El océano es el principal sumidero del CO_2 atmosférico, y su secuestro hacia el océano profundo por parte de los organismos empieza con los productores primarios. El microzooplancton consume entre el 60 y el 70% de la producción primaria, y actúa de intermediario entre el fitoplancton y el mesozooplancton. En esta tesis, se han estudiado los efectos físico-químicos y biológicos que influyen en la distribución y composición de comunidades planctónicas, influyendo en las relaciones tróficas que mantienen los organismos entre si. Un ejemplo de ello es el efecto en cascada observado en aguas subtropicales, donde el mesozooplancton depredó sobre los ciliados, liberando a los autótrofos de la presión de pastaje. El estudio del bloom primaveral en un fiordo danés puso de manifiesto la sucesión de ciliados mixotróficos a heterotróficos a medida que se agotaban los nutrientes, y el cambio producido en la comunidad fitoplanctónica. Además, el estudio integrado de variables físico-químicas, la comunidad planctónica y las relaciones tróficas en el océano Atlántico permitió obtener una imagen detallada del desarrollo de las comunidades y los roles tróficos de los organismos, poniendo de manifiesto la dicotomía que existe entre las zonas oligotróficas y productivas.

Laia Armengol Bové | TESIS DOCTORAL
Microzooplankton marine foodwebs
Top-down effects, Bloom control and Grazing at ocean basin scale

Microzooplankton in marine foodwebs

Top-down effects, Bloom control and Grazing at ocean basin scale

TESIS DOCTORAL

Laia Armengol Bové

IOCAG Instituto de Oceanografía y Cambio Global
MINISTERIO DE ECONOMÍA, INDUSTRIA Y COMPETITIVIDAD
AGENCIA ESTATAL DE INVESTIGACIÓN
ULPGC
UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA
Doctorado en Oceanografía y Cambio Global

Las Palmas de Gran Canaria
diciembre 2018

UNIVERSIDAD DE LAS PALMAS
DE GRAN CANARIA

D. SANTIAGO HERNÁNDEZ LEÓN COORDINADOR DEL PROGRAMA DE DOCTORADO DE OCEANOGRAFÍA Y CAMBIO GLOBAL DE LA UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA,

INFORMA,

Que la Comisión Académica del Programa de Doctorado, en su sesión de fecha 22 de noviembre de dos mil dieciocho tomó el acuerdo de dar el consentimiento para su tramitación, a la tesis doctoral titulada "Microzooplankton in marine foodwebs: Top-down effects, Bloom control and grazing at ocean basin scale" presentada por la doctoranda Dª Laia Armengol Bové y dirigida por el Dr. Santiago Hernández León.

Asimismo, se acordó el informar favorablemente la solicitud para optar a la Mención Internacional del Título de Doctor, por cumplir los requisitos reglamentarios.

Y para que así conste y a efectos de lo previsto en el Artº 11 del reglamento de Estudios de Doctorado (BOULPGC 7/10/2016) de la Universidad de Las Palmas de Gran Canaria, firmo la presente en Las Palmas de Gran Canaria, a 22 de noviembre de dieciocho.

UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA

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MICROZOOPLANKTON IN MARINE FOODWEBS: TOP-DOWN EFFECTS, BLOOM CONTROL, AND GRAZING AT OCEAN BASIN SCALE

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La doctoranda

El director

Las Palmas de Gran Canaria, 23 de noviembre 2018

UNIVERSIDAD DE LAS PALMAS
DE GRAN CANARIA

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Microzooplankton in marine foodwebs:
Top-down effects, bloom control, and grazing at ocean basin scale

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A la meva família.

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de los “nuevos” doctorandos el pensamiento de “esto no me va a pasar a mi”, no cambiaría estos años dedicados a la investigación.

Es difícil empezar esta parte de la tesis pues son muchas las personas que directa o indirectamente han hecho posible este proyecto. Una vez, alguien dijo que lo mejor es empezar por el principio. Quiero agradecer al programa de Formación de Personal Investigador (FPI) del Ministerio de Economía y Competitividad (Gobierno de España) por la financiación recibida para el desarrollo de esta tesis y la ayuda de movilidad que me concedió para la realización de la estancia. También quiero agradecer al Programa de Formación de Personal Investigador de la Universidad de Las Palmas de Gran Canaria por las ayudas recibidas para estancias y asistencia a congresos nacionales e internaciones.

Creo que es de justicia agradecer en primer lugar a mi director, al Dr. Santiago Hernández León, la supervisión de esta tesis, así como la oportunidad y la confianza depositada en mi. Cuando entré en tu aula el primer año de licenciatura poco me podía imaginar que acabaría compartiendo todos mis años de formación contigo. Has sido unas de las personas clave para que hoy esté aquí. Cuando terminé la carrera no tenía muy claro que camino tomar, y recuerdo una conversación contigo frente a la máquina de café de la facultad (¡qué grandes decisiones se han tomado con un café!) donde me propusiste hacer el máster en Oceanografía. Allí aún no era consciente, pero me enganchaste a la “droga” de la ciencia. Gracias por formarme como estudiante y como científica, y sobretodo por confiar en que podía conseguirlo.

