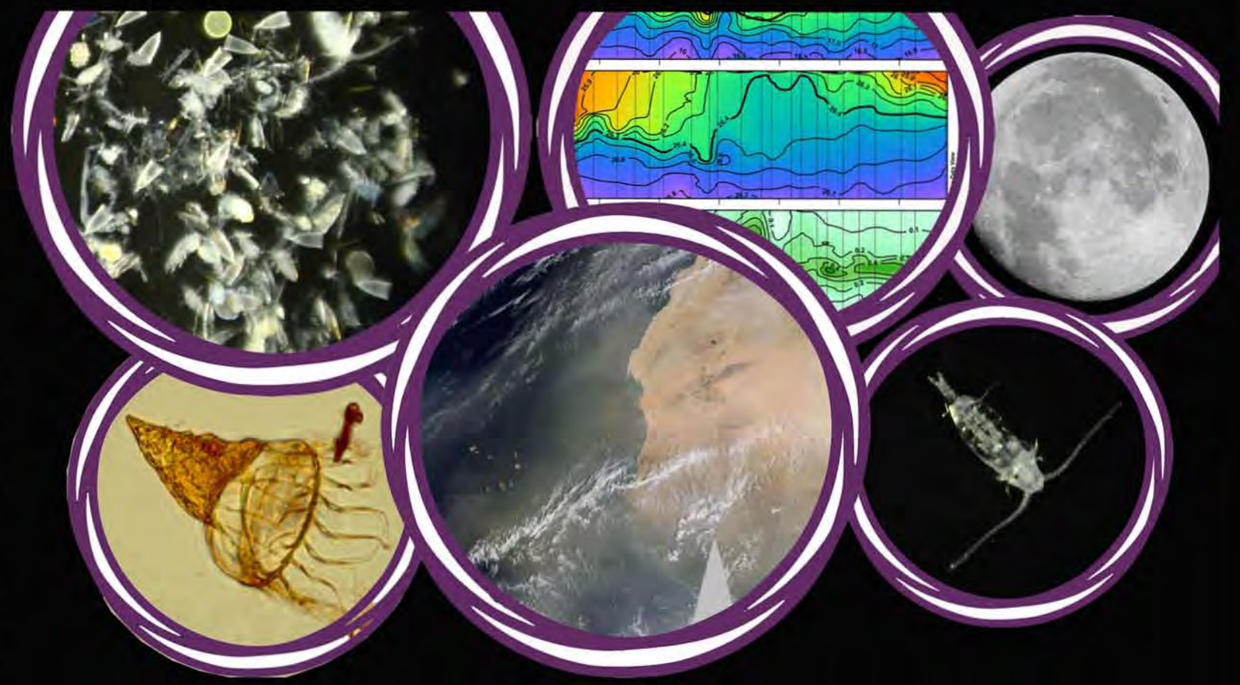
Subtropical gyres comprise large areas of the world's oceans where the recycling of energy and matter sustain the productivity of the ecosystem.The planktonic community inhabiting these systems is shaped by the interplay between resource availability and consumption by higher trophic levels.However, the knowledge about the temporal variability of the planktonic components or their role within the trophic web is not well characterized in subtropical waters. In this thesis we assessed the short-term variability of different components of the planktonic community. Picoplankton dominated the community excepting during productive periods, when larger autotrophic organisms could play a prominent role.Our results showed how seasonal variability was related to bottom-up forces, while top-down processes dominated in a short-time scale. We found a great impact of microzooplankton on regulating microbial communities, not only over autotrophic, but also on heterotrophic organisms. Furthermore, a close coupling between consumers and their prey was observed. Another mechanism regulating the planktonic structure is predation by diel vertical migrants upon zooplankton. The variability of the epipelagic mesozooplankton was driven by a predatory cycle linked to the lunar illumination. We simulated this variability and obtained the values of community mortality from which we derived the active carbon flux to the mesopelagic realm. This active carbon export was in the same order than gravitational sinking in subtropical waters. In the northeast Atlantic the marine community could also be influenced by the frequent Saharan dust storms affecting the area. We studied an intense dust period in 2010, during which a clear response of primary production was not observed. However, within the planktonic community, diatoms and mesozooplankton stocks increased after a strong Saharan dust storm. On the contrary, smaller autotrophic fractions were negatively affected. The outcome of this work supposes an important contribution to the understanding of the complex planktonic dynamics within the subtropical ecosystems and highlights the need to carry out oceanographic samplings at short-time scales.

Short-term variability and trophic interactions within the planktonic community in subtropical waters of the Canary Islands (Northeast Atlantic)

GARA FRANCHY GIL

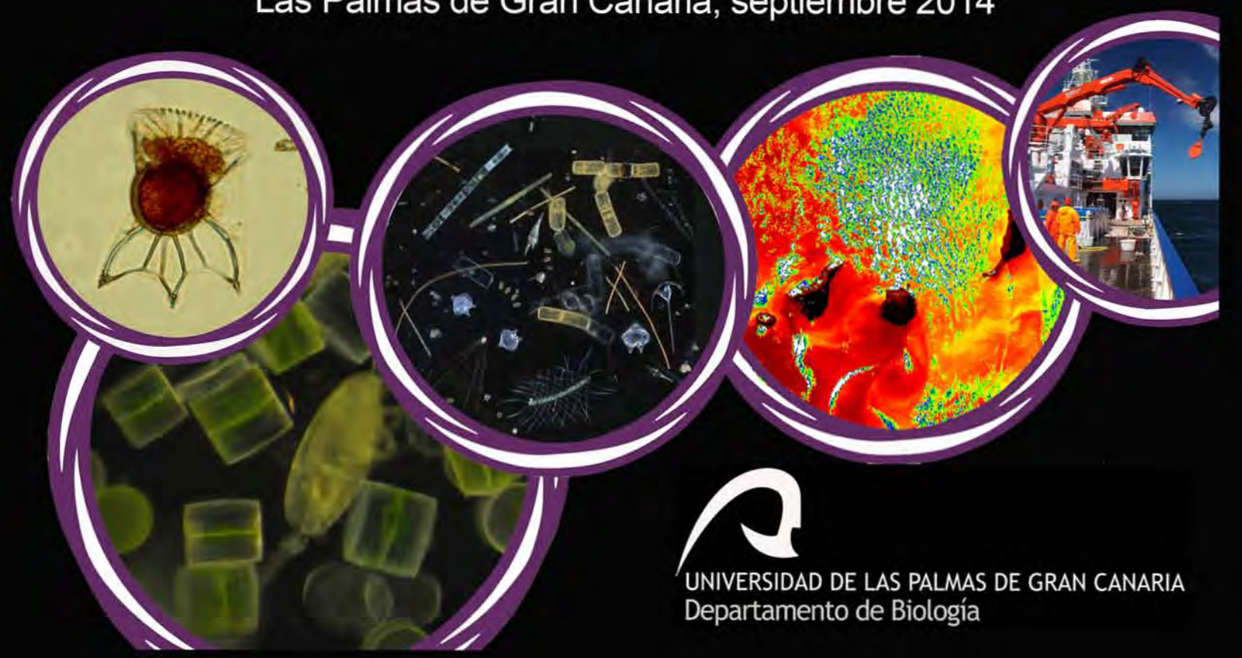


TESIS DOCTORAL

Short-term variability and trophic interactions within the planktonic community in subtropical waters of the Canary Islands (Northeast Atlantic)

GARA FRANCHY GIL

Las Palmas de Gran Canaria, septiembre 2014



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D. JOSÉ MANUEL VERGARA MARTÍN SECRETARIO DEL DEPARTAMENTO DE BIOLOGÍA DE LA UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA,

CERTIFICA,

Que el Consejo de Doctores del Departamento en su sesión de fecha ..... tomó el acuerdo de dar el consentimiento para su tramitación, a la tesis doctoral titulada "Short-term variability and trophic interactions within the planktonic community in subtropical waters of the Canary Islands (Northeast Atlantic)" presentada por la doctoranda Dª. Gara Franchy Gil y dirigida por el Doctor D. Santiago Hernández León.

Y para que así conste, y a efectos de lo previsto en el Artº 6 del Reglamento para la elaboración, defensa, tribunal y evaluación de tesis doctorales de la Universidad de Las Palmas de Gran Canaria, firmo la presente en Las Palmas de Gran Canaria, a ..... de Septiembre de 2014.

Fdo. : D. José Manuel Vergara Martín
Departamento de Biología
Universidad de Las Palmas de Gran Canaria

UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA
Departamento de Biología

TESIS DOCTORAL

Programa de Doctorado en Oceanografía

SHORT-TERM VARIABILITY AND TROPHIC INTERACTIONS WITHIN THE PLANKTONIC COMMUNITY IN SUBTROPICAL WATERS OF THE CANARY ISLANDS (NORTHEAST ATLANTIC)

(VARIABILIDAD A CORTO PLAZO E INTERACCIONES TRÓFICAS DE LA COMUNIDAD PLANCTÓNICA EN AGUAS SUBTROPICALES DE LAS ISLAS CANARIAS, NORESTE ATLÁNTICO)

Tesis doctoral presentada por Dª. Gara Franchy Gil para la obtención del grado de Doctora en Oceanografía por la Universidad de Las Palmas de Gran Canaria.

Esta tesis doctoral ha sido dirigida por el Dr. D. Santiago Hernández León.

La Doctoranda
Fdo.: Dª. Gara Franchy Gil

El Director
Fdo.: Dr. D. Santiago Hernández León

En Las Palmas de Gran Canaria, a , de Septiembre, de 2014.

RESUMEN

Los giros subtropicales abarcan grandes áreas del océano donde la productividad del ecosistema se sostiene a través del reciclado de materia y energía. En estas aguas, la interacción entre la disponibilidad de recursos y la presión de los niveles tróficos superiores determina la dinámica de la comunidad planctónica. Sin embargo, en aguas subtropicales, el conocimiento de la variabilidad temporal o el papel de los diferentes componentes de la comunidad dentro de la red trófica es bastante limitado. En esta tesis se evalúa la variabilidad a corto plazo de los diferentes componentes de la comunidad planctónica. El picoplancton dominó la comunidad salvo durante la época productiva, en la que los organismos autótrofos de mayor tamaño desempeñaron un papel destacado. Nuestros resultados muestran como la variabilidad estacional está relacionada con fuerzas “bottom-up”, mientras que los procesos “top-down” dominan a una escala de tiempo más corta. Encontramos que el microzooplancton ejerce un gran impacto sobre la comunidad microbiana, en organismos tanto autótrofos como heterótrofos. Además, observamos un acoplamiento muy estrecho entre estos consumidores y sus presas. Otro mecanismo que regula la estructura planctónica es la depredación de los migradores verticales sobre el zooplancton. Así, la variabilidad del mesozooplancton epipelágico está controlada por un ciclo de depredación vinculado a la iluminación de la luna. En este trabajo realizamos una simulación de esta variabilidad con la que se obtuvieron valores de mortalidad comunitaria de los que derivamos el flujo de carbono activo hacia la zona mesopelágica. Estos valores calculados de transporte activo de carbono son del mismo orden de magnitud que el flujo gravitacional en aguas subtropicales. En el Atlántico noreste la comunidad marina también podría estar influenciada por las tormentas de polvo sahariano que ocurren con gran frecuencia en la zona. En este sentido, se estudió la respuesta de la comunidad planctónica en un período de deposición de polvo atmosférico de gran intensidad, en el año 2010, sin observar una clara respuesta en términos de producción primaria. Por el contrario, la biomasa de diatomeas y mesozooplancton sí se vio aumentada en gran medida tras el paso de una fuerte tormenta de polvo del Sáhara, mientras que los organismos autótrofos de menor tamaño se vieron afectados negativamente. Los resultados de esta tesis suponen una contribución importante para entender la dinámica planctónica tan compleja en los ecosistemas subtropicales, y además, pone de manifiesto la necesidad de llevar a cabo muestreos oceanográficos a escalas de tiempo más cortas.

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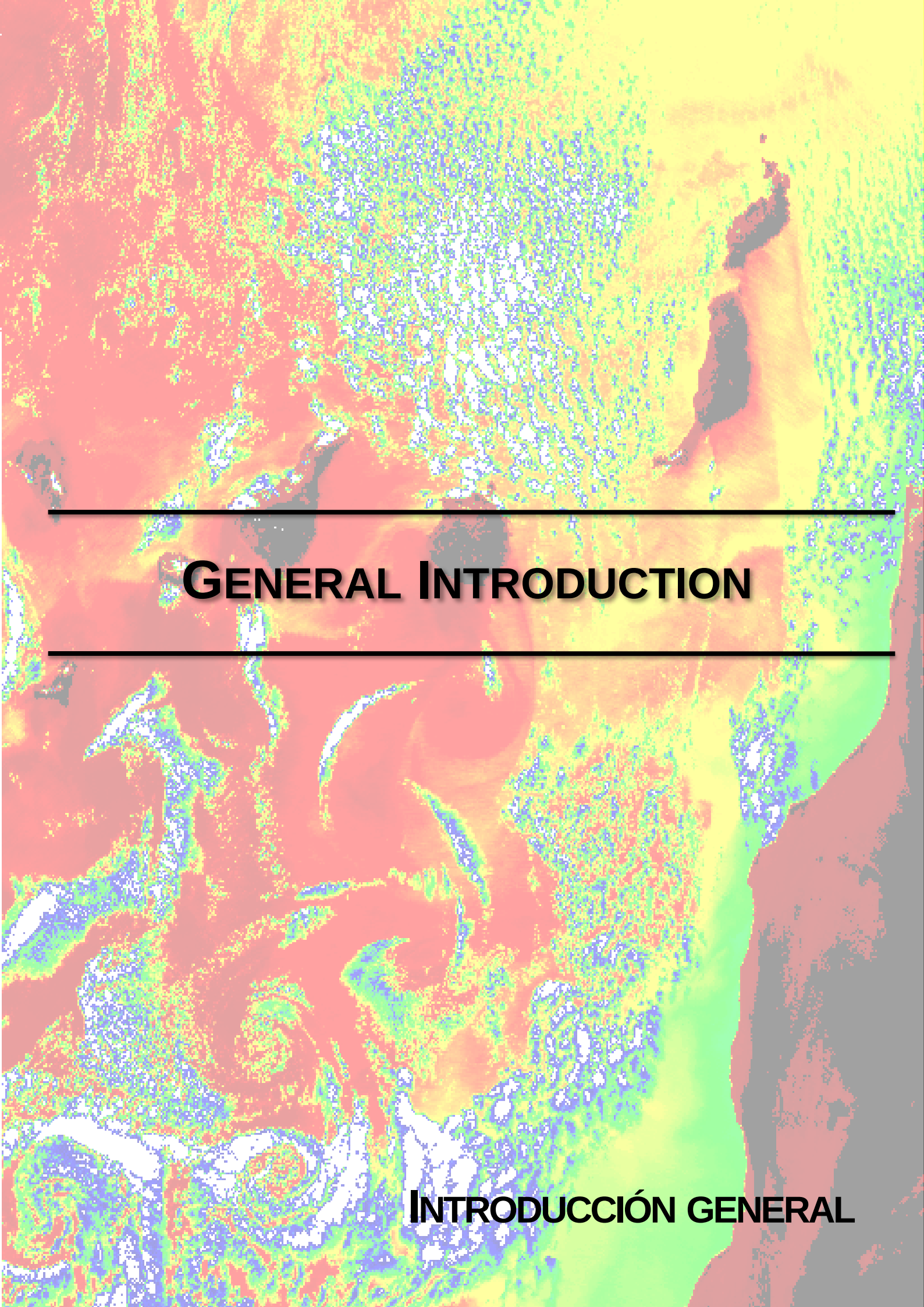

General Introduction

Introducción general

I. INTRODUCTION

I.1. BASIC CONCEPTS ABOUT PLANKTON

The term plankton comes from the Greek word *planktos* that means errant or drifter, alluding to the limited swimming ability of the organisms belonging to this community. It is a heterogeneous group of organisms including a wide variety of sizes, morphologies and types of feeding. The most widespread classification is from Sieburth et al. (1978), a general division in several categories according to size and feeding behavior (Fig. 1). Thus, four categories are distinguished according to size: picoplankton (0.2-2 μm), nanoplankton (2-20 μm), microplankton (20-200 μm) and mesoplankton (200-20000 μm). Two other size groups are frequently used for smaller or larger organisms: femtoplankton (<0.2 μm) and macroplankton (>20 mm). Among these categories we found two types of feeding: autotrophy and heterotrophy. Autotrophic organisms, called phytoplankton, obtain their energy requirements through the photosynthesis metabolism, while heterotrophs, called zooplankton, feed on other organisms (auto- or heterotrophic) to cover their metabolic needs. Although not included in the classification of Sieburth, it is now widely known that there are organisms, called mixotrophs, that are able to use both types of feeding. That is, they ingest other prey but they can also photosynthesize organic matter.

Picoplankton include autotrophic picoeukaryotes, two types of cyanobacteria (*Prochloroccocus* and *Synechoccocus*) and heterotrophic bacteria. Nanoplankton is mainly composed by unicellular flagellates, both autotrophic and heterotrophic. The major autotrophic components of microplankton are diatoms, dinoflagellates and coccolithophorids, although some species of dinoflagellates are now well recognized as mixotrophic (Sherr & Sherr 2002). Within microzooplankton, the principal components are protozoan organisms: ciliates, foraminiferans, radiolarians and acantharids; although metazoans as copepod nauplii, copepodites or meroplanktonic larvae are also included in this group. Mesoplankton is usually conformed by heterotrophs, although some autotrophic cells such as large dinoglagellates or diatom chains could be larger than 200 μm. Copepods are the main group within mesozooplankton, although other metazoans as pteropods, salps, chaetognaths, cnidarians or fish larvae are also important components.

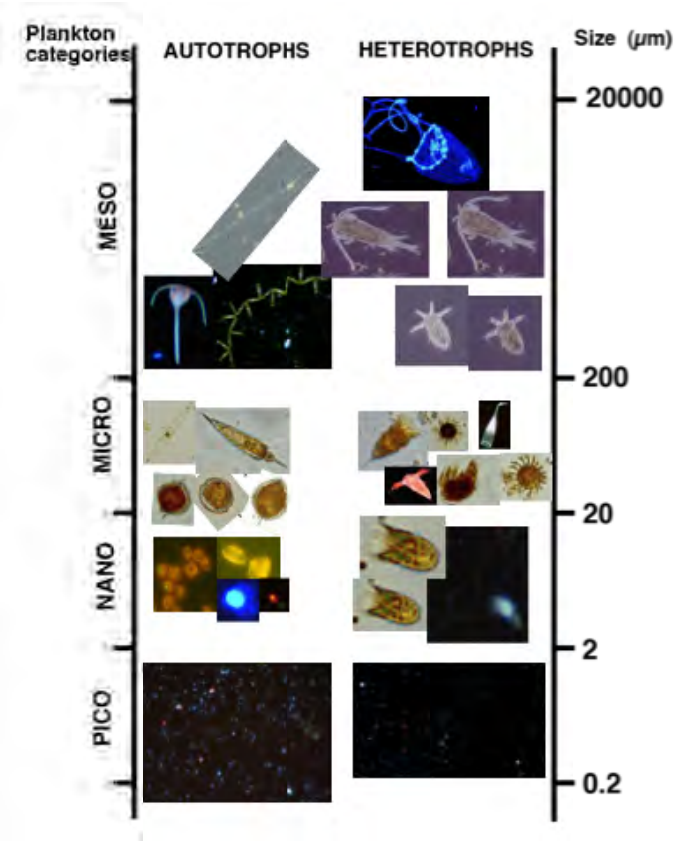

Figure 1. Classification of plankton components by size and feeding behavior from Schmoker (2011), redrawn from Sieburth et al. (1978).

I.2. THE CANARY CURRENT SYSTEM

The Canary Current System is located in the eastern branch of the North Atlantic subtropical gyre (Fig. 2). This system is characterized by oligotrophic waters in the oceanic area (De León & Braun 1973, Braun 1980), whilst to the east, we find the productive waters produced by the African coastal upwelling (Barton et al. 1998). Furthermore, productive pulses are frequent at the south of the Canaries, where cyclonic and anticyclonic eddies are formed by the interaction between the current and the islands (Arístegui et al. 1994, Barton et al. 2004).

The oceanic area, unaffected by the African upwelling and the eddy influence, is characterized by a strong stratification of the water column during most of the year that precludes the input of new nutrients into the photic layer (De León & Braun 1973, Braun 1980, Barton et al. 1998). The quasi-permanent seasonal thermocline is only eroded in January-March because of the winter convection resulting from the slight sea-surface cooling. At that time, the nutrient entrance into the photic layer promotes the so-called “late winter bloom” (De León & Braun 1973, Braun 1980, Barton et al. 1998). During the winter bloom, maximum values of chlorophyll *a* concentration and primary production are

reached in surface waters (De León & Braun 1973, Braun 1980, Arístegui et al. 2001), coinciding with the maximum mixed layer depth and nutrient availability (Cianca et al. 2007). During this productive period, the enhanced phytoplankton growth is followed by an increase of mesozooplankton biomass (Arístegui et al. 2001, Hernández-León et al. 2004, Hernández-León et al. 2007).

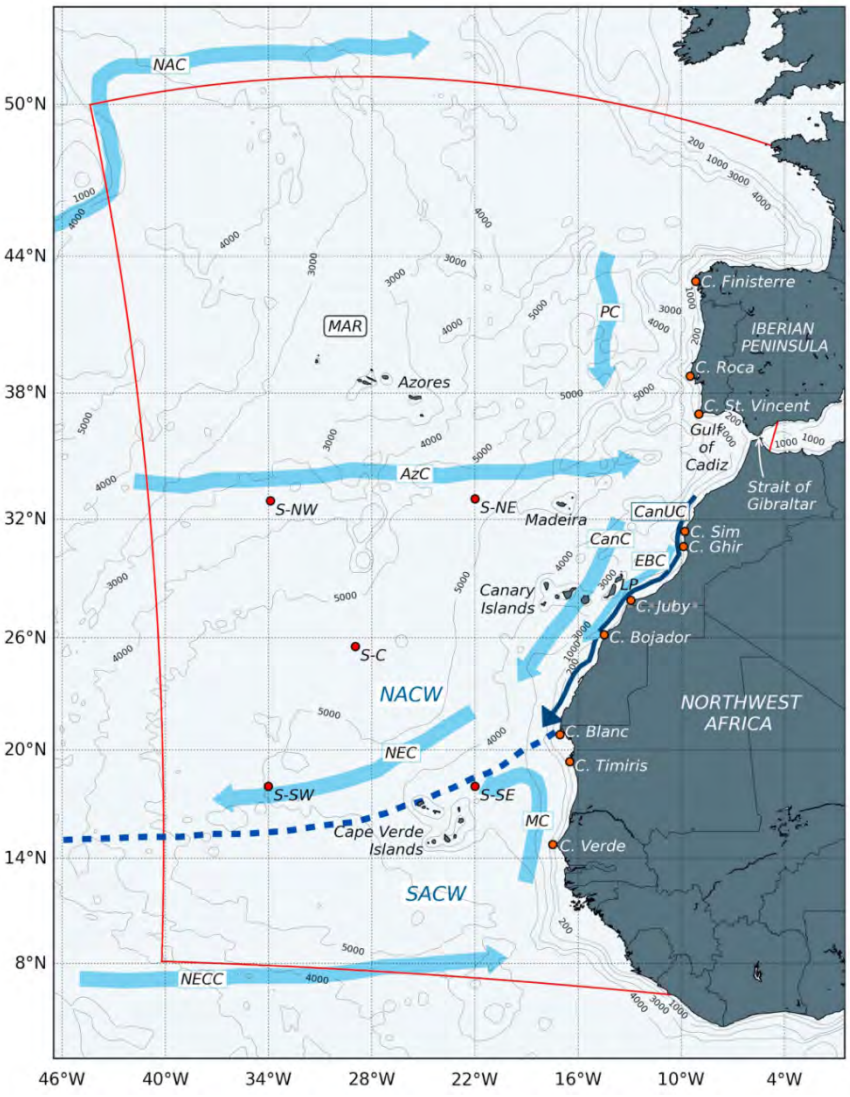

Figure 2. Scheme of surface circulation of the Canary Basin from Mason et al. (2011). AzC, Azores Current; CanC, Canary Current; CanUC, Canary Upwelling Current; EBC, Eastern Boundary Current; MC, Mauritania Current; NEC, North Equatorial Current; NECC, North Equatorial Countercurrent; PC, Portugal Current; MAR, Mid-Atlantic Ridge; LP, Lanzarote Passage.

I.3. PLANKTONIC COMPOSITION AND VARIABILITY IN SUBTROPICAL WATERS OF THE CANARY ISLANDS

A common feature of subtropical gyres is that primary production is mainly supported by nutrient recycling and the flow of energy and matter is controlled by the microbial loop (Pomeroy 1974, Azam et al. 1983, Longhurst 1998). That is, bacteria and small autotrophic cells (<2 μm) are consumed by nano- and microheterotrophic organisms, which in turn excrete dissolved organic matter used by the former for their metabolic requirements (Fig. 3). This is the case in oligotrophic waters of the Canary Current System, where phytoplankton is mostly composed by picoautotrophs, which are largely consumed by micrograzers (nano- and microheterotrophs), that controls more than 80% of primary production (Arístegui et al. 2001, Marañón et al. 2007).

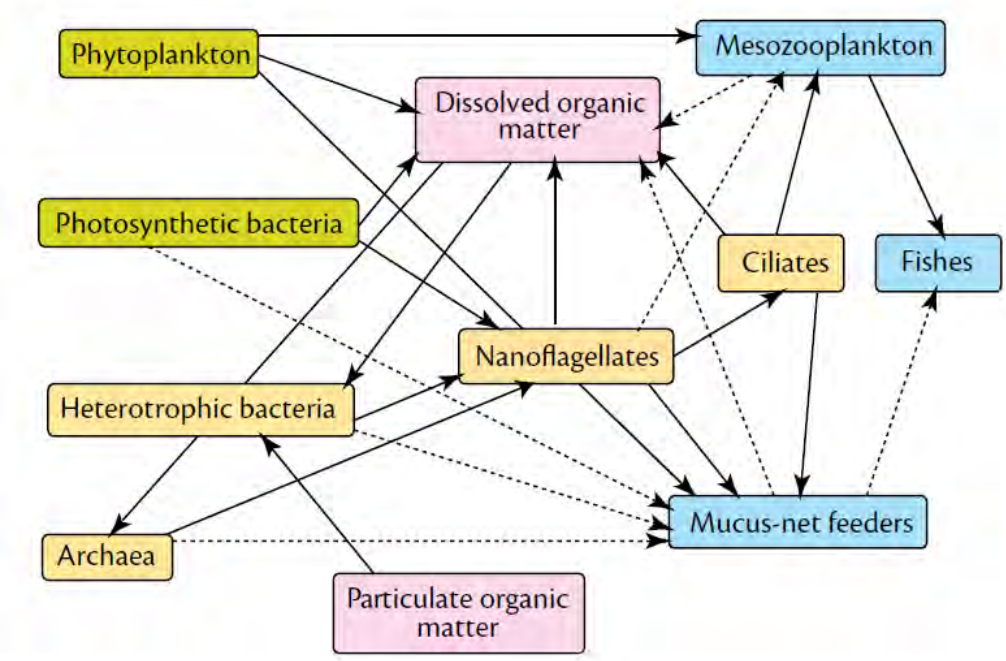

Figure 3. Diagram from Pomeroy et al. (2007) showing the principal role of the microbial components (green and yellow boxes) in the trophic web. Major fluxes of energy and matter are represented by continuous lines.

However, the seasonal variability of the planktonic community composition or the role of different planktonic groups throughout the year is poorly known in subtropical gyres, and also in the northeast Atlantic. To our knowledge, there are only two detailed studies about the temporal variability of the planktonic composition in the Canary Islands (Schmoker et al. 2012, Schmoker & Hernández-León 2013). These authors described a seasonal pattern for picophytoplankton consisting of the dominance of eukaryotic cells and *Synechococcus* during winter-spring while *Prochlorococcus* reached their maximum abundance in summer-fall. This seasonal variability has

also been found in subtropical gyres at the northwest Atlantic and in the north Pacific (DuRand et al. 2001, Giovannoni & Vergin 2012). On the contrary, any seasonality has been observed for heterotrophic bacteria in the northeast Atlantic (Schmoker & Hernández-León 2013), whilst in the northwest the biomass of bacteria was the highest after the winter-spring productive period (Steinberg et al. 2001).

A seasonal increase has also reported for nano- and microphytoplankton during the bloom period in the Canaries (Schmoker et al. 2012, Schmoker & Hernández-León 2013). However, a clear signal has not been found for all microplanktonic groups. For example, in the case of diatoms, they bloom occasionally in winter-spring or summer. Finally, a seasonal increment of the mesozooplankton stock during the productive season has also been described in these waters (Hernández-León et al. 2004, 2007).

I.4. TOP-DOWN PROCESSES REGULATING PLANKTONIC COMMUNITIES

Planktonic variability in subtropical waters is not only driven by physical conditions (light, temperature, stratification, etc.) or nutrient availability (bottom-up processes), but also top-down forces, namely grazing and predation, play an important role regulating planktonic populations.

Nano- and microzooplankton (heterotrophic organisms ranging 2-200 μm, hereinafter microzooplankton) are key components in tropical and subtropical areas where they account for 72-76% of the daily primary production grazed (Calbet & Landry 2004). Additionally, heterotrophic consumption of microzooplankton seems to be common, especially in subtropical waters because of the complexity of the food web (Calbet & Saiz 2013). Heterotrophic nanoflagellates are known to be effective bacterivores and they could be in turn preyed by ciliates (Azam et al. 1983, Guillou et al. 2001, Sherr & Sherr 2002, Pomeroy et al. 2007). Despite their importance, the knowledge about the feeding impact of microzooplankton is limited in subtropical gyres (Schmoker et al. 2013). In the subtropical waters of the Canary Current direct measurements are practically non-existent to our knowledge, and only some studies in the south-east of Azores (Gaul et al. 1999, Stelfox-Widdicombe et al. 2000, Quevedo & Anadón 2001) and in the south of the Canary Islands (Gutiérrez-Rodríguez et al. 2011) have assessed the microzooplankton grazing impact on primary production. The measured impact on productivity is highly variable according to these studies ranging between 37 and >100% of the daily production consumed by micrograzers. Furthermore, potential differences of the feeding impact upon the diverse autotrophic community inhabiting these waters have only been evaluated by Quevedo & Anadón (2001) and Gutiérrez-Rodríguez et al. (2011).

On the other hand, microzooplankton support the predator pressure exerted by mesozooplankton. Actually, microzooplankton contribute up to 50% to the daily copepod diet in oligotrophic waters (Calbet 2008). Mesozooplankton stocks, in turn, are also controlled and shaped by predation. In the Canary Islands waters, variability of the epipelagic mesozooplankton (non-migrant organisms inhabiting the upper water column) has been related to predation by diel vertical migrants (DVM) (Hernández-León 1998, Hernández-León et al. 2001a, 2002, 2004, 2010). Vertical migration of zooplankton and micronekton has been explained as a strategy to avoid predation (Stich & Lampert 1981, Ringelberg 2010). They keep at deeper and unlit waters between 200-1000 meters during the day, and rise to shallower layers for feeding during the night (Moore 1950, Uda 1956, Angel 1989, Longhurst et al. 1989), when they are less visible to their visual predators (Fig. 4). The magnitude of this process is partly determined by the lunar illumination (Uda 1956, Benoit-Bird et al. 2009), as these animals do not reach the upper layers when maximum illumination takes place (full moon) to avoid predation. In fact, the influence of the moonlight on the DVM behavior has been proposed as the mechanism that explains the variability of the epipelagic mesozooplankton biomass observed in the Canaries (Hernández-León 1998, Hernández-León et al. 2001a, 2002, 2004, 2010). Furthermore, this predatory cycle affects not only the planktonic dynamic in the upper water column, but significantly contributes to the active carbon export to the mesopelagic realm (Longhurst & Harrison 1988, Longhurst et al. 1989).

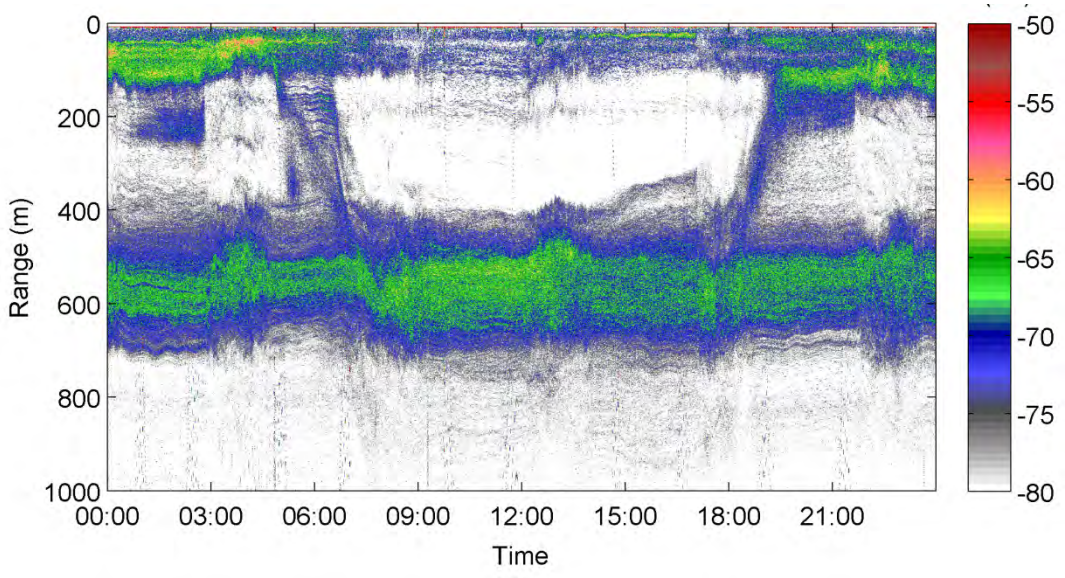

Figure 4. Diel vertical migration observed in an echogram (image by A. Ariza). Migrants descended from the surface to the deep scattering layer (600 m) at dawn (06:00 local time), and they moved up to upper layers at dusk (18:00 local time).

I.5. THE ROLE OF SAHARAN DUST DEPOSITION

The Canary Islands are located in the eastern subtropical North Atlantic close to the Sahara desert, which is the most important dust source for the world's oceans (Goudie & Middleton 2001). The Saharan dust contents high values of crustal elements as silicate, aluminum, manganese or iron (Goudie & Middleton 2001, Viana et al. 2002), as well as nitrate and phosphorus (Bonnet et al. 2005, Herut et al. 2005, Duarte et al. 2006, Pulido-Villena et al. 2010). Thus, atmospheric dust deposition is one of the major sources of nutrients to the open ocean (Donaghay et al. 1991, Duce & Tindale 1991, Guerzoni et al. 1999, Jickells 1999). In fact, the experimental addition of Saharan dust, releasing iron, phosphate, nitrate and silicate, has resulted in the stimulation of primary productivity in low nutrient low chlorophyll waters (Bonnet et al. 2005, Herut et al. 2005, Duarte et al. 2006, Marañón et al. 2010, Ridame et al. 2014). Therefore, the high potential nutrient inputs produced by the frequent African dust events (Sarthou et al. 2007) reaching the stratified waters of the Canary Islands could be a major factor affecting primary production. However, the effect of the Saharan dust deposition in the field has been rarely studied and is still not clear.

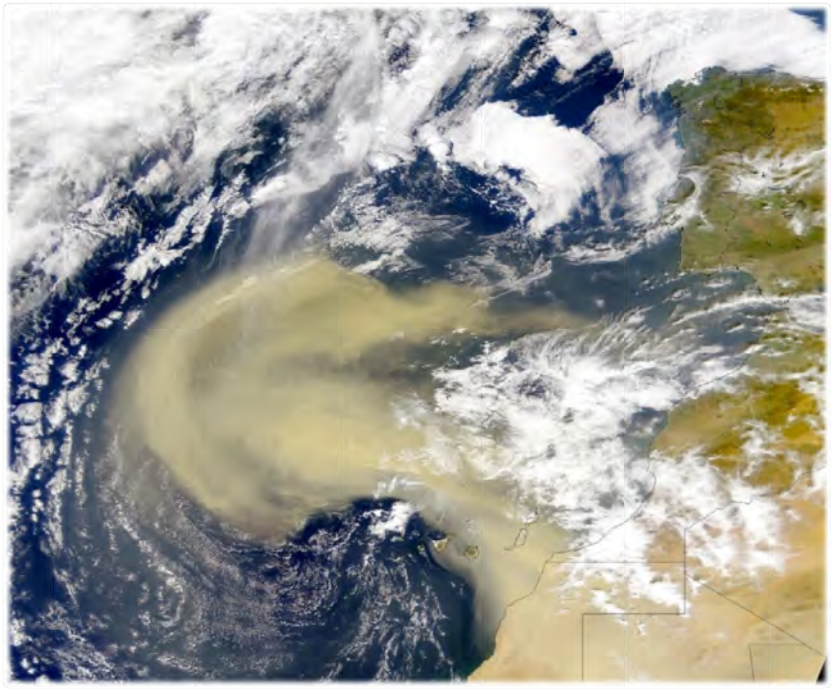

Figure 5. Satellite image of a Saharan dust storm over the Canary Islands on February 26, 2000 (provided by the SeaWIFS Project, NASA/GSFC).

II. OBJECTIVES

This thesis is based on the following main objectives:

1) To characterize in more detail the composition of the planktonic communities in the subtropical waters of the Canary Islands and to study the short-term variability of the planktonic components. This objective is addressed in Chapter I through the study of the abundance and biomass of pico-, nano- and microplankton assessing differences between productive and non-productive periods.

2) To quantify the potential impact of the Saharan dust deposition on the planktonic community inhabiting the oligotrophic surface waters of the Canary Islands. For that, the *in situ* effect of the Saharan dust events observed during the winter-spring 2010 on planktonic abundance and biomass was studied and results are detailed in Chapter II.

3) To assess the role of microzooplankton in controlling microbial communities through their feeding impact in these waters. For this purpose, we performed several dilution experiments to measure the impact of nano- and microheterotrophs over different planktonic groups. These results are shown in Chapter III. Furthermore, an alternative methodological approach to estimate microzooplankton feeding rates is evaluated in Chapter IV.

4) To estimate the magnitude of the zooplankton predatory cycle linked to the lunar illumination previously observed in the area and to evaluate its potential contribution to the active carbon export to mesopelagic waters. This point is assessed in Chapter V.

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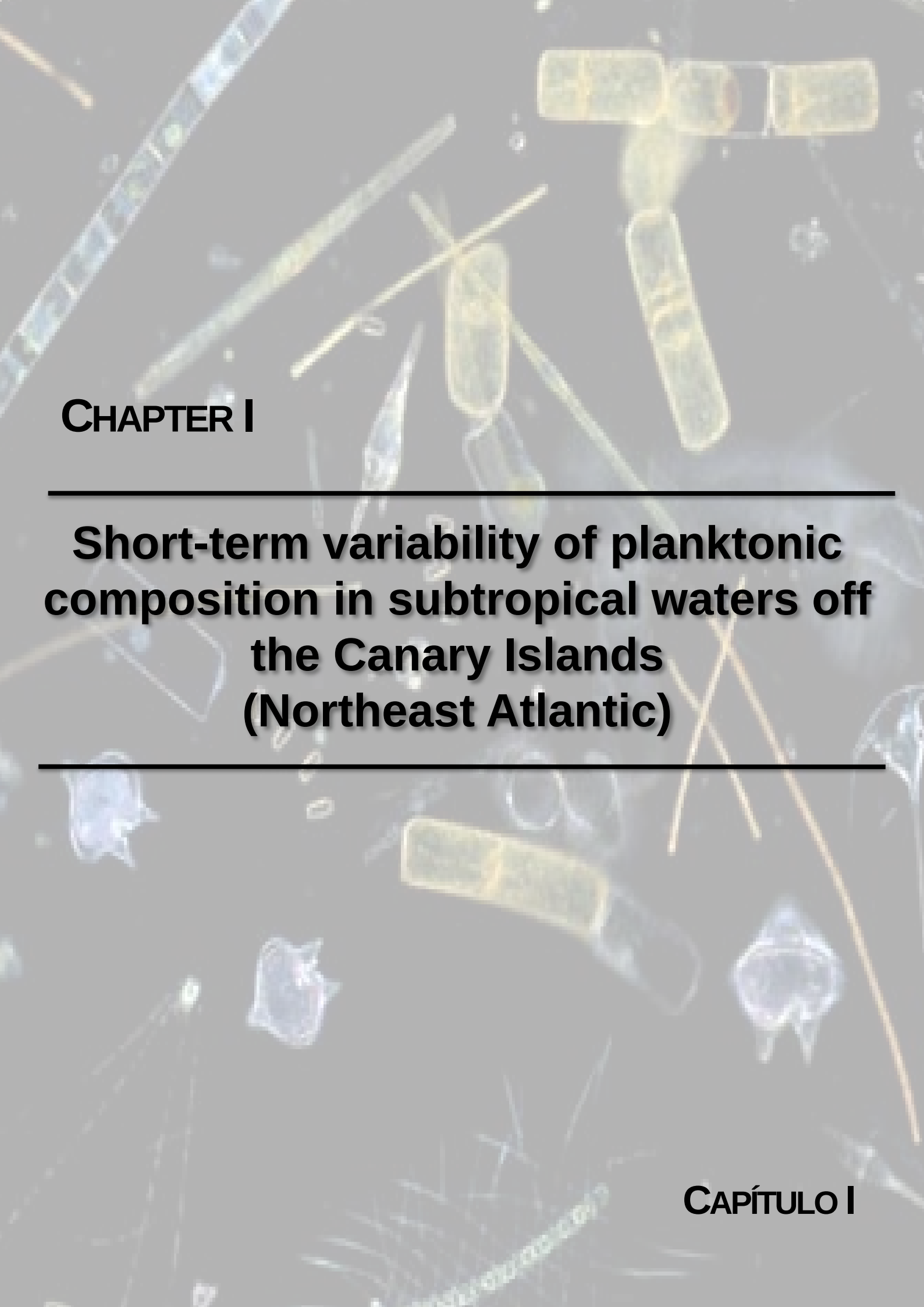

CHAPTER I

Short-term variability of planktonic composition in subtropical waters off the Canary Islands (Northeast Atlantic)

CAPÍTULO I

ABSTRACT

The planktonic community composition in oligotrophic waters off the Canary Islands was studied from November 2010 to June 2011 carrying out a weekly sampling and covering the productive period in these waters. The characteristic late winter bloom took place from February to April 2011, when the cooling of surface waters promoted the deepening of the mixed layer, and chlorophyll *a* concentration and primary production increased at the surface. Planktonic community was dominated by picoplankton, especially *Prochlorococcus* and heterotrophic prokaryotes, except during the productive period, when *Synechococcus* and picoeukaryotes dominated the picophytoplankton. During the bloom, diatoms were the major contributors to total autotrophic biomass and they were probably responsible for the highest rates of primary production. The planktonic variability was driven by bottom-up forces at a seasonal scale through the nutrient enrichment during winter. Additionally, a short-scale variability of phytoplankton biomass and productivity was observed even during stratified conditions. Short-term variability would also result from top-down processes such as feeding and grazing by nano- and microheterotrophs. In this sense, a significant correlation was found between the latter and their potential preys: heterotrohic prokaryotes and autotrophic picoeukaryotes and nanoflagellates. These results showed the need to sample at short-time scales to account for the total variability of the planktonic communities in subtropical waters. As an example, the striking and ephemeral diatom peak observed here, which would be probably not detected in a monthly sampling.

I. INTRODUCTION

Subtropical gyres comprise large oligotrophic areas of the world ocean where, despite the low nutrient conditions, complex trophic webs are common (Longhurst 1998) and annual production rates could be as high as in temperate ecosystems (Menzel & Ryther 1960). The seasonal and interannual production cycle in north subtropical gyres is fairly known from the Ocean Long-Term Time-Series Stations in the Pacific (HOT: Hawaii Ocean Time-Series) and the western Atlantic (BATS: Bermuda Atlantic Time-Series). The productive cycle in these subtropical waters, where light is not a limiting factor, is mainly supported by nutrient recycling, as the input of new nutrients into the photic layer only occurs during winter (Menzel & Ryther 1960, Karl et al. 1996, Cianca et al. 2007). Sea surface cooling during this season produces a deepening of the mixed layer, reaching the highest surface nutrient concentration and promoting the highest annual production rate.

The microbial loop (Pomeroy 1974, Azam et al. 1983) controls the flow of energy and matter in subtropical gyres (Pomeroy 1974, Longhurst 1998). It is now well known that picophytoplankton (<2 μm cells) accounts for most of the primary productivity (Li et al. 1983), which is mostly consumed by nano- and micrograzers (Calbet & Landry 2004). However, the seasonal variability of the planktonic community composition or the role of different planktonic groups throughout the year remains poorly known. Regarding to picophytoplankton composition, a seasonal pattern has been found in the Sargasso Sea and the HOT station (DuRand et al. 2001, Giovannoni & Vergin 2012). Eukaryotic cells and *Synechococcus* dominated in winter-spring while *Prochlorococcus* reached their maximum values in late summer and fall. This seasonal variability was also observed in the eastern subtropical North Atlantic gyre (Schmoker et al. 2012, Schmoker & Hernández-León 2013). These authors also described a seasonal pattern in nano- and microphytoplankton that increased their biomass during the bloom period. Within autotrophic microplankton, a seasonal variability has been observed for coccolithophores at BATS (Steinberg et al. 2001), where the highest concentrations were found during the productive period (January-March). However, other groups do not show a clear pattern, as in the case of diatoms, which bloom occasionally in winter-spring or summer at both eastern (Schmoker et al. 2014) and western north Atlantic gyre (Steinberg et al. 2001). Any seasonality has been observed for heterotrophic prokaryotes or nanoflagellates in the northeast Atlantic (Schmoker & Hernández-León 2013), whilst in the northwest the biomass of the former was the highest after the winter-spring productive period (Steinberg et al. 2001). Finally, a seasonal increase of the mesozooplankton stock during the productive season has been also described at both edges of the north Atlantic gyre (Madin et al. 2001, Hernández-León et al. 2004, 2007).

The Canary Current System is located in the eastern branch of the North Atlantic subtropical gyre. This system is characterized by oligotrophic waters in the oceanic area (De León & Braun 1973, Braun 1980), and like other subtropical areas, is characterized by a strong stratification of the water

column during most of the year. The seasonal thermocline is only eroded in winter because of the slight sea surface cooling, allowing a nutrient input into the photic layer and promoting the so-called late winter bloom (De León & Braun 1973, Braun 1980, Barton et al. 1998). During the winter bloom (January-March), maximum values of chlorophyll *a* concentration and primary production are reached in surface waters (De León & Braun 1973, Braun 1980, Arístegui et al. 2001), coinciding with the maximum mixed layer depth and nutrient availability (Cianca et al. 2007). Phytoplankton, mostly composed by picoautotrophs, are largely consumed by micrograzers that controls more than 80% of primary production in these waters (Arístegui et al. 2001, Marañón et al. 2007). Microplankton in turn are controlled by mesozooplankton (Schmoker & Hernández-León 2013), which mainly prey on non-pigmented organisms (Arístegui et al. 2001, Hernández-León et al. 2004) and are influenced by the predator pressure of diel vertical migrants (Moore 1950, Uda 1956, Angel 1989). Hence, all these trophic interactions modulate the planktonic variability and composition. In this sense, Schmoker et al. (2012) described how the interplay between different planktonic groups resulted in a succession of biomass peaks during the late winter bloom in the Canaries. The cycle, observed several times during the bloom, consisted in the increase of autotrophic picoeukaryotes, followed by heterotrophic nanoflagellates, microplankton and mesozooplankton.

In order to better understand the complex trophic interactions and successions described for subtropical waters, we studied the planktonic community composition in oligotrophic waters of the Canary Islands. For that, a weekly sampling covering the productive period was carried out during 8 months. Thus, differences between bloom and non-bloom conditions were assessed, as well as the short-term variability in terms of abundance, biomass and primary productivity.

II. MATERIAL AND METHODS

A weekly sampling was carried out in the Canary Islands waters to the north of Gran Canaria Island from November 2010 to June 2011, on-board the R/V Atlantic Explorer. Four stations 10 nautical miles equidistant (Fig. I.1) were sampled from surface to 300 m depth. Pressure, salinity, temperature and fluorescence were measured using a SBE25 CTD and a Turner Scufa Fluorometer coupled to an oceanographic rosette equipped with six 4-L Niskin bottles. Seawater samples were taken at the mixed layer (20 m) and at the deep chlorophyll maximum (DCM) depth to characterize the pico-, nano- and microplankton communities. A SBE19 plus CTD was eventually used during some cruises because of the main CTD failure. From temperature data, the mixed layer depth (MLD) was calculated as the level with a temperature difference of 0.5ºC from the 10 m depth (Cianca et al. 2007).

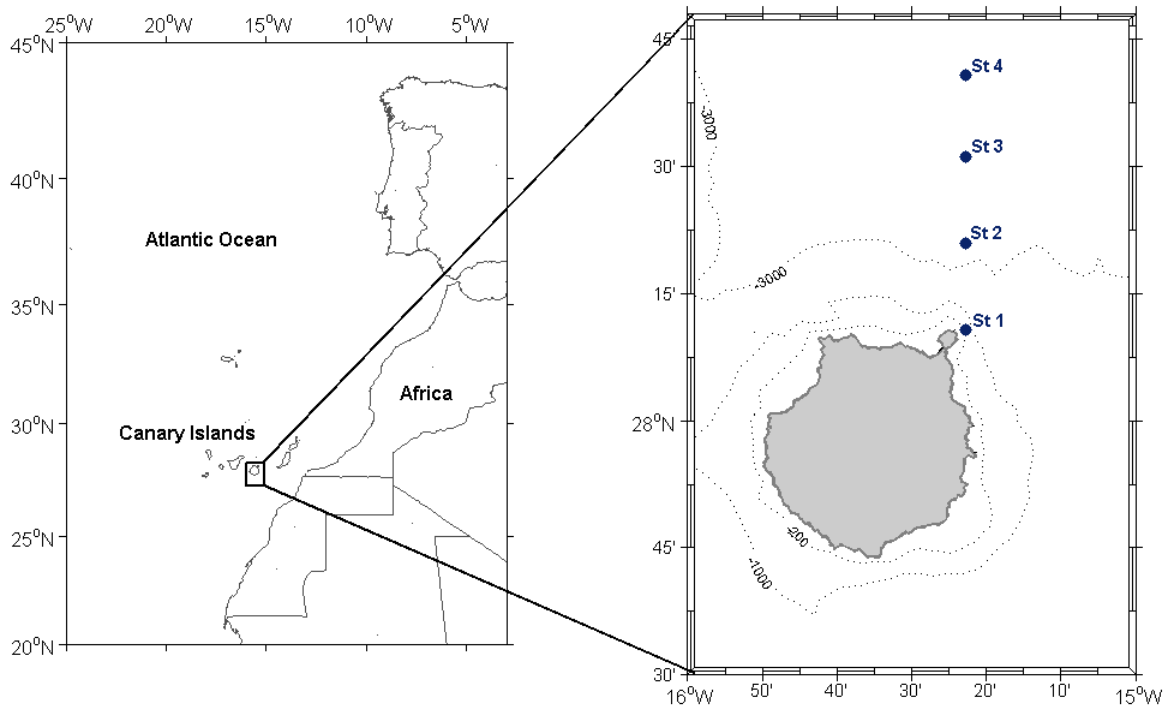

Figure I.1. Location of the four oceanographic stations (St) at the north of Gran Canaria Island (Canary Islands).

Chlorophyll *a* (Chl *a*) was measured by filtering 500 mL of seawater through a 25 mm Whatman GF/F filter and freezing it at -20ºC until its analysis at the laboratory. The extraction procedure consisted in placing the filter in 90% acetone at -20ºC in the dark, during at least 20 hours, and following the acidification method by Strickland & Parsons (1972b). Pigments were measured on a Turner Design 10A Fluorometer, previously calibrated with pure Chl *a* (Yentsch & Menzel 1963). From these data at 20 m, fluorescence in the whole profile was converted to Chl *a* (Chl *a* = 0.004 + 0.29 · fluorescence, r^2=0.54, p<0.001 for the SBE25 and Chl *a* = 0.034 + 15.78 · fluorescence, r^2=0.45, p<0.001 for the SBE19 plus) as an indicator of phytoplankton biomass. Size-fractionated Chl *a* (<2 µm, 2-20 µm, >20 µm) was obtained filtering additional 500 mL samples serially through 20 and 2 µm polycarbonate filters (25 mm). The smallest fraction was obtained subtracting the sum of 2-20 and >20 µm fractions from total chlorophyll values.

Picoplanktonic organisms (0.2-2 µm) were sampled in 1.6 mL tubes, fixed immediately with 100 µL of 20% paraformaldehyde, incubated at 4ºC during half an hour, frozen in liquid nitrogen, and finally kept at -80ºC until further analysis. Samples were analyzed later by flow cytometry using a FACScalibur Cytometer (Beckton and Dickinson). Side scatter (SSC) and fluorescence parameters

were obtained to distinguish between autotrophic picoeukaryotes (APE), cyanobacteria (*Prochlorococcus*, Pro, and *Synechococcus*, Syn) and heterotrophic prokaryotes (HP). Samples were run until 10000 events were reached or after 2 minutes at high speed to measure phototrophic organisms (APE, Pro and Syn), and at low speed for HP samples which were pre-stained with SYTO-13.

Auto- and heterotrophic nanoflagellates (ANF and HNF) were fixed using 540 µL of 25% glutaraldehyde in a tube containing 45 mL of seawater. Samples were kept at 4ºC in dark until the sample was filtered onto a 0.6 µm black polycarbonate filter placed over a backing filter and stained by diamidino-2-phenylindole (DAPI) for 5 minutes. The filter was immediately mounted on a microscope slide with low-fluorescence immersion oil and kept at -20ºC. Finally, it was analyzed by epifluorescence microscopy with a Zeiss Axiovert 35 microscope (Haas 1982). Only samples from 20 m were analyzed.

Microplanktonic organisms were kept in 500 mL dark bottles fixed with 1.5 mL of acid lugol and analyzed afterwards by the Utermöhl technique. A 100 mL subsample was settled for 48 h in a composite chamber. The bottom chamber was then examined by an inverted Zeiss Axiovert 35 microscope to identify the main microplanktonic groups: diatoms (Dia), dinoflagellates (Din), ciliates (Cil), and copepod nauplii or copepodites. Only samples from station 3 at 20 m were analyzed.

