2 µm Whatman Polycap to obtain particle-free seawater used as the diluent. Both treatments were then transferred to 3.4 L Tedlar bags (6 bags for each treatment). Three different nutrient concentrations were added to the Tedlar bags during the course of the experiments. Then, we sampled picoplankton for time t_0. Once the first sampling time t_0 was obtained, the 12 Tedlar plastic bags were set into a mesh bag and incubated in the

pier with the same light and temperature conditions as the mesocosms. Twenty four hours later we stopped the experiment for t_{24} and repeated the same sampling procedure. A total of 10 experiments were performed. Picoplankton organisms such as *Synechococcus* and picoeukaryotes were counted to obtain abundance and biomass by flow cytometry using a FACScalibur instrument (Becton and Dickinson). Samples of 1.6 mL were fixed with paraformaldehyde, kept for 25 min at 4°C in darkness, frozen in liquid nitrogen, and then stored frozen at -80°C until analysis. *Synechococcus* and autotrophic picoeukaryote abundances were converted to biomass using conversion factors of 250 fgC·cell^{-1} (Kana and Glibert, 1987; Li et al., 1992) and 2100 fgC·cell^{-1} (Campbell et al., 1997), respectively.

Results

A considerably increase in the mesocosm temperature values (about 1ºC) was observed at the beginning of the experiment (Fig. 1a), and remained rather constant (18.8±0.2) all along the experiment. Salinity values, however, fell after time -3, and increased dramatically after time 11. A quite high salinity (38.6) was measured at the end of the experiment (Fig. 1b). The lowest pCO2 treatment (M5) maintained practically a constant concentration of 400 µatm, whereas the highest pCO2 mesocosm (M6) varied during the experiments (Fig. 1c) from 1450 µatm at the begining to approximately 1000 µatm at the end of the experiment.



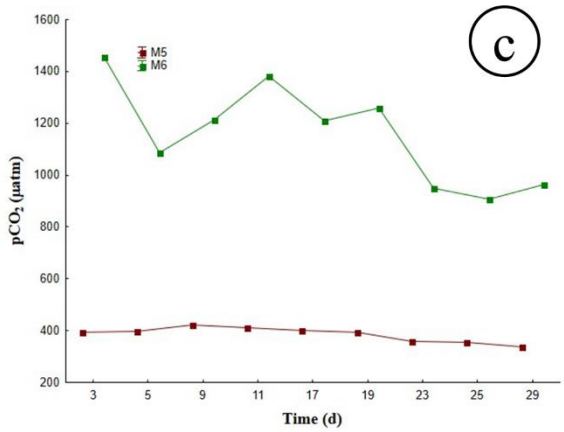
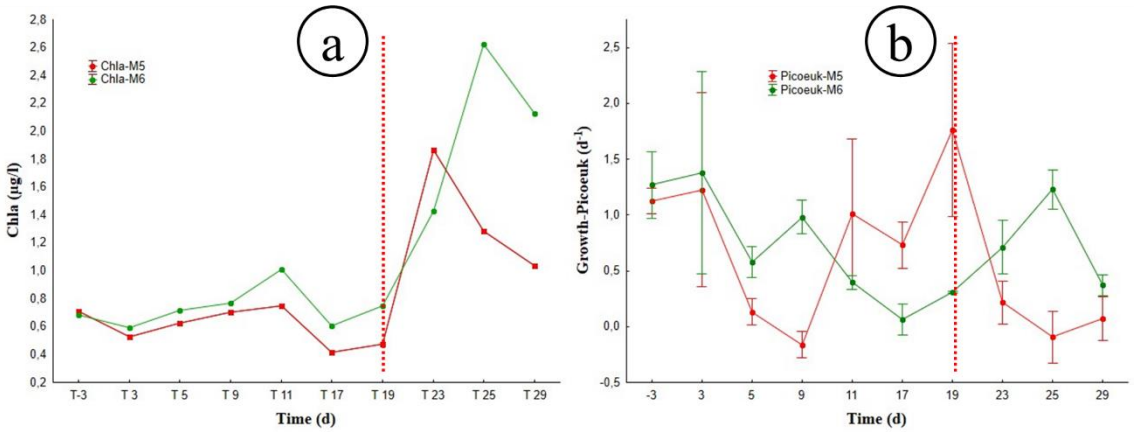