The abundance of organisms obtained by flow cytometry was converted to biomass using the carbon conversion factor of: 17 fgC $cell^{-1}$ for HP (Bode et al. 2001), 29 fgC $cell^{-1}$ for Pro, 100 fgC $cell^{-1}$ for Syn (Zubkov et al. 2000) and 1500 fgC $cell^{-1}$ for APE (Zubkov et al. 1998). After biovolume estimation by microscopy, nanoflagellates were converted to carbon using the factor of 220 fgC μm^{-3} for HNF (Borsheim & Bratbak 1987) and the equation $0.433(BV)^{0.863}$ pgC $cell^{-1}$ for ANF (Verity et al. 1992). Finally, microplanktonic abundance was converted to biomass from total biovolume data obtained directly by microscopy for the most abundant cells or from previous measurements in these waters (A. Ojeda, unpublished data). If these data were not available, an average size from the literature for every organism or group (Tomas 1997, Horner 2002, Ojeda 2006, Ojeda 2011) was assumed, fitting them to the suitable shape following Hillebrand et al. (1999). The corresponding parameters were used for Dia (log a = -0.541, b = 0.811 for V<3000 μm^3; log a = 0.933, b = 0.881 for V>3000 μm^3), Din (log a = -0.353, b = 0.864) and Cil (log a = -0.639, b = 0.984 for aloricate ciliates; log a = -0.168, b = 0.841 for tintinnids) to obtain the biovolume to biomass conversion factor (Menden-Deuer & Lessard 2000) (log pgC $cell^{-1}$ = log a + b · log V). Some species were not taken into account to calculate the microbial biomass because of the impossibility of finding their size range or biovolume in the literature, but these species were always practically negligible in terms of abundance. Average Chl *a* concentration, abundance and biomass were calculated without station

1 as a significant difference (Kruskal-Wallis test, $p<0.05$) was found between this coastal station and the oceanic stations 2, 3 and 4 for APE.

Primary production (PP) was measured using the ^{13}C method (Hama et al. 1983) in station 2 at 20 m and DCM depth. Water samples were transferred to 2L polycarbonate bottles previously rinsed with 10% HCl and distilled-deionized water and $NaH^{13}CO_3$ was added at about 10% of total inorganic carbon in the ambient water. Samples were incubated in a tank on-deck for between 3 and 7 hours depending on the cruise. Running surface seawater and appropriate meshes simulated *in situ* temperature and light intensity. Initial and final particulate organic carbon (POC) and particulate material used for isotope analysis were filtered through precombusted GF/F filters (5 h, 450ºC). These filters were frozen and stored at -20ºC until analysis. POC was measured using a CHN analyzer (Carlo Erba EA 1108) and isotopes in a mass spectrometer equipped with an elemental analyzer (Flash EA 11 ThermoFinnigan with Deltaplus).

III. RESULTS

The characteristic late winter bloom was developed from February to April 2011 when the cooling of surface waters occurred and a minimum temperature of 19ºC was measured in the mixed layer (Fig. I.2A). At that time the average depth of the mixed layer (ML) reached maximum values (around 140 m), promoting the shoaling of the 26.4 kg m^{-3} isopycnal surface (Fig. I.2B) and a modest increase in the Chl *a* concentration at the ML reaching around 0.3 mg m^{-3} (Fig. I.2C). Before February and after April higher surface temperatures were observed (Fig. I.2A) and the seasonal thermocline was well developed resulting in a thinner ML, especially during May and June (above 60 m). Within this period a deep chlorophyll maximum appeared between 70 and 100 m (Fig. I.2C).

Integrated Chl *a* concentration increased in February and March (Fig. I.3A), reaching average values of 34 and 50 mg m^{-2}, respectively. These maxima matched the deepest ML and a positive correlation between them was observed (Spearman's rank $r=0.38$, $p<0.01$). Relatively high values were also maintained during April, when the maximum chlorophyll concentration was found at the DCM (Fig. I.2C).

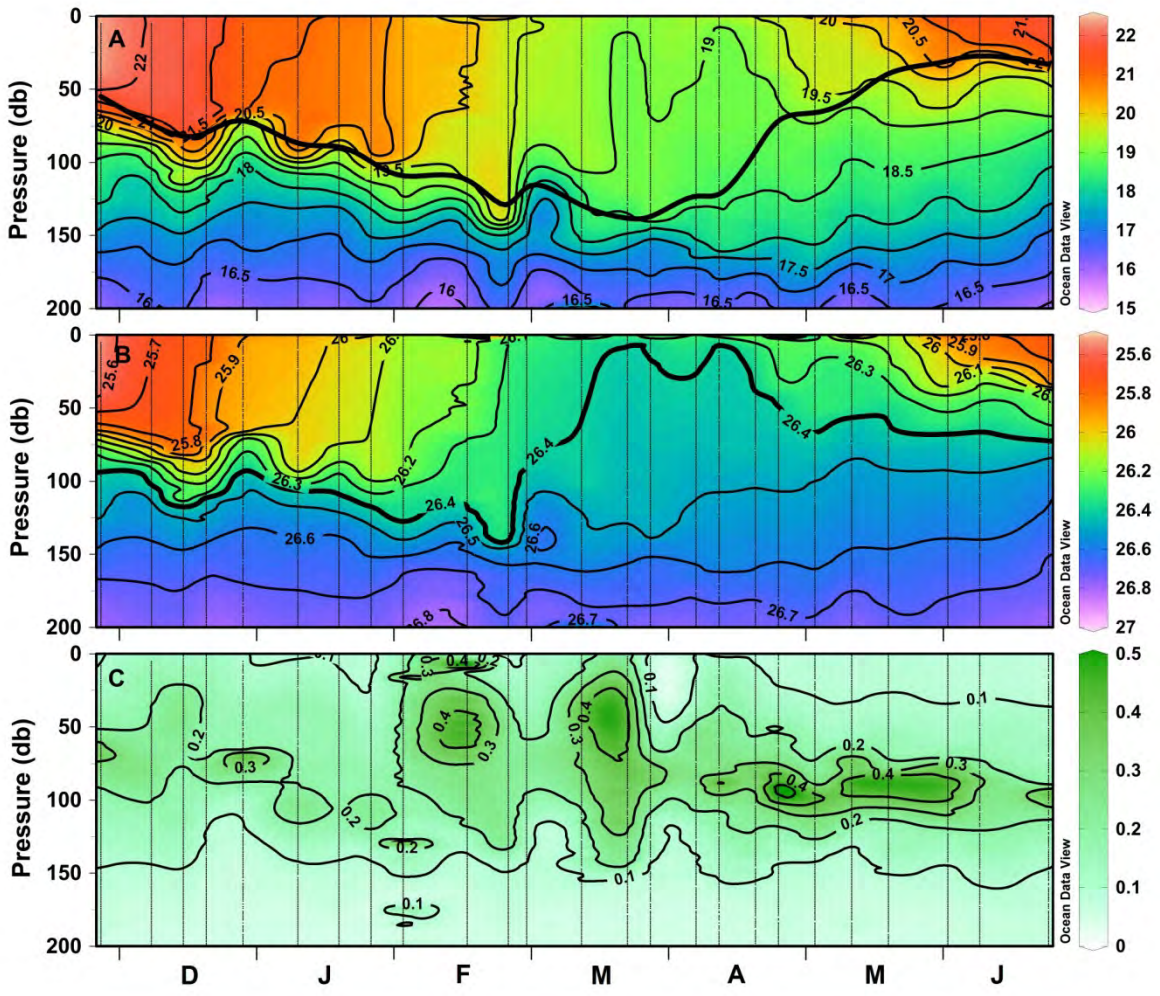

Figure I.2. Temporal variability of temperature (2A, ºC), potential density (2B, kg m^{-3}) and Chl *a* (2C, mg m^{-3}) from surface to 200 db. ML depth is drawn in the upper panel (black solid line). Data correspond to average from stations 2, 3 and 4; from November 2010 to June 2011.

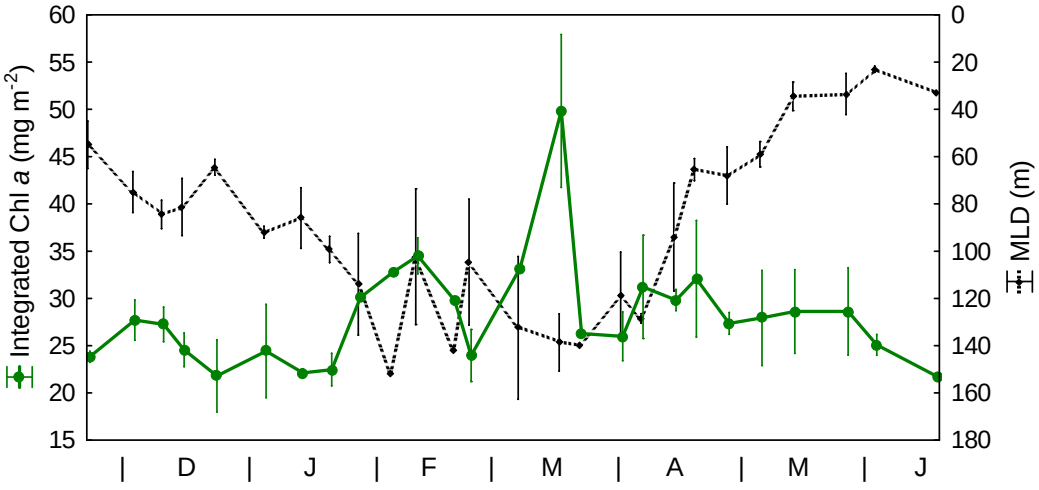

Figure I.3. Average (±SD) integrated Chl *a* concentration (mg m^{-2}) and MLD (m) from November 2010 to June 2011.

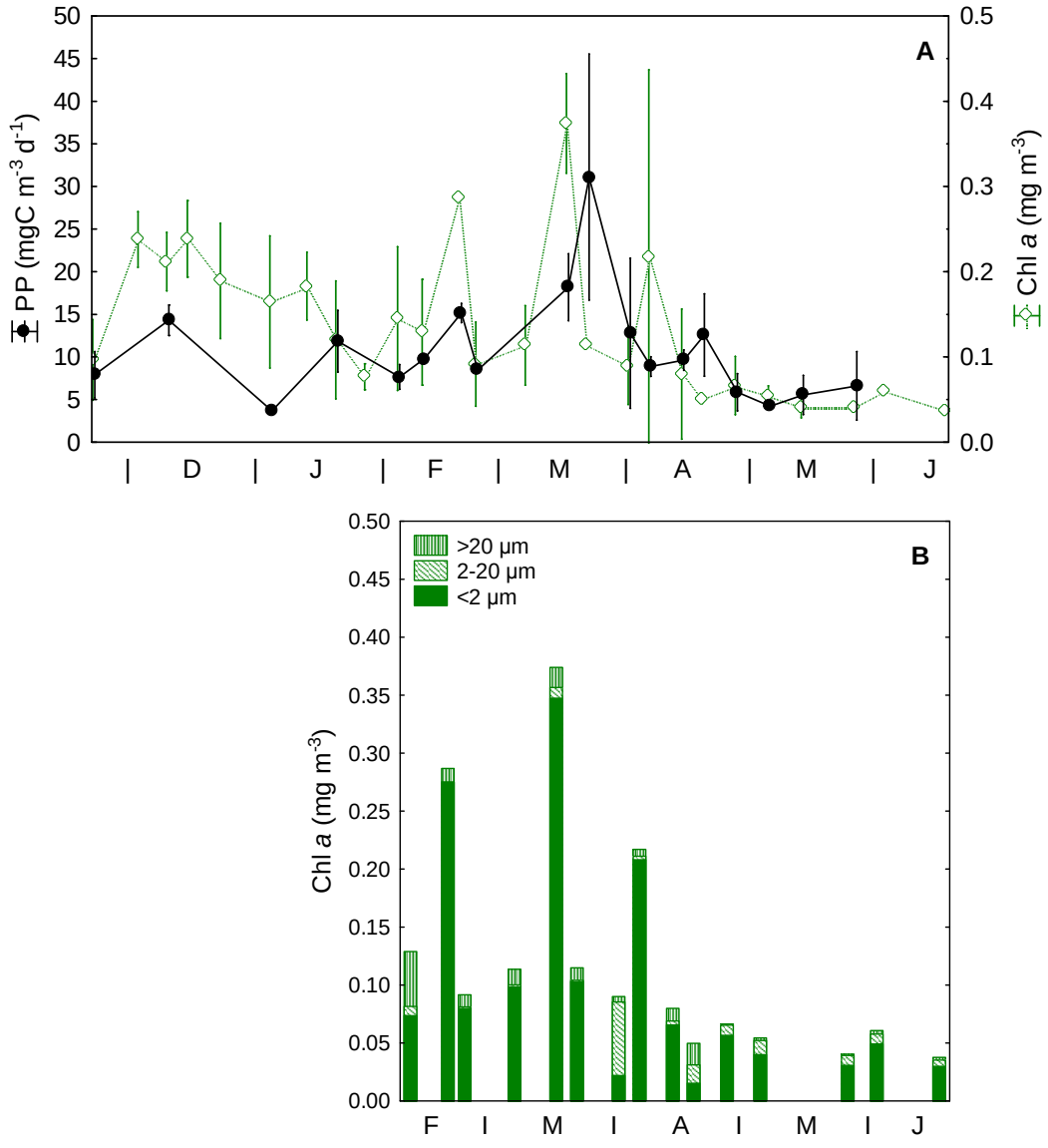

Figure I.4. Average (±SD) primary production (PP, mgC m^{-3} d^{-1}) and Chl a concentration (mg m^{-3}) measured in the ML during the period studied (4A); and average fractionated Chl *a* (mg m^{-3}) measured in the ML from February to June 2010 (4B).

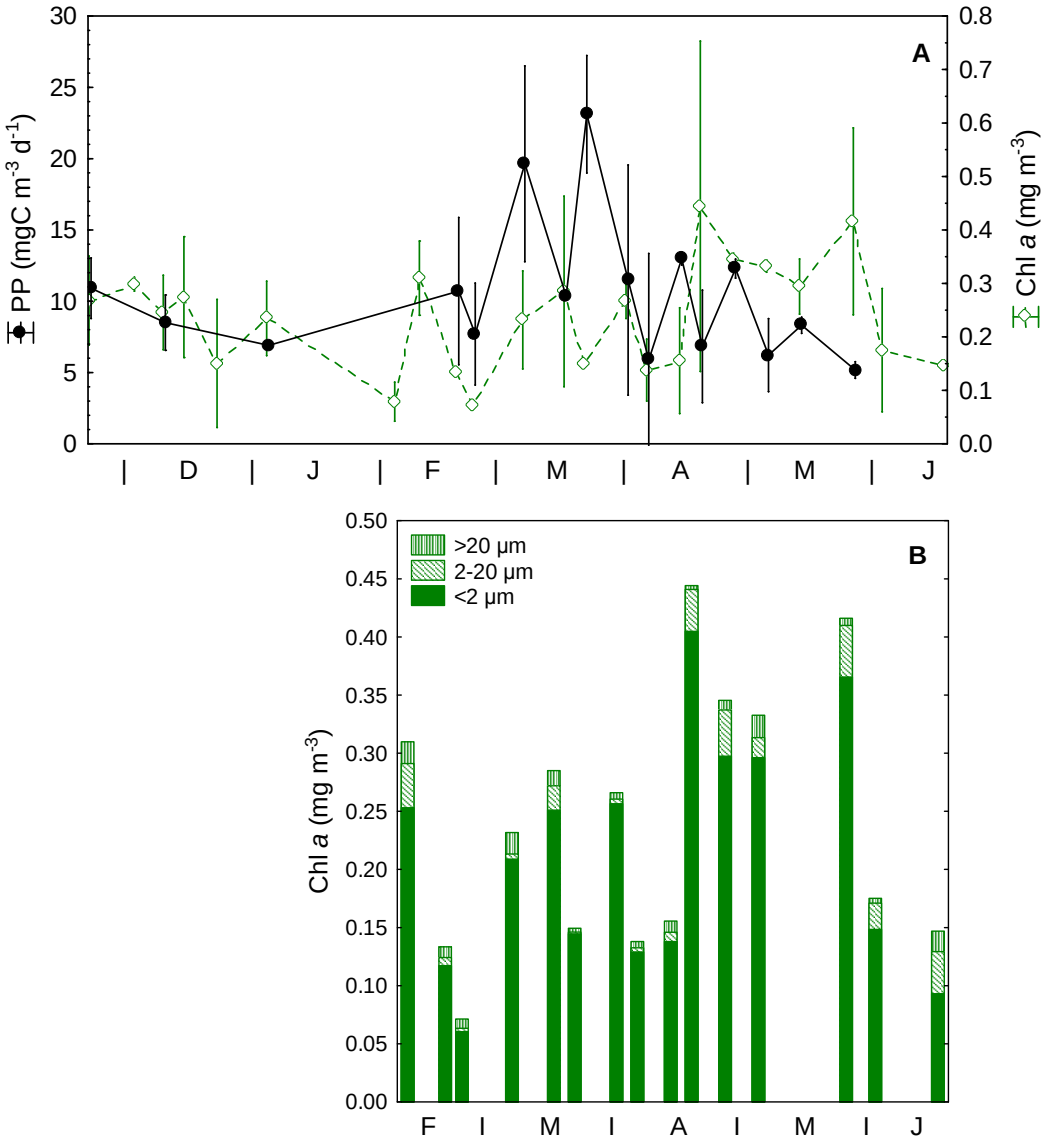

Figure I.5. Average (±SD) primary production (PP, mgC m^{-3} d^{-1}) and Chl a concentration (mg m^{-3}) measured in the DCM during the period studied (5A); and average fractionated Chl *a* (mg m^{-3}) measured in the DCM from February to June 2010 (5B).

Primary production relatively increased every month at the ML, except in May, and showed a similar pattern to Chl *a* (Fig. I.4A, Spearman's rank r=0.47, p<0.01). The highest value of 31 mgC m^{-3} d^{-1} was reached in March, matching the highest Chl *a* concentration. Maximum PP at the DCM also occurred in March when two maxima of around 20 mgC m^{-3} d^{-1} were observed (Fig. I.5A). However, at this depth, PP and Chl *a* seemed to be uncoupled and no correlation was found between them (Spearman's rank r=-0.10, p>0.05). In fact, higher Chl *a* concentrations were found after the bloom period, when PP rate was the lowest. In both, the ML and the DCM, the small size-fraction (<2 μm) generally contributed more than 50% to total Chl *a* (Figs. I.4B and I.5B). At the ML (Fig. I.4B) the smallest fraction ranged between 24 and 96%, while larger fractions rarely accounted for more than 20%. At the DCM the contribution of the smallest autotrophic cells was even slightly higher, between 63 and 97% of total Chl *a* (Fig. I.5B).

Within the most productive period from February to March an increase in abundance of Syn and APE was observed in the ML (Fig. I.6), whilst Pro decreased compared to previous months. Despite this moderate decrease, two maxima of 5.8 and $5.4 \cdot 10^4$ cells mL^{-1} appeared during this period, and Pro clearly dominated in terms of abundance during the whole period. In January Pro reached the maximum abundance ($9.9 \cdot 10^4$ cells mL^{-1}) and in March and June the lowest (1.1 and $0.3 \cdot 10^4$ cells mL^{-1}). Syn abundance was less than $1.0 \cdot 10^4$ cells mL^{-1} except during the productive period, reaching up to $3.1 \cdot 10^4$ cells mL^{-1}. APE were the less abundant organisms of autotrophic picoplankton, and although they slightly increased up to $2.5 \cdot 10^3$ cells mL^{-1}, it was an order of magnitude below Pro and Syn. ANF were less abundant than picophytoplankton cells in the ML, ranging between 0 and $11.8 \cdot 10^1$ cells mL^{-1} (Fig. I.7), and any seasonal pattern was observed. Dia abundance was less than 4 cells mL^{-1}, except in February and March, reaching 31.4 and 9.0 cells mL^{-1}, respectively. Din did not vary as much as diatoms, ranging between 4.1 and 16.2 cells mL^{-1}, although its abundance was higher from April to June.

HP dominated the abundance of heterotrophs in the ML and peaked in February up to $6.8 \cdot 10^5$ cells mL^{-1} (Fig. I.8). HNF showed lower abundances between 0.1 and $4.5 \cdot 10^2$ cells mL^{-1}. Cil were the less abundant heterotrophic group showing low variability with values between 0.7 and 2.9 cells mL^{-1}.

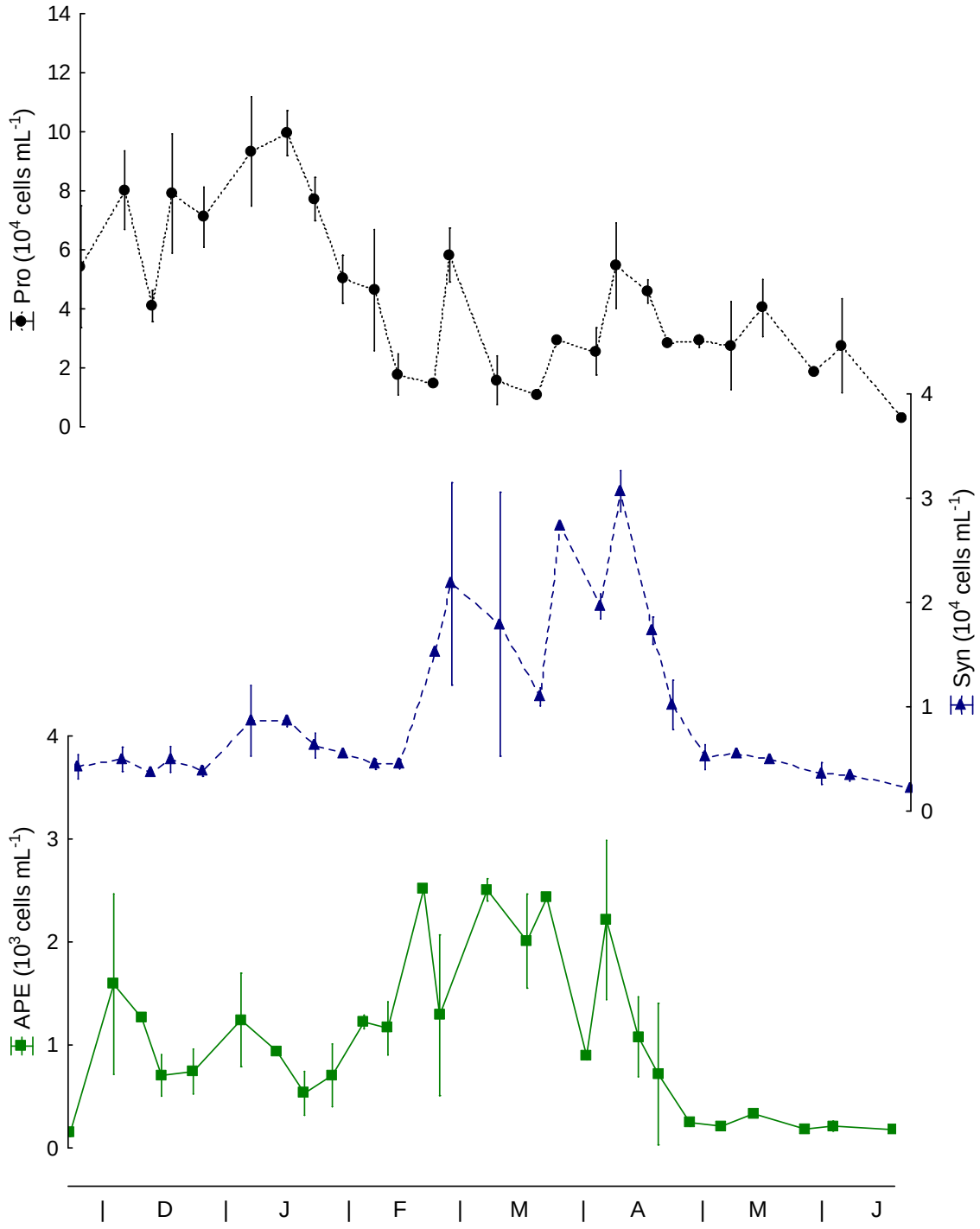

Figure I.6. Temporal variability of average (±SD) abundance (cells mL^{-1}) of *Prochlorococcus* (Pro), *Synechococcus* (Syn) and autotrophic picoeukaryotes (APE) in the ML from November 2010 to June 2011. Note the different scales.

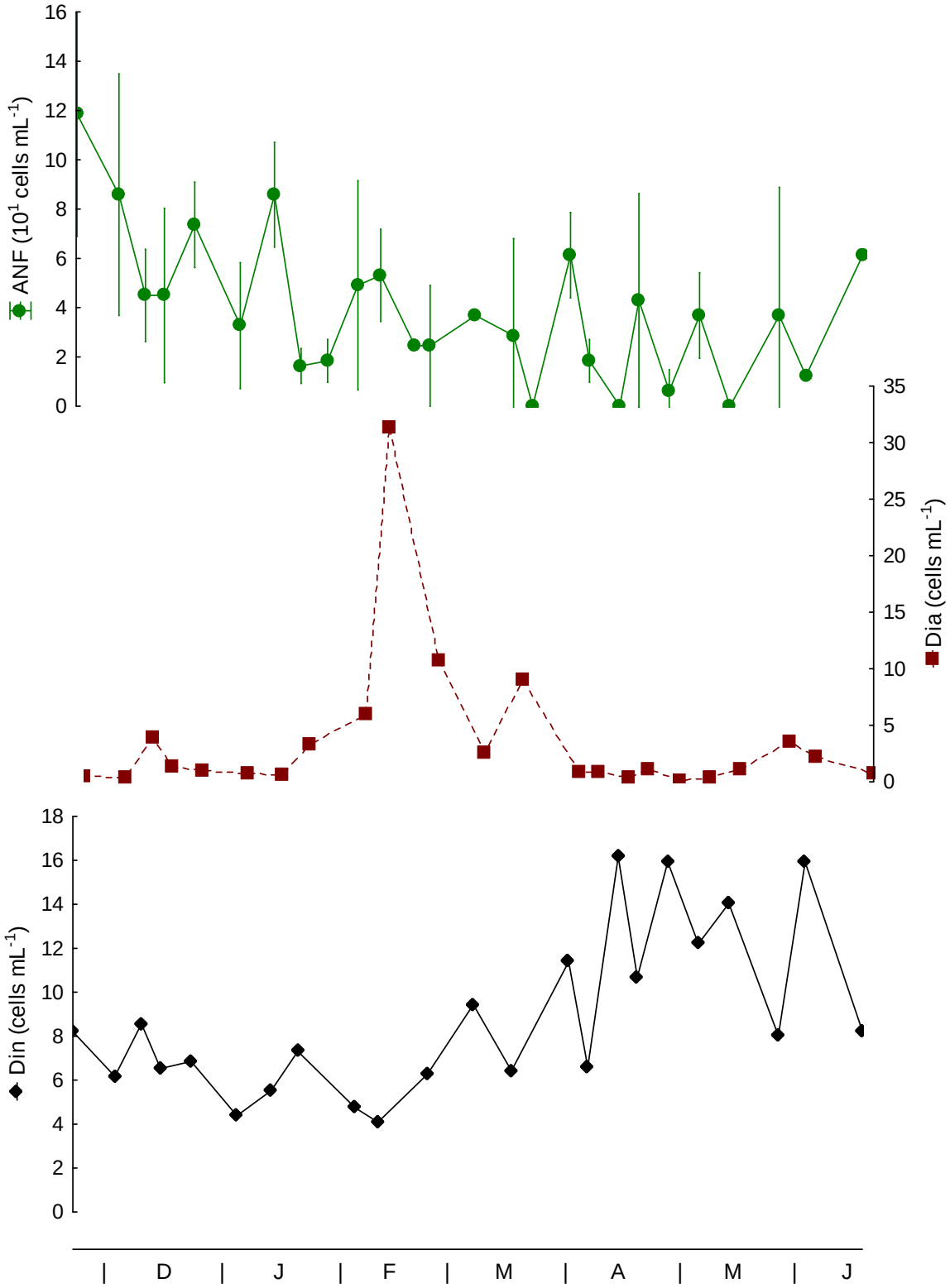

Figure I.7. Temporal variability of average (±SD) abundance (cells mL^{-1}) of autotrophic nanoflagellates (ANF), diatoms (Dia) and dinoflagellates (Din) in the ML from November 2010 to June 2011. Note the different scales.

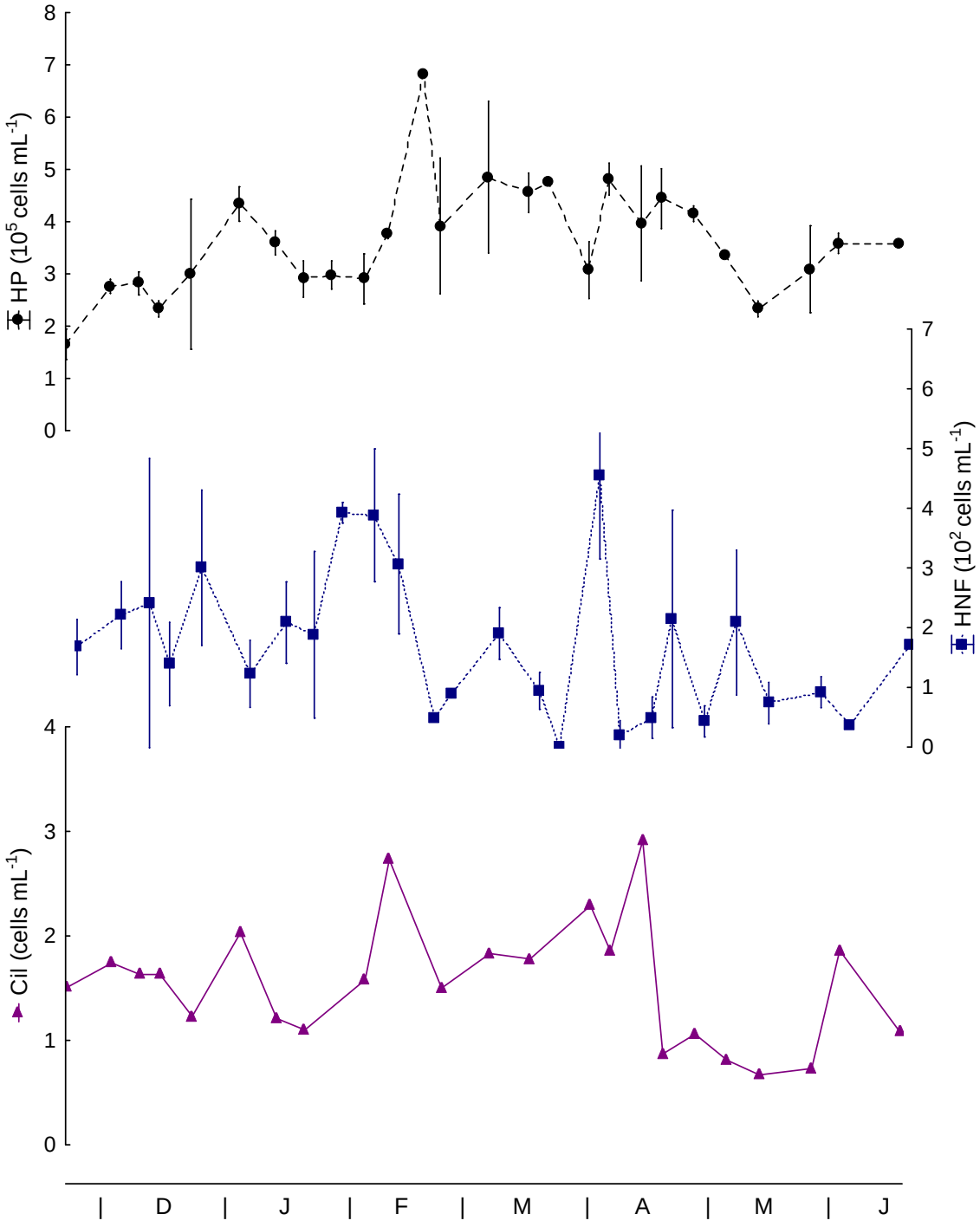

Figure I.8. Temporal variability of average (±SD) abundance (cells mL^{-1}) of heterotrophic prokaryotes (HP), heterotrophic nanoflagellates (HNF) and ciliates (Cil) in the ML from November 2010 to June 2011. Note the different scales.

Autotrophic pico- and nanoplankton biomass was higher from December to April (Fig. I.9A) at the ML, being Pro the major contributors (around 50%) except in February, March and April. During these months, APE (35 and 62%) and Syn to a lesser extent (25 and 38%) accounted for the majority of biomass, summing up between 73 and 91% of total autotrophic biomass of pico- and nanoplankton. ANF biomass was almost negligible (below 5%) during the period studied, except during November, when it was 17% of pico- and nanoautotrophic biomass. However, contribution of picophytoplankton to total autotropic biomass was lower than Dia and Din (Fig. I.9B), which supposed more than 50% in all months apart from January. Dia were the main contributors in February and March, and they were responsible for the highest autotrophic biomass value of 119 mgC m^{-3} reached in February, accounting for 79% of total biomass. Din biomass by opposite did not show any seasonal pattern, but a higher biomass contribution was observed in December, May and June, accounting for 74, 61 and 87% of total phytoplankton biomass.

Heterotrophic biomass was also higher from December to April, reaching more than 9 mgC m^{-3} (Fig. I.9C) during the productive months, February and March. It was dominated by HP, which ranged between 61 and 84% of total heterotrophic biomass. Cil biomass, although lower than HP, accounted for 9-18% of heterotrophic biomass from December to June, and reached a 26% in November. HNF were minor biomass contributors, being below 10% except in November and December.

Heterotrophic biomass exceeded autotrophic biomass during the whole period when pico- and nanoplanktonic community was only considered ($A_{Pico,ANF}$:$H_{HP,HNF}$ ratio was always below 1, Table I.1). However, when microplankton was included considering that dinoflagellates were half autotrophs and half heterotrophs, A:H ratio was above 1 in December, February and March. In both cases, the lowest ratios were measured in May and June. By opposite, the lowest P:B ratios were found during December (0.16), February (0.08), and March (0.20), and the same was observed when Din were included in autotrophic biomass ($P:B_{+Din}$). The highest ratios were measured in April and May.

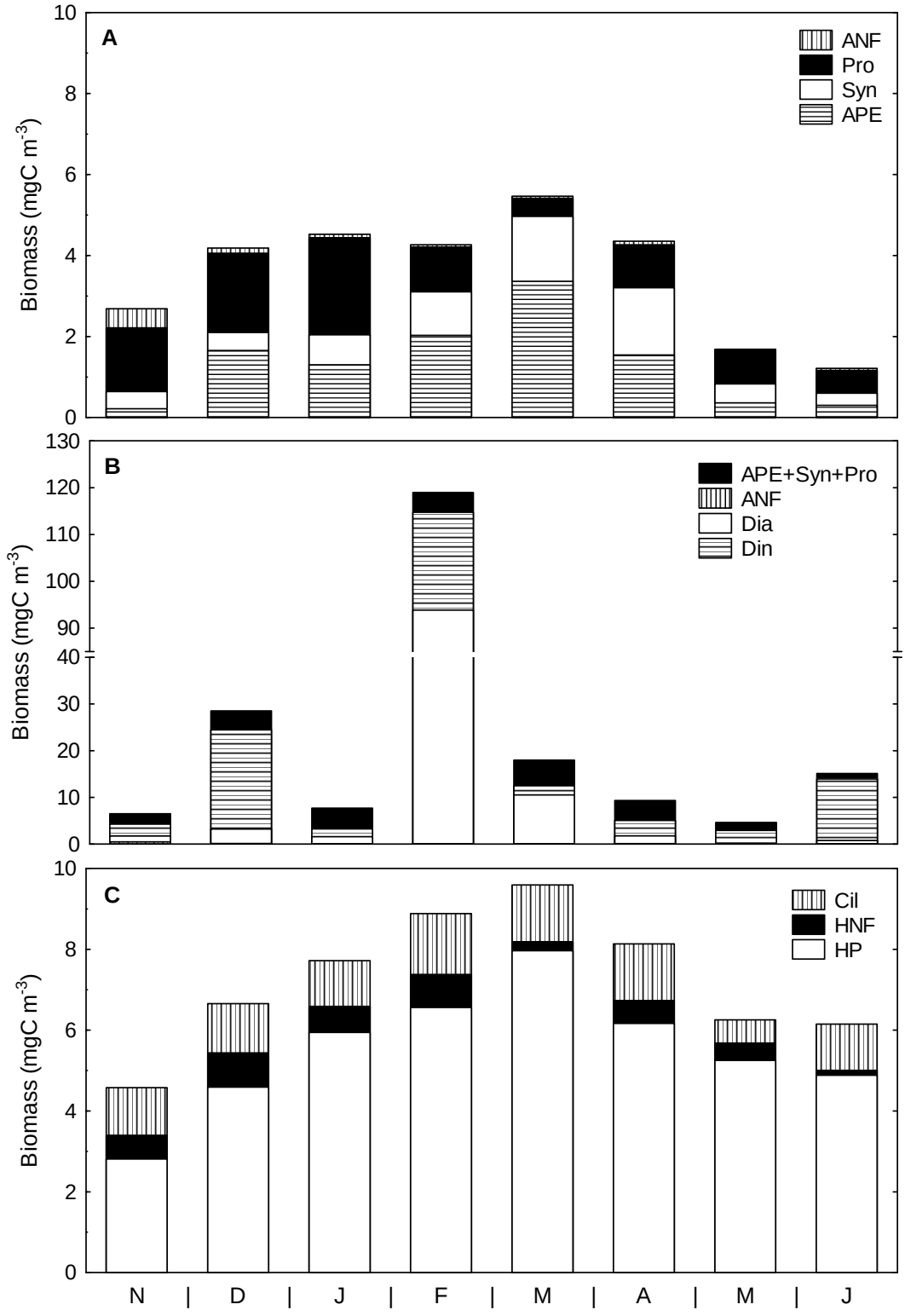

Figure I.9. Monthly average biomass (mgC m^{-3}) of Pro, Syn, APE, ANF (9A), Dia, Din (9B), HP, HNF and Cil (9C) in the ML from November 2010 to June 2011.

Month	$A_{Pico,ANF}:H_{HP,HNF}$	A:H	P:B	$P:B_{+Din}$
Nov	0.79 (±0.00)	0.89 (±0.00)	0.34 (±0.00)	0.45 (±0.00)
Dec	0.78 (±0.22)	1.03 (±0.13)	0.16 (±0.00)	0.31 (±0.00)
Jan	0.72 (±0.06)	0.82 (±0.14)		
Feb	0.60 (±0.23)	5.79 (±4.74)	0.08 (±0.00)	0.11 (±0.00)
Mar	0.63 (±0.10)	1.58 (±0.44)	0.20 (±0.00)	0.21 (±0.00)
Apr	0.62 (±0.24)	0.78 (±0.25)	0.52 (±0.20)	0.74 (±0.30)
May	0.31 (±0.14)	0.43 (±0.16)	0.51 (±0.07)	0.90 (±0.13)
Jun	0.23 (±0.04)	0.57 (±0.29)		

Table I.1. Monthly average (±SD) of autotrophic:heterotrophic biomass (A:H) and primary production:autotrophic biomass (P:B) ratios at the ML during the period studied. A:H ratios are shown for pico- and nanoplankton ($A_{Pico,ANF}:H_{HP,HNF}$) and including microplankton (Dia, Cil, Din) considering 50% Din as autotrophs and 50% as heterotrophs (A:H). P:B ratios (d^{-1}) are shown without (P:B) or with 50% of Din ($P:B_{+Din}$) in computing autotrophic biomass. Biomass in mgC m^{-3} and primary production in mgC m^{-3} d^{-1}.

IV. DISCUSSION

The characteristic late winter bloom previously described for subtropical gyres (Menzel & Ryther 1960, Karl et al. 1996) and also for the Canary Islands waters (De León & Braun 1973, Braun 1980, Arístegui et al. 2001) was observed to the north of the Canaries from February to April 2011. The cooling of surface waters promoted the deepening of the ML and the consequent input of new nutrients into the euphotic zone. Although nutrients were not measured here, it is well known that nutrient concentration significantly increases at a potential density of 26.4 kg m^{-3} in the eastern North Atlantic subtropical waters (Cianca et al. 2007). Thus, the shoaling of the 26.4 kg m^{-3} isopycnal surface (Fig. I.2B) indicates that nutrient-enriched deeper waters reached the surface, especially during March and April. The availability of new nutrients promoted the increase of Chl *a* concentration and the enhancement of PP at the ML during those productive months (Figs. I.2C, I.3 and I.4). In fact, both parameters were positively correlated to the ML depth (Chl *a*: Spearman's rank $r=0.42$, $p<0.001$; PP: Spearman's rank $r=0.55$, $p<0.01$). After the bloom, when surface waters became warmer, autotrophic biomass decreased in the ML and increased at the DCM (Fig. I.3C). The composition of autotrophs in this layer, as at the surface, was dominated by small cells (<2 μm).

The magnitude of the winter-spring bloom was similar to previous studies in these waters in terms of Chl *a* (Schmoker & Hernández-León 2013). These authors found maximum Chl *a* values of 0.25 mg m^{-3} at the mixed layer in 2007. They observed a decrease in Chl *a* within the bloom period as winter temperatures increased from year 2005 to 2007. Actually, the characteristic bloom did not take place during 2010, when winter temperatures above 19ºC were measured and Chl *a* remained below 0.1 mg m^{-3} in the ML (see Chapter II). In spite of low Chl *a* concentration (up to 0.3 mg m^{-3}), PP showed quite high values not only within the bloom, but during the whole period studied. Average productivity rates measured here (3.7- 31.1 mgC m^{-3} d^{-1}) were higher than rates measured at the BATS station during the same period, which ranged 2.4-15.5 mgC m^{-3} d^{-1} (BATS interactive data access, http://bats.bios.edu/index.html, last access: July 2013). Absolute rates of PP could be overestimated because of the ^{13}C method used here. However, a rather good agreement between both ^{13}C and ^{14}C isotopic methods has been referred in the literature (Slawyk et al. 1977, Hama et al. 1983). Furthermore, values and variability of the P:B ratios shown here are similar to other measurements in the area (Arístegui et al. 2001). On the other hand, a higher variability of our data may be unveiled by the frequency of the sampling, as we carried out a weekly sampling whereas data for the time-series station at the Sargasso Sea were obtained once a month. Additionally, the enhancement of PP could be caused by the fertilization effect of Saharan dust storms, frequent in the area (Goudie & Middleton 2001, Viana et al. 2002, Sarthou et al. 2007). A dust deposition event occurred in mid-January (data not shown), matching the peak of PP within that month. However, any dust event took place in December, when high rates were also measured. Nevertheless, relatively high values of PP and Chl *a* even when stratification conditions would preclude the entrance of nutrient-rich deeper waters, point out the existence of other short-scale events apart from winter convection that would enhance primary productivity in the ML.

The planktonic community at the ML was dominated by picoplankton, especially autotrophic and heterotrophic prokaryotes, as expected in subtropical waters (Buck et al. 1996, Longhurst 1998). *Prochlorococcus* dominated picophytoplankton abundance and biomass (Figs. I.6 and I.9A), except during the productive period, when Syn and APE abundance increased and comprised the majority of the autotrophic biomass. The seasonality observed for cyanobacteria and picoeukaryotes has already been described for these waters (Baltar et al. 2009b, Schmoker et al. 2012, Schmoker & Hernández-León 2013) and it is a common feature of subtropical gyres (Zubkov et al. 2000, DuRand et al. 2001, Giovannoni & Vergin 2012). HP also reached the highest abundance and biomass during the productive period, maintaining the predominance over picophytoplankton even at that time ($A_{Pico,ANF}$:$H_{HP,HNF}$<1, Table I.1). The heterotrophy of the pico- and nanoplankton community in the Canary Islands waters during both spring and summer seasons has also been reported by other authors (Arístegui & Montero 2005, Baltar et al. 2009a). By opposite, the dominance of autotrophic pico- and nanophytoplankton was measured during the colder winter-

spring period in 2005 (Schmoker et al. 2012). It is likely that less oligotrophic conditions in 2005 (up to 0.5 mg Chl *a* m^{-3}) favored A:H ratios above 1, whereas lower Chl *a* concentration, as observed here, promoted the heterotrophic biomass dominance (Buck et al. 1996).

However, during the bloom period, in February-March, diatoms substantially rose in abundance and dominated total autotrophic biomass (Figs. I.7 and I.9B). Moreover, only when diatoms increased in December, February and March, autotrophic biomass was higher than heterotrophic biomass (A:H>1, Table I.1). The appearance of a peak of diatoms at the beginning of the bloom period has been previously observed in Canary Islands waters (Ojeda 1998, Schmoker et al. 2014) but contrary to our results, their contribution to autotrophic biomass even at that time remained low. The large increase of diatom biomass was a striking result as higher temperatures than previous years (see Schmoker & Hernández-León 2013) would expect less mixing and nutrient availability and, thus, a less favorable scenario for these large algae. However, recent research has contradicted the traditional view that diatoms are favored by mixing and high nutrient conditions (Kemp & Villareal 2013). These authors argue that diatom species involved in diazotrophic diatom symbioses could enhance primary production in stratified and oligotrophic waters. This view would support our results, although we were not able to assess if within *Chaetoceros* sp, the major contributor to the high diatom biomass in February (Fig. I.10), some species would be involved in diazotrophic diatom assemblages. On the other hand, the increase in abundance and biomass of *Chaetoceros* sp could also be related to Saharan dust deposition events in this area (see Chapter II). In this sense, a dust storm observed in January (data not shown) could contribute to previously fertilize the ML, and thereby enhancing the availability of nutrients due to the subsequent deep water input and thus favoring the extraordinary increase of diatoms.

Apart from the productive period, microplankton abundance was dominated by dinoflagellates, as it has been previously found in the area (Buck et al. 1996, Bode et al. 2001, Schmoker & Hernández-León 2013), although we observed lower abundances. Schmoker and Hernández-León (2013) also found that Cil dominated microplanktonic biomass, whilst our results showed higher Din (1.6-21.2 mgC m^{-3}) than Cil (0.6-1.5 mgC m^{-3}) biomass (Fig. I.9B and C). However, higher values in December, February and June were due to the presence of only few cells of *Kofoidinium velleloides,* a rather large dinoflagellate. Furthermore, microplankton biomass data should be taken with caution as we did not make direct measurements of biovolumes in some cases (see *Material and methods*) and different conversion factors than previous years were used.

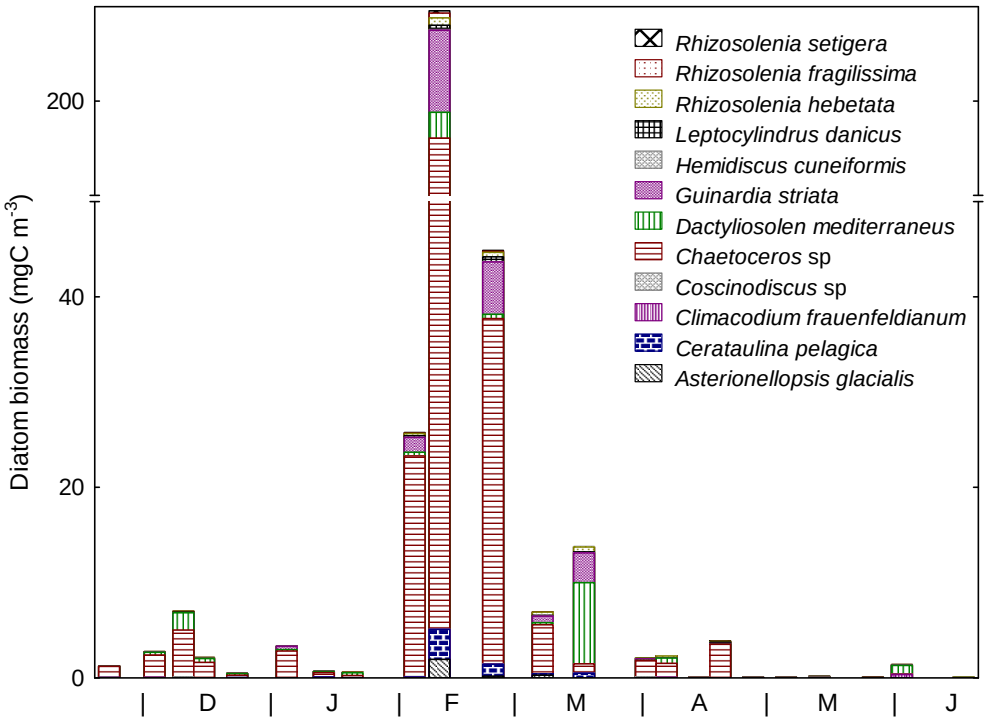

Figure I.10. Contribution of different species to diatom biomass (mgC m^{-3}) in the ML from November 2010 to June 2011.

The planktonic community dynamic was clearly controlled by bottom-up forces at a seasonal scale. Actually, the abundance of pico- and nanoplankton was significantly correlated to average temperature in the ML (Table I.2). However, trophic interactions also influence the variability of the different planktonic groups at a short-term scale. HP could be consumed by HNF as a clear inverse pattern was observed between them (Fig. I.8 and Table I.2). Flagellates are known to be effective bacterivores (Azam et al. 1983) and not only upon HP, but also upon cyanobacteria. Here, a significant correlation between HNF and Syn was also measured (Table I.2). Similarly, a significant relationship between HNF and HP, as well as between HNF and Syn, has been observed in the Canaries (Arístegui & Montero 2005). An inverse relationship between autotrophic picoeukaryotes and nanoflagellates, and Din was also observed (Figs. I.6 and I.7 and Table I.2), suggesting that Din would be grazing upon APE and ANF. These results are partly supported by Schmoker & Hernández-León (2013) who found a strong correlation between microplankton and autotrophic picoplankton. They suggested that small-sized microplankton in these waters would be able to control small autotrophic cells. In any case, we are aware of the limitation to totally understand the relationships between different trophic levels of complex webs in subtropical waters through linear regressions alone.

	Syn	Pro	APE	HP	ANF	HNF	Dia	Din	Cil
Pro	0.083								
APE	**0.489*** **	-0.035							
HP	**0.541*** **	**-0.320***	**0.530*** **						
ANF	**-0.218***	0.162	-0.089	**-0.319***					
HNF	**-0.278***	0.089	-0.061	**-0.310***	**0.575*** **				
Dia	-0.122	-0.255	0.217	-0.077	0.142	0.031			
Din	0.004	-0.302	**-0.498***	-0.063	**-0.473***	-0.277	-0.367		
Cil	0.274	0.132	**0.536** **	0.272	-0.066	-0.064	0.101	-0.209	
Temp	**-0.369** **	**-0.720*** **	**0.448*** **	**-0.636*** **	**0.484*** **	**0.307***	-0.165	-0.190	-0.199

Table I.2. Correlation (Spearman's rank, r) between abundance (cells mL^{-1}) of *Synechoccocus* (Syn), *Prochloroccocus* (Pro), autotrophic picoeukaryotes (APE), heterotrophic prokaryotes (HP), autotrophic nanoflagellates (ANF), heterotrophic nanoflagellates (HNF), diatoms (Dia), dinoflagellates (Din) and ciliates (Cil); and average temperature in the ML (Temp). Significant correlations at $p<0.05$ (*), $p<0.01$ (**) and $p<0.001$ (***) levels are in bold.

In conclusion, the oligotrophic waters of the Canary Current were dominated by picoplankton during the period studied, especially *Prochlorococcus* and heterotrophic prokaryotes. However, during the productive period *Synechococcus* and picoeukaryotes dominated the picophytoplankton, and diatoms became the major contributors to total autotrophic biomass. Thereby, diatoms were probably responsible for the highest rates of primary production in February and March. This variability of the phytoplanktonic community was driven by bottom-up forces at a seasonal scale, so that the availability of new nutrients during winter-spring allowed the enhancement of primary production at that period. However, other short-scale events such as Saharan dust deposition could be enhancing the primary productivity in the ML at a short-time scale even during stratified periods. We emphasize the observed and unexpected role of diatoms in these subtropical waters, which needs further research. Short-term variability would also be driven by top-down forces through feeding and grazing by nano- and microheterotrophs, as significant correlations were found between the latter and their potential preys: heterotrophic prokaryotes and autotrophic picoeukaryotes and nanoflagellates. Finally, the results presented here highlight the need to carry out oceanographic samplings at short-time scales to account for the total variability of the planktonic communities in subtropical waters.

V. ACKNOWLEDGMENTS

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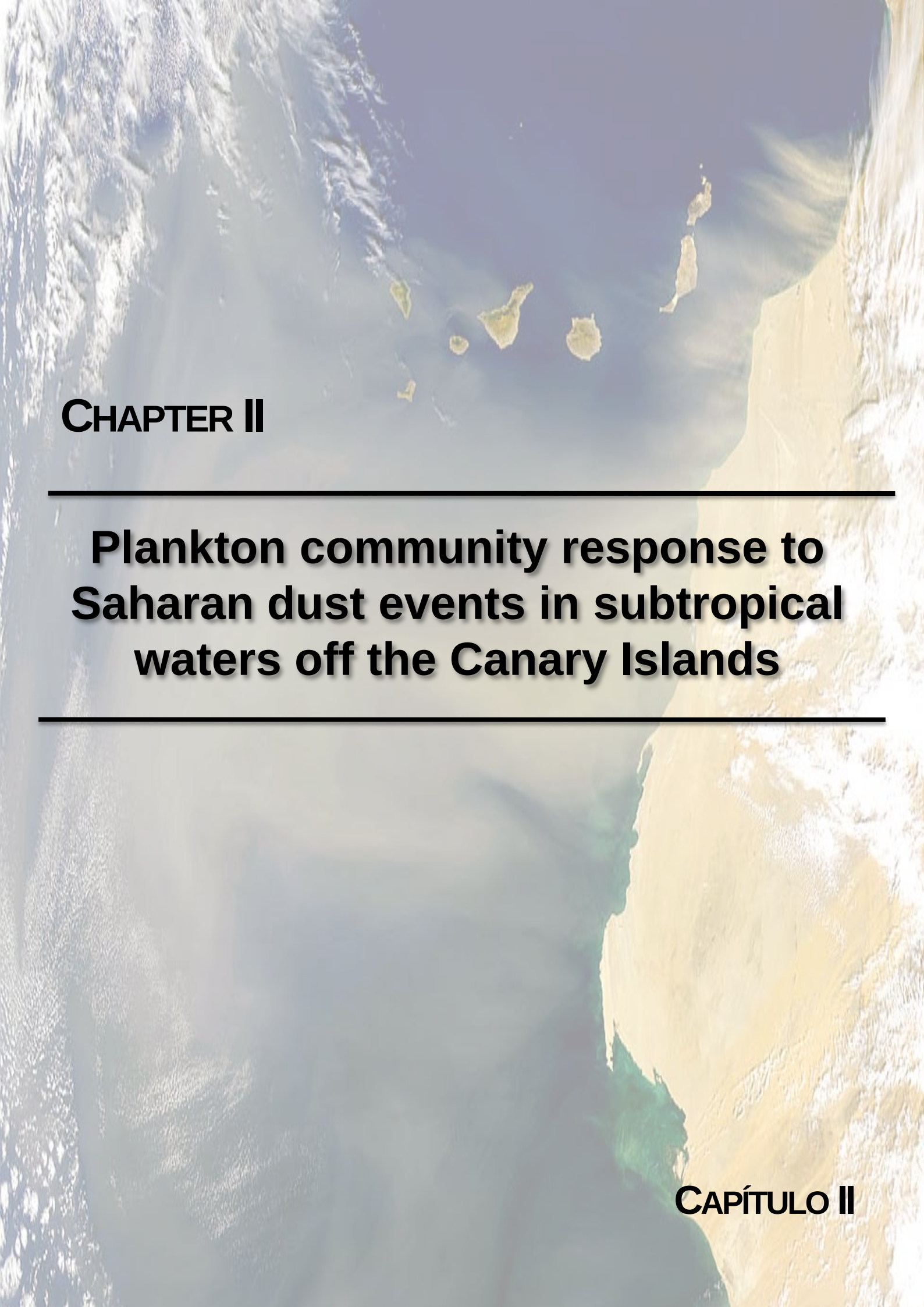

CHAPTER II

Plankton community response to Saharan dust events in subtropical waters off the Canary Islands

CAPÍTULO II

ABSTRACT

The plankton community response to Saharan dust deposition was studied in the Canary Islands waters during winter-spring 2010. For this, a weekly sampling was carried out to characterize the pico-, nano- and microplankton communities. During this period several dust events were identified from atmospheric suspended matter and metal composition. Temperatures above 19ºC in the mixed layer, high stratification and a very low concentration of chlorophyll *a*, indicated the absence of the characteristic late winter bloom during this year. However, high seawater nutrient concentration was observed during this period, probably result of the release from the deposited atmospheric dust. In fact, this winter-spring was one of the most intense dust periods during the last years and Saharan dust events took place every month. The effect of the Saharan dust was negligible in terms of chlorophyll *a* and productivity, whereas relative increases of primary production rates at the surface seemed to be related to every dust event. The response of the planktonic community consisted mainly in the increase of diatom and mesozooplankton stocks by more than 1000 and 100%, respectively, whereas cyanobacteria and autotrophic picoeukaryotes were negatively affected. These results suggest that the Saharan dust deposition could be affecting the planktonic community in these oligotrophic waters of the northeast Atlantic, especially during stratified periods, when the atmospheric dust would be the most important nutrient source.

I. INTRODUCTION

The productivity of the oceans is largely determined by nutrient availability for autotrophic organisms living in the euphotic zone. In this sense, it is now rather clear that the iron deficiency limits primary production in the so-called high nutrient low chlorophyll (HNLC) ecosystems from polar to tropical regions (Boyd et al. 2007). In oligotrophic waters, where macronutrients are scarce, phytoplankton growth is usually limited by nitrogen or co-limited by nitrogen and phosphorus (Moore et al. 2013). However, it seems that iron could be also limiting in low nutrient low chlorophyll (LNLC) waters where its addition has promoted the increase in chlorophyll *a* and primary productivity (Bonnet et al., 2005). Therefore, primary productivity is stimulated when nutrients are available for phototrophic organisms. This was observed in the oligotrophic gyre of the North Pacific (McAndrew et al. 2007, Mahaffey et al. 2012) where a positive response of phytoplankton biomass and primary production was found when nutrient-replete deep water was added to surface water. Similarly, the experimental addition of Saharan dust, releasing iron, phosphate, nitrate and silicate, has resulted in the stimulation of primary productivity in low nutrient low chlorophyll waters (Bonnet et al. 2005, Herut et al. 2005, Duarte et al. 2006, Marañón et al. 2010, Ridame et al. 2014).

Atmospheric deposition is one of the major sources of limiting nutrients to the open ocean, mainly iron (Duce & Tindale 1991, Jickells 1999) but also nitrogen and phosphorus (Donaghay et al. 1991, Guerzoni et al. 1999, Duarte et al. 2006). In this sense, the Sahara desert is the most important dust source for the world's oceans, particularly in the North Atlantic. The Saharan dust contents high values of crustal elements as silicate, aluminum, manganese or iron (Goudie & Middleton 2001, Viana et al. 2002), as well as nitrate and phosphorus (Bonnet et al. 2005, Herut et al. 2005, Duarte et al. 2006, Pulido-Villena et al. 2010). The release of these nutrients from Saharan dust has a positive effect over phytoplankton growth that has been experimentally observed (Bonnet et al. 2005, Herut et al. 2005, Duarte et al. 2006, Marañón et al. 2010, Giovagnetti et al. 2013, Ridame et al. 2014). However, the effect of the Saharan dust deposition in the field has been rarely studied and is still not clear. The enhanced biological response after a dust storm was observed by Bishop et al. (2002) based on *in situ* particulate organic carbon and chlorophyll data in HNLC waters in the North Pacific. Also in oligotrophic waters of South China Sea, chlorophyll *a* concentration was significantly increased during heavy dust deposition years (Wang et al. 2012). However, Chami et al. (2012) showed a negative effect of dust on primary production in the subtropical Atlantic Ocean and they ascribed it to the attenuation of PAR radiation produced by the dust aerosol layer in the atmosphere. In the Mediterranean Sea, a relationship between phytoplankton and dust was only observed when atmospheric events coincided with the stratified season (Eker-Develi et al. 2006, Volpe et al. 2009). Hence, it has been hypothesized (Guerzoni et al. 1999) that Saharan dust would

only have a major influence on primary production at local and short-time scales, just when an atmospheric deposition event takes place.

There is another key factor regulating the biological response when nutrients are available, the control exerted by grazers (Donaghay et al. 1991, Landry et al. 1997). Microzooplankton grazing has been pointed out as the principal mechanism limiting phytoplankton growth in both artificial iron injections (Landry et al. 2000a, Landry et al. 2000b, De Baar et al. 2005, Boyd et al. 2007, Henjes et al. 2007) and dust addition experiments (Herut et al. 2005, Marañón et al. 2010). Thereby, larger diatoms are the most favored organisms when primary production is artificially induced (Landry et al. 2000b, De Baar et al. 2005, Boyd et al. 2007, Henjes et al. 2007), because of their higher growth rates and a lower grazing pressure compared to prokaryotic algae (Landry et al. 2000a, Landry et al. 2000b, Henjes et al. 2007). Furthermore, although mesozooplankton grazing is also enhanced (Bollens & Landry 2000, Tsuda et al. 2007), its impact upon primary production seems to be low (Tsuda et al. 2005). The coupling between phytoplankton and their grazers, as well as predation of mesozooplankton upon microzooplankton, and the composition of the planktonic community are essential aspects determining the flux of organic matter from the euphotic zone to deeper waters. In this sense, the community biomass change observed from nano- to microplankton and the success of diatoms in artificial fertilization (De Baar et al. 2005, Boyd et al. 2007) would favor a higher carbon export flux. However, if the microbial loop is enhanced, the major carbon respiration occurs in surface waters and a less effective export of carbon takes place (Azam et al. 1983, Legendre & Le Fèvre 1995). This has been observed in the equatorial Pacific after the increase in primary production promoted by iron addition. The coupled response between phytoplankton growth and microzooplankton grazing maintained the usual low carbon export scenario of these waters (Landry et al. 2000a).

The Canary Islands are located in the eastern subtropical North Atlantic within the Canary Current and close to the Sahara desert. In these oligotrophic waters, a quasi-permanent thermocline prevents the entrance of nutrients from deeper waters into the mixed layer, and only the cooling of surface waters during winter erodes the thermocline. This allows the increment of nutrient concentrations in the upper water column, stimulating the phytoplankton growth (De León & Braun 1973, Braun 1980). In this scenario, the nutrient input produced by the frequent African dust events reaching these stratified waters (Sarthou et al. 2007) could be a major factor affecting primary production, especially during winter, because of the highest intensity of the events (Viana et al. 2002). Therefore, the Canary Islands are a suitable place to study the effect of Saharan dust deposition in the ocean, not only because of their location in oligotrophic waters and their proximity to the most important dust source, but also because their distant position from intense anthropogenic influences.

In this work, we studied the *in situ* effect of the Saharan dust deposition on the planktonic community within the oligotrophic waters of the northeast Atlantic Ocean (Canary Islands) during the winter-spring 2010.

II. MATERIAL AND METHODS

A weekly sampling was carried out in the Canary Islands waters to the north of Gran Canaria Island from February to June 2010, on-board the R/V Atlantic Explorer. Four stations 10 nautical miles equidistant (Fig. II.1) were sampled from surface to 300 m depth. Pressure, salinity, temperature and fluorescence were measured using a SBE25 CTD and a Turner Scufa Fluorometer coupled to an oceanographic rosette equipped with six 4-L Niskin bottles. Seawater samples were taken at the mixed layer (20 m) to characterize the pico-, nano- and microplankton communities. A SBE19 plus CTD was eventually used during some cruises because of the main CTD failure. From temperature data, the mixed layer depth (MLD) was calculated as the level with a temperature difference of 0.5ºC from the 10 m depth (Cianca et al. 2007).

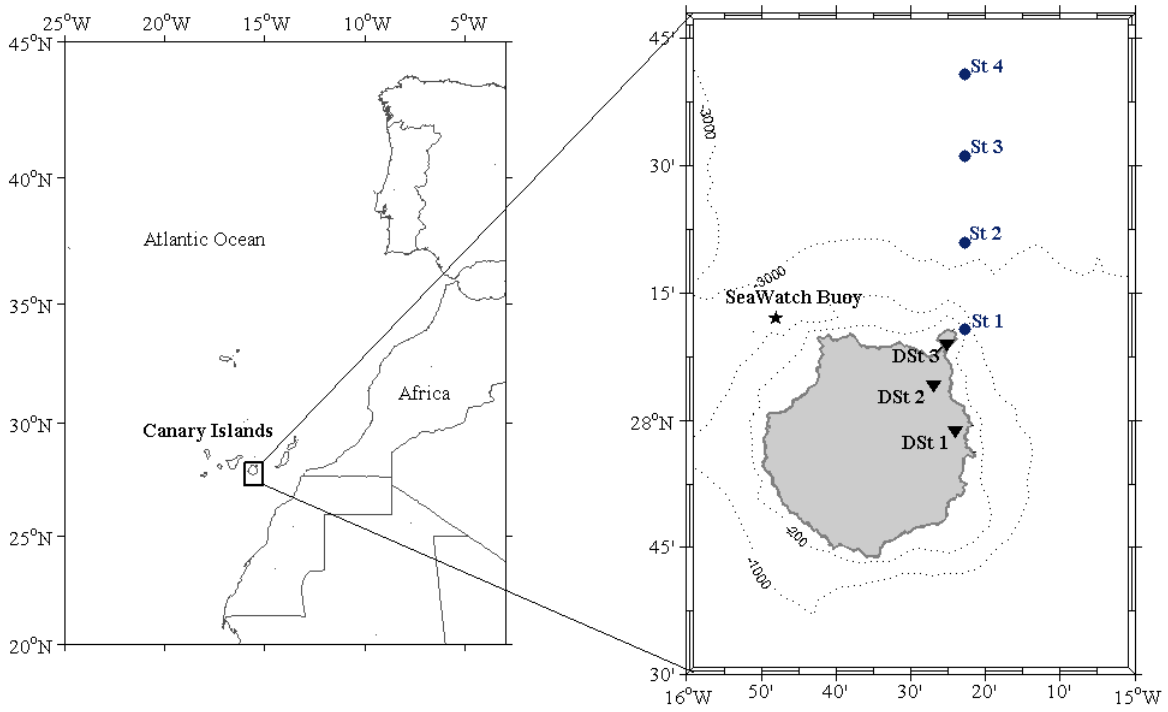

Figure II.1. Location of the four oceanographic stations (St) at the north of Gran Canaria Island (Canary Islands) and the three dust stations (DSt) in the northeast of the island. SeaWatch buoy position is also plotted.

Sea surface temperature was obtained from the Deep Sea Buoy Network dataset (REDEXT, http://www.puertos.es/oceanografia_y_meteorologia/redes_de_medida/index.html, last access: April 2013) belonging to Puertos del Estado (Spanish Government). Hourly data were taken at 3 m depth by a SeaWatch buoy located to the northwest of Gran Canaria (28.20°N, 15.80°W, Fig. II.1) from February to June 2010.

Chlorophyll *a* (Chl *a*) was measured by filtering 500 mL of seawater through a 25 mm Whatman GF/F filter and freezing it at -20ºC until its analysis at the laboratory. The extraction procedure consisted in placing the filter in 90% acetone at -20ºC in the dark, during at least 20 hours, and following the acidification method by Strickland and Parsons (1972a). Pigments were measured on a Turner Designs 10A Fluorometer, previously calibrated with pure Chl *a* (Yentsch & Menzel 1963). From these data at 20 m, fluorescence in the whole profile was converted to Chl *a* (Chl *a* = -0.001 + 26.05 · fluorescence, r^2=0.65, p<0.001 for the SBE19plus and Chl *a* = 0.001 + 0.21 · fluorescence, r^2=0.12, p>0.05 for the SBE25) as an indicator of phytoplankton biomass. The relationship between fluorescence and Chl *a* for the SBE25 was not significant because of the scarcity of data and the fact that all concentrations measured using that sensor were very low. However, it was the only way to obtain chlorophyll data for all cruises in May and until June, 9.

Picoplanktonic organisms (0.2-2 µm) were sampled in 1.6 mL tubes, fixed immediately with 100 µL of 20% paraformaldehyde, incubated at 4ºC during half an hour, frozen in liquid nitrogen, and finally kept at -80ºC until further analysis. Samples were analyzed later by flow cytometry using a FACScalibur Cytometer (Beckton and Dickinson). Side scatter (SSC) and fluorescence parameters were obtained to distinguish between autotrophic picoeukaryotes (APE), cyanobacteria (*Prochlorococcus*, Pro, and *Synechococcus*, Syn) and heterotrophic prokaryotes (HP). Samples were run until 10000 events were reached or after 2 min at high speed to measure phototrophic organisms (APE, Pro and Syn), and at low speed for HP samples which were pre-stained with SYTO-13.

Auto- and heterotrophic nanoflagellates (ANF and HNF) were fixed using 540 µL of 25% glutaraldehyde in a tube containing 45 mL of seawater. Samples were kept at 4ºC in dark until the sample was filtered onto a 0.6 µm black polycarbonate filter placed over a backing filter and stained by diamidino-2-phenylindole (DAPI) for 5 min. The filter was immediately mounted on a microscope slide with low-fluorescence immersion oil and kept at -20ºC. Finally, it was analyzed by epifluorescence microscopy with a Zeiss Axiovert 35 microscope (Haas 1982).

Microplanktonic organisms were kept in 500 mL dark bottles fixed with 1.5 mL of acid lugol and analyzed afterwards by the Utermöhl technique. A 100 mL subsample was settled for 48 h in a composite chamber. The bottom chamber was then examined by an inverted Zeiss Axiovert 35

microscope to identify the main microplanktonic groups: diatoms (Dia), dinoflagellates (Din), ciliates (Cil), and copepod nauplii or copepodites. Only samples from station 3 at 20 m were analyzed.

The abundance of organisms obtained by flow cytometry was converted to biomass using the carbon conversion factor of: 17 fgC $cell^{-1}$ for HP (Bode et al. 2001), 29 fgC $cell^{-1}$ for Pro, 100 fgC $cell^{-1}$ for Syn (Zubkov et al. 2000) and 1500 fgC $cell^{-1}$ for APE (Zubkov et al. 1998). After biovolume estimation by microscopy, nanoflagellates were converted to carbon using the factor of 220 fgC μm^{-3} for HNF (Borsheim & Bratbak 1987) and the equation $0.433(BV)^{0.863}$ pgC $cell^{-1}$ for ANF (Verity et al. 1992). Finally, microplanktonic abundance was converted to biomass from total biovolume data obtained directly by microscopy for the most abundant cells or from previous measurements in these waters (A. Ojeda, unpublished data). If these data were not available, an average size from the literature for every organism or group (Tomas 1997, Horner 2002, Ojeda 2006, Ojeda 2011) was assumed, fitting them to the suitable shape following Hillebrand et al. (1999). The corresponding parameters were used for Dia (log a = -0.541, b = 0.811 for V<3000 μm^3; log a = 0.933, b = 0.881 for V>3000 μm^3), Din (log a = -0.353, b = 0.864) and Cil (log a = -0.639, b = 0.984 for aloricate ciliates; log a = -0.168, b = 0.841 for tintinnids) to obtain the biovolume to biomass conversion factor (Menden-Deuer & Lessard 2000) (log pgC $cell^{-1}$ = log a + b · log V). Some species were not taken into account to calculate the microbial biomass because of the impossibility of finding their size range or biovolume in the literature, but these species were always practically negligible in terms of abundance. Average Chl *a* concentration, abundance and biomass were calculated without station 1 as a significant difference (Kruskal-Wallis test, p<0.001) was found between this coastal station and the stations 2, 3 and 4 for Chl *a*.

Primary production (PP) was measured using the ^{13}C method (Hama et al. 1983) in station 2 at 20 m depth. Water samples were transferred to 2L polycarbonate bottles previously rinsed with 10% HCl and distilled-deionized water and $NaH^{13}CO_3$ was added at about 10% of total inorganic carbon in the ambient water. Samples were incubated in a tank on-deck for between 6.5 and 10 h depending on the cruise excepting on March 10, when the incubation lasted 22 h because of logistical issues. Running surface seawater and appropriate meshes simulated *in situ* temperature and light intensity. Initial and final particulate organic carbon (POC) and particulate material used for isotopic analysis were filtered through precombusted GF/F filters (5 h, 450ºC). These filters were frozen and stored at -20ºC until analysis. POC was measured using a CHN analyzer (Carlo Erba EA 1108) and isotopes in a mass spectrometer equipped with an elemental analyzer (Flash EA 11 ThermoFinnigan with Deltaplus).

Additional PP rates from satellite data were obtained from the Ocean Productivity web page (http://www.science.oregonstate.edu/ocean.productivity/index.php, last acces: June 2014). These PP values are based on the original description of the Vertically Generalized Production Model

(VGPM) (Behrenfeld & Falkowski 1997), surface chlorophyll concentrations (MODIS), sea surface temperature data (MODIS 4), and cloud-corrected incident daily photosynthetically active radiation (PAR) (MODIS). Euphotic depths are calculated from surface chlorophyll concentrations following Morel and Berthon (1989). Average surface PP rates were obtained every 8 days from 10 locations close to the area studied (28.08ºN, 15.25ºW; 28.08ºN, 15.42ºW; 28.25ºN, 15.25ºW; 28.25ºN, 15.42ºW; 28.42ºN, 15.25ºW; 28.42ºN, 15.42ºW; 28.58ºN, 15.25ºW; 28.58ºN, 15.42ºW; 28.75ºN, 15.25ºW; 28.75ºN, 15.42ºW), from January to July 2010.

Atmospheric total suspended particulate matter (TSM) was collected every 6 days from November 2009 to June 2010. A high volume sampler pumping system (MCV) was used at a flow rate of 50 m^3 h^{-1} and onto Whatman GF/A 20 cm x 25 cm fiberglass filters. Each sampling period started at 08:00 h local time and lasted 24 h. Three collectors were located in the northeast of the island (Fig. II.1) and placed 10 m above the ground. For Fe, Al and Mn analysis, filters were treated with nitric and hydrochloric acid, according to the Beyer modified method (López-Cancio et al. 2008). These elements were determined by atomic emission spectrophotometry using an inductively coupled plasma optical emission spectrometer (Perkin Elmer 3200 DV).

The zooplankton sampling procedure is explained in detail elsewhere (Herrera et al. in prep.). Briefly, organisms were captured in vertical hauls with a double WP-2 net equipped with 100 µm mesh nets. One of the samples was used for measuring biomass as dry weight using a standard protocol (Lovegrove 1966). The average zooplankton biomass was calculated from the four stations data as no statistical differences were found (Herrera et al. in prep.).

III. RESULTS

A highly stratified water column was characteristic during the whole period from February to June 2010. Average temperature and potential density were above 19ºC and 26.4 kg m^{-3}, respectively, in the upper 100 m layer (Fig. II.2A and B). Average MLD ranged between 110 m in April to 38 m in June, with no differences among stations (Kruskal-Wallis test, $p>0.05$). Very low values of Chl *a* (<0.3 mg m^{-3}) were measured within the mixed layer, and at the deep chlorophyll maximum (DCM) the highest average chorophyll concentration was 0.4 mg m^{-3} at the end of March and during April (Fig. II.2C).

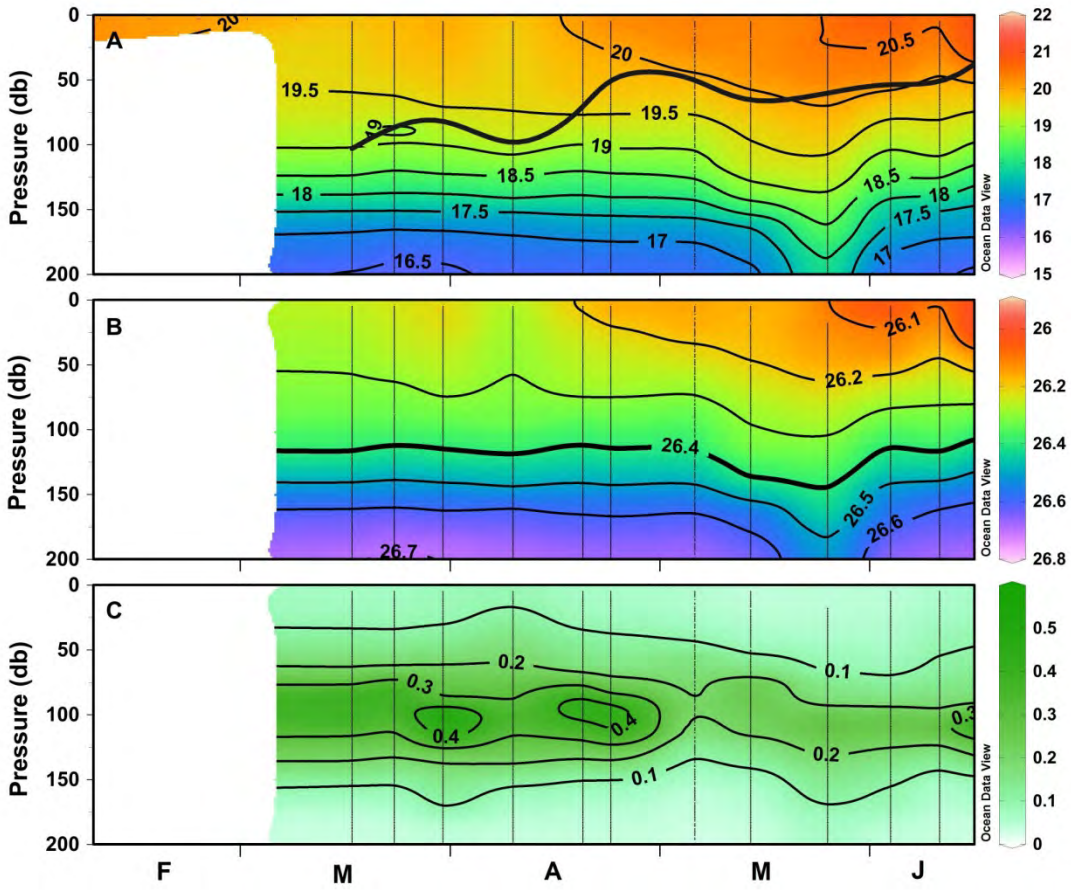

Figure II.2. Temporal variability of temperature (2A, ºC), potential density (2B, kg m^{-3}) and Chl a (2C, mg m^{-3}) from surface to 200 db. MLD is drawn in the upper panel (black solid line). Data correspond to average from station 2, 3 and 4; from February to June 2010. CTD data were available after March 17. Before that date sea surface temperature is showed since February 2 (from SeaWatch Buoy data).

Saharan dust events were identified in every month from TSM data, from February to June (Fig. II.3, upper panel). The highest value (521 ± 71 µg m^{-3}) was reached during March, coinciding with maximum concentrations of iron and aluminum (Fig. II.3, central and lower panels). Less intense TSM maxima also coincided with relative maxima in metal concentrations, but the magnitude of the increment was not proportional in all cases. In May, an intense maximum of aluminum was found without observing a similar increase of iron. In June, a rather high value of iron concentration was reached without observing a parallel increase in aluminum. Nevertheless, a significant correlation (Spearman's rank $r>0.41$, $p<0.001$) was found for TSM and the two metals.

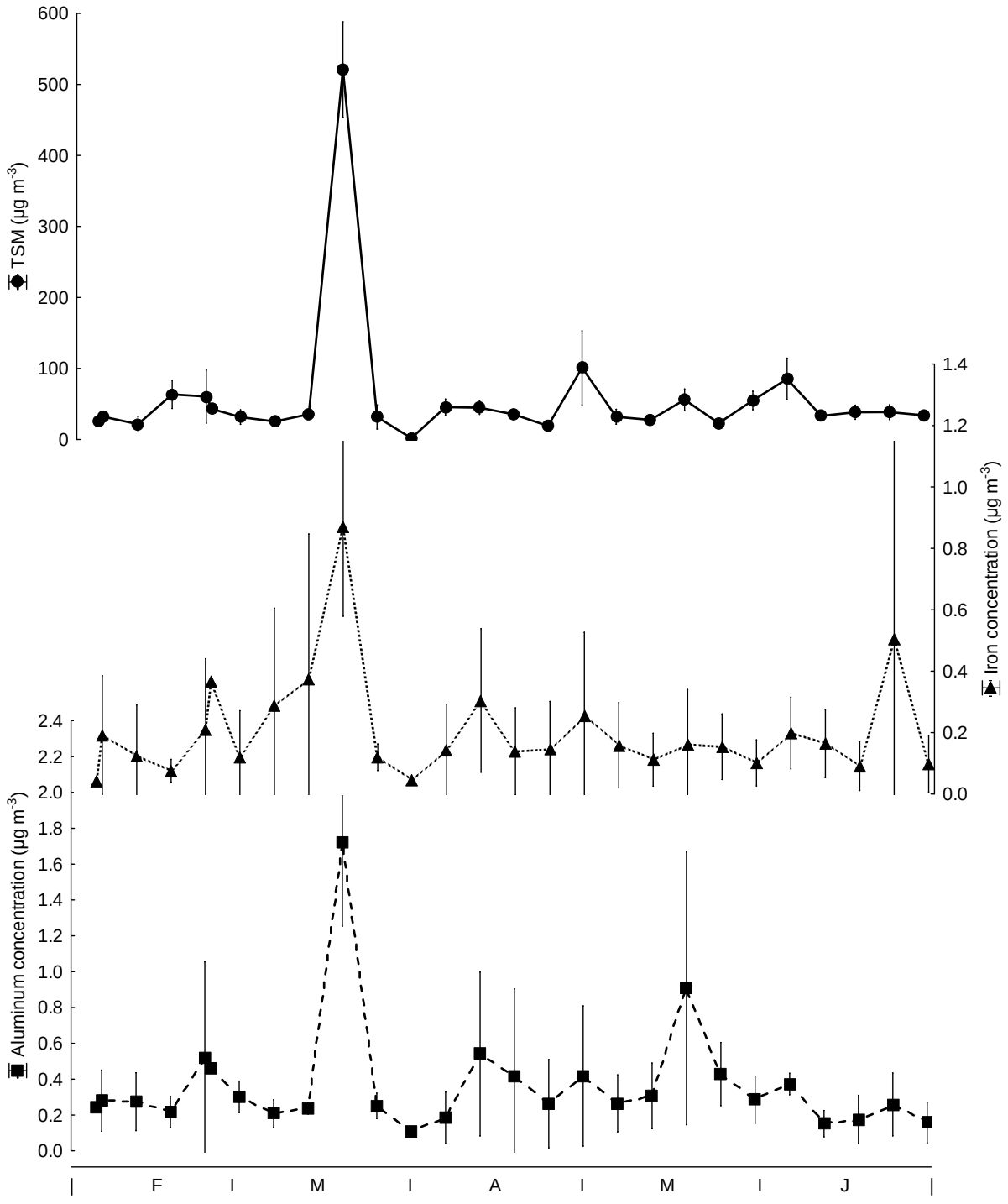

Figure II.3. Atmospheric total suspended matter (TSM, µg m^{-3}) and iron and aluminum concentration (µg m^{-3}) from February to June 2010. Data showed as average and standard deviation.

In spite of the quite low Chl *a* concentration observed at 20 m from February to June (Figs. II.2C and II.4, upper panel), unrealistic high values of PP were measured. Rates were maximal at the end of March (56.2 ± 6.9 mgC m^{-3} d^{-1}) and in May and June (Fig. II.4, central panel), reaching rates up to 97.4 ± 53.3 mgC m^{-3} d^{-1}. Given these unlikely rates measured, we used surface PP rates from satellite data (Fig. II.4, lower panel). Surface PP rates were rather low during the period studied, but higher rates were also observed at the end of March (414.9 ± 60.3 mgC m^{-2} d^{-1}) and June (449.8 ± 60.6 mgC m^{-2} d^{-1}). Furthermore, a relative increase of surface PP rates was observed almost every month from January to May after every dust event observed from TSM data (Fig. II.5).

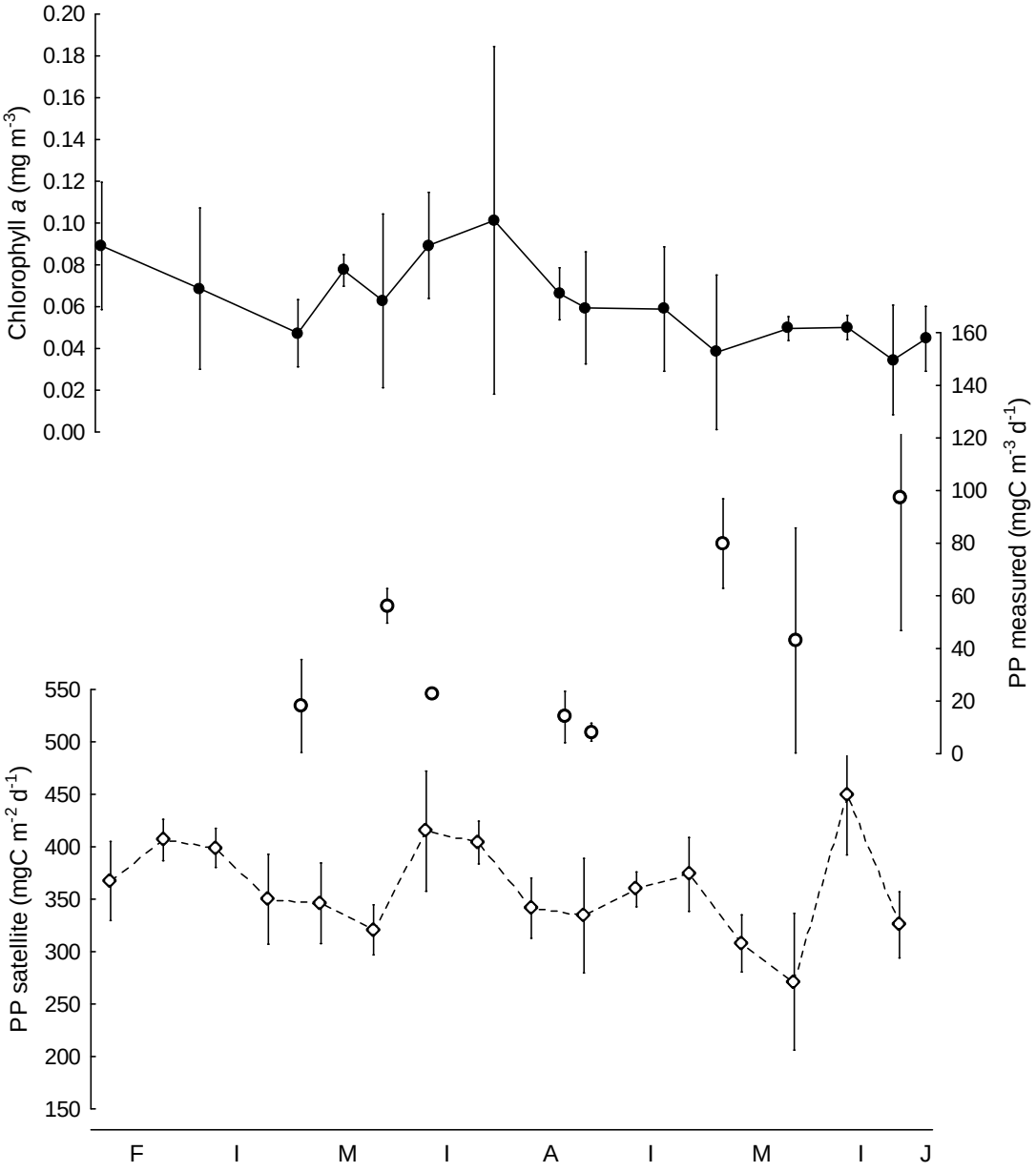

Figure II.4. Average (±SD) Chl *a* concentration (mg m^{-3}) and primary production measured at the mixed layer (PP measured, mgC m^{-3} d^{-1}) and from satellite data (PP satellite, mgC m^{-2} d^{-1}) from February to June 2010.

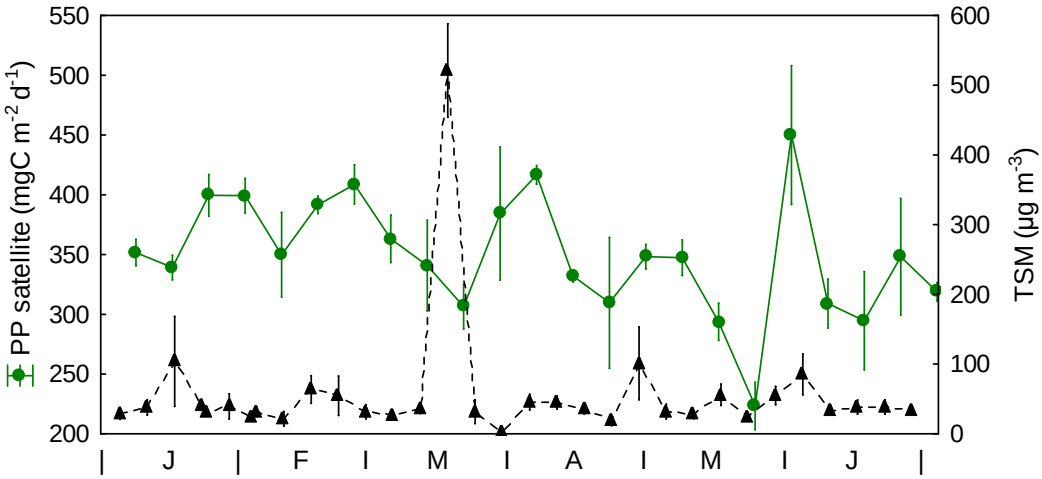

Figure II.5. Primary production from satellite data (PP satellite, mgC m^{-2} d^{-1}) and atmospheric total suspended matter (TSM, µg m^{-3}) from February to June 2010.

Both autotrophic and heterotrophic organisms kept quite low abundances (Fig. II.6). Average values were below $4{\cdot}10^3$, $4{\cdot}10^4$ and $0.3{\cdot}10^3$ cells mL^{-1} for Syn, Pro and APE respectively. Dia showed cell numbers below 1 cell mL^{-1}, while ANF reached more than $3{\cdot}10^2$ cells mL^{-1}. Dinoflagellates varied between 4 and 27 cells mL^{-1}. HP were below $16{\cdot}10^4$ cells mL^{-1}, HNF varied between 1 and $6{\cdot}10^2$ cells mL^{-1} and Cil were below 2 cells mL^{-1}. Most of planktonic groups showed a low variability during the period studied and a clear signal was not observed after every dust event. However, the abundance of Syn and APE was minimal after the deposition event on 18 March, and maximal for ANF and HNF (Fig. II.6).

The effect of dust upon the planktonic community was estimated from the dust deposition event observed on 18 March. For that, the difference between parameters, before (two samplings before) and after (two samplings after) this date was calculated. Negligible and non-significant changes (*t* test, $p>0.05$) in PP and Chl *a* were measured (Fig. II.7). The response of the different planktonic groups considered was quite different, finding an increase in abundance (Fig. II.8A) and biomass (Fig. II.8B) for nano- and microplankton, excluding Dia. The latter organisms decreased in abundance but increased significantly their biomass. In the case of picoplankton, autotrophic organisms (Syn, Pro, APE) showed a negative change in abundance and biomass (Fig. II.8A and B), while HP increased. Mesozooplankton (Meso) also showed a positive change in biomass (Fig. II.8B). However, the changes observed after the dust event were only significant (*t* test) in the case of Syn and APE for abundance and biomass, and for Dia biomass. The positive changes of HNF abundance and Meso biomass were also close to the level of significance ($p=0.06$).

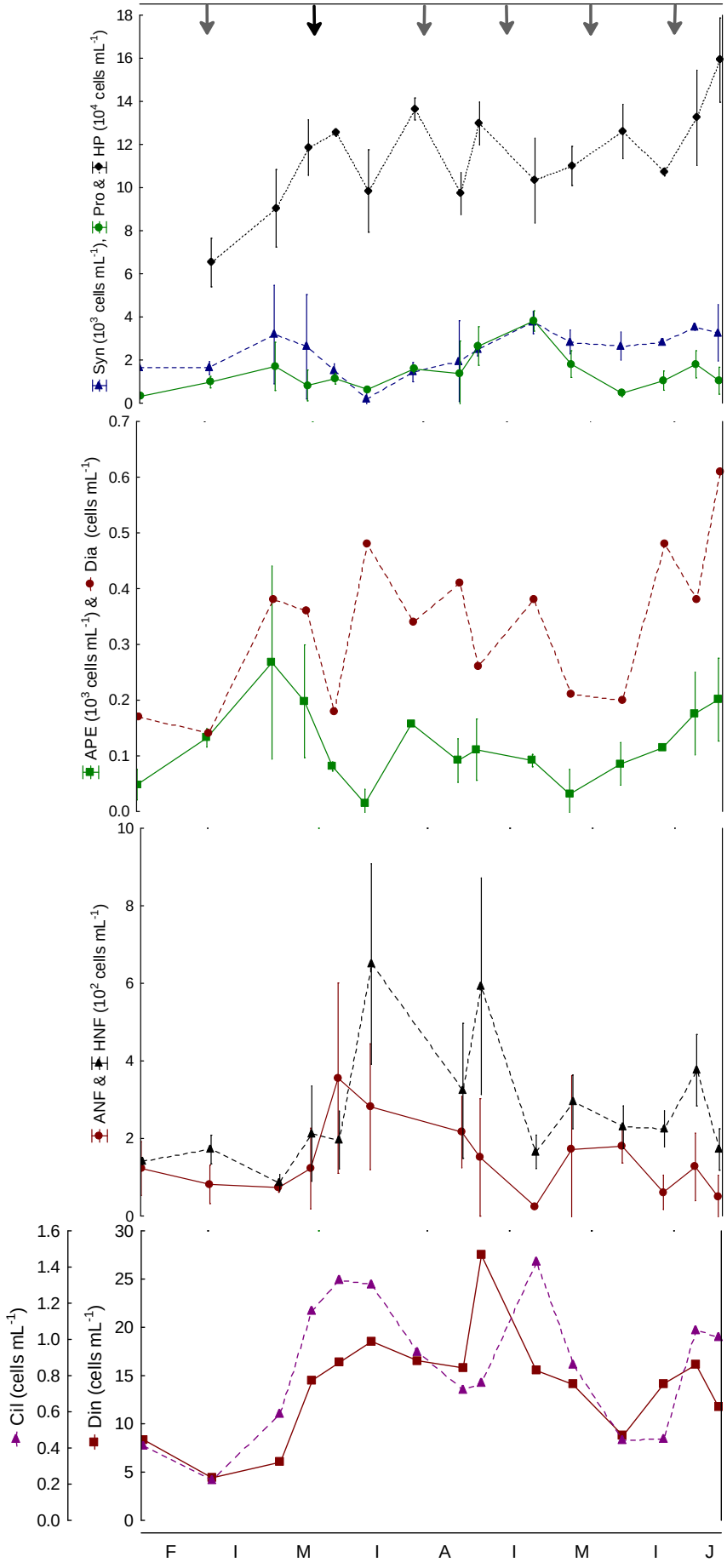

Figure II.6. Average (±SD) abundance (cells mL^{-1}) of HP, Syn, Pro, APE, Dia, ANF, HNF, Din and Cil during the period studied in 2010. Dust deposition is marked at the top with grey arrows for relative low dust events, and a black arrow for the highest dust event in March.

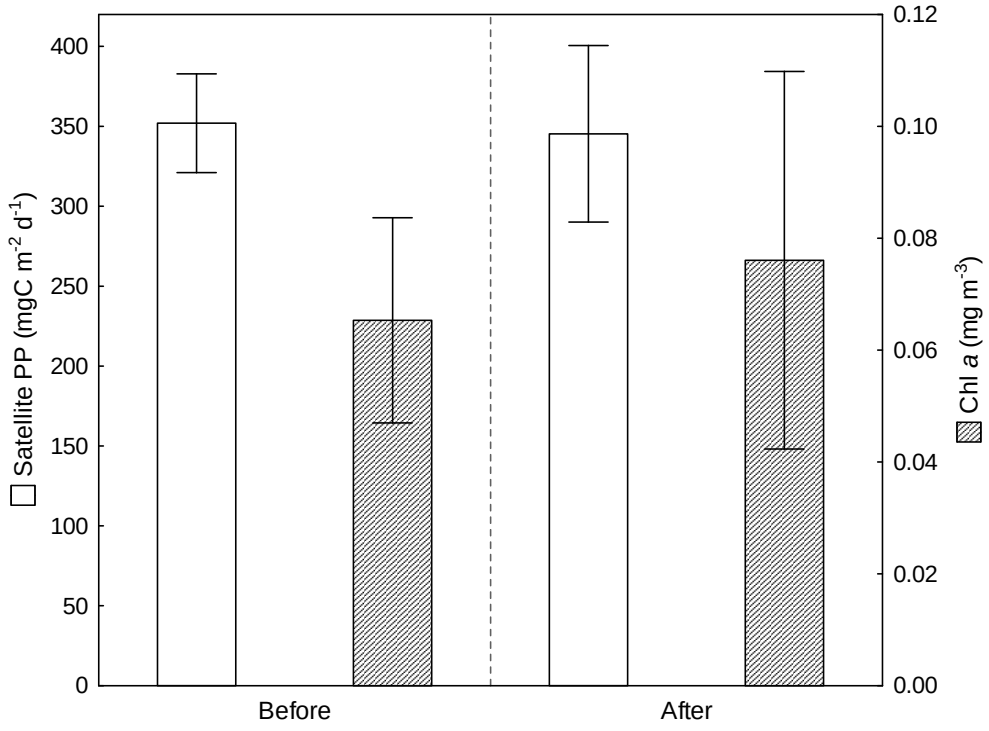

Figure II.7. Comparison between average (±SD) PP (Satellite PP, mgC m^{-2} d^{-1}) and Chl a (mg m^{-3}) values before and after the highest dust event on 18 March.

These changes in abundance and biomass of some planktonic groups entailed considerable relative changes (Fig. II.9). The negative effect of the dust event upon APE and Syn supposed a reduction in their abundance and biomass of 79 ± 154 and 70 ± 164 %, respectively (Fig. II.9A and B). HNF abundance increased more than 100% (Fig. II.9A) and the positive change in Meso biomass supposed an increase of 95 ± 213 % (Fig. II.9B). The highest relative change was observed for Dia biomass which supposed an increment of 1308 ± 1885 % (Fig. II.9B), despite its abundance decreased by 11 ± 68 % (Fig. II.9A).

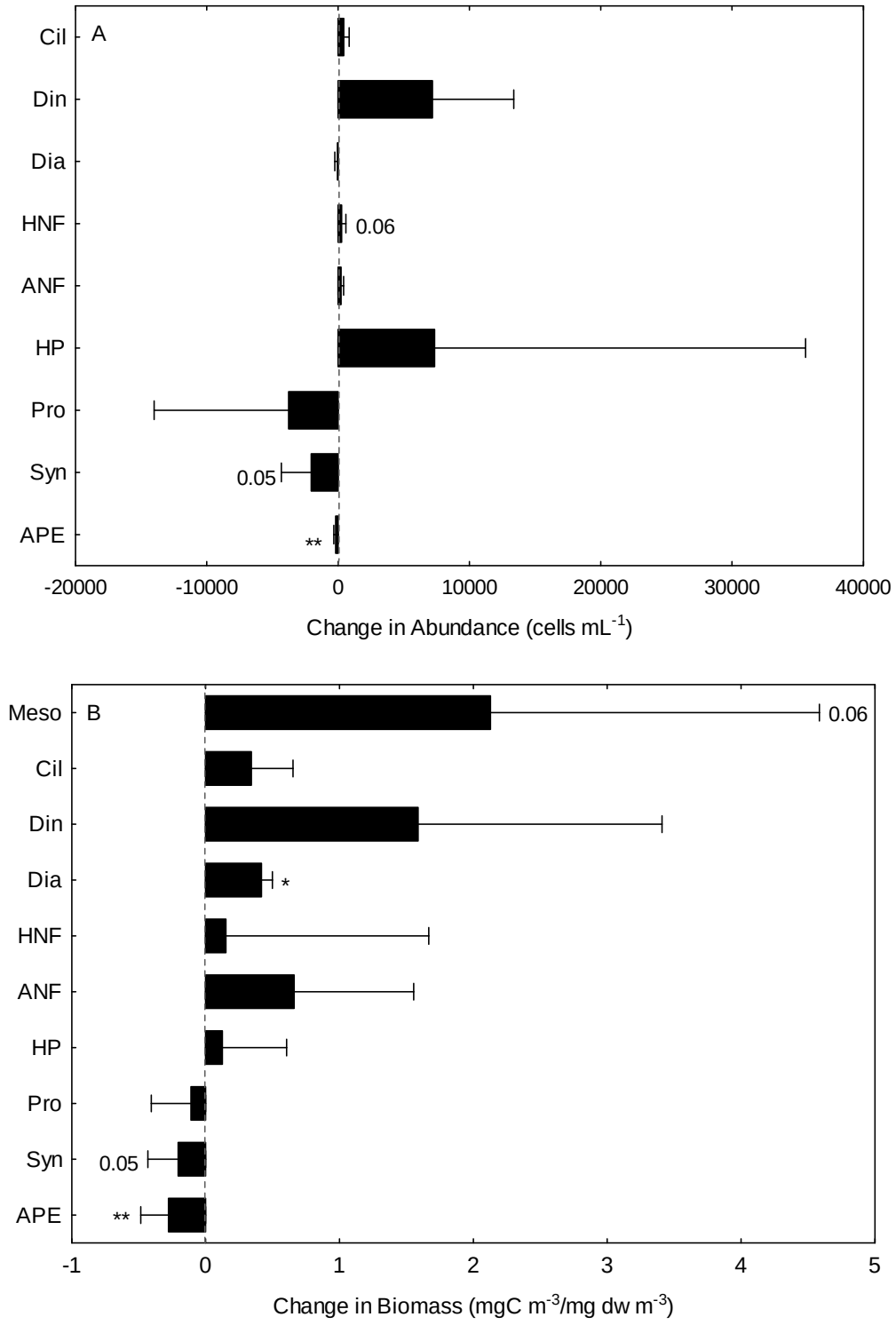

Figure II.8. Average (+SD) change in abundance (8A, cells mL^{-1}) and biomass (8B, mgC m^{-3}) for pico-, nano- and microplankton; mgDW m^{-3} for mesozooplankton). * $p<0.05$, ** $p<0.01$ and *** $p<0.001$. p-values below or equal to 0.06 are also shown.

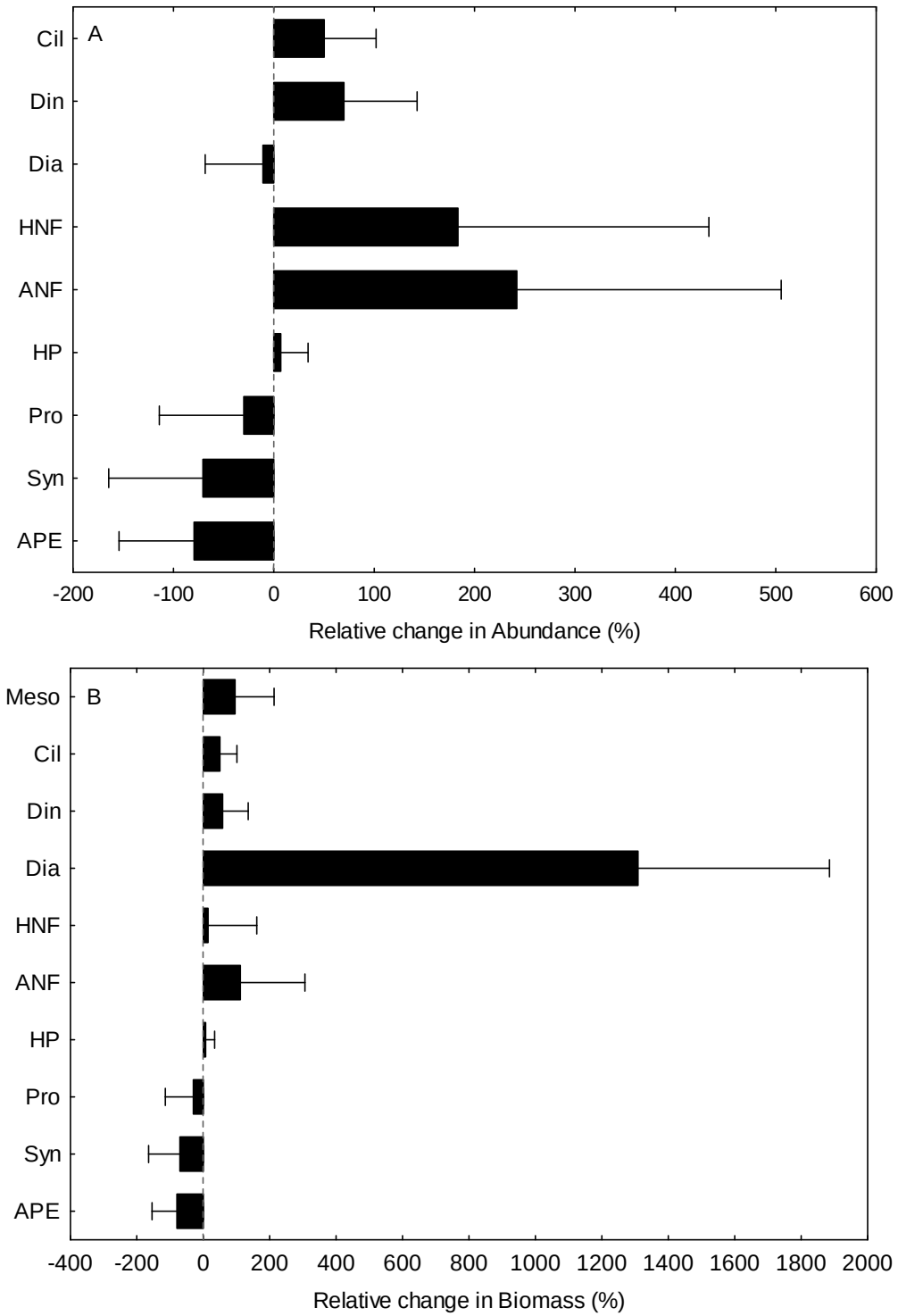

Figure II.9. Average (+SD) relative change (%) in abundance (9A) and biomass (9B) after the dust deposition event on March 18. Relative change was calculated as (A-B/B)*100, where A and B are the average values for every parameter after and before the event, respectively.

IV. DISCUSSION

High stratification (Fig. II.2A and B) and low Chl *a* (Figs. II.2C and II.4) conditions indicated that the characteristic late winter bloom (De León & Braun 1973, Braun 1980, Arístegui et al. 2001) did not take place during the period studied. Furthermore, in order to discard the possibility of the bloom occurrence before we started to sampling, we showed that sea surface temperature was higher before February 2010 (Fig. II.2A). Therefore, the winter surface cooling, and consequently the characteristic winter bloom, did not take place during 2010.

The low values of phytoplankton abundance and biomass were common for all autotrophic organisms (Fig. II.6) but extremely low for cyanobacteria and picoeukaryotes comparing to previous years in the area (Schmoker & Hernández-León 2013) and the next year, 2011 (see Chapter I). Biomass was also lower than in previous years for both HNF and Cil. Despite the low biomass values for both autotrophs and heterotrophs, the latter dominated during the whole period (A:H<1, Table II.1), which seems to be a common feature in the North Atlantic gyre waters when Chl *a* is below 0.1 mg m^{-3} (Buck et al. 1996).

Month	$A_{Pico,ANF}$:$H_{HP,HNF}$	A:H
Feb	0.66 (±0.00)	0.78 (±0.00)
Mar	0.58 (±0.29)	0.67 (±0.14)
Apr	0.49 (±0.14)	0.77 (±0.11)
May	0.78 (±0.34)	0.81 (±0.13)
Jun	0.57 (±0.08)	0.69 (±0.09)

Table II.1. Monthly average (±SD) of autotrophic:heterotrophic biomass (A:H) ratio at the mixed layer from February to June 2010. Ratios are shown for pico- and nanoplankton ($A_{Pico,ANF}$:$H_{HP,HNF}$) and including microplankton (Dia, Cil, Din) considering 50% Din as autotrophs and 50% as heterotrophs (A:H). Biomass in mgC m^{-3}.

Surprisingly, we measured unrealistic PP rates (0.43-12.46 mgC m^{-3} h^{-1}) much higher than maximum rates found in these waters by De León & Braun (1973) during a characteristic late winter bloom (2.4-3.3 mgC m^{-3} h^{-1}). Moreover, our mean rates (50.05 mgC m^{-3} d^{-1}) were 5-fold over mean rates measured at BATS station (10.94 mgC m^{-3} d^{-1}) during the same period (BATS interactive data access, http://bats.bios.edu/index.html, last access: July 2013). The difference is not expected to result by a methodological issue, as good agreement has been described between the ^{13}C isotopic

technique and the ^{14}C method (Slawyk et al. 1977, Hama et al. 1983). On the other hand, high PP rates resulted from the extremely high POC concentrations measured within the incubation bottles and used to compute them. Actually, POC concentrations measured at the ML (data not shown) were in many cases much higher than previous measurements in this area (Alonso-González et al. 2009). These authors measured concentrations around 4 µM in the surface waters at the northeast of the Canary Islands. Our data showed a high variability ranging between 0.99 to 25.66 µM (7.47 ± 7.85 µM) and reaching values higher than 4 µM during March, May and June. However, we are not able to explain the cause behind these POC values, although a methodological issue cannot be totally discarded.

In addition to unusual hydrographic and biological properties, 2010 was one of the most intense dust periods during the last years, especially in March (Aerosol Optical Thickness data collected by the Moderate Resolution Imaging Spectroradiometer, MODIS, from NASA Earth Observations Project, http://earthobservatory.nasa.gov/, last access: April 2013). This was also observed in the frequent dust events occurred over Gran Canaria Island during the whole period. Furthermore, the most intense event took place in March, when the TSM concentration reached the highest value (Fig. II.3, upper panel). The presence of significant concentrations of iron and aluminum in dust and their correlation with TSM confirmed the crustal nature of the dust and its Saharan origin (Viana et al. 2002). This fact, together with the strong water column stratification observed during this winter (Fig. II.2) suggests that the major source of nutrients to surface waters in this area during the period studied was the atmospheric deposition of Saharan dust. In this sense, it is important to note the location of the sampling site, to the north of the islands, away from intense mesoscale activity prevalent leeward (Arístegui et al. 1994, Barton et al. 2004) which could be an important source of nutrients into the mixed layer (Barton et al. 1998).