Fig. 1 Temporal variability of both treatments, M5 (~400 µatm) and M6 (~1450 µatm); a) Temperature (°C); b) Salinity; c) pCO2 (µatm)

Chla values showed a similar pattern for both mesocosms, although M5 showed lower values than M6 at the end of the experiment. Maximum values for M5 appeared during day 23, whereas for M6 the maximum value was observed during day 25 (Fig. 2a). Picoeukaryote growth values were similar at the beginning of the experiment for both mesocosms. Picoeukaryotes at 400 µatm showed an increase in growth from time 9 through time 19, which was the date of ferlitilization, whereas at >1000 µatm treatment they increased gradually after day 17 reaching maximum values during day 25 (Fig. 2b). *Synechococcus* growth values showed a similar pattern in both treatments, showing maximum values during days 9 and 11, respectively (Fig. 2c). Negative values were assumed as no growth.



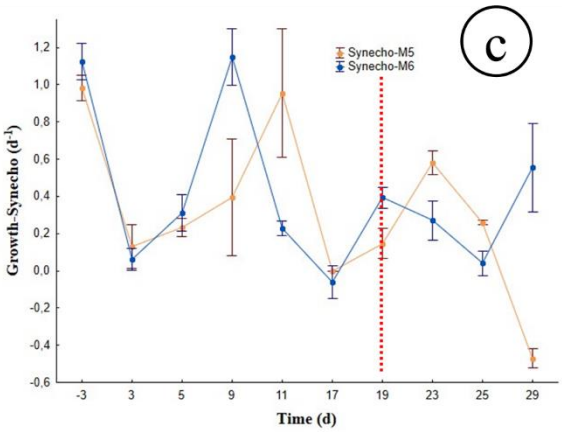


Fig. 2 Chla and growth variation trough time at the two different mesocosms; a) chla (µg/l); b) Picoeukaryote growth (d^{-1}); c) Synechococcus growth (d^{-1}). The dotted line represents the nutrient fertilization day.

Grazing values also showed a similar pattern for both treatments, although grazing rates for picoeukaryotes in the >1000 µatm were higher than those in the 400 µatm (Fig. 3a). The latter showed maximum values matching the time in which nutrients were added, whereas for the high pCO_2 treatment the maximum value was at time 23 (reaching a huge value of 5 d^{-1}). *Synechococcus* organisms also showed a similar grazing pattern, but these grazing rates increased considerably after fertilization (Fig. 3b). Negative values were assumed as no grazing.

We represented the highest pCO2 concentrations (M6) against the growth of picoeukaryoyes and *Synechococcus.* We found that there was not correlation between these two variables, (r^2 =0.015) for picoeukaryotes and (r^2 =0.001) for *Synechococcus*.