Data on nutrient concentration in the mixed layer were not available for the whole period, but some measurements were performed during the sampling (see Benavides et al. 2013). Surprisingly, rather high values of nitrate+nitrite (0.3-0.6 µM) and exceptionally high phosphate concentration (0.5-2.5 µM) were found. These concentrations are higher than previous measurements at the time-series ESTOC station (Neuer et al. 2007) and at the northeast subtropical gyre (Marañón et al. 2000, 2003). This high nutrient level was measured even in May, a typically stratified period with low nutrient concentrations (<0.1 µM nitrate+nitrite, <0.05 µM phosphate) in these waters (Neuer et al. 2007). It is unlikely that these high concentrations were promoted by the enrichment from deeper waters given the hydrographic conditions observed and the knowledge about nutrient dynamics in the area. Cianca et al. (2007) showed that in subtropical waters of the northeast Atlantic (European Station for Time Series, ESTOC) nitrate concentration remained low (< 0.5 µM) at potential densities below 26.4 kg m^{-3}, and it increased significantly above that isopycnal surface. During our sampling, the MLD was never deeper than the 26.4 kg m^{-3} isopycnal surface (Fig. II.2A and B).

Another possible source of nitrogen could be nitrogen fixation, which would be favored by high temperatures and stratification. However, it was not the case as low fixation rates were measured on average by Benavides et al. (2013) during our sampling.

On the other hand, the release of nutrients (nitrate, phosphate and iron) from Saharan dust has been demonstrated by other studies (Bonnet et al. 2005, Herut et al. 2005, Duarte et al. 2006, Pulido-Villena et al. 2010, Ridame et al. 2014). Therefore, there are enough evidences (atmospheric suspended matter, hydrographic data and seawater nutrient concentration) that strongly support that the intense atmospheric dust deposition during the period studied promoted the high nitrate and phosphate concentrations in the mixed layer. In the case of silicate, an element also contained in Saharan dust (Goudie & Middleton 2001, Viana et al. 2002), rather low concentrations (0.02-0.03 μM) were measured by Benavides et al. (2013). We suggest this could be the result of diatom uptake, as silicate concentration was minimum in March (Benavides et al. 2013), when diatom abundance increased (Fig. II.6).

The effect over the plankton community was unequal, as it was shown for the heavy dust deposition during March. A negligible change in Chl *a* and PP was observed after the event (Fig. II.7). This is partly consistent with experimental results when Saharan dust was added to oligotrophic waters of the Atlantic (Marañón et al. 2010) and the Mediterranean (Bonnet et al. 2005). In both cases little changes in phytoplankton biomass were observed. Contrary, a considerable response in primary production was measured. Marañón et al. (2010) used dust concentrations that mimicked a high atmospheric deposition event, obtaining an increment in PP rates of 1-1.5 mgC m^{-3} d^{-1}, which supposed a mean relative change of 25% in PP. After the simulation of a medium dust event, Bonnet et al. (2005) found a positive change of 48% in PP rates. In our case, an average decline of 1.89% in surface PP was measured, accounting for a decrease of 6.65 mgC m^{-2} d^{-1} (Fig. II.7). Herut et al. (2005) measured an increment of more than 9 mgC m^{-3} d^{-1} in PP in experiments adding Saharan dust. However, they argued that in the field, the likely low nutrient release during an atmospheric event would promote a minor phytoplankton response, as they measured *in situ* a very low increase in Chl *a* after a dust storm. This result has been explained by other authors arguing that despite the enhancement of primary productivity after an artificial or natural fertilization, phytoplankton stocks do not increase because of the control exerted by grazers (Landry et al. 2000a, Landry et al. 2000b, De Baar et al. 2005, Herut et al. 2005, Boyd et al. 2007, Henjes et al. 2007, Marañón et al. 2010). Another possibility is that we did not measure the actual change in productivity because we took the next two samplings after the dust event for calculations, whilst the increase was observed in the second and third value of PP measured after March, 18 (Fig. II.5). Thus, the response of PP would not be immediate after the dust event, but lagged more than 10 days. If we take the second and third sampling as the average value after the event we obtain a PP increase of 48.43 mgC m^{-2} d^{-1}, which supposes an increment of 13.76%. Therefore, the frequency

and depth of the sampling used here could be determining the observation of the biological response measured.

Due to the scarcity of studies about the effect of dust deposition in the field, neither the time of biological response nor the depth to which this response could be found after a dust event is well established. The response in the field can be almost immediate or lagged up to several weeks. For example, in the eastern Mediterranean the bacterial activity increased just one day after a dust storm (Herut et al., 2005), while in the north Pacific an increase in POC concentration was observed after 5 days and peaked after 2 weeks (Bishop et al. 2002). A high variability in the time lag of the response of phytoplankton, between less than 8 up to 16 days, was also reported from satellite Chl *a* measurements after different dust events in the northeast Atlantic (Ohde & Siegel 2010). Regarding to the vertical extent of the response, it is difficult to know until what depth is the planktonic community affected as the response in the field has been measured from satellite data in many cases. However, it seems that the effect of dust deposition is not limited to surface waters according to some results. The increase in POC concentration in the north Pacific measured robotically by Bishop et al. (2002) reached 40 m depth. In the eastern Mediterranean the effect of a dust storm was measured up to 40 m for *Prochloroccocus* and in the first 15 m of the mixed layer for Chl *a* and heterotrophic prokaryotes (Herut et al., 2005). Thus, the impact of dust on marine communities would be longer than a few days and they could be affected not only in surface waters, but deeper in the mixed layer.

In this study, Dia were the only planktonic group that was significantly and positively affected in terms of biomass (Figs. II.8B and II.9B). This is a common feature in ocean fertilization experiments (De Baar et al. 2005, Boyd et al. 2007) because of their higher growth rates compared to prokaryotic algae during the release of iron (Landry et al. 2000a), and the lower grazing pressure upon them (Landry et al. 2000a, Landry et al. 2000b, Henjes et al. 2007). In this study, Dia biomass showed a remarkable increment of 1308% (Fig. II.9B), whereas their abundance diminished by 11% (Fig. II.9A). This increase of their biomass after the dust event matched the appearance of *Chaetoceros* sp. (Fig. II.10), which supposed a 60% of total biomass despite their low abundance. The increase of larger diatoms has also been observed in artificial fertilization experiments (De Baar et al. 2005). However, the presence of *Chaetoceros* sp. cannot be directly related to the dust deposition effect as they also caused the other two maxima of Dia biomass in April and May.

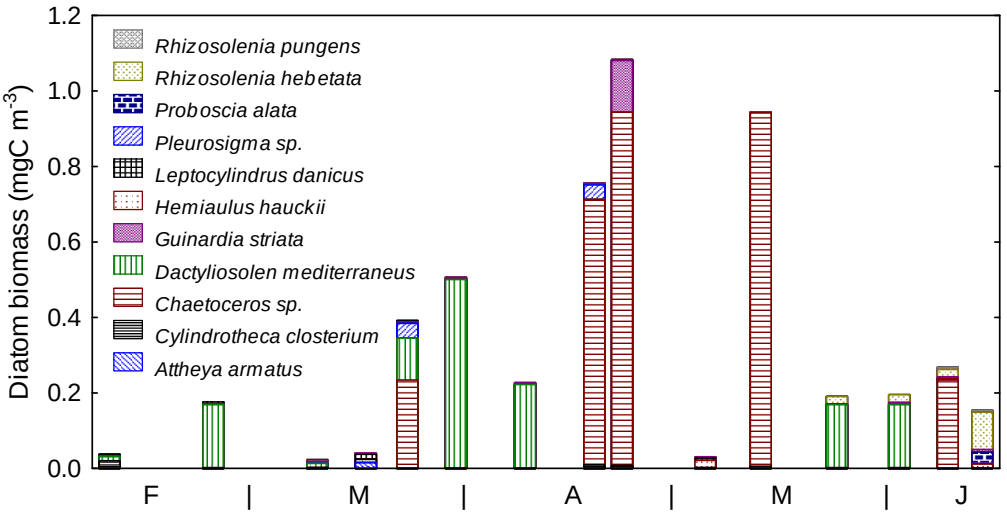

Figure II.10. Contribution of different species to diatom biomass (mgC m^{-3}) during the period studied in 2010.

Contrary, a significant negative response was observed for APE and Syn after 18 March (Figs. II.6 and II.8). Their biomass and abundance reduction suggests a higher grazing pressure after the event or a possible negative effect of dust over these organisms. Grazing has been pointed out as a major process controlling primary producers, even when phytoplankton growth is enhanced (Landry et al. 2000a, Landry et al. 2000b, De Baar et al. 2005, Herut et al. 2005, Boyd et al. 2007, Henjes et al. 2007, Marañón et al. 2010). Moreover, microzooplankton are major grazers in these subtropical waters (Calbet & Landry 2004), and an increment was found for all heterotrophic organisms (Figs. II.6 and II.8). Thus, it is not surprising that prokaryotic algae would be under a higher grazing pressure than diatoms, the latter favored by either the fertilization effect as well as lower grazing. On the other hand, a toxic effect of dust has been previously suggested for cyanobacteria (Marañón et al. 2010) and demonstrated for APE and Syn (Paytan et al. 2009). However, in this case, there was not a substantial increase in copper, the element associated to toxicity, for the period of the heavy dust event in March in comparison with the average concentration during the rest of the year (data not shown). Thus, it is not likely that picophytoplanktonic organisms would be affected by dust toxicity.

The mesozooplankton response was the highest positive change in biomass, increasing over a 100% after the dust event on 18 March. This change was not statistically significant, but the p-value was very close to the level of significance (p=0.06). Actually, mesozooplankton increased not only their biomass but also their metabolic activity after that event (Herrera et al. in prep.). This effect was previously described in the Canary Islands waters by Hernández-León et al. (2004), who measured an increase in biomass, gut fluorescence and potential respiration (ETS activity) of these

organisms after a Saharan dust storm. This enhancement has been reported previously by Boyd et al. (2007) after the enrichment promoted by mesoscale iron experiments, although not in all cases.

In summary, our results showed that the Canary Islands waters would be potentially affected by the Saharan dust deposition during the whole period studied. The effect of these events was strongly supported by TSM, hydrographic data and nutrient concentration in the mixed layer. These data suggest that in addition to the heavy dust event observed in March, the smaller but numerous dust events in April, May and June would potentially reinforce the effect of atmospheric deposition. Furthermore, despite the low values of PP observed at the surface, a relative short-term variability seemed to be related to the Saharan dust events identified. In any case, different responses were found in the planktonic community. On one hand, diatoms increased their biomass by more than 1000%, and mesozooplankton also showed an increment in biomass, as it has been observed before. On the other hand, picophytoplankton seemed to be negatively affected, but if this effect was directly caused by dust or indirectly by grazing losses remains unknown. This unequal effect upon autotrophs, favoring diatoms instead the small autotrophs, could also enhanced the biological pump due to a higher carbon export flux resulted from diatom sedimentation. Hence, the Saharan dust deposition would be partly fuelling the primary production in these oligotrophic waters, and could also enhance the carbon export, especially during stratified periods, when it would be the most important nutrient source. However, Saharan dust fertilization seems to promote a modest increase in productivity or it would not be detected as primary producers could be rapidly consumed. Nevertheless, further research is needed to better understand the potential influence of this process in the subtropical northeast Atlantic. In this sense, an intensive temporal sampling would help to properly quantify the timing and intensity of the response of the different planktonic groups in the field, especially in subtropical waters, given the complexity and quickness of the biological interactions.

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CHAPTER III

Microzooplankton feeding impact on the microbial community of the Canary Islands waters

CAPÍTULO III

ABSTRACT

Microzooplankton are major grazers in the ocean but the knowledge about their feeding impact in subtropical gyres is rather limited. In order to assess the grazing activity of these organisms in the subtropical waters of the Northeast Atlantic we carried out several dilution experiments in 2010 and 2011 to the north of the Canary Islands. During the period studied heterotrophic prokaryotes and *Prochloroccocus* were the most abundant preys and heterotrophic nanoflagellates were the dominant consumers. We found an unequal impact of microzooplankton feeding on the different microbial components. These differences were not related to temperature, chlorophyll *a* or community composition, whereas a significant correlation was found between microzooplankton grazing and the growth rate of their prey. We found the highest grazing rates upon large-sized cells, autotrophic nanoflagellates (2.14 ± 0.74 d^{-1}) and diatoms (1.93 ± 0.30 d^{-1}), which also showed the highest growth. Contrary, small autotrophic cells, *Synechoccocus* (0.22 ± 0.10 d^{-1}), *Prochloroccocus* (0.27 ± 0.26 d^{-1}) and autotrophic picoeukaryotes (0.89 ± 0.00 d^{-1}), were grazed at lower rates. Heterotrophic consumption was prevalent (1.12 ± 0.55 and 1.59 ± 0.31 d^{-1} for HP and HNF, respectively) and could be the cause of non-significant grazing values obtained. Our results showed that more than 100% of the daily primary production was removed, although the impact on the different autotrophic organisms was unequal. Consumption over eukaryotic cells was higher than the impact on prokaryotic production. The daily procaryotic production was almost totally removed by microzooplankton (96%), and more than 100% of the daily production of heterotrophic nanoflagellates was consumed.

I. INTRODUCTION

Nano- and microzooplankton (heterotrophic organisms ranging 2-200 μm according to Sieburth et al. 1978) comprise a heterogeneous group including heterotrophic nanoflagellates, ciliates and heterotrophic dinoflagellates which are key components of the food webs in the ocean (Sherr & Sherr 2002). They are major grazers not only in tropical and subtropical areas where they account for 72-76% of the daily primary production grazed, but also in productive ecosystems where the percentage of the primary production consumed per day is also high: 60-75% (Calbet & Landry 2004). In addition to their large impact on primary production, they are also an important food resource for mesozooplankton contributing up to 50% to the daily copepod diet in oligotrophic waters (Calbet 2008). Thus, these organisms play a key role in the recycling of energy and matter within the microbial loop (Pomeroy 1974, Azam et al. 1983, Sherr & Sherr 2002) and also in the flow to higher trophic levels (Sherr & Sherr 2002). Despite their importance, the knowledge about the feeding impact of micrograzers (nano- and microheterotrophs) is limited in subtropical gyres (Schmoker et al. 2013).

The dilution method (Landry & Hassett 1982) has been widely used to assess the microzooplankton feeding impact through the estimation of phytoplankton mortality rates by grazing. Furthermore, the method provides a simultaneous estimation of phytoplankton growth. This approach is based on the premise that grazing pressure is proportionally reduced with the dilution of seawater. As a result, the net growth rate of preys increases linearly with the increasing dilution of natural seawater due to the decrease of the encounter rates between preys and their grazers. Additionally, this technique entails two other assumptions: the intrinsic growth rate of preys is not density-dependent and the density of preys (P) changes with time (t) following the exponential equation $P_t = P_0 e^{(\mu-m)t}$, where μ and m are the instantaneous rates of growth and mortality by grazing, respectively. However, these assumptions are sometimes violated in dilution experiments as non-linear responses have been frequently observed (Gallegos 1989, Strom et al. 2007, Teixeira & Figueiras 2009). This non-linear relationship has been attributed to saturated feeding of microzooplankton at low dilution levels (Gallegos 1989) or to changes in microzooplankton community among the dilution treatments (Dolan et al. 2000, Agis et al. 2007). Additionally, Teixeira & Figueiras (2009) showed that the non-linear response could also be the result of food selectivity by micrograzers. More recently, Calbet & Saiz (2013) suggested that the presence of trophic cascades also promotes the non-linearity of dilution experiment results. Hence, heterotrophic consumption occurs during incubations besides grazing affecting the relationship between preys and grazers, the latter being also under predator pressure. This process would be particularly important in subtropical waters where trophic relationships are especially complex (Longhurst 1998).

In subtropical waters of the Canary Current the knowledge about the feeding impact of microzooplankton is rather limited. In these waters, direct measurements are practically non-existent to our knowledge. There are only some studies near the area, in the south-east of Azores (Gaul et al. 1999, Stelfox-Widdicombe et al. 2000, Quevedo & Anadón 2001) and to the south of the Canary Islands (Gutiérrez-Rodríguez et al. 2011). The measured impact on productivity is highly variable according to these studies ranging between 37 and >100% of the daily production consumed by micrograzers. Furthermore, potential differences in the microzooplankton feeding impact upon the diverse autotrophic community inhabiting these subtropical waters have only been evaluated by Quevedo & Anadon (2001) and Gutiérrez-Rodríguez et al. (2011).

In this work we measured the microzooplankton feeding impact not only on primary producers but also on heterotrophic organisms in the subtropical waters of the Northeast Atlantic (Canary Islands). We used the dilution technique and the analysis of different planktonic groups to be able to examine the impact of microzooplankton over every group considered.

II. MATERIAL AND METHODS

Sampling site

Seawater was collected to the north of Gran Canaria Island (Canary Islands) from February 2010 to June 2011, on-board the R/V Atlantic Explorer. Two different stations 10 nautical miles equidistant were sampled depending on the experiment (Fig. III.1). Data on pressure, temperature and fluorescence were measured from surface to 200 db using a SBE25 CTD and a Turner Scufa Fluorometer coupled to an oceanographic rosette. Fluorescence in the whole profile was converted to chlorophyll *a* from the relationship between both parameters at 20 m.

Experimental set up

Seawater was sampled at the mixed layer (20 m depth) using a 30-L Niskin bottle and transferred to two 24-L carboys. One was filled with unfiltered seawater and the other with gravity-filtered seawater (Whatman capsule filter, 0.2 µm). Both were maintained in dark while the experiment was performed.

Experiments were performed following the dilution technique (Landry & Hassett 1982, Landry et al. 1995). Fifteen 2.3-L bottles (Nalgene) were used to obtain three replicates of 5 different seawater dilution levels for experiments D1-D6: 100, 75, 50, 25 and 10% of natural seawater. For experiments D6 and D7 twelve bottles of 4 different dilution levels were used: 100, 70, 40 and 5% of seawater. Nutrients were added to every bottle (except in experiment D2) to avoid phytoplankton

growth limitation in the more diluted treatments. For that, 1 ml of a nutrient solution containing NH_4Cl and Na_2HPO_4 was added to every container to reach a final concentration of 0.5 and 0.03 µM of NH_4 and PO_4, respectively. Other four bottles were filled with natural seawater, one of them was used to obtain the initial samples, and the others were incubated without adding nutrients to take into account possible nutrient effects upon phytoplankton growth or microzooplankton mortality. In some experiments a 100% filtered seawater bottle was also incubated to verify the filtering system checking that neither phytoplankton nor bacteria growth took place.

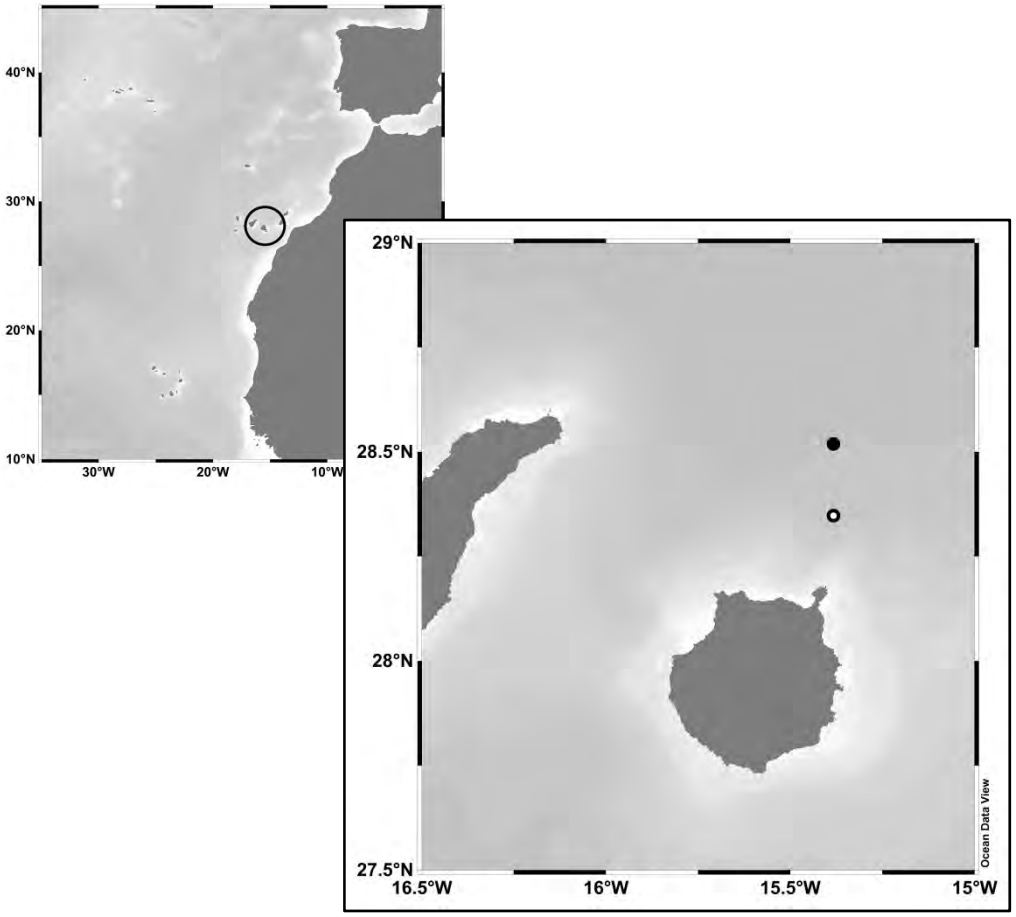

Figure III.1. Location of the two stations sampled at the north of Gran Canaria Island (Canary Islands). Seawater sampling for experiments D1, D2, D3, D4, D5 and D6 was carried out at the stations marked by a full circle, and only for experiment D7 the sampling was at the station marked by an open circle.

Incubation was carried out for 24 h in a tank on-deck with a continuous superficial water flux to keep *in situ* temperature and proper meshes on the top to simulate *in situ* light conditions. After incubation, every bottle was sampled to measure chlorophyll *a* (Chl *a*) concentration and picophytoplankton and heterotrophic procaryote abundance. In some cases the abundance of autotrophic and heterotrophic nanoplankton and of the main microplanktonic groups (diatoms, dinoflagellates and ciliates) was also measured.

Sample analysis

Chl *a* was measured by filtering 500 mL of sample through a 47 mm Whatman GF/F filter and freezing it at -20ºC until its analysis at the laboratory. The extraction procedure consisted in placing the filter in 90% acetone at -20ºC in the dark during at least 20 h, and following the acidification method by Strickland & Parsons (1972c). Pigments were measured on a Turner Design 10A Fluorometer, previously calibrated with pure Chl *a* (Yentsch & Menzel 1963).

Picoplanktonic organisms (0.2-2 µm) were sampled in a 1.6 mL tube, fixed immediately with 100 µL of 20% paraformaldehyde, incubated at 4ºC during half an hour, freezed in liquid nitrogen, and finally kept at -80ºC until their analysis. Samples were analyzed later by flow cytometry using a FACScalibur Cytometer (Beckton and Dickinson). Side scatter (SSC) and fluorescence parameters were obtained to distinguish between autotrophic picoeukaryotes (APE), cyanobacteria (*Prochlorococcus*, Pro, and *Synechococcus*, Syn) and heterotrophic prokaryotes (HP). Samples were run until 10000 events were reached or after 2 min at high speed to measure phototrophic organisms (APE, Pro and Syn), and at low speed for HP samples which were pre-stained with SYTO-13.

Auto- and heterotrophic nanoflagellates (ANF and HNF) were fixed using 540 µL of 25% glutaraldehyde in a tube containing 45 mL of seawater. Samples were kept at 4ºC in dark until the sample was filtered onto 0.6 µm black polycarbonate filter placed over a backing filter and stained by diamidino-2-phenylindole (DAPI) for 5 min. The filter was immediately mounted on a microscope slide with low-fluorescence immersion oil and kept at -20ºC. Finally, it was analyzed by epifluorescence microscopy with a Zeiss Axiovert 35 microscope (Haas 1982).

Microplanktonic organisms were kept in 500 mL dark bottles fixed with 1.5 mL of acid lugol and analyzed afterwards by the Utermöhl technique. A 100 mL subsample was settled for 48 h in a composite chamber. The bottom chamber was then examined with an inverted Zeiss Axiovert 35 microscope to identify the main microplanktonic groups: diatoms (Dia), dinoflagellates (Din) and ciliates (Cil).

Biomass conversion factors

The abundance of organisms obtained by flow cytometry was converted to biomass using the carbon conversion factor of: 17 fgC $cell^{-1}$ for HP (Bode et al. 2001), 29 fgC $cell^{-1}$ for Pro, 100 fgC $cell^{-1}$ for Syn (Zubkov et al. 2000) and 1500 fgC $cell^{-1}$ for APE (Zubkov et al. 1998). After biovolume estimation by microscopy, nanoflagellates were converted to carbon using the factor of 220 fgC μm^{-3} for HNF (Borsheim & Bratbak 1987) and the following equation for ANF: $0.433(BV)^{0.863}$ pgC $cell^{-1}$ (Verity et al. 1992). Finally, microplanktonic abundance was converted to biomass from total biovolume data obtained directly by microscopy for the most abundant cells or from previous measurements in these waters (A. Ojeda, unpublished data). If these data were not available, an average size from the literature for every organism or group (Tomas 1997, Horner 2002, Ojeda 2006, Ojeda 2011) was assumed, fitting them to the suitable shape following Hillebrand et al. (1999). The corresponding parameters were used for Dia (log a = -0.541, b = 0.811 for V<3000 μm^3; log a = 0.933, b = 0.881 for V>3000 μm^3), Din (log a = -0.353, b = 0.864) and Cil (log a = -0.639, b = 0.984 for aloricate ciliates; log a = -0.168, b = 0.841 for tintinnids) to obtain the biovolume to biomass conversion factor (Menden-Deuer & Lessard 2000) (log pgC $cell^{-1}$ = log a + b · log V). Some species were not taken into account to calculate the microbial biomass because of the impossibility of finding the size range or biovolume in the literature, but these species were always practically negligible in terms of abundance. Chl *a* concentration was converted to carbon using a C:Chl a ratio of 50 (Harris 1986).

Rates and feeding impact calculations

Growth (μ) and mortality by microzooplankton consumption (m) were determined for the different planktonic groups considered according to Landry et al. (1995) by linear regression between the net growth rate (k_i) and the dilution factor (D_i):

$$k_i = \ln (C_t / C_0) / t = \mu - m \cdot D_i$$

where C_t and C_0 are concentration (Chl *a*) or abundance at final and initial time, respectively, and t is the incubation time (~1 day). The mortality rate (m) is the slope of the regression, while the y-intercept is the growth rate obtained from the nutrient-amended series (μ_n). The intrinsic growth rate without nutrients (μ_0) was then obtained adding m to the net growth rate in natural seawater treatments (k_0):

$$\mu_0 = k_0 + m$$

Daily production (P, μgC L^{-1} d^{-1}) and consumption (G, μgC L^{-1} d^{-1}) of every group was determined according to calculations from Landry et al. (2000a):

$P = \mu \cdot C_m$

$G = m \cdot C_m$

$C_m = C_0 (e^{(\mu - m)t} - 1) / (\mu - m)\, t$

where C_0 and C_m are the initial and the mean carbon concentration during the incubation, respectively. Finally, the impact of microzooplankton consumption on production (%) was estimated as follows:

$\%P = (G / P) \cdot 100 = (m / \mu) \cdot 100$

III. RESULTS

Experiments were performed at different months of the year (Table III.1) trying to cover both the productive period in January, March and April (D1, D2, D6, D7), and less productive months: May and June (D3, D4, D5). However, hydrographic conditions of the water column were quite similar along the whole period of sampling (Fig. III.2). The mixed layer was characterized by warm temperatures (19.5-20.6ºC) and a quite low Chl *a* concentration (0.04-0.09 µg L^{-1}) in all cases (Table III.1).

Potential preys within picoplankton were mainly composed by heterotrophs, being HP the most abundant organisms (Fig. III.3A). Pro followed HP while APE showed lower cell abundance (Fig. III.3B). Nanoautotrophs (ANF) were more abundant than Dia (Fig. III.3C), which were the less abundant autotrophic cells. HNF were the most abundant potential consumers (Fig. III.3B), followed by Din (Fig. III.3C), which dominated microplankton. Among experiments, D6 and D7 showed a higher abundance of picoplankton than the other experiments (Figs. III.3A and B). By opposite, a lower abundance of nanoplankton was found for D6 and D7 (Fig. III.3B).

Experiment	Date	Temperature (ºC)	Chl *a* (µg L^{-1})
D1	03/10/10	-	0.035
D2	03/17/10	19.46	0.077
D3	05/24/10	20.58	0.044
D4	06/02/10	20.65	0.051
D5	06/09/10	20.08	0.007
D6	01/20/11	20.64	0.045
D7	04/27/11	19.66	0.041

Table III.1. Date and environmental conditions: temperature (ºC) and Chl *a* concentration (µg L^{-1}) at the mixed layer (20 m) for each experiment. Temperature data were not available for D1.

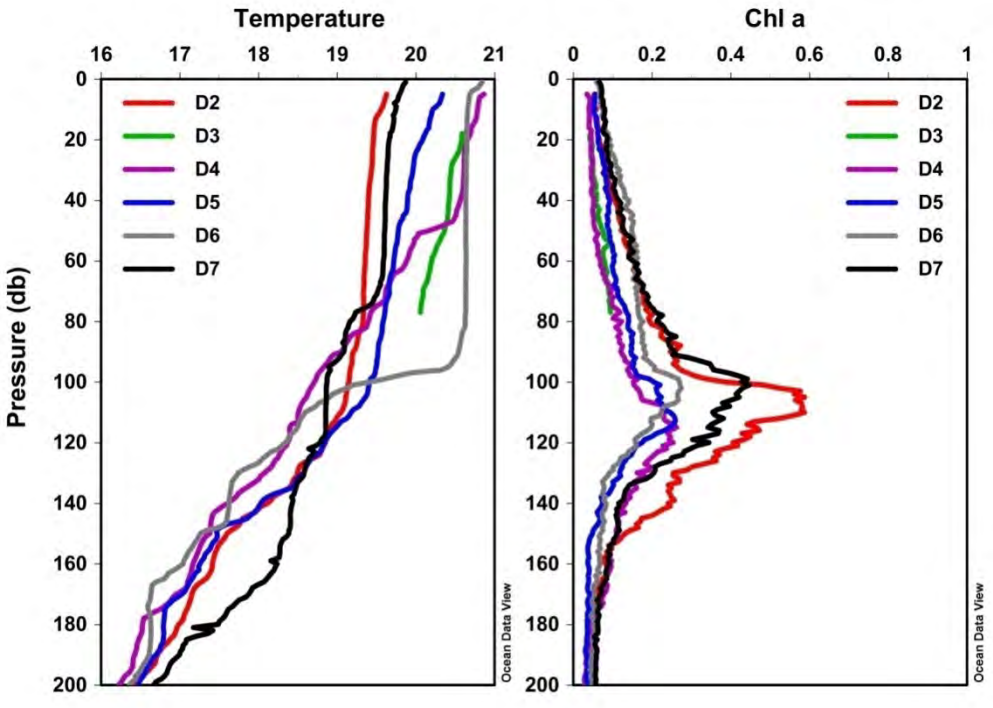

Figure III.2. Vertical profiles of temperature (ºC, left panel) and Chl *a* (µg L^{-1}, right panel) up to 200 db in the stations sampled. No data were available for D1.

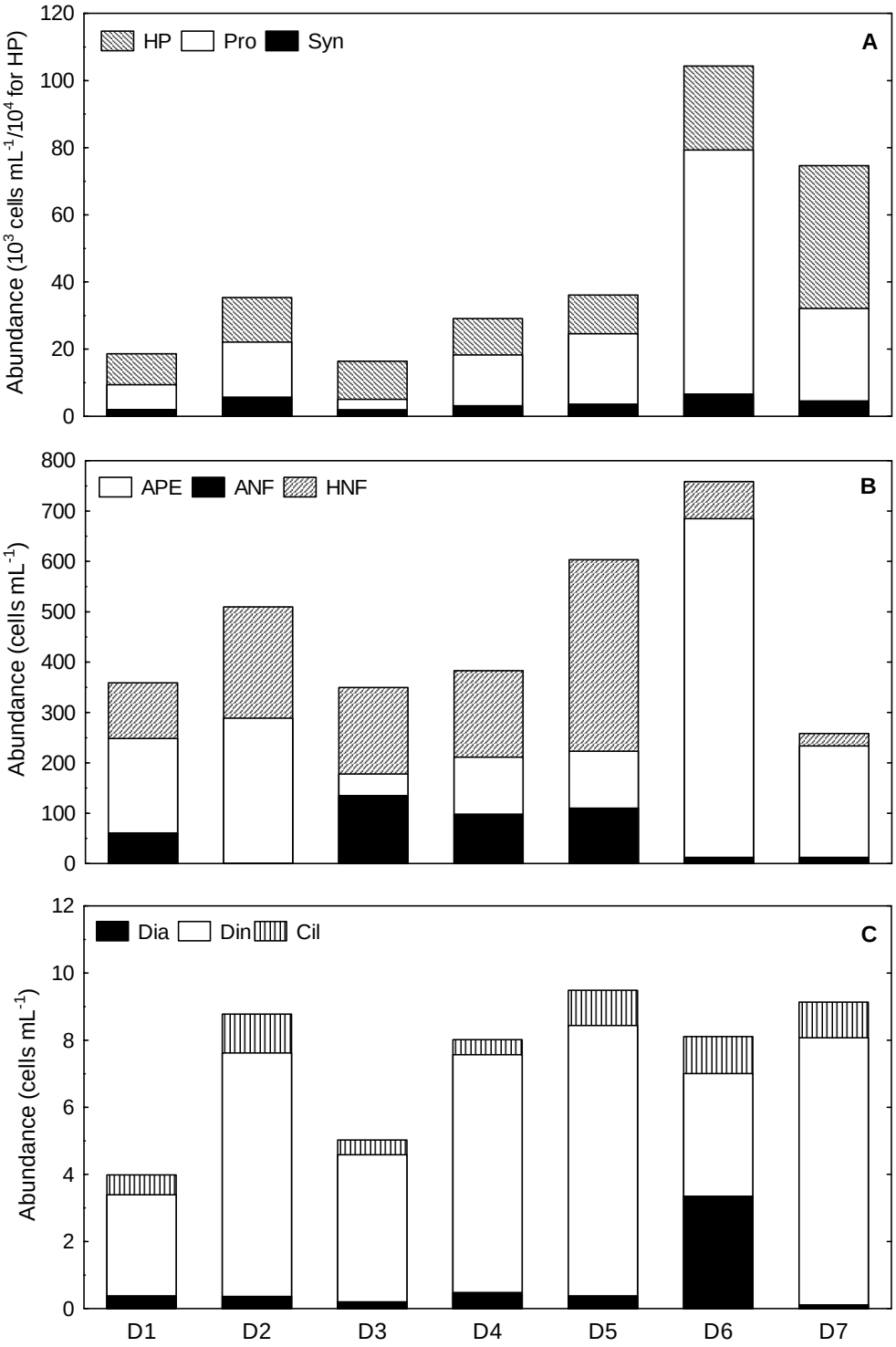

Figure III.3. Planktonic community composition at the mixed layer (20 m) in the station sampled for every experiment. Abundance (cells mL^{-1}) of heterotrophic prokaryotes (HP), Prochlorococcus (Pro), Synechococcus (Syn) (3A), autotrophic picoeukaryotes (APE), autotrophic (ANF) and heterotrophic nanoflagellates (HNF) (3B), diatoms (Dia), dinoflagellates (Din) and ciliates (Cil) (3C). Note the different concentrations of HP, Pro and Syn; and the different scales among panels.

A large number of non-significant results were found when total phytoplankton (as Chl *a*) or the different autotrophic groups were considered (Table III.2). Thus, the microzooplankton grazing (m) on autotrophic organisms was unequal depending on the experiment or group of organisms. In almost all cases (excepting D2), significant grazing rates were measured on at least one of the autotrophic groups despite the non-significant grazing observed from Chl *a* data. Regarding to heterotrophic organisms, significant results were obtained in all cases analyzed (Table III.3). Nevertheless, the microzooplankton feeding rate on heterotrophic organisms was also variable depending on the experiment.

The average grazing rate upon total phytoplankton (Fig. III.4) was 0.94 ± 0.13 d^{-1}, higher than the mean growth rate of 0.57 ± 0.43 d^{-1}. Within the different autotrophic groups considered, the smaller cells (picoeukaryotes and cyanobacteria) showed lower mortality rates by grazing, especially Syn and Pro, which presented average rates lower than 0.5 d^{-1} (APE: 0.89 ± 0.00, Syn: 0.22 ± 0.10, Pro: 0.27 ± 0.26 d^{-1}). Contrary, ANF and Dia supported higher grazing rates above 1.5 d^{-1} (ANF: 2.14 ± 0.74, Dia: 1.93 ± 0.30 d^{-1}). Mortality by grazing (m) was higher than growth rate (μ) for eukaryotic cells (APE, ANF and Dia), while it was lower for autotrophic prokaryotes (Syn and Pro). Higher growth rates were observed for larger cells (ANF: 1.58 ± 0.10, Dia: 1.56 ± 0.44 d^{-1}) but also for Pro (1.12 ± 0.95 d^{-1}). In the case of APE and Syn their average growth rates were below 1 d^{-1} (APE: 0.34 ± 0.00, Syn: 0.54 ± 0.28 d^{-1}). On average, the feeding rate upon heterotrophs was above 1 d^{-1} (Fig. 4), being 1.12 ± 0.55 and 1.59 ± 0.31 d^{-1} for HP and HNF, respectively. Din showed an average mortality rate of 0.98 ± 0.70 d^{-1}. Average growth rate of these groups balanced mortality by consumption as values around 1 d^{-1} were measured (HP: 1.09 ± 0.60, HNF: 0.93 ± 0.27, Din: 1.04 ± 0.92 d^{-1}).

	Chl *a*		APE		Syn		Pro		ANF		Dia	
	m	μ_0	m	μ_0	m	μ_0	m	μ_0	m	μ_0	m	μ_0
D1	n.s.	n.s.	n.s.	n.s.	0.15*	0.74*	0.23*	0.63*	n.s.	n.s.	-	-
D2	n.s.	n.s.	0.89*	0.34*	0.29**	0.35**	n.s.	n.s.	-	-	-	-
D3	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.62***	2.20***	-	-	-	-
D4	n.s.	n.s.	n.s.	n.s.	-0.33*	-0.01*	n.s.	n.s.	-	-	-	-
D5	1.03*	0.88*	n.s.	n.s.	n.s.	n.s.	0.24*	1.58*	2.67**	1.51**	n.s.	n.s.
D6	0.85**	0.27**	-0.48*	-0.42*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	2.14***	1.87***
D7	n.s.	n.s.	n.s.	n.s.	-0.54**	-0.25**	-0.55*	0.07*	1.62*	1.65*	1.72*	1.25*

Table III.2. Mortality (m) and growth (μ_0) rates (d^{-1}) of total phytoplankton (Chl *a*), autotrophic picoeukaryotes (APE), *Synechococcus* (Syn), *Prochlorococcus* (Pro), autotrophic nanoflagellates (ANF) and diatoms (Dia) for each dilution experiment. No data were available for ANF in D2-4 and for Dia in D1-4. *p<0.05, **p<0.01, **p<0.001, n.s. not significant.

	HP		HNF		Din	
	m	μ_0	m	μ_0	m	μ_0
D1	1.81***	1.73***	1.26*	0.76*	-	-
D2	1.59***	1.32***	-	-	-	-
D3	1.32***	1.45***	-	-	-	-
D4	0.36**	-0.10**	-	-	-	-
D5	0.57***	0.87***	1.87**	1.24**	0.34*	0.22*
D6	0.76***	0.75***	n.s.	n.s.	1.73***	2.03***
D7	1.45***	1.54***	1.64*	0.78*	0.86***	0.86***

Table III.3. Mortality (m) and growth (μ_0) rates (d^{-1}) of heterotrophic prokaryotes (HP), heterotrophic nanoflagellates (HNF) and dinoflagellates (Din) for each dilution experiment. No data were available for HNF in D2-4, and for Din in D1-4. *p<0.05, **p<0.01, **p<0.001, n.s. not significant.

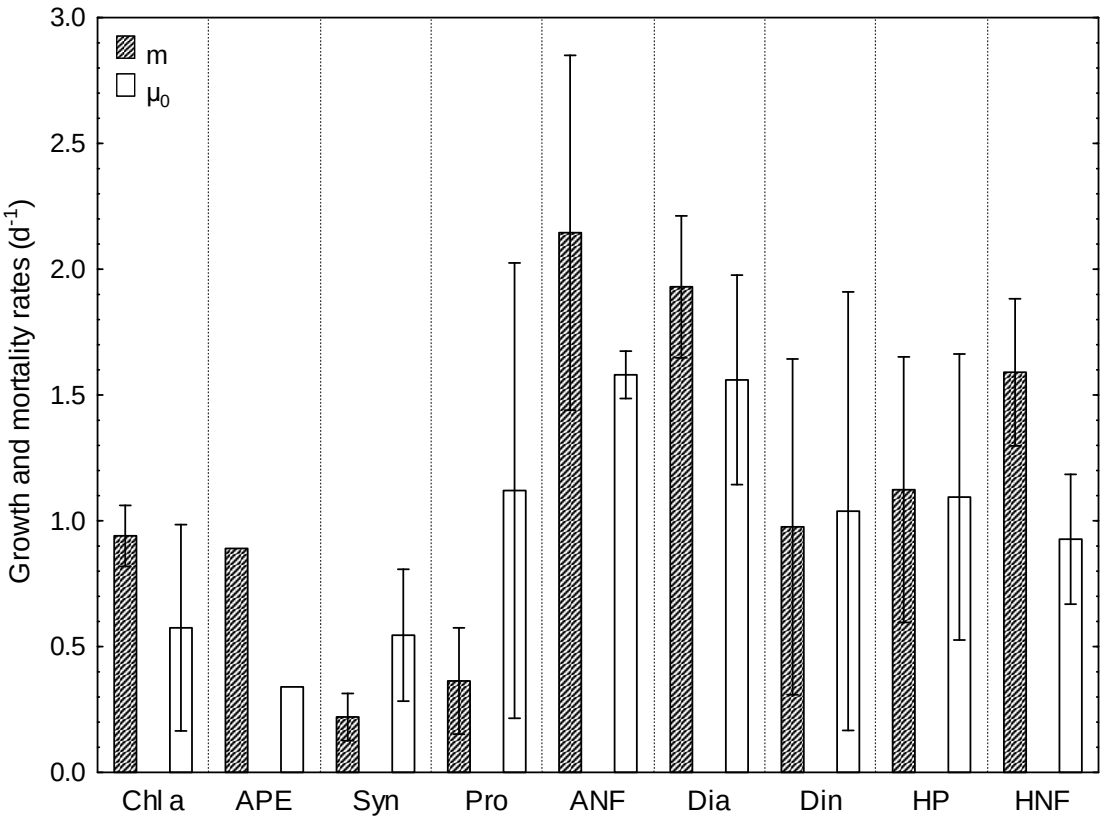

Figure III.4. Average (±SD) rates (d^{-1}) of mortality (m) and growth (μ_0) of total phytoplankton (Chl a, n=2), APE (n=1), Syn (n=2), Pro (n=3), ANF (n=2), Dia (n=2), Din (n=3), HP (n=7) and HNF (n=3). Abbreviations as in Fig. III.3. Negative rates were not included in average calculations.

Growth and mortality rates did not show any relationship with neither temperature nor Chl *a* or initial abundance or biomass (Spearman correlation, $p>0.05$). Mortality rate was only significantly correlated to growth rate (Spearman's rank $r=0.62$, $p<0.001$) considering both autotrophs and heterotrophs (Fig. III.5). This correlation was even higher when Pro were excluded (Spearman's rank $r=0.80$, $p<0.0001$).

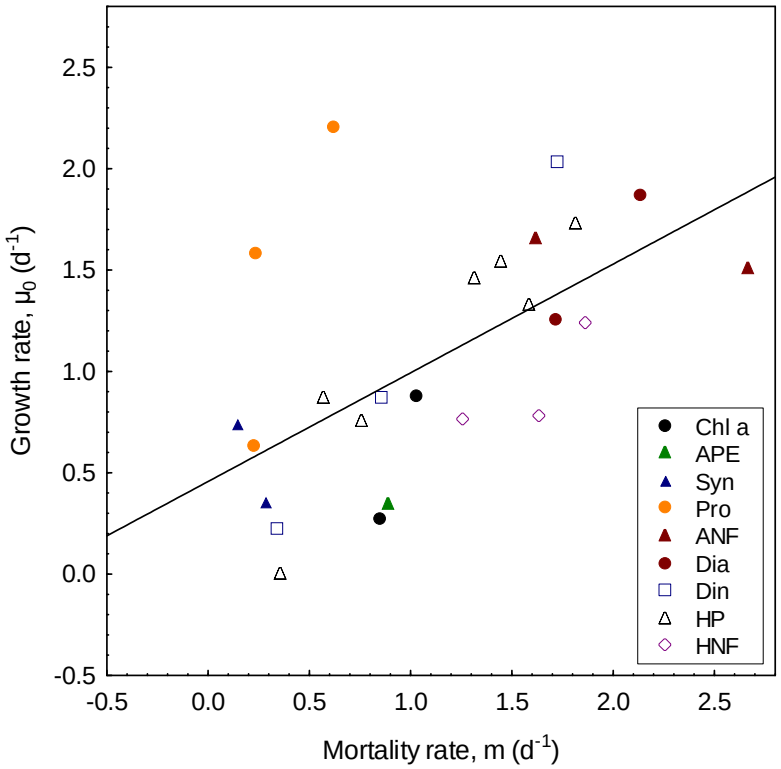

Figure III.5. Relationship between growth (μ_0) and mortality (m) rates (d^{-1}) obtained for all planktonic groups (abbreviations as in Fig. III.3). The line represents the linear regression including all groups ($y = 0.457 + 0.536 \cdot x$, $r=0.62$, $p<0.001$, $n=26$). Negative rates were not included.

A possible effect of nutrient limitation on growth rates was assessed using the nutrient limitation index ($N_L = \mu_0 / \mu_n$). Only Syn and ANF showed average values of N_L below 1 (Table III.4), indicating that the growth of these groups could be limited by nutrient availability. The mean N_L value for autotrophs (from Chl *a*) was also below 1 although its variability was high. In fact, autotrophs were only nutrient-limited in D6, in which N_L was below 1.

Phytoplankton group	μ_0 (d^{-1})	μ_n (d^{-1})	N_L	n
Syn	0.74 (0.00)	0.82 (0.00)	0.90 (0.00)	1
Pro	1.12 (0.95)	0.78 (0.64)	1.43 (1.69)	4
APE	-	-	-	
ANF	1.58 (0.10)	2.11 (0.18)	0.75 (0.08)	2
Dia	1.56 (0.44)	1.30 (0.65)	1.20 (0.69)	2
Chl *a*	0.58 (0.43)	0.62 (0.09)	0.93 (0.71)	2

Table III.4. Nutrient limitation index (N_L) (Landry et al., 1995; Landry et al., 1998) calculated from growth rates without (μ_0) and adding (μ_n) nutrients, $N_L = \mu_0 / \mu_n$ (SD). D2 was not included because nutrients were not added to the dilution series. Abbreviations as in Table III.2.

Non-significant or negative values of mortality (significant positive slopes for Syn in D4, APE in D6 and cyanobacteria in D7) were observed (Tables III.2 and III.3). To evaluate if these responses were due to changes in the microzooplankton community during incubations data on net growth rates of potential consumers were also analyzed (Fig. III.6). Unfortunately, we only obtained data on microplankton abundance in D5, D6 and D7. In these cases, the net growth rates of HNF, Din and Cil were negatively correlated to the dilution factor except in D5 for Cil and D6 for HNF.

In terms of daily production (Fig. III.7A), HP and Din accounted for the highest values (HP: 3.57 ± 3.71, Din: 3.15 ± 1.89 µgC L^{-1} d^{-1}) and they also supported the highest daily consumption (HP: 3.52 ± 3.43, Din: 3.10 ±1.38 µgC L^{-1} d^{-1}) (Fig. III.7B). The average daily production and consumption for the other organisms were below 1 µgC L^{-1} d^{-1}. Autotrophic production (from Chl *a*) was on average 0.37 ± 0.12 µgC L^{-1} d^{-1}, while a higher value of 0.89 ± 0.79 µgC L^{-1} d^{-1} was measured for the daily feeding on autotrophic biomass. Thus, more than 100% of primary production was removed on average (Fig. III.8), although a rather high variability was observed. The impact on the different autotrophic organisms was unequal. Consumption over eukaryotic cells (APE, ANF, Dia, Din) production was higher than 100%, while the impact on prokaryotic production was lower, only 52% and 20% of the daily production of Syn and Pro, respectively. In the case of heterotrophs, daily prokaryotic production (HP) was almost totally removed by microzooplankton (96%), and more than 100% of the daily production of HNF was consumed.

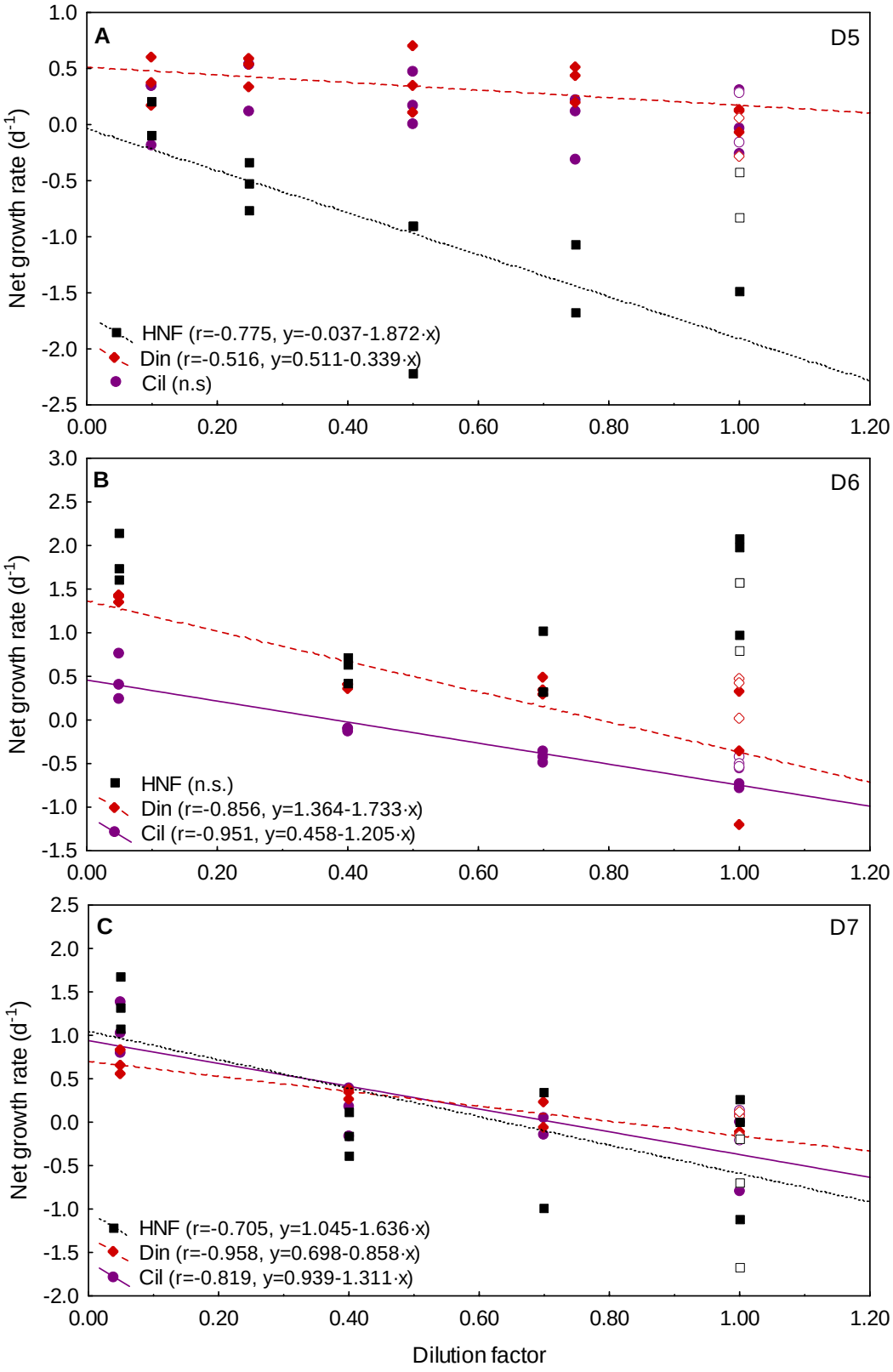

Figure III.6. Linear regression between the net growth rates of potential consumers (HNF, Din and Cil) and the dilution factor in D5 (6A), D6 (6B) and D7 (6C). Fitting line was plotted when the relationship between variables was significant ($p<0.05$, n.s.: not significant). Open symbols stand for net growth rates in natural seawater treatments incubated without nutrients. Abbreviations as in Fig. III.3.

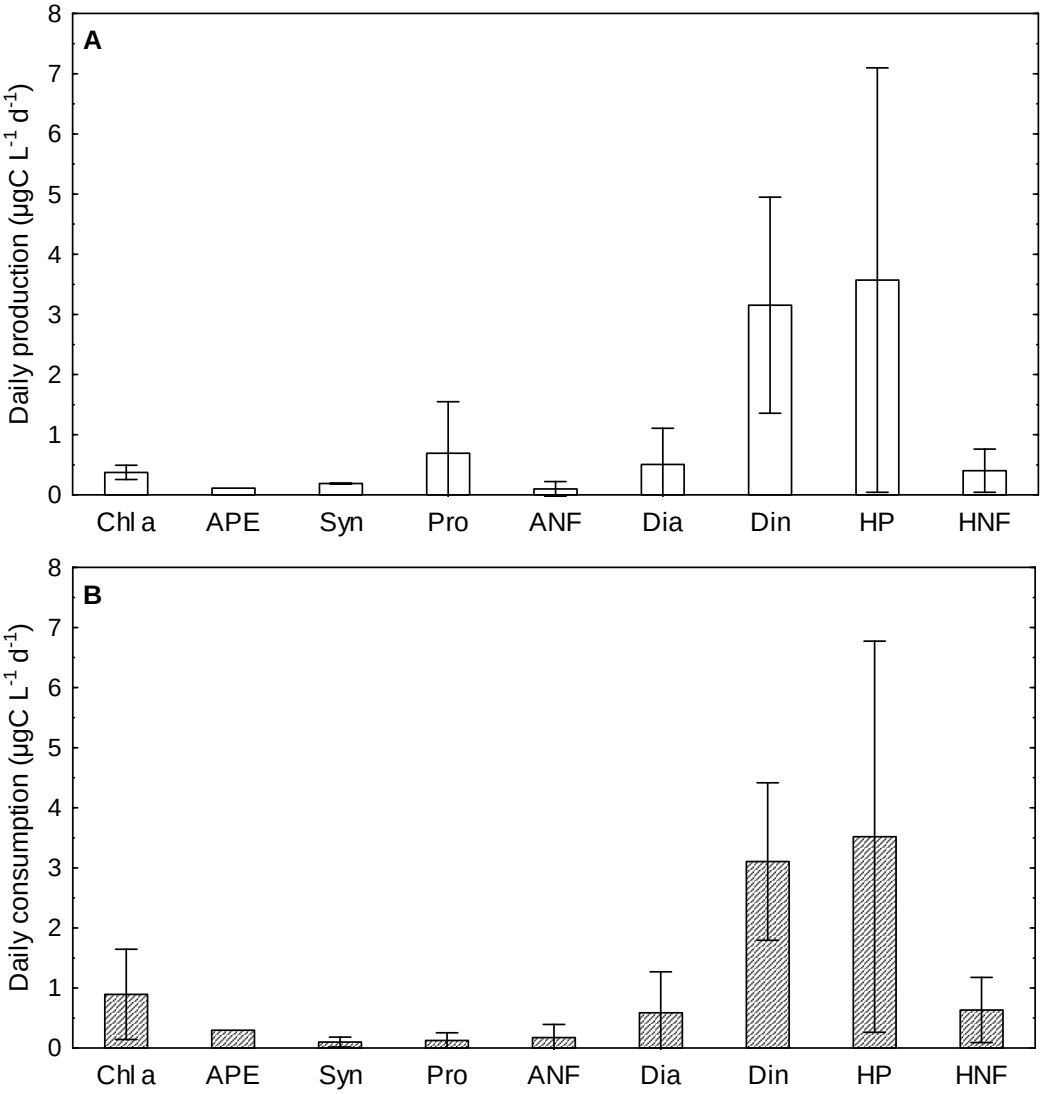

Figure III.7. Average (±SD) daily production (7A) and consumption (7B) (µgC L^{-1} d^{-1}) of each planktonic group (abbreviations as in Fig. III.3). Negative rates were not included in average calculations. n as in Fig. III.4.

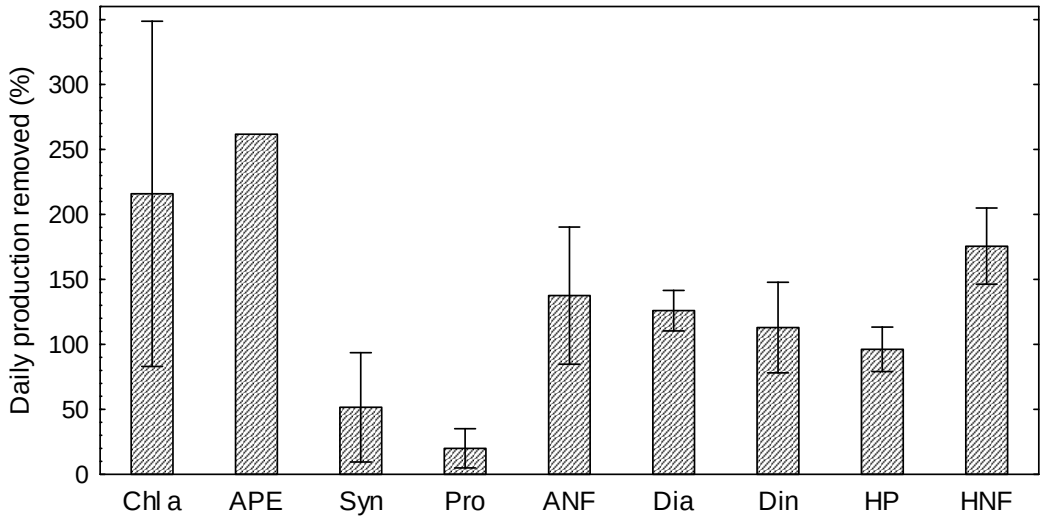

Figure III.8. Average (±SD) percentage of daily production of each planktonic group (abbreviations as in Fig. III.3) removed by microzooplankton consumption. Negative rates were not included in average calculations. n as in Fig. III.4.

IV. DISCUSSION

We attempted to quantify the temporal variability of the microzooplankton feeding impact covering the productive and non-productive periods in the subtropical waters off the Canary Islands. However, low variability in temperature and Chl *a* was observed suggesting rather stable environmental conditions (Fig. III.2 and Table III.1). Neither the microbial community composition showed a clear temporal pattern, although during 2011 a higher abundance of picoplankton and a lower abundance of nanoplankton were observed (Fig. III.3). This relatively higher availability of potential preys did not result in clear differences on grazing rates between 2011 (D6 and D7) and 2010 (D1, D2, D3, D4 and D5) (Tables III.2 and III.3).

The variability of grazing pressure has been previously associated to changes in the planktonic community composition (Strom et al. 2007, Calbet et al. 2008, Schmoker et al. 2013). However, we did not find any relationship between growth or mortality rates and abundance or biomass of prey or potential consumers. Probably, growth and mortality changes were not related to temperature, Chl *a* or community composition because of the extremely low variability of these parameters. Indeed, HP and Pro were the most abundant preys and HNF were the dominant consumers during the whole period (Fig. III.3). The numerical dominance of nanoflagellates within microzooplankton assemblages has been reported in the subtropical northeastern Atlantic (Quevedo & Anadón 2001,

Quevedo et al. 2003) and it is a common feature in oligotrophic systems where nanograzers are the major consumers of primary production (Calbet 2008).

Microzooplankton grazing rate was only significantly correlated to the growth rate of preys (Fig. III.5). The linear relationship obtained between both rates indicated the close coupling between consumers and producers, with a higher grazing pressure over faster-growing cells. The coupled relationship between phytoplankton growth and microzooplankton grazing is common in the ocean (Schmoker et al. 2013) and it has also been observed in the area (Quevedo & Anadón 2001). Gaul & Antia (2001) found that grazers selectively consumed the taxa with the highest growth rates, even although they accounted for minor fractions of autotrophic biomass. Here, ANF and Dia showed the highest average growth rates and they were heavily grazed, whilst the lowest grazing rates were on Syn and APE, whose growth rates were also the lowest (Fig. III.4). Additionally, we found this close relationship for heterotrophic preys, at least for HP, as few data on HNF rates were obtained. Although a considerable variability was observed (Table III.2), the exception was the case of Pro, which showed on average the lowest mortality rate by grazing despite their high average growth rate. Gutiérrez-Rodríguez et al. (2011) also found that Pro supported the lowest grazing pressure comparing to other autotrophic groups in the northeastern Atlantic. This suggests that micrograzers would be selectively feeding not only upon faster-growing autotrophs but also on eukaryotic cells more than prokaryotes (Fig. III.4). A negative selectivity on small autotrophs has also been observed by several authors in both eutrophic (Teixeira & Figueiras 2009) and oligotrophic environments (Berninger & Wickham 2005). On the other hand, a positive selection over cyanobacteria and dinoflagellates instead of diatoms was observed in the temperate Northeast Atlantic (Gaul & Antia 2001).

The microzooplankton feeding impact on every planktonic group was unequal among experiments as disparate results were obtained (Tables III.2 and III.3). First, grazing rates measured from Chl *a* were only significant in two experiments (D5 and D6), although significant grazing rates on autotrophic groups were found in the other experiments. Therefore, calculating microzooplankton grazing impact on primary production from Chl *a* data alone could be a great source or error, at least under low Chl *a* conditions. A detailed analysis of every autotrophic group is needed to properly quantify the actual impact of microzooplankton grazing.

In addition, significant positive slopes (negative values of m) were observed (Tables III.2 and III.3). This type of response could be due to changes in microzooplankton community during incubations (Dolan et al. 2000, Agis et al. 2007). In this sense, we observed higher net growth rates of potential consumers (HNF, Din and Cil) in the more diluted treatments (Fig. III.6). The increment in the abundance of HNF in highly diluted treatments has been previously attributed to Cil mortality (Agis et al. 2007). Contrary, we found that Cil also increased in diluted samples after incubation in D6 and

D7. Actually, the increase of Cil and Din in those experiments matched the negative values of prey mortality (APE in D6, Syn and Pro in D7). Hence, it seems that all consumers including Cil would be under predator pressure which would be released in highly diluted samples. These results would be in agreement with the existence of trophic cascades in dilution experiments (Calbet & Saiz 2013). The complexity of the trophic web in subtropical ecosystems supports the existence of trophic cascades as the relationship between autotrophs and grazers is altered by heterotrophic consumption of predators.

Our results showed that heterotrophic consumption was considerable and occurred in all experiments (Table III.3). Significant consumption rates on HP were measured during the whole period and feeding upon HNF was also significant in the experiments analyzed. HNF are known to be effective bacterivores (Azam et al. 1983, Guillou et al. 2001, Sherr & Sherr 2002) and a significant relationship between them has been found in these waters (Arístegui & Montero 2005, Vázquez-Domínguez et al. 2005). HNF could be preyed by Cil (Azam et al. 1983, Sherr & Sherr 2002, Pomeroy et al. 2007), which in turn would be consumed by copepod nauplii or copepodites frequently observed in the samples (data not shown) or even by heterotrophic dinoglagellates (Sherr & Sherr 2002). Hence, the prevalence of carnivory among experiments could also be the cause of non-significant or negative grazing rates observed (Calbet & Saiz 2013). On the other hand, microzooplankton food selectivity could promote the positive relationship of cyanobacteria and the dilution factor (Teixeira & Figueiras 2009). In this case, the negative selection of consumers on prokaryotic autotrophs released the grazing pressure upon these cells, which were in all cases under a lower grazing pressure than eukaryotic cells (Fig. III.4).

The finding of higher growth and mortality rates in large than small autotrophic cells was an unexpected result. Normally, small autotrophs support a higher grazing pressure than larger cells (Strom et al. 2007, Calbet et al. 2008, Gutiérrez-Rodríguez et al. 2011, Teixeira et al. 2011). Furthermore, microalgae are supposed to be nutrient-limited in oligotrophic marine environments (Berninger & Wickham 2005). In the northeast Atlantic the limitation of growth by nutrient availability was observed for both diatoms and prokaryotes (Gutiérrez-Rodríguez et al. 2011), although it was more severe for the former. Conversely, no nutrient limitation was observed for phytoplankton in other areas of the northeast Atlantic (Cáceres et al. 2013). Moreover, these authors also found higher growth and mortality rates (above 1 d^{-1}) for larger phytoplankton (>5 µm) despite the low nutrient conditions measured. Our results are in agreement with that study as we did not find that microalgae were nutrient-limited (Table III.4). Thus, despite its low abundance, Dia were growing at high rates and not limited by bottom-up forces, although controlled by microzooplankton grazing. Nevertheless, we are not able to know if this result could be extrapolated to all experiments as data on autotrophic nano- and microplankton rates were obtained only for D5, D6 and D7.

In spite of the higher growth rates of nano- and microautotrophs, the higher biomass of HP and Din caused that they were the major daily producers and showed the highest values of biomass consumption (Fig. III.7). Nevertheless, the daily production of both autotrophs and heterotrophs was totally removed by the microzooplankton feeding impact, except for cyanobacteria.

In summary, our results showed the unequal impact of microzooplankton feeding on the different microbial groups considered. The microzooplankton consumption was closely coupled to the growth of their prey, for both autotrophic and heterotrophic organisms. On average, the highest grazing rates were upon ANF and Dia, whilst Syn and APE were grazed at lower rates. Nonetheless, heterotrophic consumption was prevalent and could be influencing the impact of grazers on primary production. Thus, the complexity of the trophic web in these subtropical waters makes more difficult to measure the actual impact of microzooplankton on the different microbial components. For this reason, the measurements based on Chl *a* concentration alone could be a great source of error in oligotrophic ecosystems. Here, significant grazing rates were found upon different autotrophic groups although grazing was not significant from Chl *a* data. Moreover, the feeding impact on heterotrophic organisms should also be quantified to better understand the relationship between the different components of the food web. In this sense, time-course experiments (Agis et al. 2007) would provide more detailed results and avoid long time incubations that suppose a high risk of changes in community composition during incubation.

V. ACKNOWLEDGEMENTS

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CHAPTER IV

Discriminating between dilution, 2-point, and Frost methods to estimate grazing by microzooplankton

CAPÍTULO IV

ABSTRACT

Grazing by microzooplankton is normally obtained through the dilution method. However, this technique is tedious to carry out because many treatments (dilutions) and replicates are needed for statistical confidence. Large volumes of water are also required which implies a quite heavy restriction for its use in oceanography. The method is also sometimes difficult to interpret because of non-linear effects, feeding at saturation, thresholds feeding response, and top-down effects. A commonly used alternative is the so-called 2-point method in which only the natural seawater (nsw) and one dilution level (37, 33, 20, 10, or 5% of nsw) are used to assess grazing. Saturation and threshold feeding responses can also make this approach rather difficult to interpret. Here, we discriminate between dilution, 2-point, and Frost (1972) methods in order to estimate grazing rates. We used the 5% nsw dilution to assess the intrinsic phytoplankton growth (μ) as an independent estimate in order to apply the equations by Frost. We found a close relationship between grazing rates estimated by the dilution and Frost methods from a review of published data, and experiments performed in the Canary Island waters and the Namibian upwelling. We also assessed grazing rates in oligotrophic and eutrophic zones of the Atlantic and Pacific Oceans, performing time-series experiments based on the Frost approach, and we observed an important variability at short-term intervals.

I. INTRODUCTION

Nano- and microzooplankton (hereinafter micrograzers, organisms in the 2-200 µm size class) are a heterogeneous community mainly formed by protists with heterotrophic and mixotrophic energy intake. They are the main grazers in the ocean (Calbet & Landry 2004) and therefore play an important role in the flux of energy and matter through the marine food web. Despite their importance, their study is constrained by the difficulty to know their composition, abundance, feeding, metabolism and growth. Among this, knowledge of feeding and their impact on primary production is of paramount importance in order to understand the fate of organic carbon in the water column.

Most of the information about the feeding pressure exerted by micrograzers has been obtained through the so-called dilution method (Landry & Hassett 1982). The procedure involved in this method is theoretically simple and no especial equipment is required. Briefly, a series of dilutions (bottles with increased proportion of filtered seawater in relation to the natural sample) in which grazers are diluted respect to autotrophs are carried out, promoting a reduction of encounter rates between grazers and their prey. The slope of the increased phytoplankton apparent growth as dilutions increase is the mortality rate (m) of autotrophs by grazing, and the intercept with the y-axis, the phytoplankton net growth rate (µ). Other methods (fluorescence microspheres, fluorescently labeled algae, ^{14}C or ^{3}H labeling …) are based on artificial or manipulated food (far from nature), are difficult to carry out, tedious to measure, and/or difficult to interpret (e.g., food of different sizes).

The dilution technique is a simple manipulation of a complex food web (Gallegos 1989) and it is based on three main assumptions (see Landry & Hassett 1982). Briefly, (1) growth of prey cells is not affected by the presence or absence of other phytoplankton cells, (2) the probability of a phytoplankton cell being consumed is a direct function of the rate of encounter of consumers with prey cells, and (3) the change in the density of phytoplankton over time can be represented by an exponential equation. However, this method is tedious to carry out because at least 4-5 dilution levels (each replicated) are needed for statistical confidence. Besides, large volumes of water (filtered and unfiltered) are also required for the experimental set-up. This implies the performance of only one or two estimations per day and, therefore, the impossibility to have sufficient resolution for the oceanographic work.

To interpret the results of the dilution method is sometimes difficult because of non-linear responses observed in the apparent growth rate of phytoplankton. While in low diluted treatments feeding at saturation is a common result, in highly diluted treatments thresholds feeding responses (similar apparent growth rates at the higher dilutions) are also observed (Gallegos 1989). Moreover, top-down effects of unknown consequences could lead to unexpected results (Calbet & Saiz 2013).

A simplification of this procedure is the so-called 2-point method in which the undiluted sample and a dilution level of 33 (Landry et al. 2009), 37 (Landry et al. 2011), or 10% (Lawrence & Menden-Deuer 2012, Sherr et al. 2013) of nsw is used to measure m. This is a compromise between the use of this method for the oceanographic work and the expected errors due to the lack of statistical confidence or the possible non-linear responses. Therefore, possible saturation and threshold feeding responses that cannot be resolved with this modified approach make the results rather difficult to interpret.

According to Frost (1972), microzooplankton grazing is estimated assuming an exponential phytoplankton growth, as in the dilution method. The apparent growth rate (k) is the result of the intrinsic growth rate (μ) and mortality by grazing (m):

$$k = \mu - m$$

In this case, k and m are not computed from linear regression analysis between the net growth rate and the dilution factor. Instead, they are measured separately (Frost 1972), k being the apparent phytoplankton growth rate measured from the incubation of natural seawater, and μ an independent estimate of the intrinsic growth rate of phytoplankton, both estimated as:

$$\mu\ (h^{-1}) = (1 / t) \cdot \ln (C_1 / C_0)$$

$$k\ (h^{-1}) = (1 / t) \cdot \ln (C_1' / C_0')$$

where t is incubation time, C_0 and C_1 the initial and final phytoplankton concentration without grazers, and C_0' and C_1' the initial and final phytoplankton concentration in presence of grazers. Grazing rate, therefore, is

$$m\ (h^{-1}) = \mu - k$$

The apparent growth rate of autotrophs in presence of grazers (k) is, in fact, the result of incubating the natural sample (the undiluted treatment in the dilution method). However, the problem is to know the intrinsic rate of autotrophic growth (μ). In this sense, Gallegos & Jordan (1997) found a strong relationship between the apparent phytoplankton growth in the 5% nsw dilution (in their terminology μ_e) and the intrinsic phytoplankton growth, μ (see their Figure 1C). Later, Dolan et al. (2000) found high grazer mortalities at high dilutions, and the grazing rate in the 5% nsw dilution was even closer to 0 than it would be predicted for a dilution factor of 0.05. Strom & Fredrickson (2008) also carried out the 2-point method and they assumed the growth measured at the 5% nsw dilution as an estimate of the intrinsic phytoplankton growth rate (μ). They supported this assumption by a previous comparison between highly diluted treatments and μ values from dilution experiments (Strom et al. 2006).

Thus, it is expected that knowing μ (as μ_e) and k, reliable estimates of m could be obtained using the equations given by Frost (1972). Conceptually, this method measures grazing by subtracting k from a known phytoplankton growth (μ) from two different (independent) estimates, providing an error in both assessments. Conversely, the dilution method is performed to derive a value of μ (the y-axis intercept) from m, which is the slope of the linear regression between the dilution factor and the apparent phytoplankton growth at each dilution. This is also the basis for the 2-point method. The difference between the dilution and 2-point, and Frost methods is simply conceptual and they provide different but similar estimates (see Fig. IV.1). However, the latter method is not based on the assumption of a decrease in encounter rates between phytoplankton and micrograzers, but it is based on an independent measurement of μ (in this approach as μ_e).

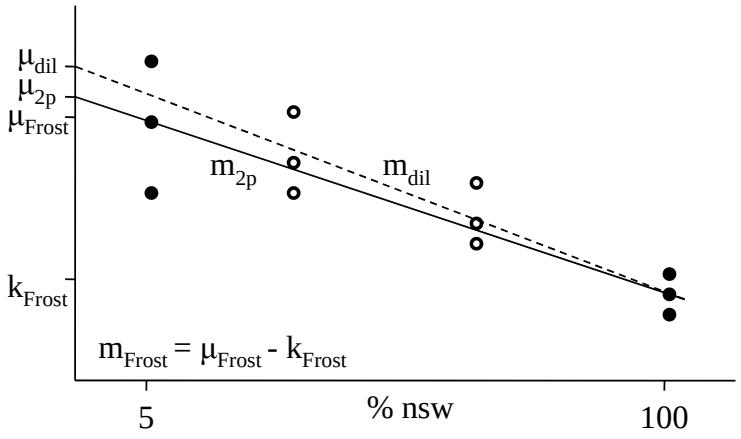

Figure IV.1. Conceptual scheme comparing the three approaches: dilution (dil), 2-point (2p) and Frost methods. Apparent phytoplankton growth is plotted versus the dilution level (% of natural seawater, nsw) to estimate μ and m. For the Frost method, the apparent net growth rate for the natural sample (k_{Frost}) and 5% nsw (μ_{Frost}) is the average of replicates (see text).

In the dilution method the regression between the apparent growth of phytoplankton and the dilution factor should be statistically significant. The 2-point method also theoretically needs a significant difference between the apparent growth of phytoplankton in natural seawater and the one obtained in the selected dilution (37, 33, 10 or 5% of nsw). By opposite, the method of Frost is based on subtracting k from μ from two different estimates, so both could be similar as they are independently estimated. Therefore, this approach could assess grazing values around zero (see discussion by Landry 2014, Latasa 2014).

As the dilution technique, the method of Frost is theoretically simple and still assumes that growth of individual phytoplankton is not affected by the presence or absence of other phytoplankton. However, as serial dilutions are not needed and the highly diluted treatment is not affected by grazers (Dolan et al. 2000), the other assumptions of the dilution series based on the rate of encounter of consumers with prey cells does not apply.

The Frost method, therefore, has the advantage of the 2-point method for the oceanographic work as several measurements could be done per day (e.g., to observe changes along transects, day and night rates, or vertical distribution of grazing), but makes the use of only one dilution level conceptually robust. Thus, in order to test this approach, we performed a series of experiments in the Atlantic and Pacific Oceans, seeking to measure grazing rates upon picoplanktonic phytoplankton in a gradient of contrasting productivities.

II. MATERIAL AND METHODS

Samples for grazing experiments were obtained in oceanic waters at different oceanographic stations in the Pacific and Atlantic Oceans (Table IV.1). The former sampling was performed on-board the R/V Hespérides from April to May 2011 (Fig. IV.2A) under the Malaspina Expedition. Twelve experiments (St 84 to 124) were performed covering latitudinal and longitudinal gradients in the tropical and subtropical zones of the Pacific Ocean. Seven experiments (CTB.01 to 07) were also carried out in the subtropical Atlantic on-board the R/V Cornide de Saavedra in the western Canary Islands (Fig. IV.2B) during the Cetobaph cruise from April 6 to 18, 2012. Other two additional experiments (DG6 and DG7) were carried out to the north of Gran Canaria Island (Canary Islands, Fig. IV.2B, station LCF) in January and April 2011 on-board the R/V Atlantic Explorer. Finally, six experiments (NG1 to 6) were done in the upwelling zone off Namibia from August 23 to September 19, 2011 (Fig. IV.2C) on-board the R/V Maria S. Merian.

	Cruise	Conditions	Months	Year	Procedure	Experiments	n	Nutrient amendment
Atlantic Ocean	Lucifer	Oligotrophic	January and April	2011	Frost vs. dilution	DG6 and 7	2	Yes
	Succession	Eutrophic	August-September	2011	Frost vs. dilution	NG1 to 6	6	Yes
	Cetobaph	Oligotrophic	April	2012	Time-series	CTB.01 to 07	7	No
Pacific Ocean	Malaspina	Oligotrophic	April-May	2011	Time-series	St 84 to 124	12	No

Table IV.1. Experiments carried out to measure microzooplankton grazing rates, comparing the Frost and dilution methods and performing time-series inside the incubators. Replicates were used for experiments CTB.01 to 07 to compare the slope of time-series using the same sample in different incubators (see text). n is the number of experiments performed.

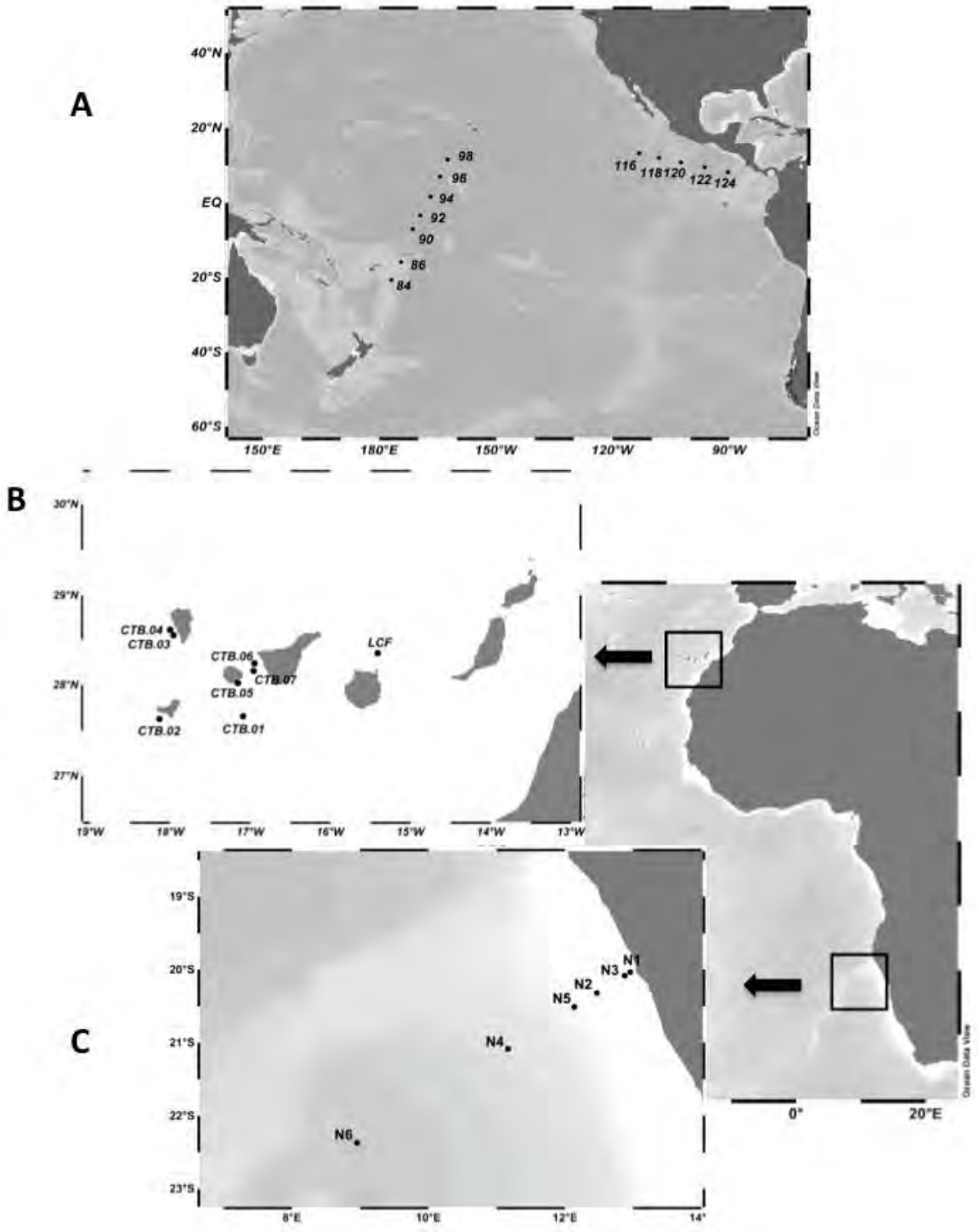

Figure IV.2. Location of oceanographic stations sampled for grazing experiments along the Pacific Ocean (2A), the subtropical waters off the Canary Islands (2B), and the upwelling zone off Namibia (2C). CTB stands for Cetobaph Cruise and LCF for station Lucifer (see text for details).

Frost vs. dilution experiments

The Canary Islands and Namibian experiments were designed to compare the method of Frost (1972) and the dilution method of Landry & Hassett (1982). In the Namibian cruise, various 20 L Niskin bottles coupled to the oceanographic rosette were used to sample at the depth of 25% of surface Photosynthetically Active Radiation (PAR) at different times along daylight hours. Sampling at station LCF was done at the mixed layer (20 m depth) around noon and using a 30 L Niskin bottle. Once on-board, water was filtered by gravity through 0.2 µm (Whatman capsule filter) to produce the dilution treatments into the 2.5 L polycarbonate (Nalgene) bottles used for experiments. In all cases, bottles were cleaned with 10% HCl, rinsed with deionized and sample water.The bottles were then slowly filled with natural seawater. The dilution method was carried out using four different seawater dilution levels: 100, 70, 40 and 5% of nsw. Nutrients (0.5 and 0.03 µM of NH_4 and PO_4, respectively) were added to the eight experiments. An additional bottle of natural seawater was used to measure the initial phytoplankton concentration (t_0). After filled, bottles were incubated on-deck using continuous surface seawater to keep temperature constant. A net was also used to simulate light conditions at the sampling depth. We measured chlorophyll *a* and cell abundance at t_0 and after 24 h to estimate the apparent phytoplankton growth.

Time-series experiments

Seawater was sampled at the surface layer (3 m depth) during the morning in the Malaspina and Cetobaph cruises using a 30 L Niskin bottle. Time-course experiments followed the same procedure to obtain the diluted treatments as the dilution experiments explained above. These experiments were also designed using the same polycarbonate bottles but slightly modified in order to sample at different intervals without producing bubbles inside the incubators (see Fig. IV.3 for details). Highly diluted (5% nsw) and natural samples were incubated on-deck simulating *in situ* temperature and light conditions as explained for dilution experiments.

Samples for determination of initial abundances were obtained after the filling of the polycarbonate bottles. In order to study the cell variability inside bottles and to have statistical confidence, time-series of abundance converted to biomass (see below) were obtained at different intervals during 30 h, every 2 h for the first 12-14 h and at 6 h intervals thereafter. No nutrients were added in any of the experiments carried out to study the time-course of cell abundance inside the incubators.The seven experiments carried out during the Cetobaph cruise (around the Canary Islands) were performed replicating the natural and 5% nsw dilution treatments with the aim to test the possible variability among incubation bottles.

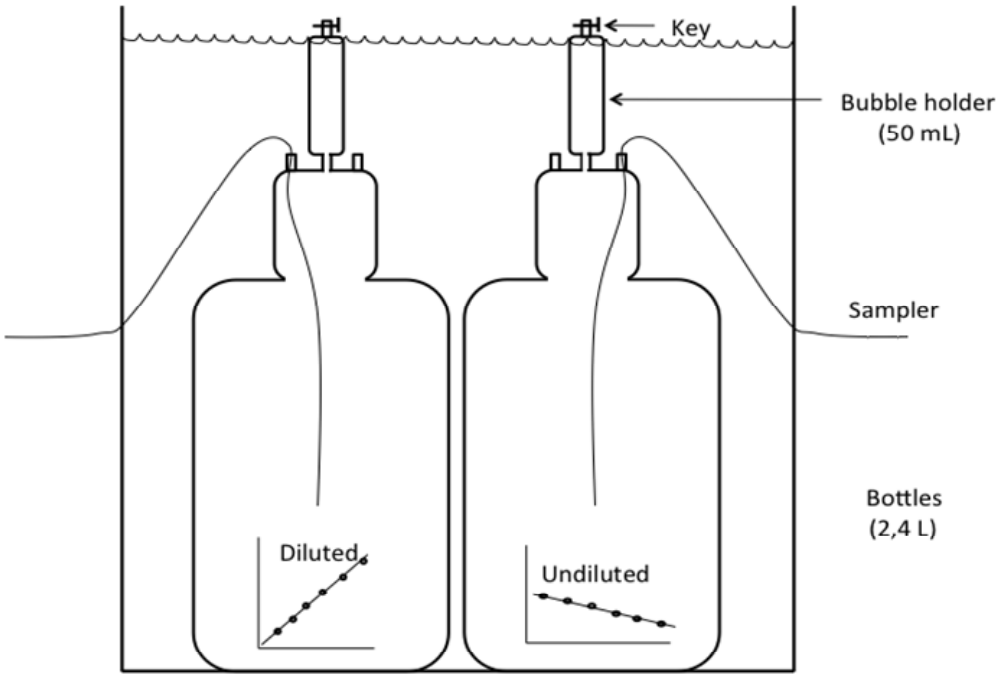

Figure IV.3. Bottle arrangement in order to sample at discrete intervals. The silicon tube sampler was 0.8 mm^2 internal diameter. Samples were slowly drained by gravity opening the key in the bubble holders, which were designed to avoid cell damage due to air inside the incubator. Before sampling, we allowed to remove the volume of water in the silicon tube (≈0.4 mL) in order to effectively sample inside the bottle. The holders were filled with the same water (dilution or natural) as the experiment. The graphs inside the bottles indicate the expected autotrophic growth (log) in the diluted and natural samples.

Sample analysis

Chlorophyll *a* (Chl *a*) was measured by filtering 500 mL of sample through a 47 mm WhatmanGF/F filter and freezing it at -20ºC until its analysis at the laboratory. The extraction procedure consisted in placing the filter in 90% acetone at -20ºC in the dark during at least 20 h, and following the acidification method by Strickland & Parsons (1972b). Pigments were measured on a Turner Design 10A Fluorometer, previously calibrated with pure Chl *a* (Yentsch & Menzel 1963).

Small photosynthetic eukaryotic cells (autotrophic picoeukaryotes), *Prochlorococcus* and *Synechococcus* type cyanobacteria, were counted by flow cytometry using a FACScalibur instrument (Becton and Dickinson). Immediately after collection, samples (2 mL) were fixed with paraformaldehyde (2% final concentration), incubated for 30 min at 4ºC, frozen in liquid nitrogen, and then stored at -80^{o}C until analysis. The identification of small phytoplankton groups was based on interactive analyses of multiple bivariate scatter plots of side scatter, red and orange

fluorescence. Samples were run at medium or high speed for autotrophic picoplankton until 10000 events were acquired or 2 min were passed.

Prochlorococcus cell numbers were converted to biomass assuming a mean biovolume of 0.1 μm^3 $cell^{-1}$ (Sieracki et al. 1995), and a conversion factor of 220 fgC μm^{-3} (Christian & Karl 1994, Zubkov et al. 2000). *Synechococcus* cell numbers were converted to biomass using a conversion factor of 250 fgC $cell^{-1}$ (Kana & Glibert 1987, Li et al. 1992). Finally, autotrophic picoeukaryote abundances were transformed to biomass using a conversion factor of 2100 fgC $cell^{-1}$ (Campbell et al. 1997).

III. RESULTS

The first approach was to review the existing data in order to test the suitability of the method by Frost (1972) comparing grazing estimates from dilution experiments in which the 5% of nsw dilution was used, with the grazing rates estimated as μ_e (growth in the 5% nsw sample) minus k (growth in the 100% nsw sample) in the same experiments. The results of this review (Fig. IV.4) showed a good agreement between the grazing assessed by the dilution (m_{dil}) and Frost (m_F) methods, except for the one in which the so-called feeding at saturation was observed (arrow in Fig. IV.4). A rather high correlation coefficient was observed ($m_F = -0.027 + 1.073 \cdot m_{dil}$, $r^2=0.886$, $p<0.001$, $n=25$, regression model II).

Experiments carried out to the north of Gran Canaria Island during winter-spring 2011 and in the upwelling zone off Namibia were used to compare the Frost and Landry & Hasset methods. Both methods were correlated for picoplankton (Fig. IV.5A) as well as between both approaches in terms of chlorophyll ($m_F = 0.057 + 0.878 \cdot m_{dil}$, $r^2=0.618$, $p<0.001$, $n=8$, regression model II). The relationship found using all chlorophyll-based data (literature review, Canary Islands, and Namibian data) was also well correlated ($m_F = 0.027 + 0.996 \cdot m_{dil}$, $r^2=0.872$, $p<0.001$, $n=33$, regression model II, Fig. IV.5B). This result is a rather good confirmation of the Frost method to assess grazing by microzooplankton in the ocean. Furthermore, this approach allowed to measure grazing values around zero (Table IV.2), something quite difficult using dilution and 2-point methods because very low slopes are not statistically significant. However, errors in some of those experiments were quite variable (Table IV.2). Large errors were mainly due to large variability in the 5% nsw dilution used to obtain phytoplankton growth. Therefore, we followed the time-course of cells inside the incubators in order to investigate this variability.

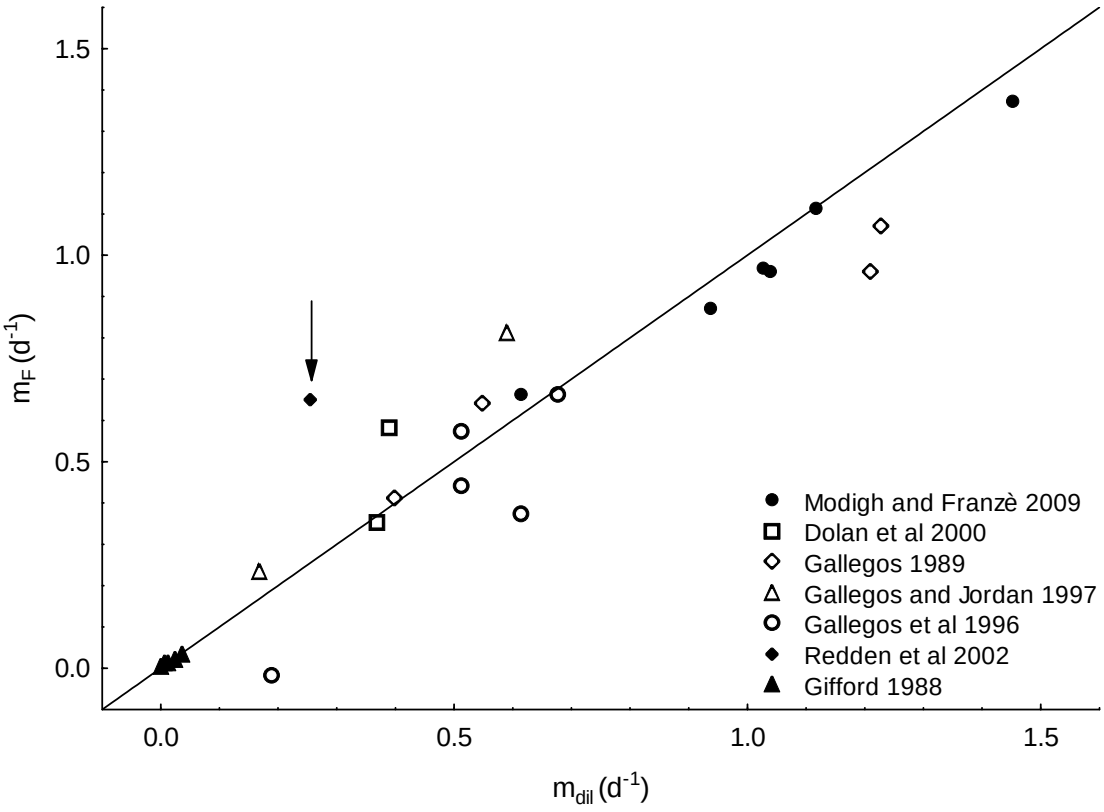

Figure IV.4. Relationship (regression model II) between published grazing rates assessed through dilution experiments (m_{dil}), and grazing rates by the method of Frost (1972) using the intrinsic autotrophic growth rates estimated by the 5% nsw dilution (μ_e) in the same experiments (m_F). The line shows 1:1 relationship. The data point marked with an arrow corresponds to an experiment where feeding at saturation was observed.

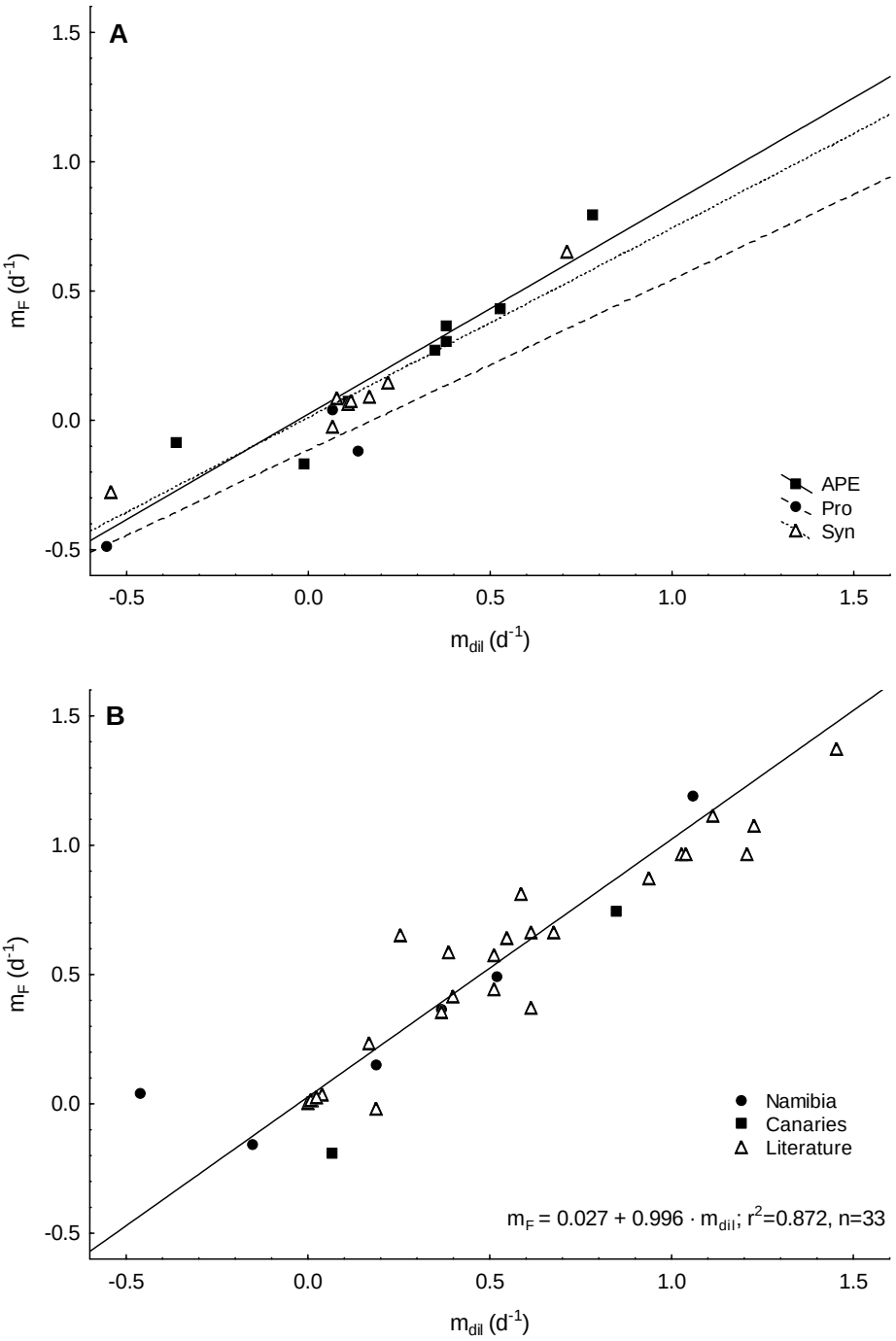

Figure IV.5. Grazing rates measured using the dilution technique of Landry & Hasset (1982), m_{dil}, and using the method of Frost (1972), m_F, at the Namibian upwelling and at the subtropical waters to the north of the Canary Islands. (5A) Relationship (regression model II) between grazing rates upon *Synechococcus* (Syn), *Prochlorococcus* (Pro) and autotrophic picoeukaryotes (APE). (5B) Relationship (regression model II) between grazing rates calculated from chlorophyll *a* data at the Namibian upwelling (Namibia), subtropical waters to the north of the Canary Islands (Canaries), and from literature data (Literature). The linear regression of all data is also shown.

Experiment	Date	Syn	Pro	APE	Chl*a*
NG1	08/27	0.13 (±0.04)		-0.13 (±0.23)	0.47 (±0.11)
NG2	09/06	0.14 (±0.06)		0.19 (±0.09)	1.22 (±0.16)
NG3	08/31	0.12 (±0.09)		0.37 (±0.08)	0.41 (±0.39)
NG4	09/12	0.01 (±0.09)		0.06 (±0.11)	0.82 (±0.38)
NG5	09/10	0.25 (±0.09)		0.27 (±0.11)	-0.06 (±0.18)
NG6	09/15	0.24 (±0.05)	0.24 (±0.01)	0.31 (±0.07)	0.43 (±0.14)
DG6	01/20	0.65(±0.11)	-0.12 (±0.09)	0.79 (±0.51)	0.68 (±0.23)
DG7	04/27	-0.56(±0.11)	-0.59 (±0.04)	-0.42(±0.31)	-0.19 (±0.36)

Table IV.2. Grazing rates (d^{-1}) upon *Synechococcus* (Syn), *Prochlorococcus* (Pro), autotrophic picoeukaryotes (APE) and total phytoplankton (Chl *a*) measured according to Frost (1972) to the north of the Canary Islands (DG6 and 7) and the Namibian upwelling (NG1 to 6). Values in parenthesis are standard errors (n=6). Note that grazing rates upon Pro are only shown for NG6 in the Namibian upwelling as these organisms were only located in oceanic waters.

Variability of cells inside the bottles in time-series treatments (highly diluted and natural samples) were measured in experiments carried out during Malaspina and Cetobaph cruises. The different autotrophic cells showed clear trends in natural and 5% nsw incubations (Fig. IV.6A), allowing also the estimation of grazing using the equations by Frost (1972). Similar trends were also observed in the Cetobaph cruise experiments in which replicates of natural and diluted samples were used (Fig. IV.6B). From the 42 replicates (3 replicates of time-series of picoeukaryotes, *Synechococcus*, and *Prochlorococcus* for natural, and 3 replicates of the same group of organisms for the diluted treatment, in each experiment) only one showed different slopes, being p=0.046, at the edge of a significant result (General Linear Model, test for comparing slopes).

Along the Pacific Ocean *Prochlorococcus* was the most abundant cell (not shown), as expected. Picoeukaryotes increased in the productive areas of the equatorial upwelling and near Central America, showing also higher biomass there. Experiments performed along the basin showed grazing rates on picoplankton varying from negative values to 1.05 d^{-1} (Fig. IV.7). Overall, we observed positive grazing rates except in those stations sampled in the most productive areas such as the stations carried out near islands in the South Pacific (St 84 and 86), and in the equatorial upwelling (St 94). These stations showed negative grazing rates upon these small cells. By opposite, higher grazing rates were found in the less productive stations suggesting an important impact on smaller cells.

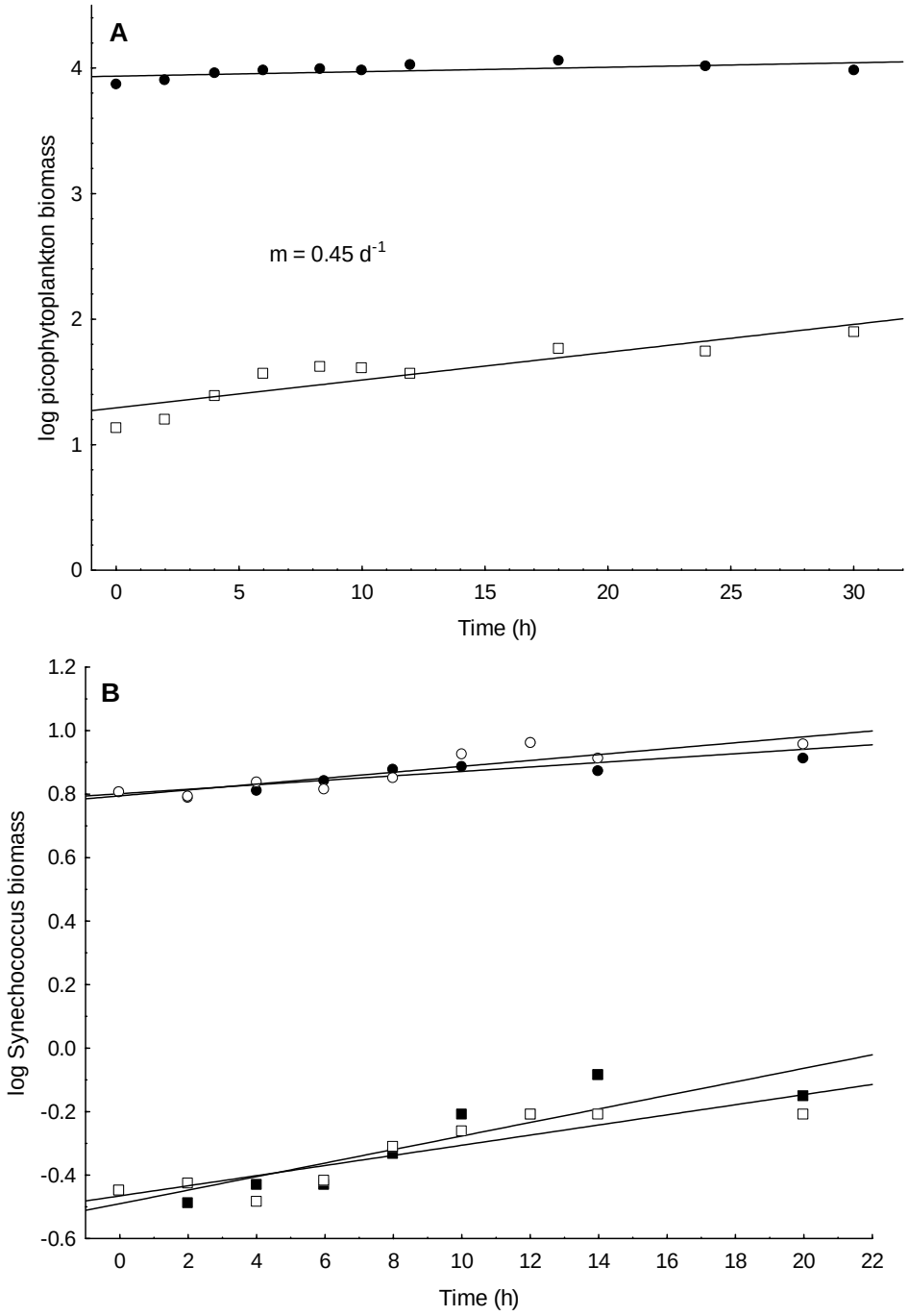

Figure IV.6. Examples of time-series measurements of biomass inside the incubator during grazing experiments. Dots are data from the natural sample (k) and squares from the 5% nsw dilution (μ). (6A) Example of experiment in station 122 at the Pacific Ocean for all three picoplanktonic groups (picoeukaryotes, *Prochlorococcus*, and *Synechococcus*) (6B) Example of replicates for each treatment in experiments carried out in the Canary Islands (Cetobaph Cruise).

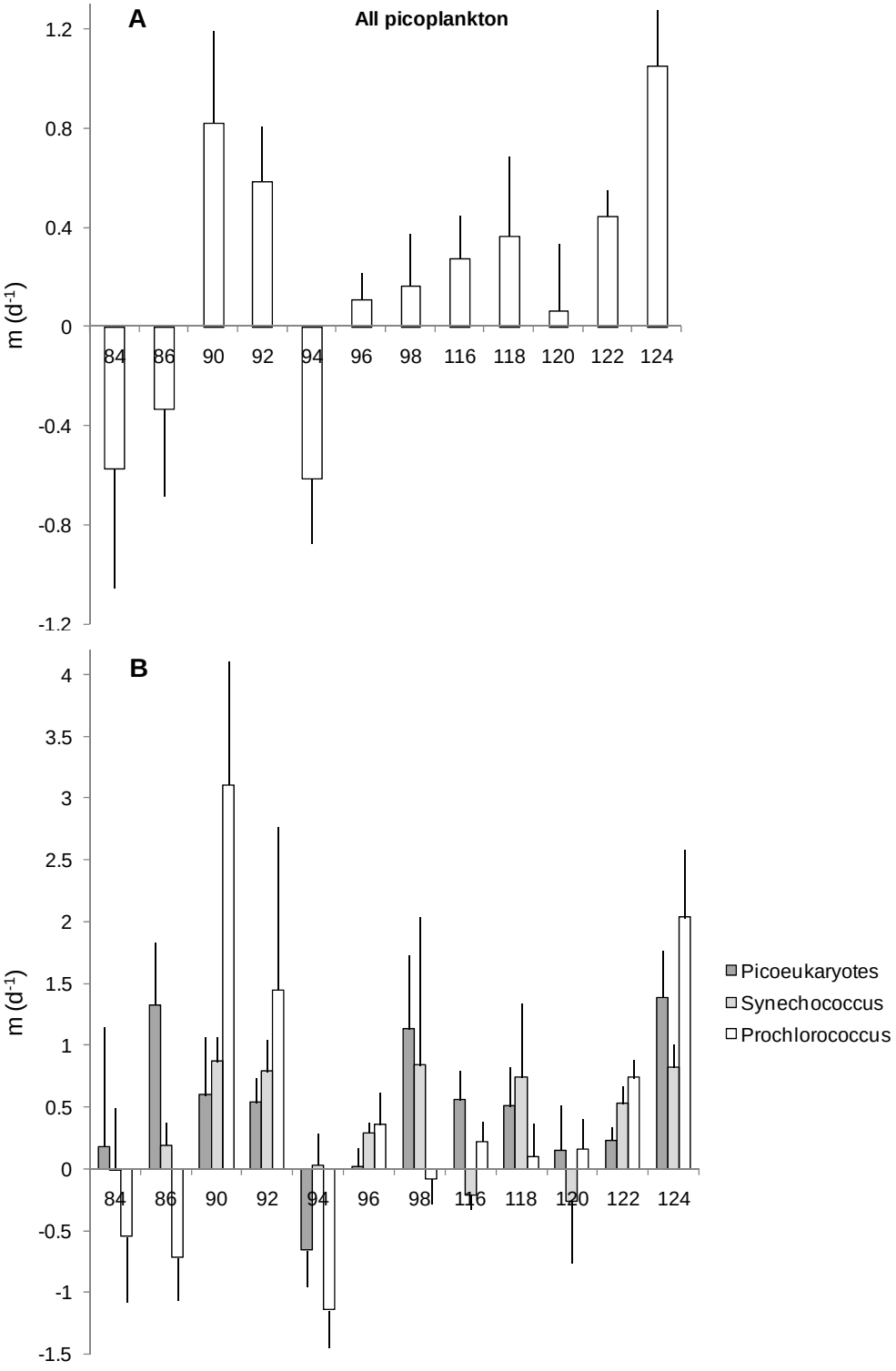

Figure IV.7. Grazing rates (d^{-1}) obtained along transects from New Zealand to Hawaii and Hawaii to Panama. (7A) Grazing rates upon the three picoplanktonic groups. (7B) Grazing rates upon picoeukaryotes, *Synechococcus*, and *Procholorococcus.*

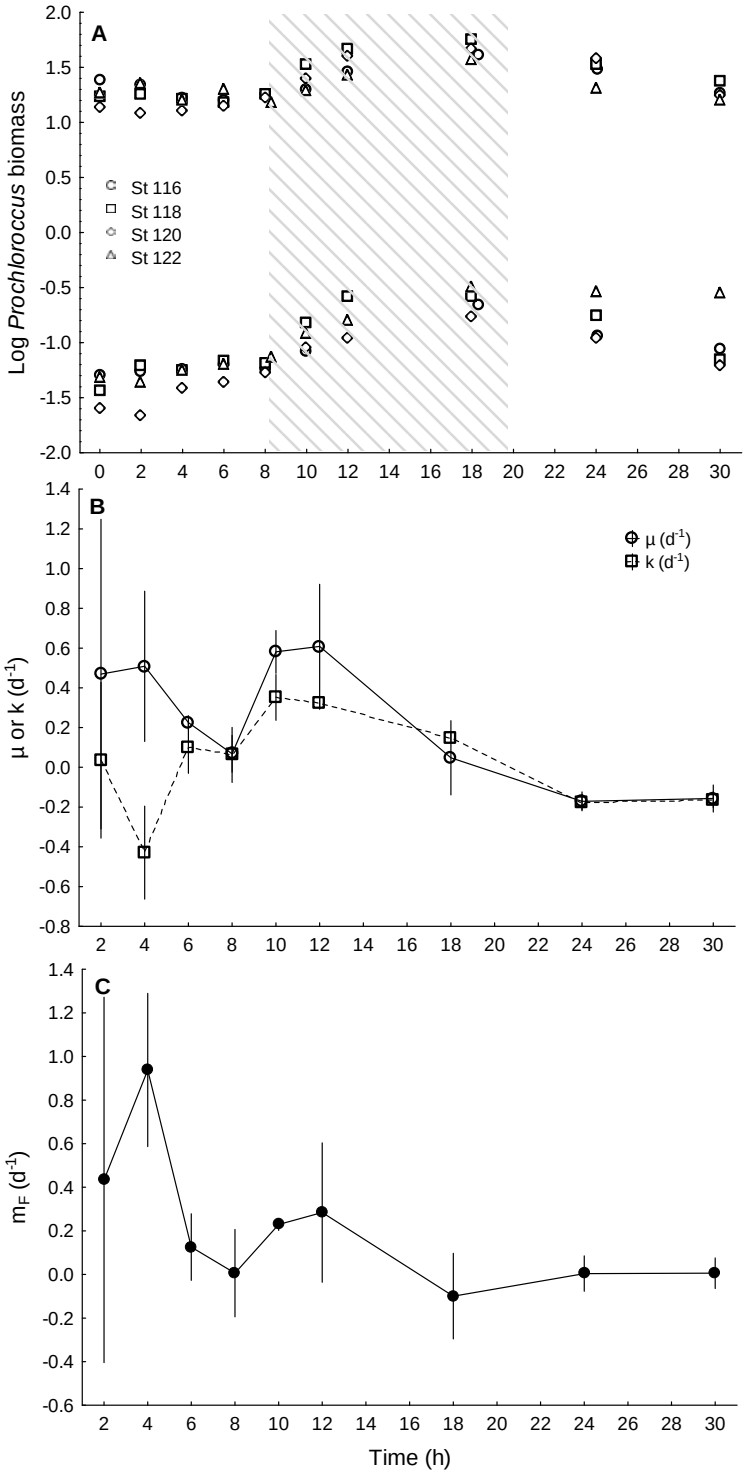

Figure IV.8. Example of short-term grazing rates along the 30 h incubation in experiments performed at similar latitudes in the Pacific Ocean (stations 116, 118, 120, 122). (8A) Biomass of Prochlorococcus. Observe the parallel trend in natural and diluted (5% nsw dilution) samples and the increase during night (shaded area, from 8 to 20 h). (8B) Short-term variability of autotrophic growth (μ) and apparent autotrophic growth (k). (8C) Time-series of grazing (d^{-1}). Observe the higher values by day, at the beginning of the experiment, and during night.