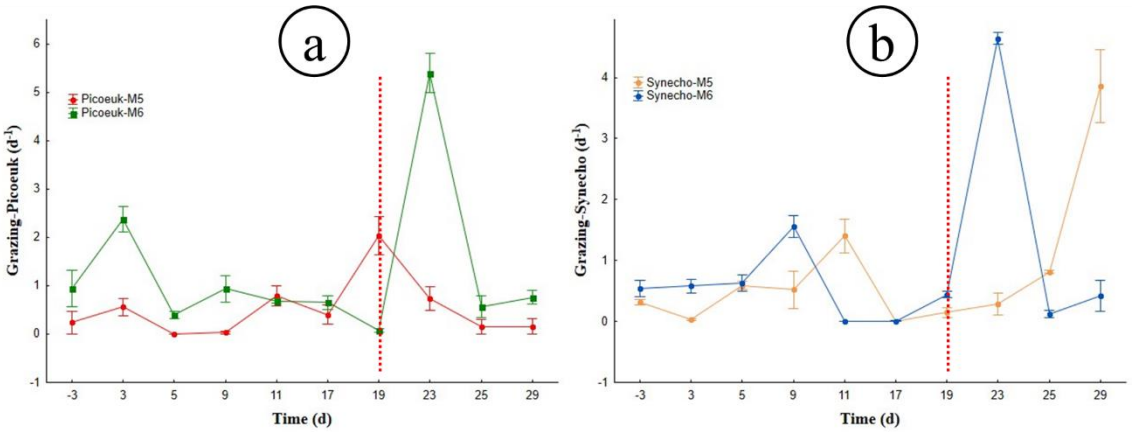


Fig. 3 Grazing rates at the two different mesocosms for a) Picoeukaryote and b) Synechococcus. The dotted line represents the nutrient fertilization day.

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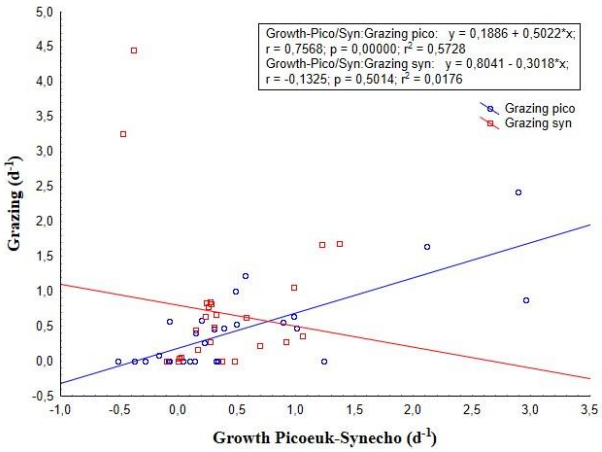

Fig. 4 Grazing rates and growth correlation for both Picoeukaryote and Synechococcus

A positive correlation between the growth of picoeukaryotes and their grazing rates ($P<0.05$) was observed (Fig. 4). However, we did not find correlation between growth and grazing for M6. Grazing and growth rates for each group of organisms separately were correlated if the huge grazing values above $3d^{-1}$ were disregarded. The results showed a similar pattern for each treatment, showing significant differences and positives correlations for picoeukaryotes ($r^2=0.497$) and for *Synechococcus* ($r^2=0.589$) (Fig. 5a-5b).

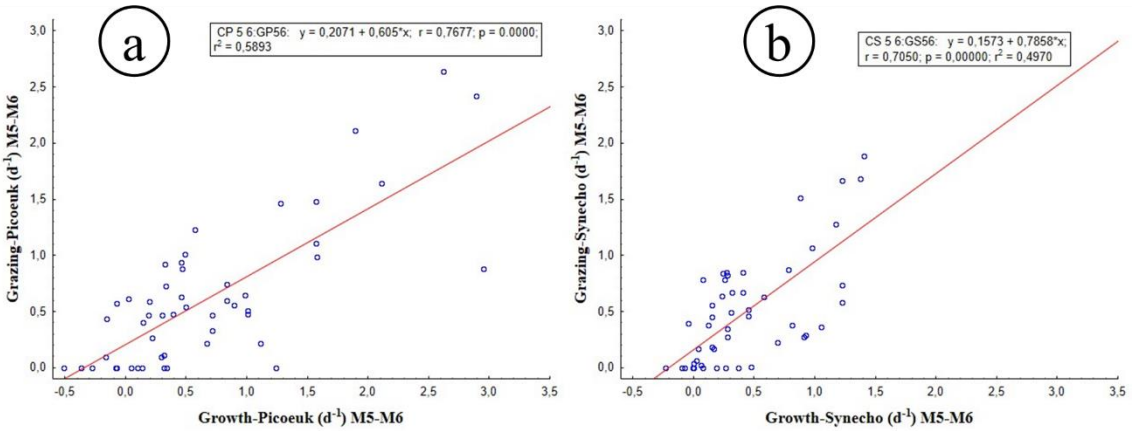

Fig. 5 Grazing rates and growth correlation without outliers for a) Picoeukaryote and b) Synechococcus

Discussion

The main goal of this work was to study the effect of how a high pCO_2 environment affects phytoplankton growth and therefore to microzooplankton grazing. A significant relationship was not found between our two treatments at different pCO_2 in picoeukaryotes and *Synechococcus* (Fig. 5). Riebesell and Tortell (2011) showed that elevated pCO_2 affects autotrophic processes, and other authors such as Suffrian et al.