In experiments carried out in a similar latitudinal band (st 116, 118, 120, and 122), showing similar oceanographic conditions, time-series of picoplankton inside the bottles showed a rather similar trend for the most abundant cells (*Prochlorococcus*, Fig. IV.8A). Cells in the four experiments increased in both natural and diluted incubations during the night. We estimated the autotrophic growth using the 0.05 dilution factor (μ_e), apparent rate of autotroph growth k (Fig. IV.8B), and grazing m (Fig. IV.8C) for short intervals using the average for the 4 stations. As observed, after quite variable rates at the start of incubation, grazing rates showed the higher values after only 4 h, decreasing thereafter and slightly increasing again during night. After 18 h, grazing became almost zero.

IV. DISCUSSION

In the present work, we made an exercise to discriminate between the concepts used in the dilution, 2-point, and Frost methods. Then, we have compared the dilution and Frost methods using a high dilution to obtain the intrinsic autotrophic growth (μ), and the measurement of the apparent autotrophic growth in the natural sample (k). We reviewed the scarce data in which dilution experiments used the 5% nsw dilution and a rather high correlation was obtained between grazing estimated by the dilution technique using the whole dilution series, and by the Frost method using only the undiluted (k) and the 5% nsw (μ) samples. This result corroborated the agreement between values of μ and μ_e observed by Gallegos & Jordan (1997). We also tested this approach in experiments performed in the oligotrophic and eutrophic waters off the Canary Islands and the upwelling zone off Namibia, respectively. Pooling experiments taken from the literature and those presented here showed a rather good agreement ($m_F = 0.027 + 0.996 \cdot m_{dil}$, $r^2=0.872$, $p<0.001$, n=33, regression model II). However, the large errors sometimes achieved (Table IV.2) encourage to look into the sources of this variability by measuring the time-course of cells.

In order to study the phytoplankton trends inside incubators, we performed time-series sampling inside the bottles as a better approach to explain processes taking place along the experiment (e.g., feeding at saturation in the natural sample). Besides, this procedure should give more statistical confidence than measuring at t_0 and after 24 h. The use of the 5% nsw dilution as μ still adopts one of the assumptions of the dilution series, the effect of the presence or absence of other phytoplankton on the growth of individual phytoplankton. However, as the highest diluted treatment is not affected by grazers (Dolan et al. 2000), the assumption based on the rate of encounter of consumers with prey cells does not apply. In time-series experiments, the true change in phytoplankton concentration was measured in the 5% nsw dilution at short intervals, estimating the actual growth. We observed the increase of the intrinsic and apparent autotrophic growth during the

night in a number of incubations (see Fig. IV.8A and B as an example). Thus, true exponential changes in density of phytoplankton, although expected, will not be longer assumed. In fact, this could be a source of underestimation of μ and k in 24 h experiments. The parallel increase during night observed in both the natural (where nutrient regeneration by micrograzers is a fact) and diluted incubations indicate a rather low importance of nutrients at least during the first 12-15 h of the experiment. Nutrient levels are not expected to change appreciably and to have a large impact during this time interval, but this will require further research. In any case, this unamended treatment will avoid the loss of certain grazers observed in dilution series (Gifford 1988) and should give a better picture of grazing in nature.

We also observed high grazing rates at the beginning of the experiment (Fig. IV.8C), similar to the finding normally observed in copepod grazing experiments (Mullen 1963, Hargrave & Geen 1970), and similar to the response of micrograzers to a pulse of prey (Schmoker et al. 2011, Calbet et al. 2013). The high grazing rate during the day is also in agreement with the circadian cycles found by Jakobsen & Strom (2004). In general, they measured higher growth and feeding rates of microzooplankton during the light period, when phytoplankton are photosynthetically active. We do not seek to go far into explaining our short-term estimations as further research is needed. However, it seems that grazing is not constant during incubations and feeding at saturation could occur as envisaged in our methodological approach. In this sense, experiments performed to study time-series of cells inside bottles (Calvo-Díaz et al. 2011) showed an important variability depending on the different organisms counted. These authors observed a marked decrease in *Prochlorococcus* and picoeukaryotes biomass during the first 6-12 h of incubation, remaining low thereafter. This seems a fingerprint, jointly with our results, to think about the variability normally observed in replicates and the suitability of large (24 h) incubations. Moreover, time-series carried out in bottles of quite different volumes (70 to 1000 mL) gave similar results (Calvo-Díaz et al. 2011), also arising the convenience or not of using rather large volumes.

Another remarkable result was the finding of impossible negative values in the grazing estimations. As we only measured grazing upon picoplankton, those results could be explained as no selection upon specific groups or organisms. In fact, we observed negative values in the most productive zones where large cells of phytoplankton are expected to grow and nutrients to be non-limiting (e.g., the Equatorial Pacific). As predators and prey in nature preferentially select large particles as observed in mesozooplankton (Mullen 1963, Richman & Rogers 1969, Frost 1972), it is clear the need for measurements of grazing upon other groups such as autotrophic nanoflagellates, dinoflagellates and diatoms in order to have the real picture of grazing in the water column. Although the problem of negative values of grazing is out of the scope of this work and needs

further research, these results suggest top-down effects in nature, releasing primary production from grazing (see Calbet & Saiz 2013).

The time-series approach presented here based on the concept of the Frost method could be fully automated using flow cytometry and image processing. Continuous measurements of cells inside different bottles using the appropriate manifold and software is nowadays possible. New technology will provide the necessary tools to automatically measure cell variability inside incubators in quite short intervals. Thus, the method should be suitable for oceanographic work as several measurements could be made per day (e.g., to observe changes along transects, day and night rates, or vertical distribution of grazing).

Finally, as we unveil here, there is a need for knowing the changes produced inside the incubators in order to explain the variability in grazing experiments. The approach presented here is still a simple manipulation of a complex food web (Gallegos 1989), but continuous measurements of cell variability along experiments could promote a better understanding of interactions between grazers and their prey in the ocean.

V. ACKNOWLEDGMENTS

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CHAPTER V

Active carbon flux estimations in relation to zooplankton lunar cycles in subtropical waters

CAPÍTULO V

ABSTRACT

The epipelagic mesozooplankton biomass was studied during bloom and non-bloom conditions along various years in the Canary Islands waters. As observed in previous works in the area, biomass was higher during the illuminated phases of the lunar cycle (second and third quarter). This occurs because during the lunar illuminated phase, to avoid predation, diel vertical migrants (DVMs) do not reach the shallower layers (0-100 m) of the ocean, while during the dark period the migrants reach the upper water column. As a consequence, the epipelagic mesozooplankton grows without predation pressure around full moon, and decrease its biomass during the dark phase of the lunar cycle because of consumption by migrants. In order to simulate this cycle of predation, a simple equation was used to model the mesozooplankton biomass. Significant correlations between the measured and predicted biomass were obtained during the period studied setting both mortality and growth rates as a function of the lunar illumination. From the modeled community mortality and biomass we estimated the active carbon flux for this period assuming that mortality was due to predation by DVMs. Average values during non-bloom (1.6 ± 0.4 mmolC m^{-2} d^{-1}) and bloom (6.7 ± 2.9 mmolC m^{-2} d^{-1}) conditions indicate that potential active flux could be of the same order of magnitude than the gravitational sinking in subtropical waters. This important flux seems of paramount importance for the assessment of the biological pump in the ocean.

I. INTRODUCTION

The active transport of organic matter by diel vertical migrants (DVMs) has been increasingly recognized as a process that significantly contributes to the biological pump (Angel 1989, Longhurst et al. 1989, 1990, Zhang & Dam 1997, Steinberg et al. 2000, Ducklow et al. 2001). The active flux involved all the biological processes driven by migrant zooplankton and micronekton that actively export organic matter from the photic layer to the mesopelagic realm (Longhurst & Harrison 1988, Longhurst et al. 1989). The active carbon flux is a rather complex mechanism that includes the gut flux (the transport due to the release of feces) (Dam et al. 1995b), carbon dioxide respiration (Longhurst *et al.*, 1990), dissolved organic carbon excretion (Steinberg et al. 2000) and mortality (Zhang & Dam 1997) below the thermocline. The few values available at present mainly based on respiration at depth indicate that the active downward carbon flux is highly variable and ranges from <1 to >100% of the gravitational flux in subtropical waters (Dam et al. 1995a, Steinberg et al. 2000, Al-Mutairi & Landry 2001, Hernández-León et al. 2001b, Steinberg et al. 2008, Putzeys et al. 2011, 2013).

The high variability of the active carbon flux has been related to changes in composition or biomass of DVMs. In some cases higher values were observed in productive waters (Yebra et al. 2005, Putzeys 2013), where the biomass of DVMs was higher than in oligotrophic regions. In the subtropical northeast Atlantic, Putzeys (2013) observed that biomass of migrants varied both spatially and seasonally, which could be an important source of the variability observed in the active transport of carbon. In other cases, the spatial variability was largely driven by the presence of larger organisms (euphausiids) within the migrant community (Stukel et al. 2013). In fact, the DVM community also includes micronektonic organisms that have been neglected in the majority of the estimations despite their important role in the active transport (Angel 1989). In this sense, Hidaka et al. (2001) showed that the flux due to micronekton accounted for 56-60% of total active flux in the western equatorial Pacific Ocean. Moreover, within the micronektonic community, mesopelagic fishes could account for a large fraction of the downward carbon flux as it has been recently measured by Davison et al. (2013). These authors estimated that the contribution of the migrant mesopelagic fishes was 15-17% of the total carbon exported. Hence, this bias in the measurement of DVM biomass supposes an important underestimation of the active flux in the ocean.

Diel vertical migration has been explained as a strategy of organisms to avoid predation (Stich & Lampert 1981, Ringelberg 2010), keeping at deeper and unlit waters between 200-1000 meters during the day and reaching the upper water column during the night (Moore 1950, Uda 1956, Angel 1989, Longhurst et al. 1989), when they are less visible to their visual predators. Additionally, the intensity of migration has been related to other factors as the water clarity or the lunar illumination. In lakes, both parameters explained the 92% of the migratory amplitude of *Daphnia* (Dodson 1990).

The influence of the moonlight has also been measured in the ocean (Uda 1956, Benoit-Bird et al. 2009) where a strong effect of the lunar phase in the vertical movement, extent and density of the scattering layer was found. Thus, migrants were closer to the surface during low illuminated nights, reaching the shallowest depth on new moon.

A relationship between zooplankton and the lunar cycle has been repeatedly observed in subtropical waters off the Canary Islands (Hernández-León 1998, Hernández-León et al. 2001a, 2002, 2004, 2010) linked to predation by DVMs. The epipelagic mesozooplankton supports a large predation pressure by these migrant organisms that reach the shallower layers at night. However, these animals do not reach the upper layers during the full moon (Uda 1956, Benoit-Bird et al. 2009), when the maximum illumination takes place, to avoid predation by nektonic organisms and thus, epipelagic mesozooplankton grow without predation pressure. Conversely, during the new moon the vertical migrants occupy the first hundred meters of the water column and feed on the epipelagic mesozooplankton, whose biomass drops (Hernández-León 1998, Hernández-León et al. 2001a, 2002, 2004, 2010).

The different predatory scenarios in the Canary Current provide an opportunity to study the predatory cycle related to the lunar phase. Active flux is usually measured by analyzing the defecation, respiration, excretion or mortality of DVMs in the mesopelagic zone. Another approach is the knowledge of feeding by DVMs in the shallower layers, assuming that a large percentage of this energy is transported to the mesopelagic zone (Hernández-León et al. 2002, 2004, 2010). Here, we simulated the observed zooplankton lunar cycle and we estimated the consumption of carbon by the migrant biota in five different years. We assessed mortality due to DVMs assuming that this value is proportional to the lunar illumination. Bloom and non-bloom periods were covered in order to evaluate potential differences in the estimated active transport. The results showed that potential active flux could be in the order of gravitational flux.

II. MATERIAL AND METHODS

Mesozooplankton biomass data were obtained from two different samplings: ConAfrica and Lucifer cruises. ConAfrica was performed from January 2005 to May 2007 at five stations around Gran Canaria Island (Canary Islands), at the edge of the island shelf. Lucifer was carried out from February to June 2010 and from November 2010 to June 2011 at four stations to the north of the island (Fig. V.1). Both cruises provided weekly data.

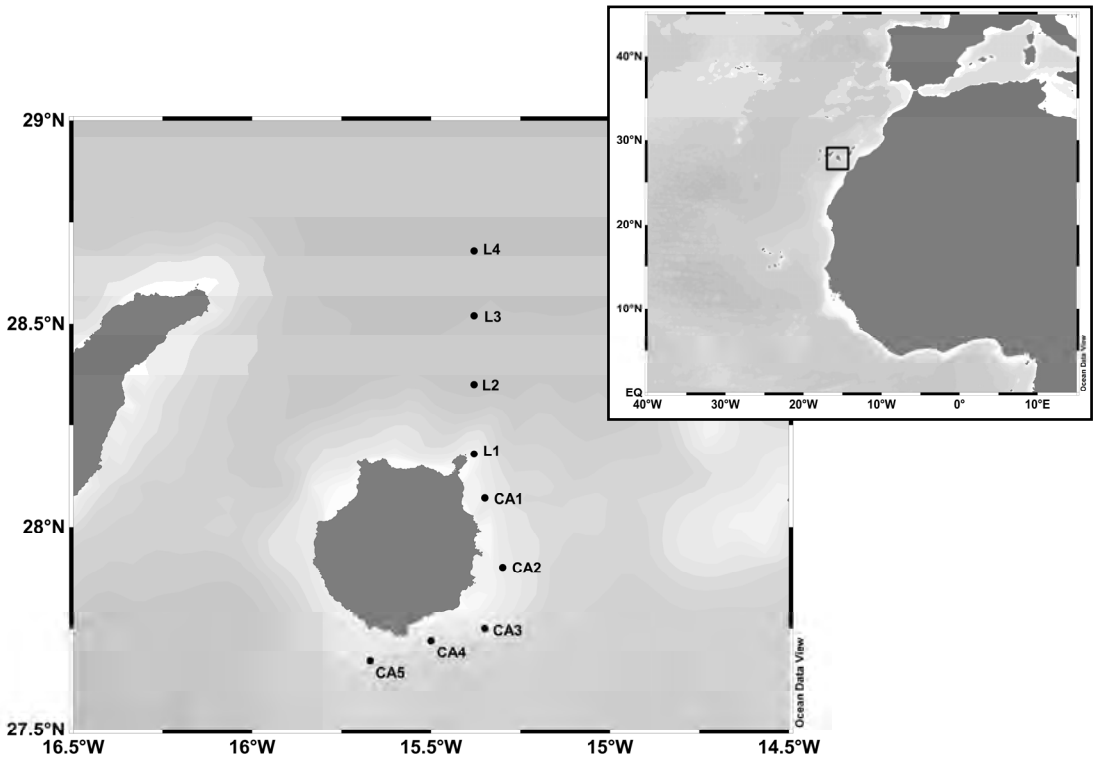

Figure V.1. Map of the sampling area showing the location of the five stations around Gran Canaria Island (ConAfrica cruise: CA1, CA2, CA3, CA4, CA5) and the four stations at the north of the island (Lucifer cruise: L1, L2, L3, L4).

Zooplankton was captured in oblique hauls with a Bongo net equipped with 200 µm mesh nets during ConAfrica, and in vertical hauls with a double WP-2 net equipped with 100 µm mesh nets during Lucifer. One of the zooplankton samples was used for measuring dry weight following a standard procedure (Lovegrove 1966). A more detailed sampling protocol is explained elsewhere (Herrera et al., in prep., Schmoker et al. 2014). Average biomass data were calculated from the five and four stations in ConAfrica and Lucifer, respectively, as no significant differences were found between different stations.

In order to estimate mortality by DVM predation, we performed a simple model to simulate the epipelagic mesozooplankton biomass using the criteria of previous works (Hernández-León et al. 2002, 2004, 2010), considering

$$P = (B_1 - B_0) + M$$

where P is production of zooplankton, B_1 and B_0 are their biomass at time 1 and 0 respectively, and M is mortality. Then,

$$B_1 = B_0 + (B_0 \cdot g) - (B_0 \cdot m)$$

being g the growth rate and m the mortality rate.

The initial biomass, B_0, was the first value measured just before the period considered for simulation, and the mortality rate, m, was set as function of the lunar illumination, i:

$$m = [(1 - i) \cdot m_{max}] + m_{min}$$

where m_{max} and m_{min} are the maximum and minimum mortality rates, respectively. For the period of new moon (i=0), DVM predation produces the maximum epipelagic mesozooplankton mortality rate. Contrary, within the full moon period (i=1) maximum illumination occurs, so that predation diminishes and the mortality rate of the epipelagic mesozooplankton is minimal. We used increasing m_{max} values to test the better correlations between the measured and simulated biomass, ascribing different values to each observed maximum.

Following Hernández-León et al. (2010), the growth rate was also set as a function of the lunar illumination, testing different g_{max} at different phases of the moon in order to find the better correlation coefficients. As for mortality, different values of g_{max} were used for each biomass maximum. g_{max} was set according to Hirst & Lampitt (1998), ranging from 0.1 to 0.3 d^{-1}, although in some cases during the bloom period higher growth rates were used to obtain a more realistic simulation. A minimum growth and mortality value of 0.01 d^{-1} was taken from the literature (Hirst & Lampitt 1998, Hirst & Kiørboe 2002).

The daily community mortality, M, was estimated from the simulated biomass (b_m) and mortality rate (m_m) for each day:

$$M = b_m \cdot m_m$$

Finally, the mean community mortality was assumed to be the average active carbon flux value during that period (see Discussion).

III. RESULTS

A periodic increase and decrease in the epipelagic mesozooplankton biomass was observed during both bloom and non-bloom conditions in every year for the period studied (Fig. V.2). The increases of biomass seemed to follow a lunar cycle pattern which was more evident when biomass was standardized taking maximum values of biomass in every lunar cycle as 100% (Fig. V.3). Biomass was lower during the first quarter of the moon (from new moon to crescent moon) and higher during the illuminated phases of the lunar cycle (second and third quarter), both in ConAfrica (Fig. V.3A) and Lucifer (Fig. V.3B). However, the difference was only statistically significant during 2006 (Fig. V.3C, Kruskal-Wallis test, $p<0.05$).

The simulated biomass was highly correlated to the measured biomass from January 2005 to May 2007 (Fig. V.4A; $r=0.738$, $p<0.001$), and from February to June 2010 and November 2010 to June 2011 (Fig. V.4B; $r=0.801$, $p<0.001$), including both bloom (2005, 2006 and 2007) and non-bloom (2006, 2010 and 2011) conditions. When considered separately, 2005 was the only year when there was no correlation between measured and predicted biomass (Table V.1).

The criterion regarding to variability of growth rate was different among years. During 2005, 2006 and 2011 g_{max} was located ten days before the full moon, during the waxing moon, while during 2007 and 2010 the setting of g_{max} at a different phase of the moon resulted in better correlation coefficients. During 2007, g_{max} was set in full moon and ten days after full moon for the first and second maxima, respectively. The same pattern was observed during 2010, when g_{max} was set in full moon for the second maximum and ten days after full moon for the third and the fourth increases.

Daily community mortality showed higher values and variability during bloom compared to non-bloom conditions (Table V.1). The highest average value (10.0 mmolC m^{-2} d^{-1}) was observed in 2007 matching the highest values of g_{max} and m_{max}, whilst community mortality was the lowest (1.1 mmolC m^{-2} d^{-1}) during 2011 when both rates were minimal.

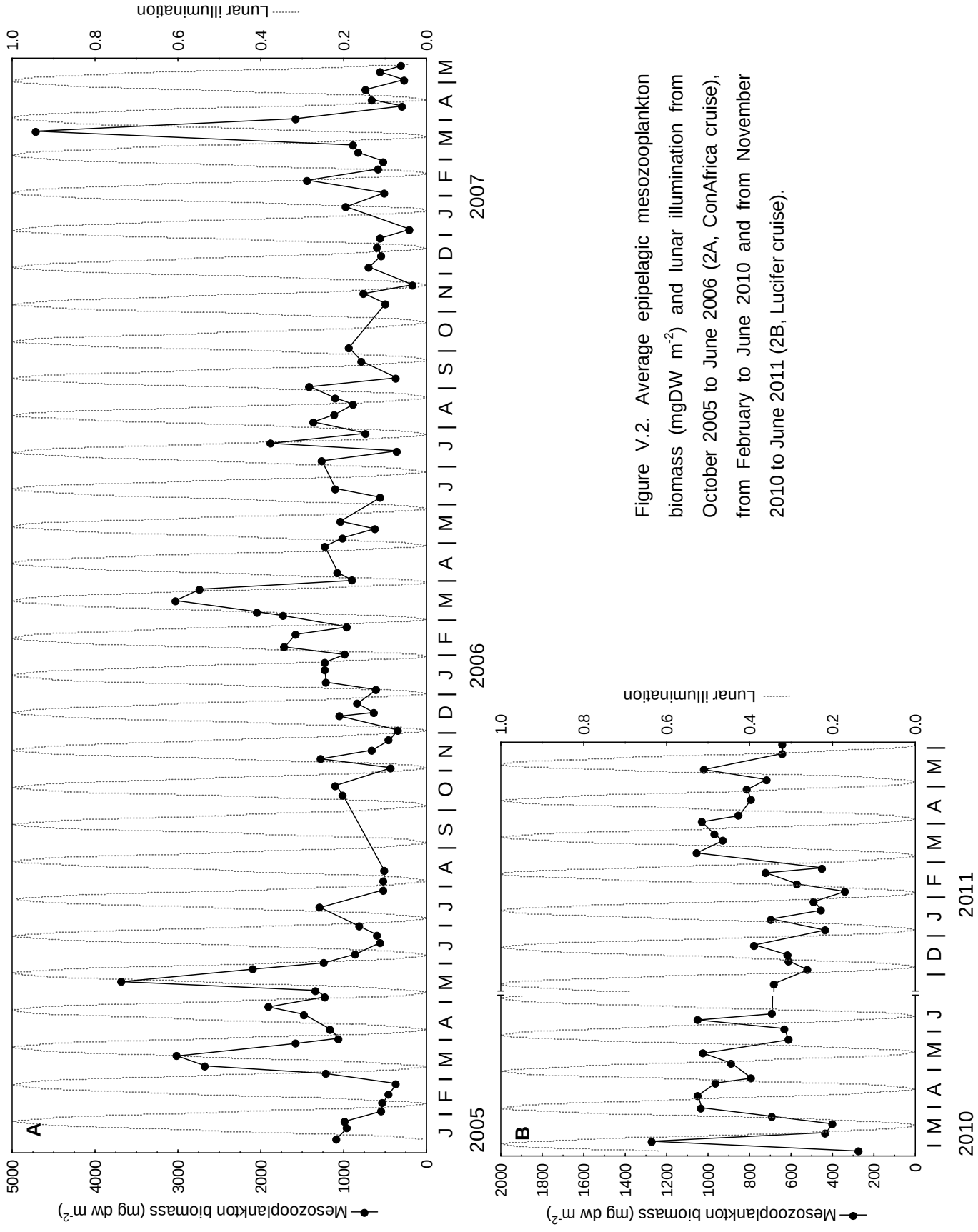

Figure V.2. Average epipelagic mesozooplankton biomass (mgDW m^{-2}) and lunar illumination from October 2005 to June 2006 (2A, ConAfrica cruise), from February to June 2010 and from November 2010 to June 2011 (2B, Lucifer cruise).

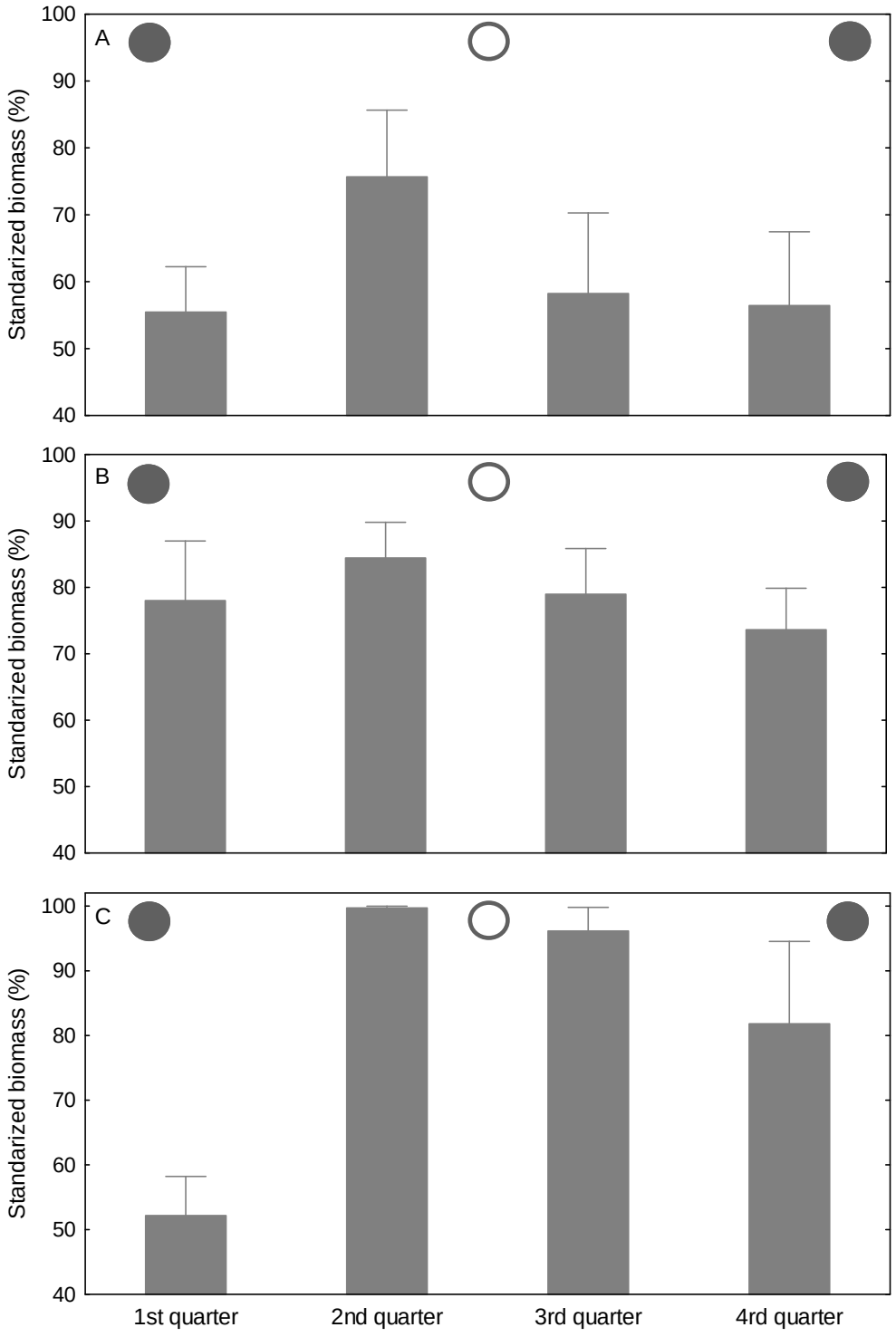

Figure V.3. Standardized biomass (maximum value of biomass in each lunar cycle converted to 100%) during ConAfrica (3A), Lucifer (3B) and data from 2006 during ConAfrica (3C). Open and full grey circles stand for full and new moon, respectively. Average data (±SE).

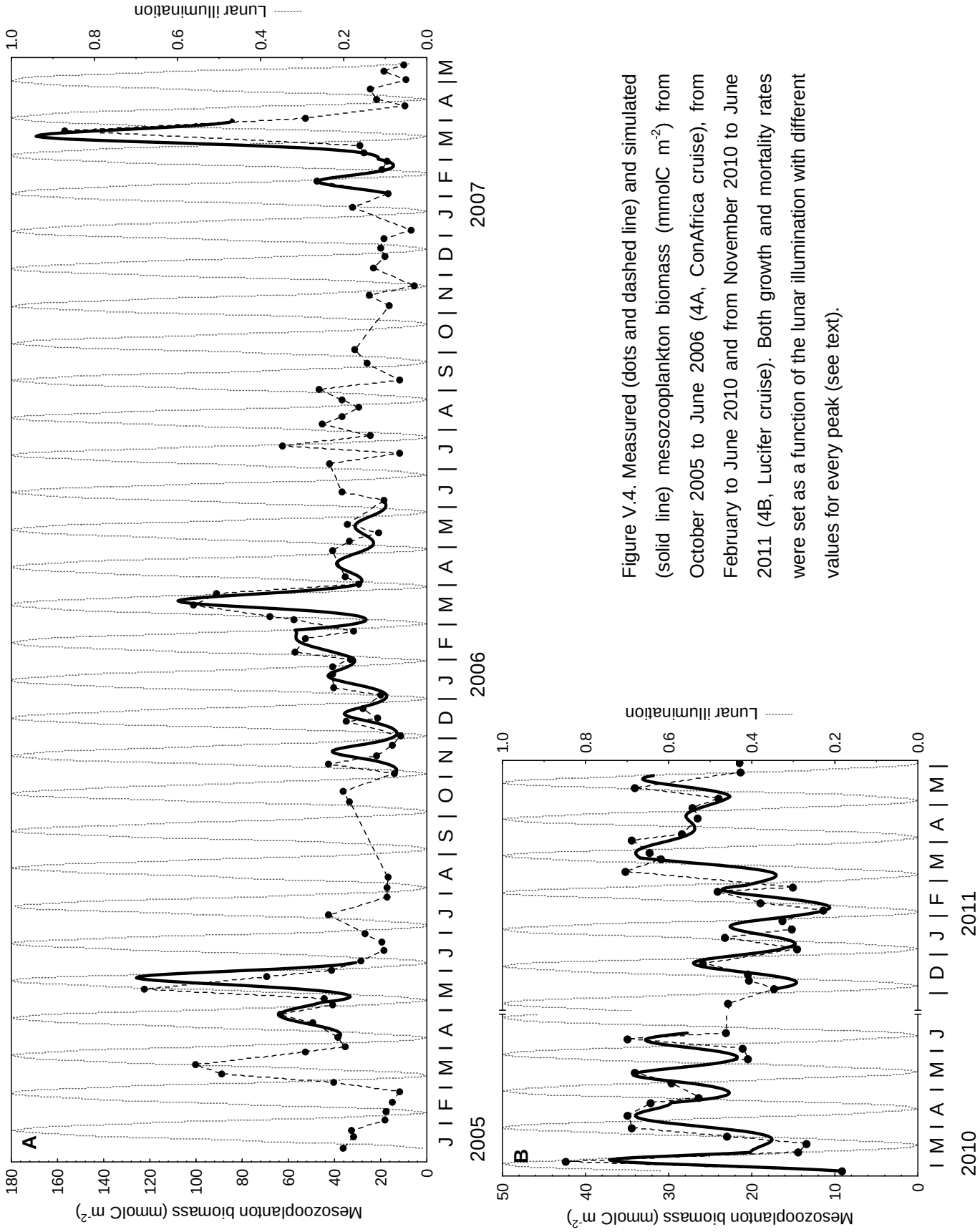

Figure V.4. Measured (dots and dashed line) and simulated (solid line) mesozooplankton biomass (mmolC m^{-2}) from October 2005 to June 2006 (4A, ConAfrica cruise), from February to June 2010 and from November 2010 to June 2011 (4B, Lucifer cruise). Both growth and mortality rates were set as a function of the lunar illumination with different values for every peak (see text).

Year	Conditions	g_{max} (d^{-1})	m_{max} (d^{-1})	r	p	**Daily community mortality** (mmolC m^{-2} d^{-1})
2005		0.23	0.21	0.19	n.s.	**6.1** (0.6-15.9)
2006	Bloom	0.18	0.16	0.75	<0.001	**4.2** (0.4-14.6)
2007		0.27	0.22	0.91	<0.01	**9.9** (0.2-47.4)
2006		0.19	0.19	0.75	<0.001	**2.0** (0.3-4.5)
2010	Non-bloom	0.23	0.16	0.89	<0.001	**1.7** (0.2-6.0)
2011		0.13	0.12	0.70	<0.001	**1.1** (0.2-2.4)

Table V.1. Average (minimum-maximum) daily community mortality (mmolC m^{-2} d^{-1}) estimated from simulated mesozooplankton biomass and mortality. Average maximum rates of growth (g_{max}) and mortality (m_{max}) used for the simulation are shown.

IV. DISCUSSION

A lunar cycle pattern was observed in the mesozooplankton biomass during the productive period and also within non-bloom conditions (Figs. V.2, V.3 and V.4), as observed by previous works in the area (Hernández-León et al. 2002, 2004). However, although biomass was always higher during the illuminated phases of the moon (2nd and 3rd quarters), the increases were not always linked to the same lunar phase (Fig. V.2 and V.4). Biomass maxima were centred near the full moon from November 2005 to March 2006 and during 2011. During 2005 and 2006 a clear pattern was not observed, whereas maximal values of biomass were located around waning moon in 2010. Hernández-León et al. (2004) found the maximum biomass near the waning moon and they explained this pattern as the effect of high growth rates of zooplankton counteracting mortality until the latter surpassed the former as darkness progressed through the lunar cycle. Thus, the interplay between both rates would promote the biomass to peak around the full moon. In fact, our results suggest that growth is also following the lunar cycle, as a better agreement was found between real and predicted biomass when this rate was simulated in function of the lunar illumination. As mortality is assumed to be always maximal with minimum moonlight conditions (new moon) and vice versa, the timing of biomass maxima is determined by the variability of maximum growth. That is, the biomass maxima matched maximal moonlight levels (full moon) when g_{max} was set ten days before the full moon (2006), while the maxima occurred near waning moon when g_{max} was set within the full moon or ten days after (2010).

The existence of a lunar rhythm in the reproductive cycle of epipelagic mesozooplankton seems to be the more plausible explanation for the possible influence of the lunar illumination on the growth rate of these organisms. In fact, potential growth rates of the epipelagic mesozooplankton, measured as enzymatic AARS activity, (Herrera, unpublished data) followed the lunar pattern described here during 2010 and 2011. That is, the potential growth rate was maximal around ten days before full moon as we set for simulating the biomass variability observed in 2011. In this sense, it has been recently observed (Mercier et al. 2011) that the reproductive cycle of various deep-sea species of invertebrates was synchronized to the lunar cycle. This evidence demonstrates that the influence of the moon is not only due to the tidal effect, as it occurs for coral reef species. However, the latter authors could not explain the nature of this lunar pattern.

The mesozooplankton biomass simulated with the simple equations used in this work showed high correlation values between the measured and simulated biomass in the whole period except during 2005 (Table V.1). An intense Saharan dust deposition event occurred just before the first maximum in 2005 probably promoting the high values of biomass, as it has been described in these waters before (Hernández-León et al. 2004). Given the higher values at the beginning of the bloom, this first maximum could not be included in the simulation and, thus, less data were available for correlation. This would likely be the reason of lower correlation values compared to other years.

Using our approach, we were able to assess mortality during the different mesozooplankton lunar cycles. The variability of the daily community mortality was considerably higher during bloom conditions ranging up to two orders of magnitude (Table V.1). The lower values observed during non-bloom conditions were not unexpected as the zooplankton biomass was lower as well as values of m_{max}. These results would be in agreement with previous works that observed higher values of carbon transport by DVMs in more productive waters (Yebra et al. 2005, Putzeys 2013). The relative low values (1.1-2.0 mmolC m^{-2} d^{-1}) during non-bloom conditions were comparable to previous estimations (Hernández-León et al. 2002, 2004) obtained north of the Canary Islands, which gave average values of 1.9 and 2.9 mmolC m^{-2} d^{-1} for non-bloom and bloom conditions, respectively. They used the same approach to estimate the community mortality, although they observed a lower variability along different conditions, which could be the result of their shorter period of study.

Mean community mortality was assumed to be the average active carbon flux, considering that the former is mainly induced by consumption of DVMs. However, as it has been pointed out by Ohman (2012), there are other causes of mortality such as parasitism, unfavourable environmental conditions (e.g. temperature or pH) or starvation. Therefore, although predation often accounts for a large fraction of zooplankton losses, assuming community mortality equivalent to active flux could be a source of overestimation.

The average active flux estimated during the non-bloom period (1.6 ± 0.4 mmolC m^{-2} d^{-1}) was of the same order of magnitude than the average value measured for gravitational flux in these waters and other subtropical regions considered (Table V.2). In the oceanic zone of the Canary Current, north of the Canaries, the average gravitational flux is 0.4 mmolC m^{-2} d^{-1} (Neuer et al. 2007, Helmke et al. 2010), whereas in Bermuda is 3.5 mmolC m^{-2} d^{-1} (Karl et al. 2001, Helmke et al. 2010), and 2.8 mmolC m^{-2} d^{-1} in Hawaii (Karl et al. 1996). Thus, our estimation of the active flux during the non-bloom period was higher than the gravitational flux in these waters (Canary Current), but slightly below the average gravitational flux in Hawaii and Bermuda. The average active flux including the bloom period (4.2 ± 3.4 mmolC m^{-2} d^{-1}) was above gravitational fluxes in these three oligotrophic locations (Table V.2), but especially in the Canary Current, where it was 10-fold greater than the average values of gravitational flux. Moreover, our average value during bloom conditions (6.7 ± 2.9 mmolC m^{-2} d^{-1}) was 5-fold greater than the highest value of gravitational flux (~1.3 mmolC m^{-2} d^{-1}) recorded in the Canary Current by Neuer et al. (2007), but in the same order than the highest records of gravitational flux observed in Bermuda and Hawaii (Karl et al. 2001) of about 5-6 mmolC m^{-2} d^{-1}.

To compare our estimations with other active flux measurements in subtropical waters, it has to be noted that these latter have been mainly obtained from migrant biomass and metabolic estimations, both accounting for a considerable level of uncertainty. Migrant biomass assessments often include only mesozooplankton organisms as it is usually calculated from the difference between night and day mesozooplankton stocks in the epipelagic zone. Regarding to metabolism, the effectiveness of the active carbon pump has been especially discussed in terms of the gut flux efficiency. Some authors suggested that given the time needed to migrate downward after feeding on surface, migrants would empty their guts before reaching the mesopelagic zone (Angel 1989, Longhurst & Glen Harrison 1989, Longhurst 1991). Actually, Dagg et al. (1989) found that only a 10% of pigments consumed during the day by migrant copepods remained at their guts when they reached deeper waters. On the other hand, low evacuation rates or longer gut residence times have been measured in diel migrant copepods compared to non-migrant species (Flint et al. 1991, Morales et al. 1993, Atkinson et al. 1996, 1999), being the time of the gut passage long enough for an effective downward transport. Moreover, gut clearance rates in micronekton were observed to be long enough for the downward migration to have been completed before evacuation occurs (Merrett & Roe 1974, Gorelova & Kobylyanskiy 1985, Angel 1989). In addition, faecal matter of mesopelagic fish show fast sinking rates (average of 1028 m d^{-1}), much higher than copepod or euphausiid faecal pellets (Robison & Bailey 1981). The latter authors also observed that the release of dissolved organic compounds is low and does not represent a significant output during sinking.

Location	Gravitational Flux (mmolC m^{-2} d^{-1})	References	Active Flux (mmolC m^{-2} d^{-1})	References
BATS	0.7 – 6.3	Karl et al., 2001	0.5 – 3.4 [a]	Dam et al., 1995
	1.3 – 2.1	Helmke et al., 2010	0.6 – 1.1 [a]	Zhang & Dam, 1997
			0.0 – 0.8 [a]	Steinberg et al., 2000
HOT	0.9 – 4.7	Karl et al., 1996	0.1 – 0.8 [a]	Al-Mutairi & Landry, 2001
Canary Current	0.4 – 0.7	Neuer et al., 2007	0.8 – 1.0 [a]	Hernández-León et al., 2001
	0.2 – 0.5	Helmke et al., 2010	1.9 [b]	Hernández-León et al., 2002
			2.9 [b]	Hernández-León et al., 2004
			1.6 – 6.3 [b]	Hernández-León et al., 2010
			0.2 – 0.4 [a]	Putzeys et al., 2011
			1.1 - 9.9	This work

[a] Estimations from metabolic calculations and migrant biomass (see *Discussion*).

[b] Estimations from mortality losses of the epipelagic zooplankton biomass.

Table V.2. Comparison between gravitational and active carbon fluxes (mmolC m^{-2} d^{-1}) in subtropical waters at the western north Atlantic gyre (BATS: Bermuda Atlantic Time Series Study), the eastern north Pacific gyre (HOT: Hawaii Ocean Time Series) and the eastern north Atlantic gyre (Canary Current).

The role of the large micronektonic fauna has been scarcely considered, giving rise to an important underestimation of the active flux that could partly explain the higher magnitude of our estimations (Table V.2). The average value obtained in this work (4.2 ± 3.4 mmolC m^{-2} d^{-1}) is 4 and 9-fold higher than the average active flux estimated from metabolic calculations in BATS (Dam et al. 1995b, Zhang & Dam 1997, Steinberg et al. 2000) and HOT (Al-Mutairi & Landry 2001), respectively, and 7-fold higher in these waters (Hernández-León et al. 2001b, Putzeys et al. 2011). On the other hand, our results are in agreement with parallel estimations of active flux obtained from measurements of migrant biota, both zooplankton and micronekton, within the same cruise in 2011 (Ariza, unpublished data). These authors obtained an average value of 0.8 mmolC m^{-2} d^{-1} comparable to the average value of 1.1 mmolC m^{-2} d^{-1} that we estimated in 2011. In any case, it is also important to note that none of these studies included all contributing fluxes (respiration, excretion and mortality) to total active flux, while the majority is based on the respiratory flux alone.

In summary, we showed that the contribution of the active flux to the downward transport of organic carbon produced in the euphotic layer could be of the same order or higher than the gravitational sinking in subtropical waters. As it has been suggested before (Steinberg et al. 2000), these results could shed some light on the uncoupling between primary production and the particle export flux in the ocean (Michaels et al. 1994, Karl et al. 1996). In addition, this active flux could explain the unaccounted downward organic flux promoting the carbon demands of bacteria and zooplankton in the mesopelagic zone (Steinberg et al. 2008). Thus, our results suggest a pivotal role of epipelagic zooplankton and DVMs in the downward carbon flux of paramount importance for the assessment of the role of the biological pump in the ocean. In any case, the lunar cycle-linked active flux described here for subtropical oligotrophic waters represents an important and unaccounted flux of carbon to the mesopelagic zone and deserves further research.

V. ACKNOWLEDGMENTS

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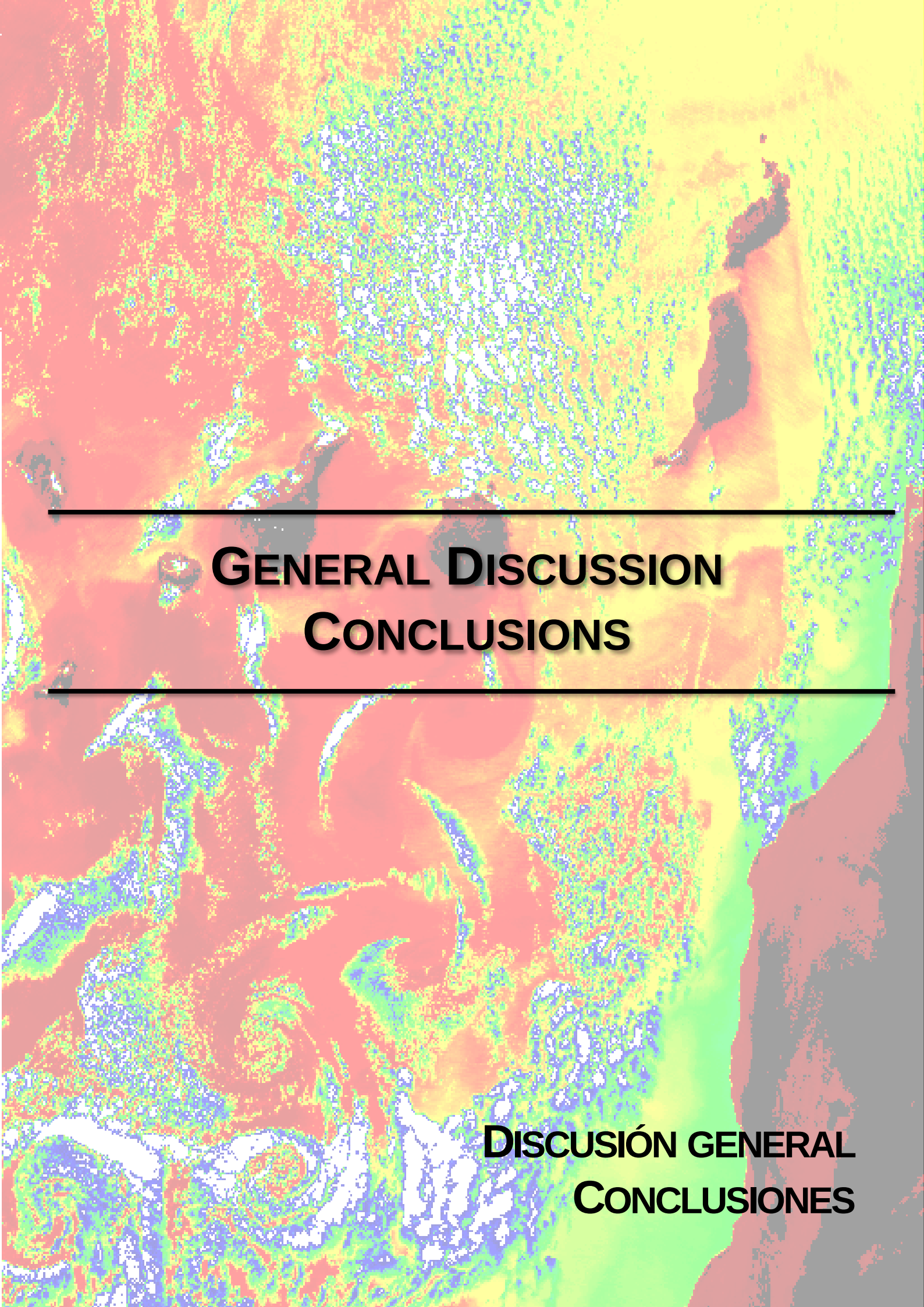

General Discussion Conclusions

Discusión general Conclusiones

I. GENERAL DISCUSSION

Characterization of the planktonic composition in subtropical waters of the Canary Islands was the first aim of this thesis besides the study of the short-term variability of the microbial components of trophic web. The results presented in Chapter I show that planktonic community was numerically dominated by small cells (< 2 µm), both autotrophic and heterotrophic, as expected in subtropical waters (Buck et al. 1996, Longhurst 1998). However, during the productive period diatoms substantially increased their contribution, dominating the autotrophic biomass.

Planktonic community dynamics were clearly controlled by bottom-up forces at a seasonal scale. The characteristic late winter bloom (De León & Braun 1973, Braun 1980, Arístegui et al. 2001) was observed from February to April 2011, when maximal chlorophyll *a* (Chl *a*) concentration and primary production rates matched the deepening of the mixed layer, as previously reported (Cianca et al. 2007). Actually, the abundance of pico- and nanoplankton was significantly correlated to average temperature in the mixed layer. Thus, *Synechococcus* and autotrophic picoeukaryotes increased their abundance and biomass during the bloom, when temperature dropped, while *Prochlorococcus* dominated during warmer and more stratified conditions of the mixed layer. This seasonality has already been described for these waters (Baltar et al. 2009b, Schmoker et al. 2012, Schmoker & Hernández-León 2013) and it is a common feature of subtropical gyres (Zubkov et al. 2000, DuRand et al. 2001, Giovannoni & Vergin 2012). The increase of diatoms during productive months has also been observed in the area by other authors (Ojeda 1998, Schmoker et al. 2014), although the substantial contribution and dominance observed in this work has not been previously reported. Furthermore, only when diatoms increased, autotrophic biomass was higher than heterotrophic biomass within the microbial community.

The large increase of diatom biomass was a striking result as higher temperatures than previous years (see Schmoker & Hernández-León 2013) would expect less mixing and nutrient availability and, thus, a less favorable scenario for these large algae. In fact, Chl *a* concentration in the mixed layer has dropped during the last years in this area, following the increase of temperature during the bloom period (Fig. 6). However, the warming of the mixed layer during 2010 and 2011 does not seem to negatively affect to large phytoplankton (Fig. 7). Moreover, recent research has contradicted the traditional view that diatoms are favored by mixing and high nutrient conditions (Kemp & Villareal 2013). These authors argue that diatom species involved in diazotrophic diatom symbioses could enhance primary production in stratified and oligotrophic waters.

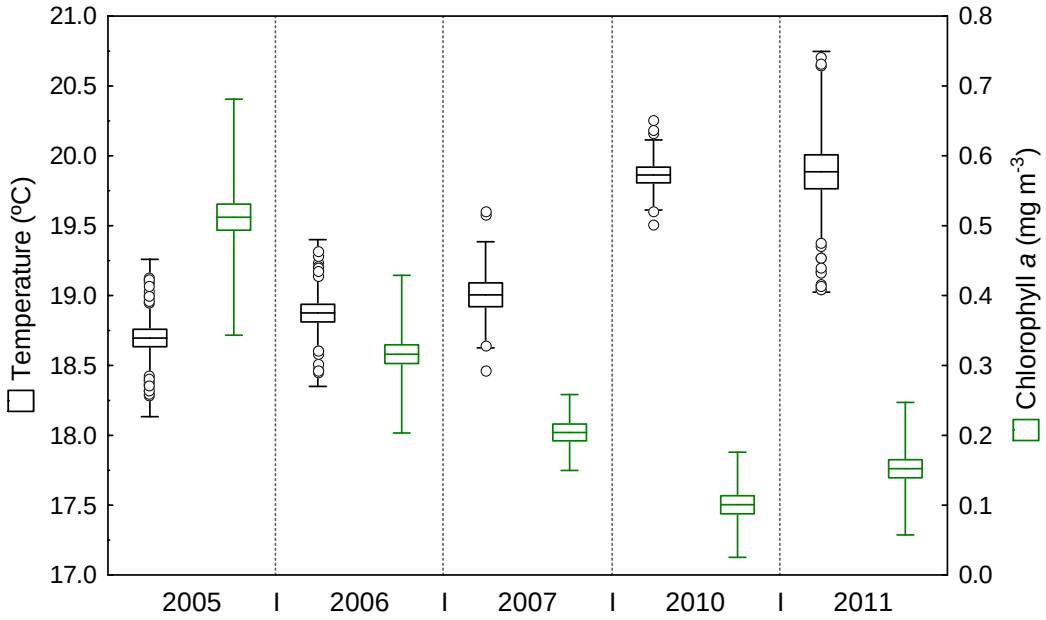

Figure 6. Average data on temperature (ºC) and chlorophyll *a* (mg m^{-3}) measured in the mixed layer during the bloom period (from January to April) in 2005, 2006, 2007, 2010 and 2011. Box and whisker account for standard error and deviation, respectively.

On the other hand, the dominance of *Chaetoceros* sp, responsible of the highest diatom biomass observed, could also be related to Saharan dust deposition in this area. In this sense, dust events could contribute to partly fertilize the mixed layer, and thereby enhancing the availability of nutrients due to the subsequent deep water input and thus favoring the extraordinary increase of diatoms. The results showed in Chapter II could partly support this point, as a significant increase in diatom biomass, also due to *Chaetoceros* sp, was measured after a Saharan dust storm.

Additionally, productivity rates were quite high not only within the more productive months, but during the whole period studied. This fact could also point out the existence of short-scale events like Saharan dust deposition events that could enhance primary productivity in the mixed layer during stratified conditions. However, we did not observed a measurable response of productivity during the study of the Saharan dust deposition effect in Chapter II.

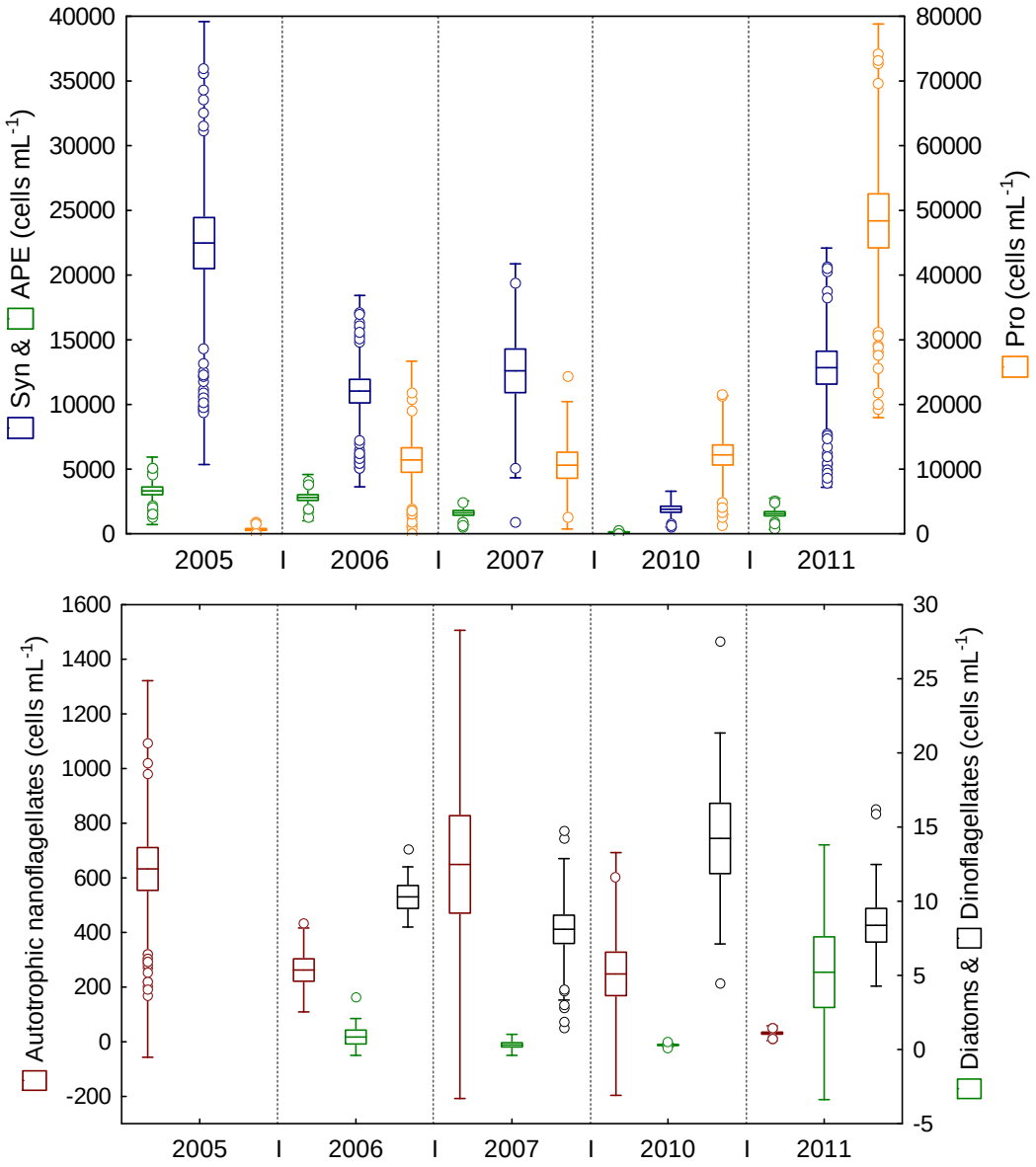

Figure 7. Average data on phytoplankton abundance (cells mL^{-1}) in the mixed layer during the bloom period (from January to April) in 2005, 2006, 2007, 2010 and 2011. (7A) Picophytoplankton including *Synechococcus* (Syn), *Prochlorococcus* (Pro) and picoeukaryotes (APE). (7B) Nanophytoplankton (Autotrophic nanoflagellates) and microphytoplankton (Diatoms and Dinoflagellates). Box and whisker account for standard error and deviation, respectively.

The variability of the different planktonic groups at a short-time scale seemed to be controlled by trophic interactions. Heterotrophic bacteria and *Synechococcus* could be consumed by heterotrohic nanoflagellates as a clear inverse pattern was observed between them. Flagellates are known to be effective bacterivores (Azam et al. 1983) and not only upon bacteria, but also upon cyanobacteria. This relationship has also been reported in these waters (Arístegui & Montero 2005). An inverse relationship between autotrophic picoeukaryotes and nano- and dinoflagellates was also observed, suggesting that the latter would be grazing upon autotrophic pico- and nanoplankton. These results are supported by the outcome of Chapter III, where the role of potential consumers and preys within the microbial community was estimated.

Quantification of the potential effect of the Saharan dust deposition over the planktonic community was the second objective of this thesis addressed in Chapter II. The period studied for this aim, from February to June 2010, was one of the most intense dust periods during the last years. Saharan dust events were identified in every month from data on total suspended matter. However, the effect over Chl *a* and primary production was negligible. Within the planktonic community different responses were observed: diatoms and mesozooplankton showed an increase in biomass, while picophytoplankton seemed to be negatively affected.

Unusual hydrographic and biological properties were observed from 2010 data. The characteristic late winter bloom (De León & Braun 1973, Braun 1980, Arístegui et al. 2001) did not take place as observed from high stratification conditions and low phytoplankton abundance and biomass. Heterotrophs also showed low biomass values, but they dominated during the whole period.

The strong water column stratification besides the frequent dust events suggested that the major source of nutrients to the surface waters in this area during the period studied was the atmospheric deposition of Saharan dust. The intense dust deposition probably promoted the high nutrient concentrations in the mixed layer (Benavides et al. 2013), which were higher than previous measurements near the area (Marañón et al. 2000, 2003, Neuer et al. 2007).

The effect of Saharan dust deposition was assessed through the change measured in the different components of the planktonic community after the highest dust event on March 18. The negligible change in Chl *a* is consistent with other experimental results when Saharan dust was added to oligotrophic waters (Bonnet et al. 2005, Marañón et al. 2010). However, contrary to our results, a substantial increase in primary productivity was measured in those experiments. In the field, some authors (Herut et al. 2005) have observed a minor phytoplankton response after a dust storm and they argue that the likely low nutrient release during an atmospheric event would promote this low increase of Chl *a*. Other authors sustain that despite the enhancement of primary productivity after an artificial or natural fertilization, phytoplankton stocks do not increase because of the control

exerted by grazers (Landry et al. 2000a, Landry et al. 2000b, De Baar et al. 2005, Herut et al. 2005, Boyd et al. 2007, Henjes et al. 2007, Marañón et al. 2010). This could explain the low values of phytoplankton in contrast to the high nutrient concentration, and agree with the high grazing rates measured in Chapter III.

Diatoms were the only autotrophic group that was positively affected, showing an increment of more than 1000% in biomass. This is a common feature in artificial fertilization experiments (De Baar et al. 2005, Boyd et al. 2007) because of the higher growth rates of diatoms compared to prokaryotic algae during the release of iron (Landry et al. 2000a), and the lower grazing pressure upon the former (Landry et al. 2000a, Landry et al. 2000b, Henjes et al. 2007). Actually, autotrophic picoeukaryotes and *Synechococcus* showed a negative response probably related to a high grazing pressure as an increment in potential grazers was observed. Thus, it is not surprising that small algae would be under a higher grazing pressure than diatoms, these latter favored by either the fertilization effect as well as lower mortality by grazing. Mesozooplankton also showed a high positive change in biomass, increasing over a 100%. Other authors have previously described this increase in mesozooplankton stock and their metabolic activity after a Saharan dust storm in the Canary Islands waters (Hernández-León et al. 2004).

The third objective of this work was to estimate the microzooplankton feeding impact on the planktonic community. For that, we presented the results obtained from dilution experiments carried out during 2010 and 2011 in Chapter III. The impact measured was unequal depending on the microbial group considered. Larger and faster-growing cells were under higher grazing pressure than smaller cells that showed lower growth rates. Thus, microzooplankton consumption was closely coupled to the growth of their prey. The impact over the heterotrophic community was also considerable and prevalent along experiments. Both autotrophic and heterotrophic daily production was totally removed by the microzooplankton feeding impact, except for cyanobacteria.

Grazing rates measured from Chl *a* concentration alone gave an elevated number of non-significant results, despite significant grazing rates were found for the same experiments when separate autotrophic groups were considered. Therefore, a detailed analysis of every autotrophic group is needed to properly quantify the actual impact of microzooplankton on primary production.

An increase in the abundance of potential consumers (heterotrophic nanoflagellates, dinoflagellates and ciliates) in highly diluted samples was found in several experiments. This point out the possible existence of trophic cascades, so that all consumers would be under predator pressure which would be released in the more diluted treatments. Recently, other authors (Calbet & Saiz 2013) have demonstrated the existence of trophic cascades in dilution experiments performed in subtropical ecosystems, given the complexity of the trophic web. Actually, heterotrophic consumption was prevalent among experiments and high feeding rates were measured over heterotrophic bacteria

and nanoflagellates and ciliates. Heterotrophic nanoflagellates are known to be effective bacterivores (Azam et al. 1983, Guillou et al. 2001, Sherr & Sherr 2002), which in turn, would be preyed by ciliates (Azam et al. 1983, Sherr & Sherr 2002, Pomeroy et al. 2007). The latter could be consumed by copepod nauplii, copepodites or even by heterotrophic dinoglagellates (Sherr & Sherr 2002).

We did not find any relationship between growth or mortality rates and temperature, Chl *a* or community composition, probably because of the extremely low variability of these parameters. Heterotrophic prokaryotes and *Prochlorococcus* were the most abundant prey and heterotrophic nanoflagellates were the dominant consumers during the whole period. This is a common feature in oligotrophic systems where nanograzers are the major consumers of primary production (Calbet 2008). Grazing rates were only correlated to the growth rate of prey, indicating the coupling between consumers and producers. This close relationship between phytoplankton growth and microzooplankton grazing has been generally observed in the ocean (Schmoker et al. 2013) and also in the area (Quevedo & Anadón 2001). Our results showed a higher grazing impact on faster-growing cells, which were autotrophic nanoflagellates and diatoms. This was an unexpected result as small cells generally support a higher grazing pressure than larger autotrophs (Strom et al. 2007, Calbet et al. 2008, Gutiérrez-Rodríguez et al. 2011, Teixeira et al. 2011). However, this was also observed in a recent study in the northeast Atlantic (Cáceres et al. 2013), in which larger phytoplankton cells showed higher growth and mortality rates.

Within the aim of assessing the role of microzooplankton in controlling microbial communities we propose an alternative methodological approach evaluated in Chapter IV. We discriminated between dilution, 2-point and Frost methods to measure microzooplankton grazing and we proposed a different approach based on the latter. Taking bibliography and our experimental data we found a high correlation between mortality rates obtained through the dilution and the Frost approach proposed.

The dilution technique (Landry & Hassett 1982) is the most widespread method to measure microzooplankton grazing. This method is based on the assumption that the encounters rates between grazers and their prey diminishes proportionally to the dilution of the natural sample. Thus, a series of dilutions is performed to measure the increment of phytoplankton apparent growth as dilution increase. However, the dilution technique is tedious to carry out as large volumes of water are required and an elevated number of replicates is needed for statistical confidence. Furthermore, results obtained through this method are sometimes difficult to interpret when non-linear responses are observed. A simplification of this procedure is the 2-point method in which the natural seawater and one diluted sample are used. Conversely, the method of Frost (1972) is based on independent measurements from natural seawater and a highly diluted sample to estimate grazing. This last

approach has the advantage of the 2-point method for the oceanographic work as several measurements could be done per day, but makes the use of only one dilution level conceptually robust.

The combination of the Frost approach with the time-series experiments performed provided us the actual change in growth and grazing rates at short intervals. Rates were quite variable at the beginning, decreasing thereafter and slightly increasing again during night. Furthermore, the highest grazing rates were observed after only 4 h and became almost zero after 18 h. This high variability of grazing rates seems a fingerprint to think about the variability observed in replicates of the dilution method and the suitability of large (24 h) incubations.

In Chapter V we evaluated the magnitude of the zooplankton predatory cycle linked to the lunar illumination, previously observed in the area, and its potential contribution to the carbon transport to the mesopelagic realm. Mesozooplankton variability was related to predation by diel vertical migrants (DVMs), a process influenced by lunar illumination. Throughout the simulation of this predatory cycle we obtained mesozooplankton mortality values. From these estimates we calculated potential values of active carbon export which were in the same order than the gravitational flux in subtropical waters.

Mesozooplankton variability showed a lunar cycle pattern, so that biomass was higher during the illuminated phases of the moon (2^{nd} and 3^{rd} quarters), as it has been previously observed (Hernández-León et al. 2002, 2004). This relationship between zooplankton and the lunar cycle has been repeatedly observed in these waters (Hernández-León 1998, Hernández-León et al. 2001a, 2002, 2004, 2010) linked to predation by DVMs. The epipelagic mesozooplankton supports a large predation pressure by these migrant organisms that reach the shallower layers at night. However, migrants do not reach the upper layer during the full moon, the period of maximum illumination, in order to avoid predation. Conversely, during the new moon DVMs occupy the first hundred meters of the water column and feed on the epipelagic mesozooplankton. Thus, the epipelagic mesozooplankton grow without this high predator pressure during the illuminated phase of the moon, but their biomass drops during new moon (Hernández-León 1998, Hernández-León et al. 2001a, 2002, 2004, 2010).

In order to estimate the magnitude of the consumption by DMVs we performed a simple model to calculate the daily community mortality of epipelagic mesozooplankton. To simulate the variability of the zooplankton biomass we set the mortality rate as a function of the lunar illumination. As mortality is assumed to be always maximal with minimum moonlight conditions (new moon) and vice versa, the timing of biomass maxima is determined by the variability of maximum growth. The growth rate was also set as a function of the lunar illumination to obtain the best correlation between real and simulated values. We hypothesize that the influence of the lunar illumination over the growth rate of

zooplankton could be the result of a lunar rhythm in the reproductive cycle of these organisms as it has been observed for deep-sea species of invertebrates (Mercier et al. 2011). Moreover, potential growth rates of the epipelagic mesozooplankton, measured as enzymatic AARS activity (Herrera, unpublished data), followed the lunar pattern described in this work during 2010 and 2011.

Variability of daily community mortality was considerably higher during bloom compare to non-bloom condition as a result of higher biomass and mortality rates within more productive periods. This is in agreement with other works that observed higher values of carbon transport by DVMs in more productive waters (Yebra et al. 2005, Putzeys 2013). The mean community mortality was assumed to be the average active carbon flux, considering that the former is mainly induced by consumption of DVMs. Thus, average active flux during the productive period was in the same order of magnitude than the average values measured for gravitational flux in these waters and other subtropical regions (Bermuda and Hawaii).

II. CONCLUSIONS

The conclusions drawn from this thesis are the following:

5) The oligotrophic waters of the Canary Current System were dominated by picoplankton during the period studied, especially *Prochlorococcus* and heterotrophic prokaryotes. However, during productive periods *Synechococcus* and picoeukaryotes dominated the picophytoplankton fraction, while diatoms became the major contributors to total autotrophic biomass. Variability of the planktonic community was driven by bottom-up forces at seasonal scale, but other short-scale events such as Saharan dust deposition could be enhancing the primary productivity in the mixed layer even during stratified periods. Short-term variability would also be driven by top-down forces through feeding and grazing by microzooplankton.

6) The Canary Islands waters were affected by the Saharan dust deposition during the whole period studied. The effect on primary production was not significant, although short-term variability seemed to be related to the Saharan dust events. Among planktonic groups a different response was found. On one hand, diatoms substantially increased their biomass, and mesozooplankton also showed an increment in biomass. On the other hand, picophytoplankton seemed to be negatively affected, probably caused by grazing losses. Hence, the Saharan dust deposition would be partly fuelling primary producers in these waters, especially during stratified periods, but the increase in productivity could not be detected as primary producers would be rapidly consumed.

7) The impact of microzooplankton feeding on the microbial community was different depending on the group. In general, microzooplankton consumption was closely coupled to the growth of their prey, for both autotrophic and heterotrophic organisms. Thus, the highest grazing rates were upon larger and faster-growing cells (autotrophic nanoflagellates and diatoms), whilst smaller cells (*Synechococcus* and autotrophic picoeukaryotes) were grazed at lower rates. Our results point out that measurements based on Chl *a* concentration alone could be a great source of error in oligotrophic ecosystems. Furthermore, the impact of grazers on primary production would be influenced by heterotrophic consumption that was prevalent in this study. Thus, the complexity of the trophic web in these subtropical waters makes more difficult to measure the actual impact of microzooplankton on the different microbial components. Therefore, the feeding impact on heterotrophic organisms should also be quantified to better understand the relationship between the different components of the food web.

8) The Frost approach proposed to measure microzooplankton feeding impact is comparable to dilution or 2-point methods, but experimentally simple and conceptually robust. The time-series approach presented here based on the concept of the Frost approach provide a better understanding of interactions between grazers and their prey in the ocean. Furthermore, this method could be fully automated using flow cytometry and image processing. Thus, the method should be suitable for oceanographic work as several measurements could be made per day.

9) The zooplankton lunar cycle linked to predation of diel vertical migrants seems to be a regular top-down process during both productive and non-productive periods in the Canary Islands waters. Daily community mortality promoted by this predatory cycle supposes that the active carbon export could be of the same order or higher than the gravitational sinking in subtropical waters. Our results could shed some light on the uncoupling between primary production and the particle export flux in the ocean. They could also explain the unaccounted downward organic flux promoting the carbon demands of bacteria and zooplankton in the mesopelagic zone. Thus, the pivotal role of epipelagic zooplankton and diel vertical migrants in the downward carbon flux is of paramount importance for the assessment of the magnitude of the biological pump in the ocean.

III. FUTURE RESEARCH

The conclusions based on the results obtained in this work highlight the need for a greater research effort in the following fields:

There is a need to carry out oceanographic samplings at short-time scales in order to account for the total variability of the planktonic communities in subtropical waters, given the complexity and quickness of the biological interactions in these waters. As an example, we emphasize the observed and unexpected role of diatoms in the Canary Islands waters, which could be determinant in terms of carbon export and, therefore, needs further research. In this sense, an intensive temporal sampling would also help to properly quantify the influence of the Saharan dust deposition in the subtropical northeast Atlantic. Thus, the timing and intensity of the response of the different planktonic groups could be better assessed.

A greater effort is especially needed in subtropical gyres in order to know the actual impact of microzooplankton on primary production. However, it is essential to quantify the impact of microzooplankton over the different planktonic components, including heterotrophs, to better understand the complex trophic interactions within the microbial community. Furthermore, time-course experiments based on the Frost concept would provide more detailed results of changes in community composition during incubation, besides this method could be automated in the future using flow cytometry and image processing.

The lunar cycle-linked active flux described here for subtropical oligotrophic waters represents an important and unaccounted flux of carbon to the mesopelagic zone and deserves further research.

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RESUMEN ESPAÑOL

SPANISH SUMMARY

I. INTRODUCCIÓN

I.1. CONCEPTOS BÁSICOS SOBRE EL PLANCTON

El término plancton proviene de la palabra griega “planktos”, que significa errante, aludiendo a la limitada capacidad natatoria de los organismos que pertenecen a esta comunidad. Es un grupo heterogéneo de organismos conformado por una amplia variedad de tamaños, morfologías y modos de alimentación, por lo que se hace necesaria su clasificación en diferentes grupos. La clasificación más extendida es la de Sieburth et al. (1978) que divide a los organismos planctónicos en diferentes categorías según su tamaño y tipo de alimentación (Fig. E.1). De esta forma, se distinguen cuatro categorías por tamaño: picoplancton (0.2-2 μm), nanoplancton (2-20 μm), microplancton (20-200 μm) y mesoplancton (200-20000 μm). Fuera de esta clasificación, se utilizan con frecuencia otras dos categorías de tamaño menor y mayor: femtoplancton (<0.2 μm) y macroplancton (>20 mm). Dentro de la clasificación de Sieburth et al. (1978) se distinguen dos modos de alimentación: autotrófica y heterotrófica. Los organismos autotróficos, a los que se denomina fitoplancton, obtienen sus requerimientos energéticos a través de la fotosíntesis, mientras que los heterótrofos, conocidos como zooplancton, se alimentan de otros organismos (autótrofos o heterótrofos) para cubrir sus necesidades metabólicas. También existen organismos que son capaces de usar ambos tipos de alimentación denominados mixótrofos, es decir, que pueden fotosintetizar materia orgánica pero también pueden ingerir otras presas.

Dentro del picoplancton se incluyen los picoeucariotas autotróficos, dos tipos de cianobacterias (*Prochloroccocus* y *Synechoccocus*) y las bacterias heterotróficas. El nanoplancton se compone principalmente de flagelados unicelulares, tanto autotróficos como heterotróficos. Con respecto al microfitoplancton, los componentes principales son diatomeas, dinoflagelados y cocolitofóridos, aunque algunas especies de dinoflagelados pueden funcionar como mixótrofos (Sherr & Sherr 2002). Dentro del microzooplancton el grupo mayoritario está formado por protozoos: ciliados, foraminíferos, radiolarios y acantáridos; aunque también lo conforman metazoos como nauplios de copépodos, copepoditos o larvas meroplanctónicas. El mesozooplancton está formado

generalmente por heterótrofos, aunque algunas células autotróficas como dinoflagelados de gran tamaño o cadenas de diatomeas pueden alcanzar tamaños mayores de 200 µm. El grupo principal dentro del mesozooplancton es el de los copépodos, aunque otros componentes importantes son pterópodos, salpas, quetognatos, cnidarios o larvas de peces.

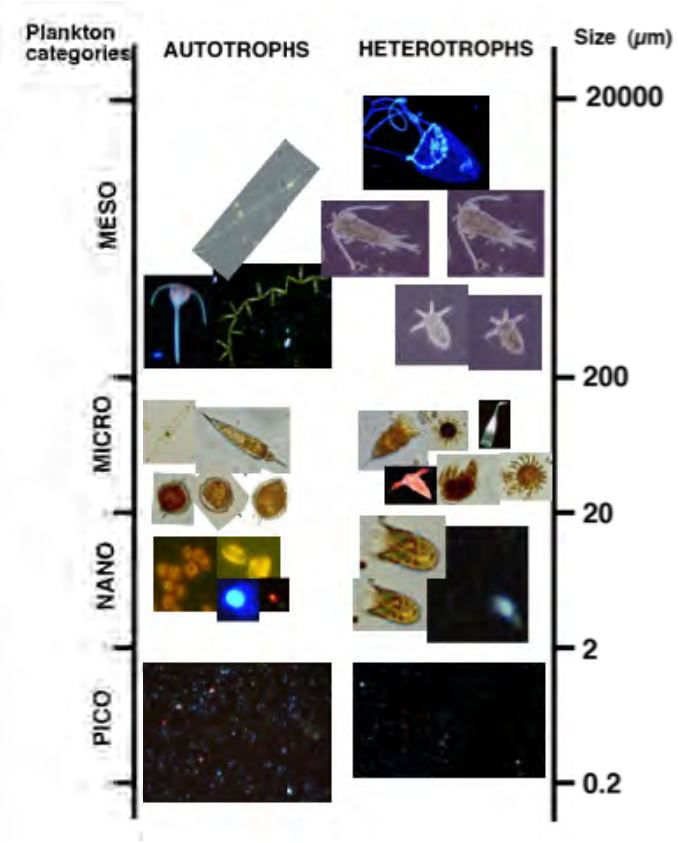

Figure E.1. Clasificación del plancton según su tamaño y modo de alimentación según Schmoker (2011), modificada a partir de Sieburth et al. (1978).

I.2. SISTEMA DE LA CORRIENTE DE CANARIAS

El sistema de la Corriente de Canarias forma parte del giro subtropical del Atlántico Norte, siendo la corriente situada al este de dicho giro y que fluye de norte a sur, encontrándose con las Islas Canarias a su paso (Fig. E.2). Este sistema se caracteriza por la presencia de aguas de carácter oligotrófico en la zona más oceánica (De León & Braun 1973, Braun 1980), mientras que más al este se encuentran aguas mucho más productivas producto del afloramiento costero africano (Barton et al. 1998). Al sur de las Islas Canarias se producen de forma periódica remolinos ciclónicos y anticiclónicos que se forman por la interacción de la corriente con las islas (Arístegui et al. 1994, Barton et al. 2004), que contribuyen a aumentar la productividad de las aguas oceánicas.

En el área oceánica, fuera de la influencia del afloramiento africano y los remolinos mesoescalares, se produce una fuerte estratificación de la columna de agua durante la mayor parte del año (De León & Braun 1973, Braun 1980, Barton et al. 1998). De hecho, la fuerte termoclina estacional sólo permite el paso de las aguas más profundas y ricas en nutrientes entre finales del invierno y principios de la primavera (Febrero-Marzo), cuando se erosiona a causa de la convección invernal por el enfriamiento de la superficie marina y se alcanza la máxima profundidad de la capa de mezcla (Cianca et al. 2007). En ese período se alcanzan los valores más altos de las tasas de producción primaria y de la biomasa de los productores primarios (Clorofila *a*) en las aguas superficiales (De León & Braun 1973, Braun 1980, Arístegui et al. 2001), y se conoce como el bloom de finales de invierno (“late winter bloom”). Durante el “late winter bloom”, al aumento del crecimiento del fitoplancton le sigue un incremento en la biomasa del mesozooplancton (Arístegui et al. 2001, Hernández-León et al. 2004, Hernández-León et al. 2007).

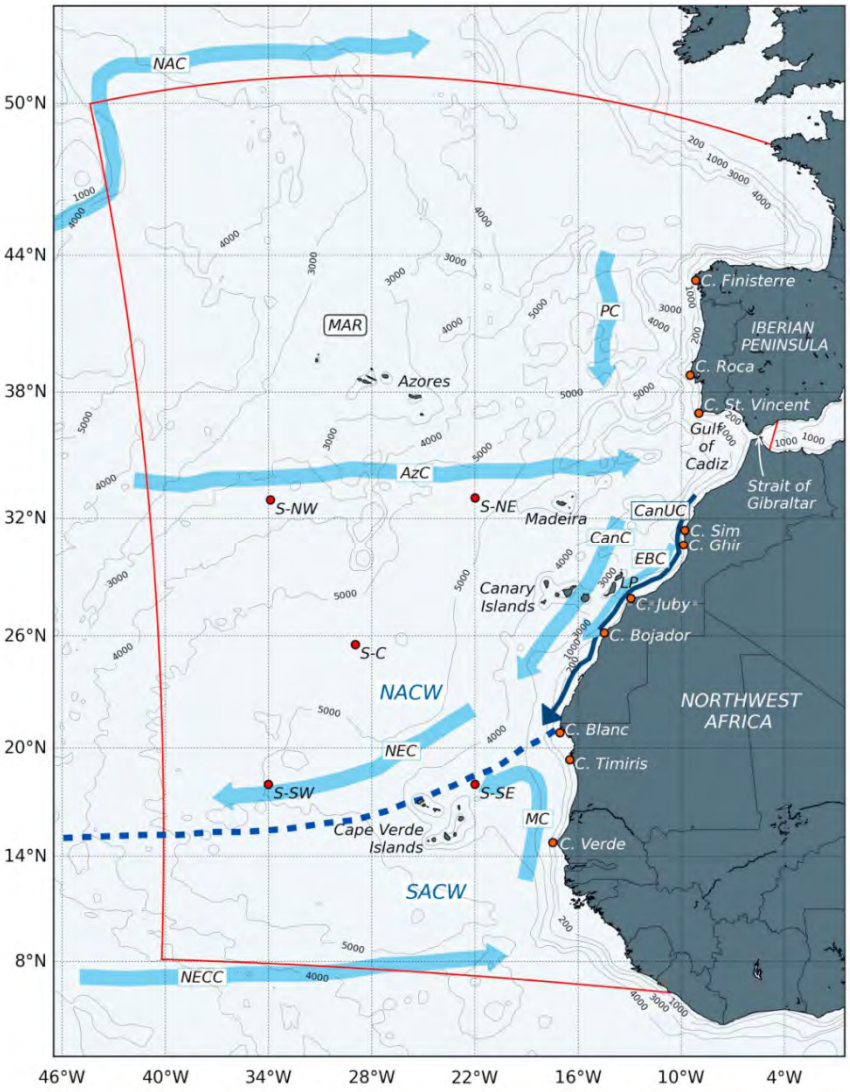

Figure E.2. Esquema de la circulación superficial en la Cuenca Canaria dibujado por Mason et al. (2011). AzC, Corriente de Azores; CanC, Corriente de Canarias; CanUC, Corriente de Afloramiento de Canarias; EBC, Corriente de Frontera Este; MC, Corriente de Mauritania; NEC, Corriente Nor-Ecuatorial; NECC, Contracorriente Nor-Ecuatorial; PC, Corriente de Portugal; MAR, Dorsal del Atlántico; LP, Pasaje de Lanzarote.

I.3. COMPOSICIÓN PLANCTÓNICA Y VARIABILIDAD EN AGUAS SUBTROPICALES DE LAS ISLAS CANARIAS

Una de las características de los giros subtropicales es que la producción primaria se sostiene principalmente a través del reciclaje de nutrientes en la capa fótica, donde el flujo de materia y energía está controlado por el bucle microbiano (Pomeroy 1974, Azam et al. 1983, Longhurst 1998). Este bucle consiste en que las bacterias y los pequeños autótrofos (<2 µm) son consumidos por los organismos heterótrofos del nano- y microplancton, que a su vez excretan materia orgánica disuelta utilizada por los primeros para sus requerimientos metabólicos (Fig. E.3). Esto es lo que sucede en las aguas oligotróficas del Sistema de la Corriente de Canarias donde el fitoplancton está compuesto por picoplancton autotrófico, que es consumido en gran medida por los "grazers" del microplancton (nano- y microheterótrofos) que controlan más de un 80% de la producción primaria en estas aguas (Arístegui et al. 2001, Marañón et al. 2007).

En los giros subtropicales se conoce poco cuál es la variabilidad estacional de la composición planctónica o el rol de los diferentes grupos planctónicos a lo largo del año. Lo mismo ocurre en el Atlántico Nororiental, donde sólo existen, según nuestro conocimiento, dos estudios detallados sobre la variabilidad temporal de la composición planctónica en las Islas Canarias (Schmoker et al. 2012, Schmoker & Hernández-León 2013). Estos autores han descrito un patrón estacional dentro del picoplancton autótrofo que consiste en la dominancia de las células eucariotas y *Synechococcus* durante el período de invierno-primavera, mientras que *Prochlorococcus* alcanza los valores máximos de abundancia en verano-otoño. Esta variabilidad estacional también se ha encontrado en otras zonas de los giros subtropicales: al noroeste del Atlántico y en el norte del Pacífico (DuRand et al. 2001, Giovannoni & Vergin 2012). Por el contrario, no se ha observado ningún patrón estacional en las bacterias heterotróficas en el noreste Atlántico (Schmoker & Hernández-León 2013), mientras que al noroeste se ha visto que la biomasa de bacterias alcanza los niveles más altos tras el período productivo de invierno-primavera (Steinberg et al. 2001).

Dentro del nano- y microplancton autótrofo también se ha descrito un incremento estacional en las Islas Canarias durante el período productivo (Schmoker et al. 2012, Schmoker & Hernández-León 2013). Sin embargo, el patrón estacional no está claro para todos los grupos del microplancton ya que, por ejemplo, en el caso de las diatomeas, se produce un incremento ocasional en invierno-primavera o en verano. Por último, la comunidad mesoplanctónica también aumenta su biomasa durante la estación productiva (Hernández-León et al. 2004, 2007).

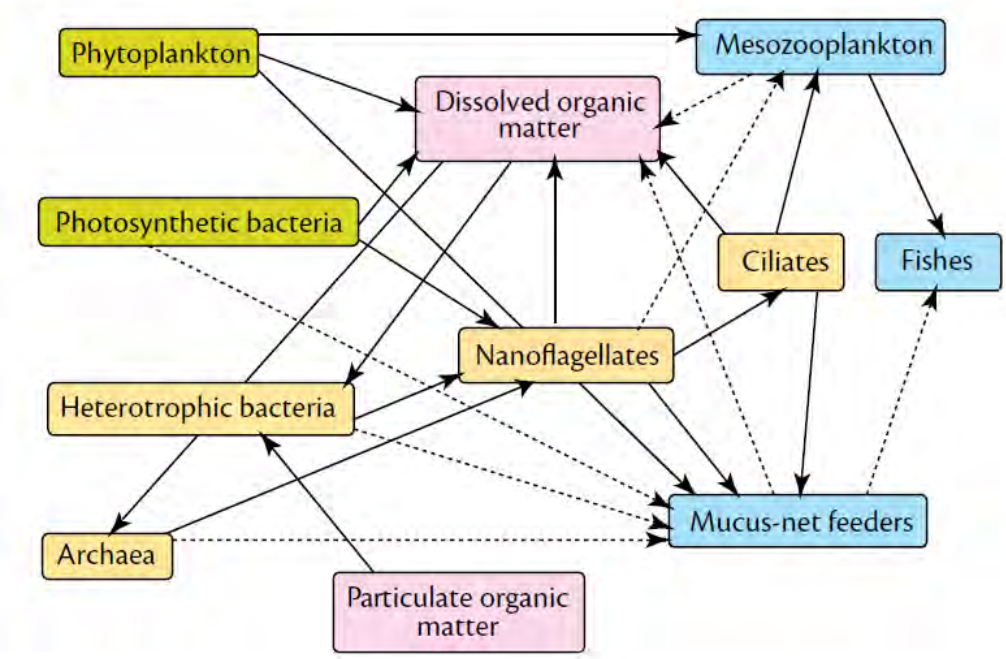

Figure E.3. Diagrama de Pomeroy et al. (2007) que muestra el papel principal de los componentes microbianos (cajas verdes y amarillas) en la red trófica. Los flujos principales de energía y materia se representan con líneas continuas.

I.4. PROCESOS "TOP-DOWN" QUE REGULAN LA COMUNIDAD PLANCTÓNICA

La variabilidad planctónica en las áreas subtropicales no se rige únicamente por factores físicos (luz, temperatura, estratificación, etc.) o por la disponibilidad de nutrientes, que constituyen los llamados procesos "bottom-up"; sino que también las fuerzas "top-down", la depredación y el "grazing", juegan un papel importante en el control de las comunidades planctónicas.

El nano- y microzooplancton (organismos heterotróficos de tamaño entre 2-200 µm, en lo sucesivo microzooplancton) conforman un grupo clave en aguas tropicales y subtropicales, donde consumen entre un 72-76% de la producción primaria diaria (Calbet & Landry 2004). Además, también es común que el microzooplancton consuma organismos heterótrofos, especialmente en zonas subtropicales, donde la red trófica es compleja (Calbet & Saiz 2013). Los nanoflagelados heterotróficos son consumidores efectivos de bacterias, y a su vez son depredados por los ciliados (Azam et al. 1983, Guillou et al. 2001, Sherr & Sherr 2002, Pomeroy et al. 2007). Pero a pesar de su importancia, el impacto del consumo del microzooplancton en la comunidad microbiana se conoce de forma limitada en los giros subtropicales (Schmoker et al. 2013). La medición directa es prácticamente inexistente en las aguas subtropicales de la Corriente de Canarias, y sólo algunos estudios al sureste de las Azores (Gaul et al. 1999, Stelfox-Widdicombe et al. 2000, Quevedo & Anadón 2001) y al sur de las Canarias (Gutiérrez-Rodríguez et al. 2011) han medido este impacto sobre la producción primaria. Los valores obtenidos son muy variables según estos estudios, que calculan que entre el 37 y más del 100% de la producción diaria es consumida por el microzooplancton. Además, las diferencias potenciales sobre los diversos grupos de la comunidad autotrófica sólo se han evaluado en dos estudios: Quevedo & Anadon (2001) y Gutiérrez-Rodríguez et al. (2011).

Por otro lado, el microzooplancton se ve afectado por la presión de depredación que ejerce el mesozooplancton. De hecho, la dieta diaria de los copépodos está constituida hasta en un 50% por microzooplancton en aguas oligotróficas (Calbet 2008). Por su parte, el mesozooplancton también está controlado por la depredación. En aguas de las Islas Canarias, el mesozooplancton

epipelágico (los organismos no migradores que habitan en la parte superior de la columna de agua) soporta una gran presión de depredación por parte de los migradores verticales diarios (DVMs) (Hernández-León 1998, Hernández-León et al. 2001a, 2002, 2004, 2010). Estos organismos migradores, formados por zooplancton y micronecton, utilizan la migración vertical como estrategia para evitar a sus depredadores (Stich & Lampert 1981, Ringelberg 2010). De esta forma, se mantienen en aguas más profundas y poco iluminadas, entre 200 y 1000 metros, durante el día, y alcanzan las capas más superficiales de la columna de agua durante la noche (Moore 1950, Uda 1956, Angel 1989, Longhurst et al. 1989), cuando son menos visibles para sus depredadores (Fig. E.4).

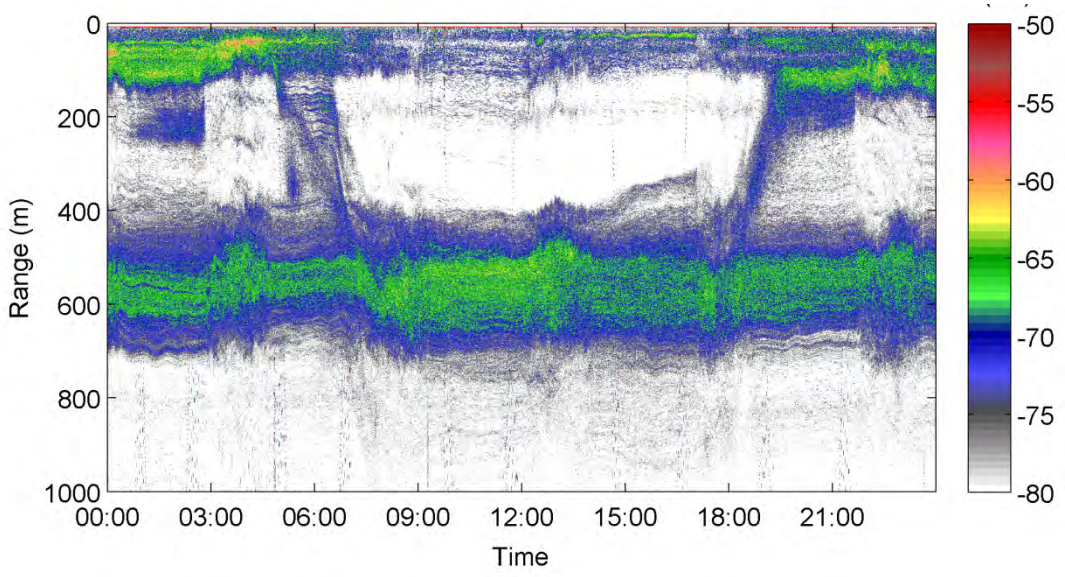

Figure E.4. Migración vertical diaria registrada en un ecograma (imagen de A. Ariza). Los migradores descienden desde la superficie hacia la capa de reflexión profunda (600 m) al amanecer (06:00 h), y se mueven hacia las aguas más superficiales al atardecer (18:00 h).

La magnitud de este proceso está en parte determinada por la iluminación lunar (Uda 1956, Benoit-Bird et al. 2009), ya que estos animales no alcanzan las aguas superficiales durante el período de máxima iluminación, la luna llena, para evitar la depredación. De hecho, la influencia de la iluminación lunar en el comportamiento de los DVMs se ha propuesto como el mecanismo que explica la variabilidad de la biomasa de mesozooplancton que se ha observado en aguas de las Islas Canarias (Hernández-León 1998, Hernández-León et al. 2001a, 2002, 2004, 2010). Además, este ciclo de depredación no sólo afectaría a la dinámica planctónica de la zona epipelágica, sino que contribuiría de forma significativa al transporte de carbono hacia la zona mesopelágica (Longhurst & Harrison 1988, Longhurst et al. 1989).

I.5. EL PAPEL DE LA DEPOSICIÓN DE POLVO SAHARIANO

Las Islas Canarias se sitúan en la zona oriental del giro subtropical del Atlántico norte, cerca del desierto del Sáhara, que constituye la fuente más importante de polvo atmosférico hacia los océanos (Goudie & Middleton 2001). El polvo sahariano contiene altas concentraciones de elementos como silicio, aluminio, manganeso o hierro (Goudie & Middleton 2001, Viana et al. 2002), así como nitratos y fosfatos (Bonnet et al. 2005, Herut et al. 2005, Duarte et al. 2006, Pulido-Villena et al. 2010). Por ello, la deposición de polvo atmosférico es una de las mayores fuentes de entrada de nutrientes en océano abierto (Donaghay et al. 1991, Duce & Tindale 1991, Guerzoni et al. 1999, Jickells 1999). De hecho, se ha visto que la adición experimental de polvo sahariano, que libera hierro, fosfato, nitrato y silicato, resulta en la estimulación de la productividad primaria en aguas de bajo contenido en nutrientes y clorofila (Bonnet et al. 2005, Herut et al. 2005, Duarte et al. 2006, Marañón et al. 2010, Ridame et al. 2014). Por tanto, la gran frecuencia de eventos de deposición de polvo sahariano que llegan a las aguas estratificadas de las Islas Canarias (Sarthou et al. 2007) podría suponer un gran aporte de nutrientes que afectaría a la producción primaria. Sin embargo, el efecto de estos eventos de deposición atmosférica no está claro ya que no existen muchos estudios que hayan medido en detalle directamente en el medio.

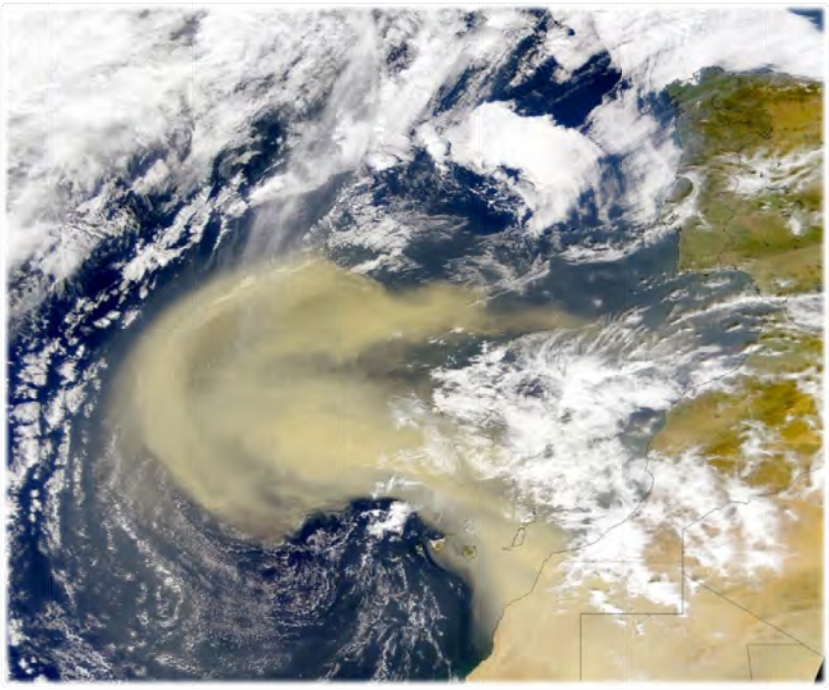

Figure E.5. Imagen de satélite de una tormenta de polvo del Sáhara sobre las Islas Canarias el 26 de Febrero del 2000 (imagen proporcionada por el proyecto SeaWIFS de la NASA/GSFC).

II. OBJETIVOS

El objetivo principal de este trabajo es caracterizar de una forma más detallada el funcionamiento de las comunidades planctónicas del sistema oligotrófico de la Corriente de Canarias, con especial énfasis en la variabilidad temporal de los diferentes grupos que constituyen este ecosistema. Además, se pretende determinar de qué forma los procesos "top-down" que ocurren en estas aguas regulan dicha variabilidad. Para ello, este estudio se desglosa en los siguientes objetivos específicos que constituyen, cada uno de ellos, un capítulo de esta tesis:

1) Caracterizar en mayor detalle la composición de las comunidades planctónicas en aguas subtropicales de las Islas Canarias y estudiar la variabilidad a corto plazo de los diferentes grupos planctónicos. Este objetivo se aborda en el Capítulo I a través del estudio de la abundancia y biomasa del pico-, nano- y microplancton evaluando las diferencias entre periodos productivos y no productivos.

2) Cuantificar el impacto potencial de la deposición de polvo del Sáhara en la comunidad planctónica que habita en las aguas superficiales y oligotróficas de las Islas Canarias. Para ello, se estudió el efecto *in situ* de los eventos de deposición de polvo sahariano que se observaron en el período de invierno-primavera de 2010 sobre la abundancia y biomasa de plancton. Los resultados se detallan en el Capítulo II.

3) Evaluar el papel del microzooplancton en el control de la comunidad microbiana a través de su impacto alimenticio en estas aguas. Con este propósito, se llevaron a cabo varios experimentos para medir el consumo del nano- y microplancton heterotrófico sobre los diferentes grupos planctónicos. Estos resultados se muestran en el Capítulo III. Además, en el Capítulo IV se evalúa el uso de una metodología alternativa para estimar las tasas de "grazing" del microzooplancton.

4) Estimar la magnitud del ciclo de depredación sobre el zooplancton relacionado con la iluminación lunar que se ha observado previamente en esta zona y valorar su contribución potencial al transporte activo de carbono hacia las aguas mesopelágicas. Este punto se aborda en el Capítulo V.

III. METODOLOGÍA

III.1. MUESTREO

La toma de muestras se realizó en aguas de las Islas Canarias, al norte de la isla de Gran Canaria en el caso de los Capítulos I, II y III, además de en la plataforma alrededor de la isla en el caso del Capítulo V (Fig. E.6). En el caso del Capítulo IV, también se utilizaron datos obtenidos en el afloramiento de Benguela y del Océano Pacífico (mirar Cap. IV para más detalle).

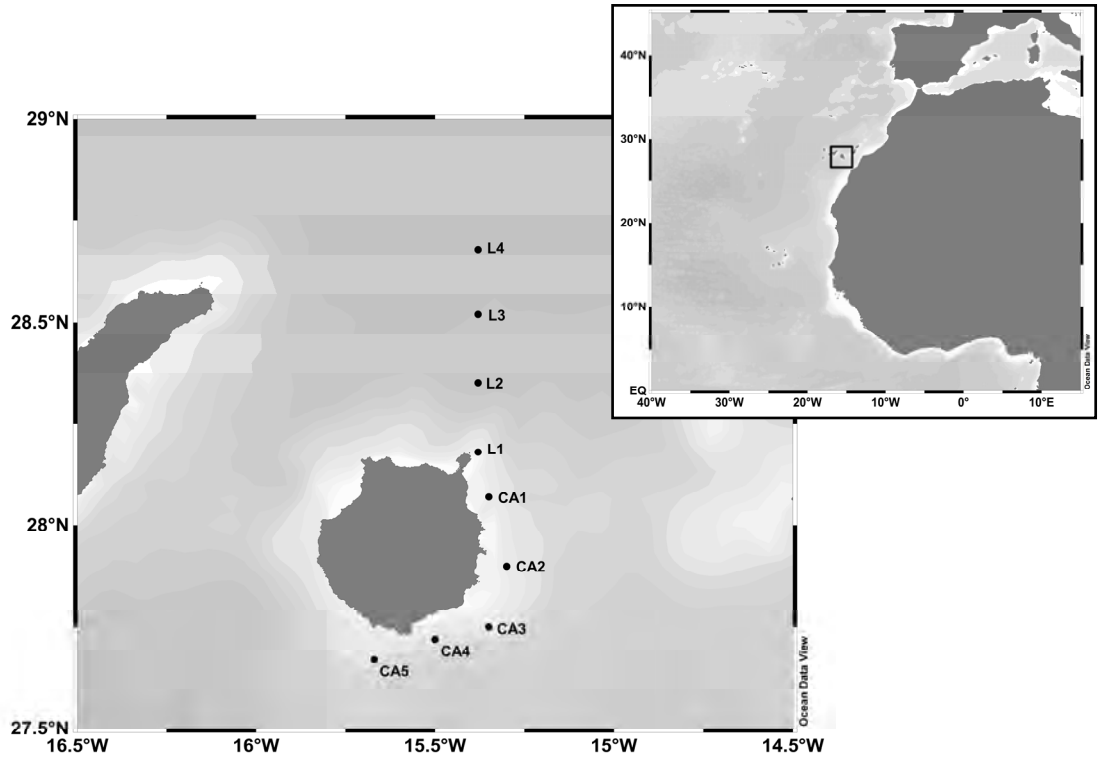

Figure E.6. Mapa del área de muestreo mostrando la localización de las cuatro estaciones al norte de la isla de Gran Canaria (Proyecto Lucifer: L1, L2, L3, L4) y las cinco estaciones alrededor de la isla (Proyecto ConAfrica: CA1, CA2, CA3, CA4, CA5).

Dentro del Proyecto Lucifer, se realizó un muestreo oceanográfico semanal de Febrero a Junio de 2010 (Cap. II) y de Noviembre de 2010 a Junio de 2011 (Cap. I) a bordo del B/O Atlantic Explorer. Las cuatro estaciones se muestrearon desde la superficie hasta 300 metros de profundidad para obtener datos de presión, salinidad, temperatura y fluorescencia usando un CTD SBE25 y un fluorímetro Turner Scufa acoplado a la roseta oceanográfica (Fig. E.7). La roseta disponía de seis botellas Niskin de 4 L con las que se tomaron muestras en la capa de mezcla (20 m) y en la profundidad del máximo profundo de clorofila (DCM) para caracterizar la comunidad planctónica. El mesozooplancton se capturó realizando arrastres verticales con una red WP-2 doble (Fig. E.7) equipada con una malla de 100 µm.

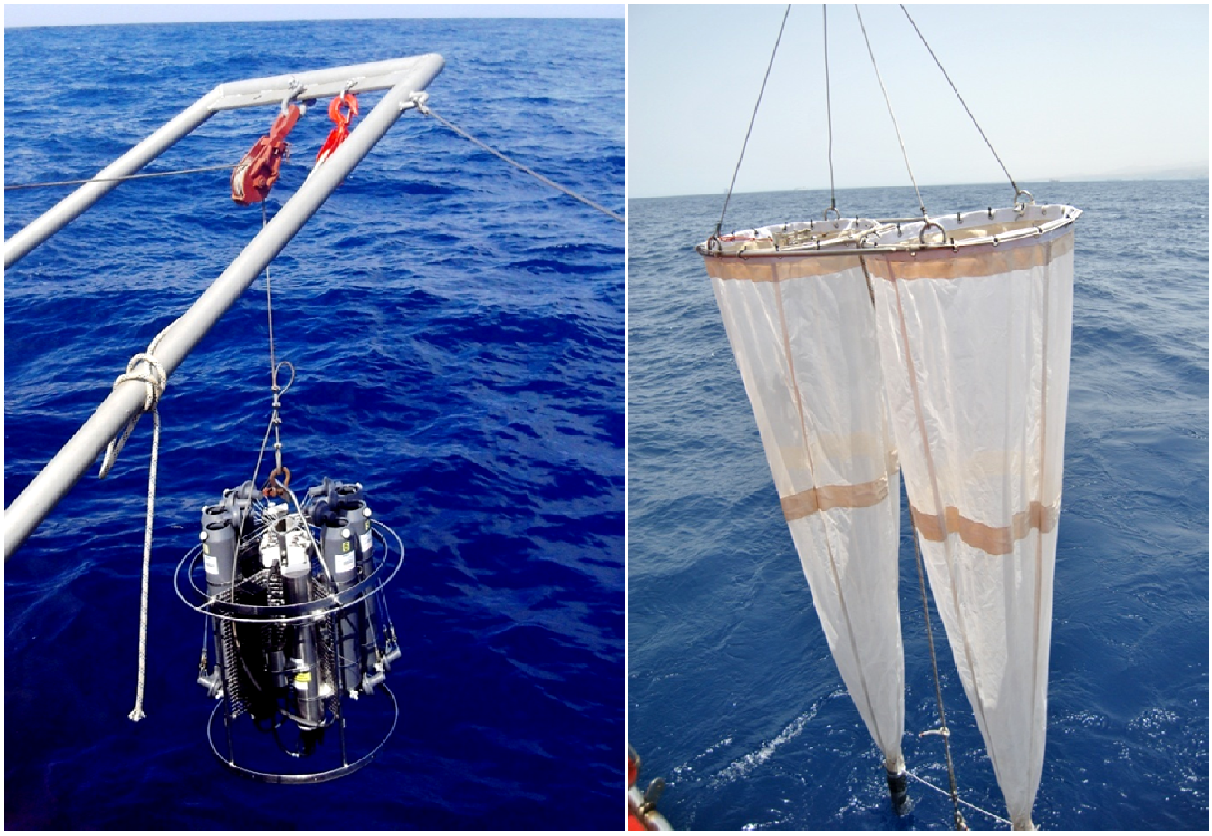

Figure E.7. Roseta oceanográfica (izquierda) y red de plancton WP-2 doble (derecha) utilizadas durante el muestreo semanal del proyecto Lucifer.

El muestreo del Proyecto ConAfrica se realizó semanalmente de Enero de 2005 a Mayo de 2007. Las estaciones se muestrearon de la misma forma que en Lucifer, salvo que sólo se tomaron muestras de agua en la capa de mezcla (15 m). El mesozooplancton se capturó realizando arrastres oblícuos con una red Bongo doble equipada con una malla de 200 µm.

Los datos de polvo atmosférico se tomaron cada 6 días desde Noviembre de 2009 a Junio de 2010 a través del muestreo de tres colectores situados al noreste de la isla (Fig. E.8).

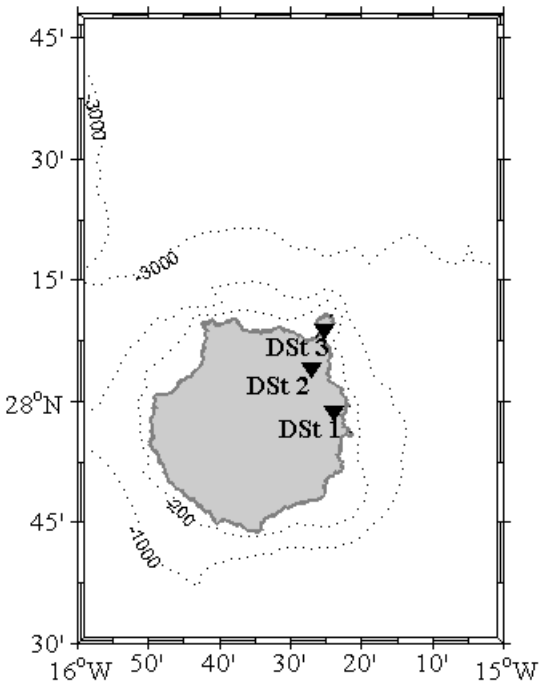

Figure E.8. Mapa de localización de las tres estaciones de muestreo de polvo atmosférico al noreste de la isla de Gran Canaria.

III.2. ANÁLISIS DE MUESTRAS

La concentración de clorofila *a* (Chl *a*) se midió filtrando 500 mL de agua de mar a través de un filtro Whatman GF/F, que posteriormente se congeló a -20ºC hasta su análisis en el laboratorio. El proceso de extracción se realizó con acetona al 90%, en oscuridad y a -20ºC, durante al menos 20

h. Los pigmentos se midieron siguiendo el método de acidificación de Strickland & Parsons (1972c) en un fluorímetro Turner Design 10A (Fig. E.9A), previamente calibrado con Chl *a* pura (Yentsch & Menzel 1963). Se obtuvieron datos adicionales de Chl *a* fraccionada (<2 µm, 2-20 µm, >20 µm) filtrando otros 500 mL en filtros de policarbonato de 20 y 2 µm colocados en serie.