(2008) and Rose et al. (2009) found high pCO_2 affecting phytoplankton communities as well as their size structure. We found a positive correlation ($P<0.05$) between the growth of picoeukaryotes and their grazing rates ($r^2=0.573$) (Fig. 4) at 400 µatm. However, at 1450 µatm we did not find such a correlation explaining the same grazing/growth pattern. Our highest grazing rates were not observed in other studies. Schmoker et al. (2013), in their review of grazing values in the ocean, showed always values lower than 3 d^{-1}. Because of this, the data analysis performed without values above 3 d^{-1}, seemed to unveil a pattern between growth and grazing rates for picoeukaryote and *Synechococcus* in both pCO_2 treatments. This pattern and these quite high values need further research.

In this sense, microzooplankton grazing rates found slightly after the nutrient fertilization during day 19 (Fig. 3a-3b) were in accordance with the results of Brussaard et al. (2013) in other mesocosms experiments. They found high microzooplankton grazing rates on pico- and nanophytoplankton just after nutrient addition. Despite a continuously high pico- and nanophytoplankton availability, microzooplankton showed a rapid decline during after nutrient addition in their experiment, just when the bloom developed. We did not analyze microzooplankton in this work, but we observed a decline on microzooplankton grazing rates just after fertilization. Whether this phenomenon is bottom-up or top-down regulated will remain. In this sense, the presense of mesozooplankton should promote a top-down effect as observed by Aberle et al. (2013).

Finally, we also ask whether the mesoscosm experiments represent the purported study of the effect of an enhanced CO_2 upon microzooplankton grazing and growth rates. Temperature increased more than 1ºC at the beginning of the experiment, changing drastically the phyto- and microplankton environment. Moreover, the continuous increase in salinity along the experiment (over values typical of Mediterranean waters) should also affect these communities and their response to high levels of CO_2. In any case, the relationship found between grazing and growth values seemed independent of these conditions and they could be used in future models about the role of microzooplankton in the ocean. However, large mesocosms are needed to avoid the important changes in the phyto- and microplankton environment.

Acknowledgedments

I wish to thank Dr. U. Riebesell, Dr. J. Arístegui, and Dr. S. Hernández León for giving me the opportunity to participate in KOSMOS experiment. I would also like to thank my co-tutor Laia Armengol for all the effort she made during last months sharing with me her knowledge on grazing experiments. Thanks also to Dr. Antonio Juan González Ramos and Dr. Lennart Thomas Bach for the help provided during key stages of the project. Many thanks to all the members of the Biological Oceanography Group,

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Appendix

Detailed description of the activities done during the FDW

El trabajo de Fin de grado (TFT) fue realizado conjuntamente con las prácticas externas ofrecidas por la Universidad de Las Palmas de Gran Canaria (ULPGC). El TFT ha sido supervisado por el Dr. Santiago Hernández León como tutor y la doctoranda Laia Armengol Bové como cotutora, en el Instituto de Oceanografía y Cambio Global (IOCAG).

Las actividades llevadas a cabo durante el TFT fueron:

- Lectura y búsqueda de artículos científicos relacionados con el objetivo de la estancia en el IOCAG, haciendo uso de numerosas bases de datos como SCOPUS o Google Scholar.
- Desarrollo de las metodologías de análisis de laboratorio y de las pautas necesarias para el muestreo en el campo.
- Desarrollo de los experimentos y obtención de resultados. Se emplearon dos diferentes citómetros de flujo para contar partículas y softwares para la interpretación de los datos.
- Tratamiento de los resultados, mediante análisis estadíticos.
- Realización de la memoria de prácticas externas
- Realización de un documento con formato de artículo científico como TFT. FDW write-up. After analysing the data, the FDW was written to present the knowledge gathered throughout the project.