La producción primaria se midió con el método del ^{13}C (Hama et al. 1983), utilizando botellas de policarbonato de 2 L con el agua de mar a la que se añadió una concentración de $NaH^{13}CO_3$ de alrededor del 10% de carbono inorgánico del agua de mar. Las muestras se incubaron en un tanque en la cubierta del barco con un flujo continuo de agua de mar superficial y una malla apropiada para simular las condiciones de temperatura y luz *in situ*. Las muestras se filtraron por filtros GF/F, tratados previamente a 450ºC durante 5 h, para obtener la concentración inicial y final de carbono orgánico particulado (POC), así como el material particulado utilizado para el análisis isotópico. Los filtros se mantuvieron congelados a -20ºC hasta su posterior análisis en un analizador CHN para el POC y por espectrometría de masas en el caso del material isotópico.

Las muestras de picoplancton se tomaron en tubos de 1.6 mL, se fijaron inmediatamente con 100 µL de paraformaldehído al 20%, se incubaron media hora a 4ºC, se congelaron en nitrógeno líquido, y finalmente se guardaron a -80ºC hasta su análisis posterior. Las muestras se analizaron por Citometría de Flujo con un citómetro FACScalibur (Beckton and Dickinson) (Fig. E.9B), usando los diferentes parámetros de fluorescencia y dispersión lateral (SSC) para distinguir entre picoeucariotas autótrofos, *Prochlorococcus*, *Synechococcus* y procariotas heterotróficos. Las muestras se analizaron hasta alcanzar 10000 eventos o 2 minutos a alta velocidad para los organismos autótrofos, y a baja velocidad para las bacterias heterotróficas. Para estos últimos organismos se realizó una tinción previa con SYTO-13.

El nanoplancton, flagelados autótrofos y heterótrofos, se analizó tomando 45 mL de agua de mar y fijándolos con 540 µL de glutaraldehído al 25%. Las muestras se mantuvieron en oscuridad a 4ºC hasta que la muestra se filtró en un filtro de policarbonato tratado con una tinción de DAPI durante 5 minutos. Inmediatamente después se montó el filtro en un portaobjetos utilizando aceite de

inmersión de baja fluorescencia y se mantuvo a -20ºC. Finalmente, el filtro se analizó por microscopía de epifluorescencia (Haas 1982) con un microscopio Zeiss Axiovert 35 (Fig. E.9C).

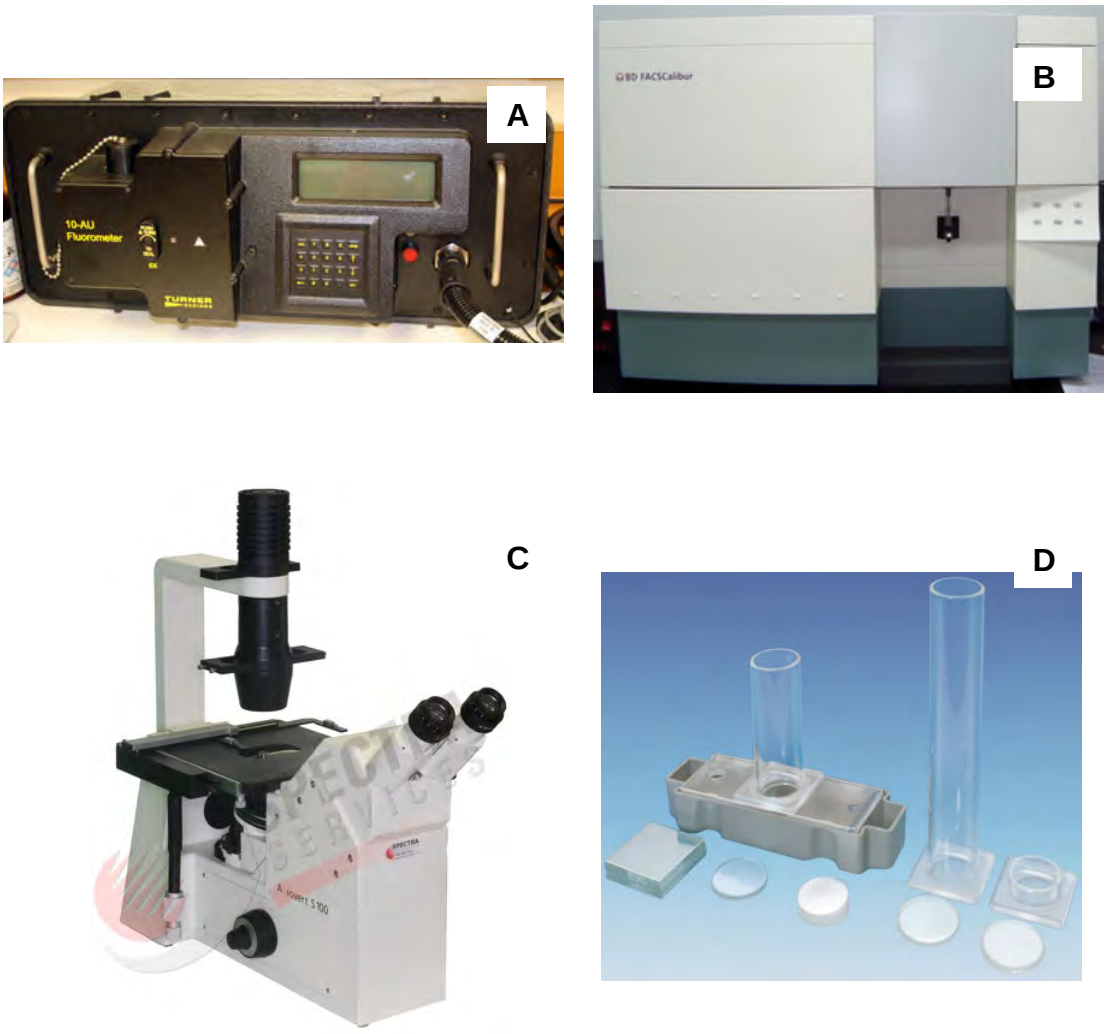

Figure E.9. Diferentes equipos utilizados para el análisis de muestras: fluorímetro (A), citómetro de flujo (B), microscopio invertido (C) y cámara de sedimentación (D).

Las muestras de agua para analizar los organismos del microplancton se tomaron en botellas ámbar de 500 mL y se fijaron con 1.5 mL de lugol ácido. Posteriormente se procesaron siguiendo la técnica Utermöhl, para lo cual se tomó una submuestra de 100 mL que se mantuvo en una cámara de sedimentación (Fig. E.9D) durante 48 h. Una vez transcurrido este tiempo el fondo de la cámara

se examinó en un microscopio invertido Zeiss Axiovert 35 para identificar los principales grupos del microplancton: diatomeas, dinoflagelados, ciliados y nauplios de copépodos o copepoditos.

La biomasa del mesozooplancton se obtuvo midiendo el peso seco de la muestra siguiendo el protocolo estándar (Lovegrove 1966).

III.3. FACTORES DE CONVERSIÓN

Se utilizaron diferentes factores de conversión para convertir la abundancia de los organismos en valores de biomasa.

Para los organismos del picoplancton se utilizaron los siguientes factores: 17 fgC cel^{-1} para los procariotas heterotróficos (Bode et al. 2001), 29 fgC cel^{-1} para *Prochlorococcus*, 100 fgC cel^{-1} para *Synechococcus* (Zubkov et al. 2000) y 1500 fgC cel^{-1} para picoeucariotas autótrofos (Zubkov et al. 1998).

Para los nanoflagelados se utilizó el biovolumen estimado por microscopía para realizar la siguiente conversión a carbono: 220 fgC μm^{-3} para los heterótrofos (Borsheim & Bratbak 1987) y la ecuación $0.433(BV)^{0.863}$ pgC cel^{-1} para los autótrofos (Verity et al. 1992).

Para el microplancton se utilizaron datos de biovolumen obtenidos directamente por microscopía en el caso de las células más abundantes, o por medidas anteriores de estas aguas (A. Ojeda, datos sin publicar). En algunos casos, al no disponer de medidas directas, se usó el tamaño medio descrito en la bibliografía para esos organismos (Tomas 1997, Horner 2002, Ojeda 2006, Ojeda 2011) asociándolos a la forma adecuada según Hillebrand et al. (1999). Finalmente se utilizó la ecuación $\log pgC\ cell^{-1} = \log a + b \cdot \log V$ (Menden-Deuer & Lessard 2000) con los correspondientes parámetros para diatomeas ($\log a$ = -0.541, b = 0.811 para $V<3000\ \mu m^3$; $\log a$ = 0.933, b = 0.881 para $V>3000\ \mu m^3$), dinoflagelados ($\log a$ = -0.353, b = 0.864) y ciliados ($\log a$ = -0.639, b = 0.984 para ciliados aloricados; $\log a$ = -0.168, b = 0.841 para tintínidos).

III.4. METODOLOGÍA EXPERIMENTAL

Experimentos de dilución

El impacto del consumo del microzooplancton se midió utilizando el método de la dilución de Landry & Hassett (1982). Para ello, usamos quince botellas de 2.3 L (Nalgene) para obtener tres réplicas de cinco niveles diferentes de dilución para los experimentos D1-D6: 100, 75, 50, 25 y 10% de agua de mar natural. Para los experimentos D6 y D7 se usaron doce botellas con cuatro niveles diferentes de dilución: 100, 60, 30 y 5% de agua de mar. El agua de mar se tomó en la capa de mezcla (20 m) en las estaciones 2 y 3 (indicadas anteriormente) usando una botella Niskin de 30 L. Este agua se transfirió a dos grandes contenedores de 24 L (Fig. E.10), uno con agua de mar natural, y otro con el agua filtrada por gravedad (filtro de cápsula Whatman, 0.2 µm), y ambos se mantuvieron en la oscuridad mientras se preparaba el experimento.

Figure E.10. Contenedor de 24 L utilizado para obtener el agua filtrada (izquierda) y botellas de 2.4 L en el tanque de incubación en cubierta (derecha).

En todos los experimentos (excepto en D2) se añadió 1 ml de solución con nutrientes a la serie de dilución para evitar limitar el crecimiento del fitoplancton en los tratamientos más diluidos. Esta solución contenía NH_4Cl y Na_2HPO_4 para alcanzar una concentración final en las botellas de 0.5 y 0.03 µM de NH_4 y PO_4, respectivamente. Otras cuatro botellas de 2.3 L se llenaron con agua de mar natural, una de ellas se usó para obtener las muestras iniciales, y las otras tres se incubaron sin añadir nutrientes para tener en cuenta la influencia de los nutrientes sobre el crecimiento del fitoplancton o el posible efecto sobre la mortalidad de microzooplancton. En algunos casos se añadió una botella al 100% de agua de mar filtrada para verificar el sistema de filtrado y comprobar que no había crecimiento de fitoplancton o bacterias en este agua.

Las botellas se incubaron durante 24 h en un tanque en cubierta con un flujo continuo de agua de mar superficial y una malla adecuada en la parte superior para mantener la temperatura y las condiciones de luz *in situ* (Fig. E.10). Tras la incubación, se muestreó cada una de las botellas para tomar medidas de concentración de Chl *a*, picoplancton autótrofo y bacterias. Además, en algunos casos también se midió la abundancia de nanoplancton autótrofo y heterótrofo y los principales grupos del microplancton: diatomeas, dinoflagelados y ciliados.

Para estos grupos planctónicos considerados se determinó el crecimiento (µ) y la mortalidad por el consumo del microzooplancton (m) según Landry et al. (1995), por regresión lineal entre la tasa de crecimiento neto (k_i) y el factor de dilución (D_i):

$$k_i = \ln (C_t / C_0) / t = \mu - m \cdot D_i$$

donde C_t y C_0 son la concentración o abundancia final e inicial, respectivamente, y t es el tiempo de incubación (~ 1 día). La tasa de mortalidad (m) sería la pendiente de la recta de regresión (Fig. E.11), mientras que el corte con el eje y sería la tasa de crecimiento obtenida de la serie de dilución con nutrientes (μ_n). La tasa de crecimiento intrínseca sin nutrientes (μ_0) se obtiene sumando m a la tasa de crecimiento neta de los tratamientos de agua de mar natural (k_0):

$$\mu_0 = k_0 + m$$

Para cada grupo se calculó la producción (P, µgC L^{-1} d^{-1}) y el consumo (G, µgC L^{-1} d^{-1}) diario según Landry et al. (2000a):

$$P = \mu \cdot C_m$$

$$G = m \cdot C_m$$

$$C_m = C_0 (e^{(\mu - m)t} - 1) / (\mu - m)\, t$$

donde C_0 y C_m son la concentración de carbono inicial y media durante la incubación, respectivamente. Por último, se estimó el impacto del microzooplancton sobre la producción planctónica de la siguiente forma:

$$\%P = (G / P) \cdot 100 = (m / \mu) \cdot 100$$

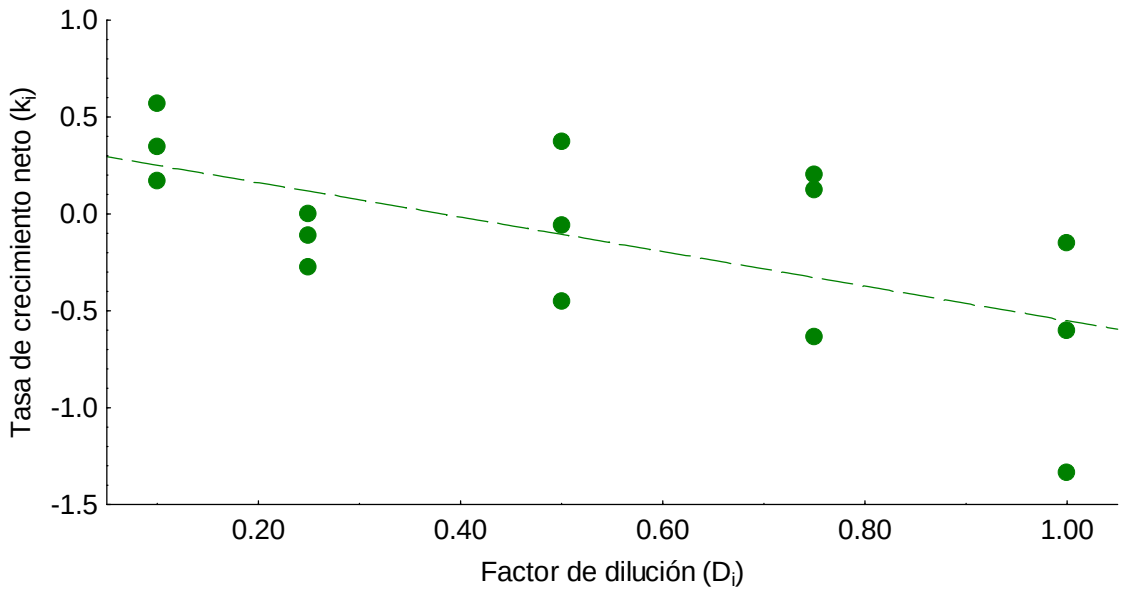

Figure E.11. Ejemplo de recta de regresión entre la tasa de crecimiento neto (k_i) y el factor de dilución (D_i).

Experimentos de Series Temporales

Para evaluar la nueva aproximación metodológica propuesta en el Capítulo IV, se realizaron algunos experimentos de series temporales siguiendo el método de Frost (1972).

El agua de mar se tomó en la capa de mezcla en diferentes estaciones oceanográficas en el Oceáno Pacífico y en el Atlántico (ver Tabla IV.1). El procedimiento fue similar al seguido para los experimentos de dilución explicado anteriormente, pero sólo se utilizó el agua de mar natural y un nivel de dilución muy alto, 5% de agua de mar natural. Además, no se añadieron nutrientes en ninguno de los experimentos.

Las botellas de 2.3 L (Nalgene) que se utilizaron estaban ligeramente modificadas para poder muestrear a diferentes intervalos de tiempo sin producir burbujas en su interior (Fig. E.12). Las muestras iniciales para determinar la concentración o abundancia se obtuvieron tras llenar las botellas, y luego se muestreó a diferentes intervalos durante 30 h. Las primeras 12-14 h se tomaron muestras cada 2 h, y después cada 6 h hasta el final de la incubación.

Las tasas de “grazing” del microzooplanton se determinaron, según Frost (1972), asumiendo el crecimiento exponencial del fitoplancton, como en el método de la dilución. La tasa de crecimiento neto (k) sería el resultado de restarle la mortalidad (m) a la tasa de crecimiento intrínseco (μ):

$$k = \mu - m$$

Sin embargo, en este caso k y m no se calculan por regresión lineal, sino de forma separada, siendo k la tasa de crecimiento neto medida en las muestras de agua de mar natural, mientras que μ sería la tasa de crecimiento neto medida en la muestra diluida, equivalente a la tasa de crecimiento intrínseco.

$$\mu\ (h^{-1}) = (1 / t) \cdot \ln (C_1 / C_0)$$

$$k\ (h^{-1}) = (1 / t) \cdot \ln (C_1' / C_0')$$

donde t es el tiempo de incubación, C_0 y C_1 la concentración de fitoplancton inicial y final en ausencia de "grazers" (agua de mar diluida al 5%), y $C_0´$ y $C_1´$ la concentración de fitoplancton inicial y final en presencia de "grazers". Por tanto, la tasa de mortalidad por "grazing", m, sería:

$m\ (h^{-1}) = \mu - k$

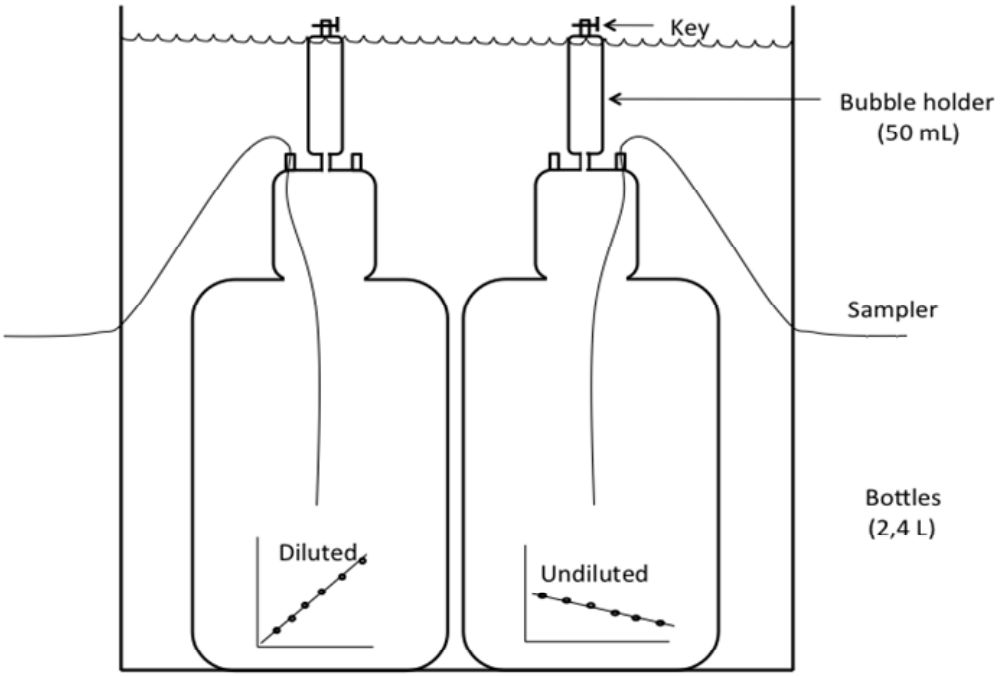

Figure E.12. Esquema que muestra el sistema utilizado para tomar muestras de las botellas a diferentes intervalos de tiempo. El muestreo se realiza a través de un tubo de silicona ("sampler") y se usa la llave instalada en la tapa de las botellas ("key") para eliminar las burbujas de aire que podrían producir daño celular.

III.5. SIMULACIÓN DEL CICLO DE DEPREDACIÓN ASOCIADO AL CICLO LUNAR

Para simular el ciclo de depredación del zooplancton relacionado con el ciclo lunar se realizó un modelo muy simple utilizando el criterio de trabajos anteriores (Hernández-León et al. 2002, 2004, 2010), considerando que

$$P = (B_1 - B_0) + M$$

donde P es la producción del zooplancton, B_1 y B_0 es la biomasa en el tiempo 1 y 0, respectivamente, y M es la mortalidad. Así,

$$B_1 = B_0 + (B_0 \cdot g) - (B_0 \cdot m)$$

siendo g y m las tasas de crecimiento y mortalidad, respectivamente.

La biomasa inicial, B_0, se tomó como el primer valor real medido justo antes del período considerado para la simulación, y la tasa de mortalidad, m, se estableció en función de la iluminación lunar, i:

$$m = [(1 - i) \cdot m_{max}] + m_{min}$$

donde m_{max} y m_{min} son las tasas de mortalidad máxima y mínima, respectivamente. Por tanto, para el período de luna nueva (i=0) la depredación de los migradores verticales produciría la tasa máxima de mortalidad del mesozooplancton epipelágico. Por el contrario, durante la luna llena (i=1), el período de máxima iluminación, la depredación, y por tanto la tasa de mortalidad del mesozooplancton sería mínima. Se usaron diferentes valores de m_{max} hasta obtener la mejor correlación entre la simulación y los datos reales, además de diferentes valores para cada uno de los picos observados durante cada período.

La tasa de crecimiento, g, también se estableció en función de la iluminación lunar, siguiendo a Hernández-León et al. (2010), probando diferentes valores de g_{max} en las diferentes fases lunares hasta obtener la mejor correlación. De la misma forma que para la mortalidad, se usaron diferentes

valores de g_{max} para cada pico de biomasa pero dentro de los valores establecidos por Hirst and Lampitt (1998), entre 0.1-0.3 d^{-1}, salvo en algún caso en que se establecieron valores de g más altos en los períodos de "bloom" para obtener una simulación más realista. El valor mínimo de g y m, 0.01 d^{-1}, se tomó de la bibliografía existente (Hirst & Lampitt 1998, Hirst & Kiørboe 2002).

Finalmente, la mortalidad comunitaria diaria, M, se estimó a partir de la biomasa (b_s) y la tasa de mortalidad (m_s) simuladas para cada día:

$M = b_s \cdot m_s$

IV. RESULTADOS PRINCIPALES

IV.1. CAPÍTULO I. VARIABILIDAD A CORTO PLAZO DE LA COMPOSICIÓN PLANCTÓNICA EN AGUAS SUBTROPICALES DE LAS ISLAS CANARIAS (ATLÁNTICO NORESTE)

La caracterización de la composición planctónica en aguas subtropicales de las Islas Canarias es el primer objetivo de esta tesis junto con el estudio a corto plazo de la variabilidad de la comunidad microbiana de la cadena trófica. Los resultados que se presentan en el Capítulo I muestran que la comunidad planctónica estuvo dominada numéricamente por células de pequeño tamaño (<2 µm), tanto autotróficas como heterotróficas (Fig. I.4, I.6 y I.8), algo común en aguas subtropicales (Buck et al. 1996, Longhurst 1998). Sin embargo, la contribución de las diatomeas aumentó sustancialmente durante el período productivo, hasta dominar la biomasa autotrófica (Fig. I.7 y I.9, Tabla I.1).

Los procesos “bottom-up” fueron claramente los que controlaron la dinámica de la comunidad planctónica a escala estacional. Observamos que el característico “bloom” de finales de invierno (De León & Braun 1973, Braun 1980, Arístegui et al. 2001) se produjo desde Febrero hasta Abril en 2011 (Fig. I.2), cuando se alcanzó la máxima concentración de Chl *a* y las tasas más altas de producción primaria, además de la profundización de la capa de mezcla, tal y como se había descrito previamente en la zona (Cianca et al. 2007). Además, se encontró una correlación significativa entre la abundancia de pico- y nanoplancton con la temperatura media de la capa de mezcla, de forma que la abundancia y biomasa de *Synechococcus* y las células picoeucariotas aumentaron durante el “bloom”, al disminuir la temperatura, mientras que *Prochlorococcus* dominaron en la capa de mezcla en períodos de mayor temperatura y estratificación (Tabla I.2). Este patrón estacional ya se había descrito en estas aguas (Baltar et al. 2009b, Schmoker et al. 2012, Schmoker & Hernández-León 2013) y es una característica común de los giros subtropicales (Zubkov et al. 2000, DuRand et al. 2001, Giovannoni & Vergin 2012). El aumento de las diatomeas en los meses más productivos también se había observado previamente en esta zona (Ojeda 1998, Schmoker et al. 2014), pero no que esto supusiera la dominancia de este grupo, tal y como se

muestra en nuestros resultados (Fig. I.7 y I.9). De hecho, la biomasa autotrófica superó a la heterotrófica dentro de la comunidad microbiana sólo en los meses en que la contribución de las diatomeas fue sustancial (Tabla I.1).

El que la biomasa de las diatomeas aumentara de forma tan importante es un resultado sorprendente ya que la temperatura en la capa de mezcla fue más elevada que en años anteriores (see Schmoker & Hernández-León 2013), con lo que cabría esperar un escenario menos favorable para estas algas al haber menor mezcla y disponibilidad de nutrientes. De hecho, la concentración de Chl *a* en la capa de mezcla ha disminuido en esta zona durante los últimos años en relación al incremento de la temperatura en los meses más productivos (Fig. E.13). Sin embargo, este calentamiento de la capa de mezcla durante 2010 y 2011 no parece afectar negativamente al fitoplancton de mayor tamaño (Fig. E.14). Es más, algunas investigaciones recientes contradicen el punto de vista tradicional que sostiene que las diatomeas se ven favorecidas por la mezcla y condiciones de alta concentración de nutrientes (Kemp & Villareal 2013). Estos investigadores argumentan que algunas especies de diatomeas implicadas en relaciones de simbiosis con organismos diazotróficos podrían aumentar la producción primaria en aguas estratificadas y oligotróficas.

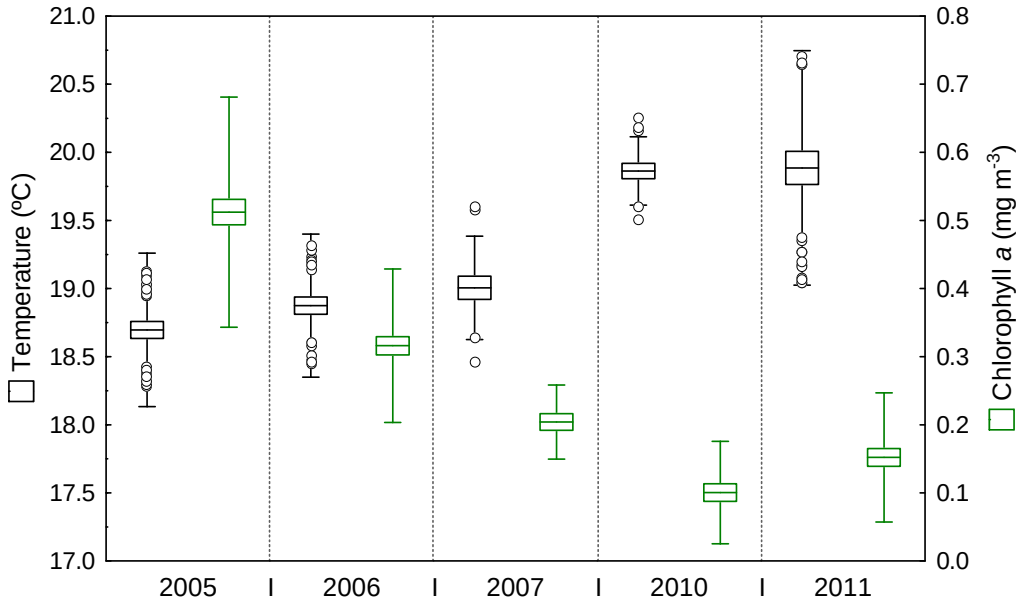

Figure E.13. Datos medios de temperatura (ºC) y clorofila *a* (mg m^{-3}) medidos en la capa de mezcla durante los meses más productivos (desde Enero hasta Abril) de 2005, 2006, 2007, 2010 y 2011.

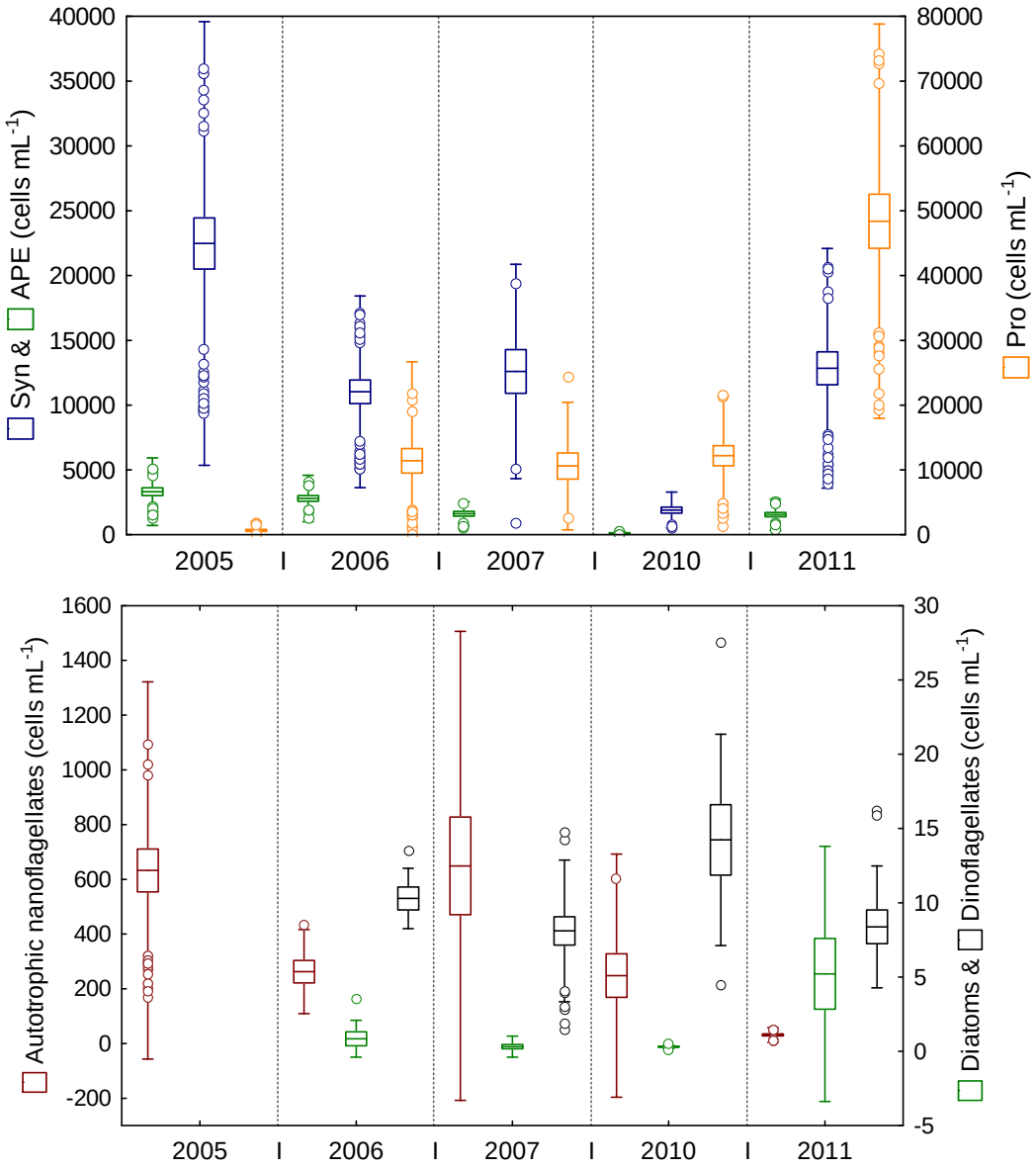

Figure E.14. Promedio de la abundancia de fitoplancton (cel mL^{-1}) en la capa de mezcla durante los meses más productivos (desde Enero hasta Abril) de 2005, 2006, 2007, 2010 and 2011. El panel superior incluye el picofitoplancton: *Synechococcus* (Syn), *Prochlorococcus* (Pro) y picoeucariotas (APE); y el panel inferior incluye al nano- ("autotrophic nanoflagellates") y al microfitoplancton ("diatoms" y "dinoflagellates").

Por otro lado, la dominancia de *Chaetoceros* sp, responsable de los valores de biomasa de diatomeas tan altos (Fig. I.10), podría estar también relacionada con los eventos de deposición de polvo en esta zona. En este sentido, la deposición de polvo podría contribuir a fertilizar en parte la capa de mezcla, aumentando así la disponibilidad de nutrientes que posteriormente se vería incrementada por la entrada de nutrientes de aguas más profundas, lo que favorecería el extraordinario aumento de las diatomeas. Esta hipótesis se sostiene en parte con los resultados del Capítulo II, donde se observa un incremento significativo en la biomasa de las diatomeas, también debido a la presencia de *Chaetoceros* sp, después del paso de una tormenta de polvo del Sáhara. Además, las tasas de producción primaria se mantuvieron bastante altas no sólo en los meses más productivos, sino durante todo el período estudiado (Fig. I.4). Esto podría indicar la existencia de eventos de menor escala temporal, como los eventos de deposición de polvo sahariano, que podrían aumentar la productividad de la capa de mezcla en condiciones de estratificación. Sin embargo, cuando estudiamos el efecto de estos eventos en la producción primaria en el Capítulo II, no se apreció una respuesta considerable.

Las interacciones tróficas también controlan la variabilidad a corto plazo de los diferentes grupos planctónicos. Los nanoflagelados heterotróficos estarían consumiendo bacterias heterotróficas y *Synechococcus* ya que se observó un claro patrón inverso entre ambos (Fig. I.6 y I.8, Tabla I.2). Los flagelados son consumidores de bacterias (Azam et al. 1983), tanto heterotróficas como autotróficas, tal y como se ha descrito en estas aguas (Arístegui & Montero 2005). Nuestros resultados también muestran una relación inversa entre picoeucariotas autótrofos y nano- y dinoflagelados (Fig. I.6 y I.7, Tabla I.2), lo que sugiere que estos últimos se estarían alimentando de pico- y nanoautótrofos. Estos resultados están de acuerdo con los resultados del Capítulo III, donde se evalúa el rol de los consumidores y las presas potenciales dentro de la comunidad microbiana.

IV.2. CAPÍTULO II. RESPUESTA DE LA COMUNIDAD PLANCTÓNICA A LOS EVENTOS DE POLVO DEL SÁHARA EN AGUAS SUBTROPICALES DE LAS ISLAS CANARIAS

El segundo objetivo de esta tesis, desarrollado en el Capítulo II, fue cuantificar el efecto potencial de la deposición de polvo sahariano sobre la comunidad plantónica. El período de estudio, de Febrero a Junio de 2010, fue uno de los períodos más intensos de eventos de polvo de los últimos años. Se identificaron eventos de deposición de polvo del Sáhara mensuales a partir de los datos de materia atmosférica suspendida total (Fig. II.3). Sin embargo, el efecto sobre la Chl *a* y la producción primaria fue insignificante, a pesar de que se observó un aumento significativo en la biomasa de diatomeas. La biomasa de mesozooplancton también aumentó, mientras que los organismos del picoplancton autótrofo se vieron afectados negativamente.

Los datos del período de estudio de 2010 muestran que las condiciones hidrográficas y biológicas fueron bastante inusuales. La columna de agua estuvo fuertemente estratificada y se observaron unos valores muy bajos de abundancia y biomasa de fitoplancton (Fig. II.2 y II.6), con lo que el característico “bloom” de finales de invierno (De León & Braun 1973, Braun 1980, Arístegui et al. 2001) no tuvo lugar ese año. La biomasa de heterótrofos también fue baja, pero dominaron durante todo el período de estudio (Fig. II.6, Tabla II.1).

La fuerte estratificación de la columna de agua, así como la alta frecuencia de eventos de deposición de polvo en el período de estudio sugieren que la fuente principal de nutrientes en las aguas superficiales fue la deposición atmosférica de polvo del Sáhara. De hecho, la concentración de nutrientes en la capa de mezcla fue muy alta (Benavides et al. 2013) en comparación con otras medidas de la zona (Marañón et al. 2000, 2003, Neuer et al. 2007), lo cual podría ser resultado de la intensidad de los eventos de polvo.

El efecto de la deposición de polvo del Sáhara se evaluó a través del cambio que se midió en los diferentes componentes de la comunidad planctónica después del evento de mayor intensidad, el 18 de Marzo. El cambio en la concentración de Chl *a* fue insignificante (Fig. II.7), tal y cómo se ha visto en algunos resultados experimentales al añadir polvo sahariano en aguas oligotróficas

(Bonnet et al. 2005, Marañón et al. 2010). Sin embargo, en estos experimentos sí que se ha medido un aumento sustancial en la productividad primaria, que nosotros no observamos en nuestros resultados (Fig. II.7). Algunos autores (Herut et al. 2005) han observado una respuesta muy débil del fitoplancton en el medio tras una tormenta de polvo y sostienen que esto se debe a que la liberación de nutrientes producida por estos eventos sería baja. Por el contrario, en otros trabajos se sugiere que el fitoplancton no muestra un incremento importante después de un proceso de fertilización natural o artificial, a pesar del aumento de la productividad primaria, por el intenso control que ejercen los "grazers" (Landry et al. 2000a, Landry et al. 2000b, De Baar et al. 2005, Herut et al. 2005, Boyd et al. 2007, Henjes et al. 2007, Marañón et al. 2010). Este último argumento podría explicar los valores bajos de abundancia de fitoplancton que contrastan con la alta concentración de nutrientes, y concuerdan con las altas tasas de "grazing" que se midieron en el Capítulo III.

El único grupo dentro de la comunidad autotrófica que mostró un cambio positivo fue el de las diatomeas, que aumentaron su biomasa en más de un 1000% (Fig. II.8 y II.9). En los experimentos de fertilización artificial esto es una característica común (De Baar et al. 2005, Boyd et al. 2007) ya que las diatomeas presentan tasas de crecimiento mayores que las algas procariotas durante los experimentos (Landry et al. 2000a), además de una menor presión de "grazing" (Landry et al. 2000a, Landry et al. 2000b, Henjes et al. 2007). De hecho, los picoeucariotas autótrofos y *Synechococcus* respondieron negativamente tras el evento de polvo (Fig. II.8 y II.9), probablemente debido a una mayor presión de los "grazers" ya que éstos también aumentaron. Por ello, no es sorprendente que las algas de menos tamaño soporten una mayor presión que las diatomeas, ya que estas últimas se ven favorecidas tanto por el efecto de fertilización, así como por una menor mortalidad a causa del "grazing". Otro grupo que mostró un cambio positivo en su biomasa fue el mesozooplancton, que incrementó alrededor de un 100% (Fig. II.8 y II.9). Este efecto sobre el mesozooplancton tras una tormenta de polvo ya había sido descrito previamente en estas aguas (Hernández-León et al. 2004). Estos autores también observaron un aumento en la actividad metabólica del zooplancton.

IV.3. CAPÍTULO III. IMPACTO ALIMENTICIO DEL MICROZOOPLANCTON SOBRE LA COMUNIDAD MICROBIANA DE LAS AGUAS DE LAS ISLAS CANARIAS

El tercer objetivo de este trabajo fue estimar el impacto de la alimentación del microzooplancton sobre la comunidad planctónica. Para ello, realizamos varios experimentos de dilución en 2010 y 2011, cuyos resultados se presentan en el Capítulo III. El impacto medido fue diferente dependiendo del grupo microbiano considerado. Así, las células de mayor tamaño y crecimiento presentaron tasas más altas de mortalidad por "grazing" que las células de menos tamaño y crecimiento más lento. Esto significa que el consumo del microzooplancton está acoplado de forma muy estrecha al crecimiento de su presa, incluso en el caso de los organismos heterótrofos, ya que el impacto sobre ellos fue también considerable y predominante en los experimentos. De hecho, la producción diaria, tanto autótrofa como heterótrofa, fue consumida en su totalidad por el microzooplancton, salvo en el caso de las cianobacterias.

Al medir las tasas de "grazing" a partir de los datos de concentración de Chl *a* se obtuvo un gran número de resultados no significativos (Tabla III.2), a pesar de que para esos mismos experimentos sí se midieron tasas significativas al considerar los diferentes grupos de autótrofos por separado. Por lo tanto, para poder cuantificar adecuadamente el impacto real del microzooplancton sobre la producción primaria es necesario realizar un análisis detallado de todos los componentes de la comunidad autotrófica.

En algunos experimentos se encontró que la abundancia de los consumidores potenciales (nanoflagelados heterotróficos, dinoflagelados y ciliados) aumentó en las muestras más diluidas (Fig. III.6), lo que podría indicar la existencia de cascadas tróficas, de manera que los consumidores estarían bajo el control de sus depredadores, y esta presión se vería disminuida en las réplicas más diluidas. Recientemente se ha demostrado la existencia de cascadas tróficas en experimentos de dilución realizados en aguas subtropicales donde la red trófica es muy compleja (Calbet & Saiz 2013). De hecho, el consumo de organismos heterótrofos resultó ser generalizado en nuestros experimentos (Tabla III.3), donde observamos tasas muy altas de mortalidad de

procariotas y nanoflagelados heterotróficos, así como ciliados. De esta forma, los nanoflagelados se estarían alimentando de bacterias (Azam et al. 1983, Guillou et al. 2001, Sherr & Sherr 2002), y a su vez serían consumidos por los ciliados (Azam et al. 1983, Sherr & Sherr 2002, Pomeroy et al. 2007), que podrían estar bajo el control de nauplios de copépodos, copepoditos o incluso dinoflagelados heterotróficos (Sherr & Sherr 2002).

No se encontró ninguna relación entre las tasas de crecimiento y mortalidad y la temperatura, concentración de Chl *a*, o la composición planctónica, probablemente por la variabilidad tan baja de estos parámetros (Fig. III.2 y III.3, Tabla III.1). Los consumidores dominantes durante todo el período fueron los nanoflagelados heterotróficos (Fig. III.3), mientras que las presas más abundantes fueron los procariotas heterotróficos y *Prochlorococcus*. Estos resultados coinciden con la situación característica de los sistemas oligotróficos donde el nanoplancton heterótrofo conforma el grupo mayoritario de consumidores de producción primaria (Calbet 2008). Las tasas de "grazing" estuvieron únicamente relacionadas con las tasas de crecimiento de las presas (Fig. III.5), lo que indica el acople entre consumidores y productores. Esta relación entre el crecimiento del fitoplancton y el consumo del microzooplancton parece ser una característica general en los océanos (Schmoker et al. 2013), así como en la zona de estudio (Quevedo & Anadón 2001). Sin embargo, el que las células autotróficas de mayor tamaño (nanoflagelados y diatomeas) presenten mayores tasas de crecimiento y mortalidad por "grazing" (Fig. III.4) es un resultado inesperado, ya que normalmente las células de menor tamaño soportan una mayor presión (Strom et al. 2007, Calbet et al. 2008, Gutiérrez-Rodríguez et al. 2011, Teixeira et al. 2011). Por otro lado, nuestros resultados estarían de acuerdo con un reciente estudio en el Atlántico noreste (Cáceres et al. 2013) en el que se observó que los organismos fitoplanctónicos de mayor tamaño presentaban las tasas más altas de crecimiento y mortalidad por "grazing".

IV.4. CAPÍTULO IV. DIFERENCIACIÓN DE LOS MÉTODOS DE LA DILUCIÓN, "2-POINT" Y FROST PARA MEDIR EL "GRAZING" DEL MICROZOOPLANCTON

Dentro del tercer objetivo de esta tesis de evaluar el papel del microzooplancton en el control de las comunidades microbianas, proponemos el uso de una metodología alternativa en el Capítulo IV. Para ello distinguimos entre el método de la dilución, el 2-point, y el de Frost, para medir el "grazing" del microzooplancton (Fig. IV.1), y proponemos una aproximación diferente basada en este último. Utilizando datos de la bibliografía existente, y nuestros propios datos experimentales, encontramos una buena correlación entre las tasas de mortalidad calculadas con el método de la dilución y el de Frost que proponemos (Fig. IV.4 y IV.5).

La técnica de la dilución (Landry & Hassett 1982) es el método más extendido para medir el "grazing" del microzooplancton y se basa en la presunción de que las tasas de encuentro entre depredador y presa disminuyen proporcionalmente al diluir una muestra de agua de mar natural. Por ello, se realiza una serie de diluciones para medir el aumento del crecimiento aparente del fitoplancton a medida que se incrementa la dilución. Sin embargo, esta técnica es tediosa ya que se requieren grandes volúmenes de agua, además de un elevado número de réplicas para obtener la confianza estadística necesaria. Además, en ocasiones, es difícil interpretar los resultados que se obtienen con este método cuando aparecen respuestas no lineales. Una simplificación de este método es el denominado método 2-point, en el que se utilizan tan sólo el agua natural y un nivel de dilución. Por el contrario, con el método de Frost (1972) se estima la tasa de "grazing" a partir de medidas independientes del agua de mar natural y una muestra muy diluida, lo que supone un concepto más robusto estadísticamente. Además, esta última aproximación tiene la misma ventaja que el método 2-point para su uso generalizado en las campañas oceanográficas, ya que se pueden realizar varias medidas al día.

La combinación de la aproximación de Frost con los experimentos de serie temporal que se realizaron nos permitió medir el cambio real en las tasas de crecimiento y mortalidad en intervalos cortos de tiempo (Fig. IV.8). Las tasas variaron bastante en las primeras horas, disminuyendo

después, e incrementándose ligeramente durante la noche. Además, las tasas más altas de "grazing" se midieron después de tan sólo 4 h, y llegaron casi a cero después de 18 h. Esta gran variabilidad en las tasas de "grazing" podría tener que ver con la gran variabilidad que se observa en ocasiones en las réplicas utilizadas en el método de la dilución, y plantea la conveniencia o no de realizar largas incubaciones (24 h) en los experimentos.

IV.5. CAPÍTULO V. ESTIMACIONES DEL FLUJO DE CARBONO ACTIVO VINCULADO A LOS CICLOS LUNARES EN EL ZOOPLANCTON EN AGUAS SUBTROPICALES

En el Capítulo V se estima la magnitud del ciclo de depredación del zooplancton relacionado con la iluminación lunar que se ha observado con anterioridad y su contribución potencial al mecanismo de transporte activo de carbono hacia la zona mesopelágica. La variabilidad del mesozooplancton se relaciona con la depredación de los migradores verticales diarios (DVMs), un proceso que se ve influenciado por la iluminación de la luna. Se realizó una simulación de este ciclo de depredación con la que calculamos los valores de mortalidad comunitaria para el mesozooplancton epipelágico, y de esta forma, estimamos los valores potenciales del flujo de carbono activo. Estas estimaciones de transporte activo de carbono fueron del mismo orden de magnitud que el flujo gravitacional medido en aguas subtropicales.

La variabilidad del mesozooplancton mostró un patrón relacionado con el ciclo lunar (Fig. V.3), de forma que los valores mayores de biomasa se observaron en las fases de mayor iluminación lunar (2º y 3er cuarto), tal y cómo se ha observado anteriormente en esta zona (Hernández-León et al. 2002, 2004). Esta relación entre el zooplancton y el ciclo lunar está relacionada con la depredación de los DMVs (Hernández-León 1998, Hernández-León et al. 2001a, 2002, 2004, 2010), que ascienden hasta las aguas más superficiales durante la noche para alimentarse, ejerciendo un intenso control sobre el mesozooplancton epipelágico. Los migradores no alcanzan la capa epipelágica durante la luna llena, el período de máxima iluminación, para evitar ser depredados, mientras que durante la luna nueva alcanzan los primeros metros de la columna de agua

alimentándose del mesozooplankton epipelágico. De esta forma, los organismos del mesozooplancton crecen sin la gran presión de los migradores durante la fase iluminada del ciclo lunar, mientras que su biomasa disminuye durante la luna nueva (Hernández-León 1998, Hernández-León et al. 2001a, 2002, 2004, 2010).

A través de una simulación muy simple (Fig. V.4) estimamos la mortalidad comunitaria del mesozooplancton epipelágico resultado del consumo de los DMVs. Para ello, establecimos la tasa de mortalidad del zooplancton en función de la iluminación lunar, es decir, la mortalidad toma el máximo valor con las condiciones de menor iluminación (luna nueva) y viceversa. De la misma forma, la tasa de crecimiento se estableció en función de la iluminación lunar para obtener la mejor correlación entre los valores de biomasa real y los simulados. La influencia de la iluminación lunar sobre el crecimiento podría explicarse si existiera un ritmo lunar en el ciclo reproductivo de estos organismos, algo que se ha observado en algunas especies de invertebrados de aguas profundas (Mercier et al. 2011). Esta hipótesis podría también sostenerse a partir de los resultados obtenidos al medir el crecimiento potencial del mesozooplancton, a través de la actividad enzimática de la AARS (Herrera, unpublished data), donde se observa el patrón lunar descrito en este trabajo en los años 2010 y 2011.

La variabilidad de la mortalidad comunitaria fue considerablemente mayor durante los períodos productivos en comparación con los no productivos (Tabla V.1), debido a los valores más altos de biomasa y mortalidad en la época más productiva. Estos resultados coinciden con otros trabajos en los que se ha observado un mayor transporte de carbono por parte de los DVMs en aguas más productivas (Yebra et al. 2005, Putzeys 2013). Para estimar el flujo de carbono activo asumimos que la mortalidad comunitaria se debe principalmente al consumo de los migradores, que estarían transportando ese carbono consumido en superficie hacia aguas más profundas. Así, el valor promedio del flujo activo estimado durante la época productiva fue del mismo orden de magnitud que el valor promedio del flujo gravitacional en estas aguas (Tabla V.2), así como en otras regiones subtropicales (Bermuda y Hawaii).

V. CONCLUSIONES

Las conclusiones principales que se pueden extraer de esta tesis son las siguientes:

1) Las aguas oligotróficas del Sistema de la Corriente de Canarias estuvieron dominadas por el picoplancton durante el período estudiado, especialmente por *Prochlorococcus* y procariotas heterotróficos. Sin embargo, durante la época productiva *Synechococcus* y picoeucariotas dominaron el picoplancton autótrofo, mientras que las diatomeas se convirtieron en los contribuidores mayoritarios a la biomasa autotrófica total. Los procesos "bottom-up" regularon la variabilidad de la comunidad planctónica a escala estacional, pero otros eventos a menor escala como la deposición de polvo sahariano podrían estar aumentando la productividad en la capa de mezcla, incluso durante períodos de estratificación de la columna de agua. Las interacciones tróficas también regulan la variabilidad a corto plazo a través de los procesos "top-down": el "grazing" y la depredación.

2) Las aguas de las Islas Canarias se vieron afectadas con frecuencia por los eventos de deposición de polvo sahariano durante todo el período de estudio. El efecto sobre la producción primaria fue insignificante, aunque la variabilidad a corto plazo de este parámetro parece estar relacionada con los eventos saharianos. Dentro de la comunidad planctónica se encontraron diferentes respuestas según el grupo considerado. Por un lado, la biomasa de diatomeas y mesozooplancton aumentó, mientras que por otro, el fitoplancton de menor tamaño se vio afectado negativamente. Por tanto, la deposición del polvo proveniente del Sáhara podría estar estimulando en parte a los productores primarios de estas aguas, especialmente durante períodos de estratificación, pero este incremento en la productividad no se estaría detectando debido posiblemente a que los productores estarían siendo rápidamente consumidos.

3) El impacto del microzooplancton en la comunidad microbiana fue diferente según el grupo considerado. En general, se observó un gran acoplamiento entre el consumo del microzooplancton y el crecimiento de sus presas, tanto autótrofos como heterótrofos, con lo que las tasas más altas de "grazing" se observaron sobre las células mayores y de crecimiento más rápido (nanoflagelados autótrofos y diatomeas), mientras que las células de menor tamaño (*Synechococcus* y picoeucariotas autótrofos) presentaron tasas de "grazing" más bajas. Por otro lado, nuestros resultados ponen de manifiesto como basándose únicamente en datos de Chl *a*, se puede introducir una gran fuente de error en las medidas de "grazing", por lo menos en ecosistemas oligotróficos. Finalmente, el impacto del microzooplancton sobre la producción primaria estaría influenciado por procesos de carnivoría, observados de forma predominante. Por lo tanto, también se debe cuantificar el impacto del microzooplancton sobre los organismos heterotróficos para tratar de comprender mejor las interrelaciones de la comunidad planctónica, sobre todo en aguas subtropicales, dada su mayor complejidad trófica.

4) La metodología alternativa que se propone en este trabajo para medir el impacto de "grazing" del microzooplancton es comparable al método de la dilución o el 2-point, pero experimentalmente simple y robusta estadísticamente. Los experimentos de serie temporal basados en el concepto de Frost suponen una mejor aproximación para entender las interacciones entre depredadores y presas en el océano. Además, la automatización del método usando técnicas de procesamiento de imagen y citometría de flujo, harían este método más adecuado para el muestreo oceanográfico ya que se podrían realizar varias medidas al día.

5) El ciclo lunar del zooplancton relacionado con la depredación de los migradores verticales diarios parece ser un proceso “top-down” común en las aguas de las Islas Canarias tanto en períodos productivos como no productivos. Los valores de mortalidad comunitaria del zooplancton a causa de este ciclo de depredación se traducen en valores de transporte activo de carbono del mismo orden de magnitud que el flujo gravitacional en aguas subtropicales. Estos resultados podrían arrojar luz sobre el desacople que se observa en el océano entre la producción primaria y el flujo de carbono particulado. También podrían explicar el flujo de materia orgánica necesario para mantener la demanda de bacterias y zooplancton de la zona mesopelágica. Por lo tanto, es esencial cuantificar el papel del zooplancton epipelágico y los migradores verticales en el transporte de carbono hacia aguas profundas para estimar la magnitud de la bomba biológica en el océano.

VI. FUTURAS LÍNEAS DE INVESTIGACIÓN

Las conclusiones extraídas de los resultados que se presentan en este trabajo subrayan la necesidad de realizar un mayor esfuerzo de investigación en los siguientes campos:

Existe la necesidad de realizar estudios oceanográficos con una mayor frecuencia de muestreo para cuantificar mejor la variabilidad a corto plazo de las comunidades planctónicas, teniendo en cuenta la complejidad y rapidez de los procesos biológicos en estas aguas. Como ejemplo, podemos destacar el rol de las diatomeas en aguas de las Islas Canarias, un resultado sorprendente e inesperado que puede ser determinante en términos de transporte de carbono y que debe ser investigado. En este sentido, un muestreo temporal intensivo también ayudaría a evaluar mejor la influencia de la deposición de polvo del Sáhara en la zona subtropical del Atlántico noreste. De esta forma, podríamos cuantificar mejor la intensidad y el tiempo de respuesta de los diferentes grupos planctónicos.

Se requiere un mayor esfuerzo de investigación para conocer el verdadero impacto del microzooplancton en la producción primaria, especialmente en los giros subtropicales. Sin embargo, es esencial cuantificar el impacto del microzooplancton sobre los diferentes componentes planctónicos, incluidos los organismos heterótrofos, para entender mejor la complejidad de las relaciones tróficas dentro de la comunidad microbiana. Por otro lado, los experimentos de series temporales basados en el concepto de Frost proporcionarían resultados más detallados sobre los cambios en la composición planctónica durante la incubación, además, este método se podría automatizar en el futuro usando citometría de flujo y técnicas de procesamiento de imagen.

Por último, el flujo activo relacionado con el ciclo lunar que se ha descrito en este trabajo para aguas subtropicales oligotróficas representa un flujo de carbono hacia la zona mesopelágica muy importante, que no se ha contabilizado hasta ahora, y que necesita ser investigado.

VII. REFERENCIAS

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