Training received

Durante el desarrollo de la práctica la formación recibida ha sido extensa y extraordinaria. Nunca imaginé que aprendería tanto durante mis prácticas, he tenido la oportunidad de trabajar todos los aspectos de un proyecto de investigación. Los experimentos de grazing son muy laboriosos ya que se realizan numerosos pasos muy delicados que si no se llevan a cabo perfectamente los resultados no tienen validez. Tuve la gran suerte de que tanto mi tutor como mi cotutora tienen mucha experiencia realizando experimentos de grazing y en todo momento me explicaron, ayudaron y aconsejaron. Los muestreos y el trabajo del laboratorio los realizaba con mi cotutora. Durante mi estancia he recibido toda la atención posible por parte de mis supervisores y en consecuencia la formación que he recibido ha sido de gran calidad. He adquirido numerosas habilidades durante mi estancia en el IOCAG, donde he aprendido a muestrear con mucha delicadeza, a tratar y conservar

las muestras para su posterior análisis y a cumplir con las normas básicas de higiene y seguridad dentro de un laboratorio de investigación. También he tenido la oportunidad de aprender a utilizar un citómetro de flujo (FACScalibur) y a interpretar los datos mediante diversos softwares (Paint-a-gate, STATISTICA). Gracias a la dedicación implicada en el TFT, he mejorado y ampliado mis conocimientos en programas con los que llevo trabajando durante años como (EXCEL y WORD)

Level of integration and implication in the organization

La integración en este grupo de investigación se hizo muy amena, ya que desde que llegué al IOCAG, el personal siempre estuvo dispuesto a ayudarme en todo lo que necesitaba. Destacar la gran labor que realizaron Santiago Hernández león y Laia Armengol durante mi estancia. En ningún momento tuve problemas para contactar con mis tutores ya que tenían plena disponibilidad. Mi implicación dentro del departamento se hizo en relación a mi trabajo, es decir, desarrolle los experimentos para obtener mis propios resultados, y así poder redactar el TFT. La integración dentro de este departamento fue muy satisfactoria. Cabe destacar que son un grupo de trabajo muy humano que se mantiene activo y que siempre trata de ayudar en la medida de lo posible.

Most significant aspects related to the development of the FDW

En primer lugar, como aspecto positivo, he de decir que el desarrollo de las prácticas y el TFT fuera del entorno de la Facultad ha sido una experiencia positiva y muy enriquecedora. He podido conocer otro centro y trabajar en un laboratorio como parte del equipo, cosa que me ha motivado mucho y me ha servido de gran ayuda para aprender cómo es la dinámica de trabajo a diario dentro de un grupo de investigación. Otro de los puntos que considero como aspecto positivo es la capacidad que he adquirido realizando todos los aspectos relacionados con un trabajo de investigación, desde la preparación y el "set up" del experimento hasta la redacción de un artículo científico. He aprendido a ser muy cuidadoso trabajando y a tomarme mi tiempo a la hora de realizar el trabajo de laboratorio, a trabajar bajo presión y con intensidad. No puedo considerar aspectos negativos, ya que todo lo surgido me ha servido para formarme y mejorar como persona, aprendiendo sobre todo que los experimentos de investigación son muy laboriosos y la dedicación tiene que ser completa y máxima.

Personal evaluation of the skills obtained during the FDW

Como evaluación personal, considero que el aprendizaje ha sido mayor del que esperaba, ya que como he comentado anteriormente, he tenido la oportunidad de colaborar en un proyecto internacional, donde he conocido a mucha gente implicada en la investigación en Oceanografía biológica. A parte de conocer y formar parte de la dinámica de trabajo del grupo de investigación del IOCAG, también pude observar y aprender del trabajo que desarrollaba el otro grupo de investigación (GEOMAR). Destacar que en ocasiones tuve que tomar yo mis propias decisiones cosa que me gustó mucho porque me hizo sentirme responsable de mis acciones. Por tanto, ha sido un aprendizaje muy positivo en todos los aspectos.