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La primera vez que entré en el laboratorio B-201 había una relación de 3:1 entre personas y mesas/sillas, y aprendí que el citómetro nunca descansa, incluso de madrugada. De este laboratorio quiero agradecer a todas las personas que pasaron por allí, porque de todas me llevé una pequeña lección. Javier, gracias por compartir conmigo las técnicas del fitoplancton; y Nandy, por preguntar siempre como iba todo. Gracias a Marta y Claire por ser mis primeras profesoras en mi primera campaña. Las dos sois geniales como investigadoras y mejor como personas. Claire, tu trabajo ha sido mi base y a ti te debo mis inicios en el “grazing”, gracias por compartir conmigo tu vida en Las Palmas y en Barcelona. Quiero recordar a Gara cuyo trabajo cimentó al

mío; a Mar que me enseñó que en las peores condiciones marinas se pueden llevar a cabo experimentos si hay voluntad; a Lidia por los años compartidos en el laboratorio buscando soluciones a todos los problemas que iban surgiendo; a Alejandro, contigo bajamos un grupo de novatas a los infiernos de la oceanografía con LUCIFER, y renacimos como "oceanógrafas imbatibles"; a Juan Carlos y Jose por vuestra calidad humana y por no desesperar ante mi impaciencia; a Acorayda y Mine porque no hay trucos que no sepáis; a Carolina, por ser mi compañera de penúrias administrativas, que guarda una gran persona en un frasco pequeño; a Isa, por no desfallecer antes las adversidades; y a Nau, por entender que las condiciones perfectas no existen. El laboratorio evolucionó, cambió y se mudó. Diletta e Ione, agradeceros vuestra comprensión, os prometo que no siempre fui así.

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He reservado para el final los agradecimientos más especiales,

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Para terminar, hay una persona que no aparecerá como co-autor de mis trabajos y sin embargo, ha vivido, sufrido y conoce cada detalle de esta tesis. David, has tenido una paciencia infinita y has antepuesto mis necesidades a las tuyas durante todos estos años. Los momentos compartidos, los recuerdos atesorados, el cariño incondicional y la complicidad eran la mejor medicina cuando las cosas no salían como quería en el laboratorio. El tiempo que hemos pasado juntos hasta ahora ha sido un suspiro, el que quiero pasar contigo no lo puedo medir, así que empecemos con un para siempre.

Laia

Noviembre 2018

Abstract

The ocean produces ca. 25% of the global primary production, capturing inorganic carbon from the atmosphere which is recirculated and/or sunk into the deep zones. Between 60 and 70% of primary production in the ocean is consumed by microzooplanktonic organisms ranging from 20 to 200 μm, which in turn are responsible for an important portion of the remineralized organic matter in the photic layer. Copepods are the major consumers of microzooplankton, the latter being key organisms between mesozooplankton and phytoplankton. Current knowledge about physical, chemical, and biological factors influencing microzooplankton, as well as their role as shapers of the planktonic community are still poorly studied. In the present work, we studied the way microzooplankton structure the planktonic community through top-down effects, bloom control, and through different physical scenarios at ocean basin scale. Our results showed how abundance of mesozooplankton modified the microzooplankton community, causing a cascade effect and releasing autotrophic picoeukaryotes, *Synechococcus*, *Prochlorococcus* and diatoms from grazing pressure. This result supports the hypotheses formulated by previous studies, suggesting that parallel increases in mesozooplankton and phytoplankton in the ocean are a consequence of a top-down control of mesozooplankton upon microzooplankton. We also studied the development of a spring bloom in a temperate area. This annual event drastically changed the phytoplankton community along with physical and chemical conditions, also promoting changes in the microzooplanktonic community. Results obtained in Roskilde fjord (Denmark) during the spring bloom showed a change in the ciliate community from mixotrophy to heterotrophy as bloom aged and nutrients became depleted. This variation in ciliates led to a change in phytoplankton, suggesting that these organisms promoted a rather fast switch in the phytoplankton community. Finally, results

obtained in tropical and subtropical Atlantic ocean showed the dominance of microzooplankton in the oligotrophic ocean, while in productive waters similar biomasses of micro- and mesozooplankton were found. The study also showed a change from dinoflagellates in oligotrophic areas to ciliates in productive systems, implying more diffusive daily grazing patterns when ciliates dominated the community. However, in both oligotrophic and productive waters microzooplankton was the main consumer of primary production. These results provides novel results on plankton dynamics influencing the productive scenario in the ocean.

Resumen

El océano produce el 25% de la producción primaria global, captando carbono inorgánico de la atmosfera que es recirculado y/o hundido hacia las zonas profundas a través de los organismos. Entre el 60 y el 70 % de la producción primaria en el océano es consumida por el microzooplancton, organismos comprendidos entre 20 a 200 μm, que a su vez son responsables de la mayor remineralización de la materia orgánica en la capa fótica. Los copépodos son los mayores consumidores de microzooplancton, siendo éstos últimos los organismos clave entre el mesozooplancton y el fitoplancton. El conocimiento actual de los factores físicos, químicos y biológicos que influyen en el microzooplancton, así como su papel en la re-estructuración de la comunidad planctónica son poco conocidos. En este trabajo se ha estudiado la forma en que el microzooplancton estructura la comunidad planctónica a través de los efectos top-down, el control del Bloom en zonas templadas y a través de distintos escenarios físicos a escala de cuenca océanica. Nuestros resultados han mostrado como como la abundancia de mesozooplancton modificó la comunidad de microzooplancton, creando un efecto en cascada, liberando a picoeucariotas, *Synechococcus*, *Prochlorococcus* y diatomeas de la presión de pastaje. Este resultado respalda las hipótesis formuladas por estudios previos, sugiriendo que el crecimiento paralelo de mesozooplancton y fitoplancton en el océano es el resultado de un control top-down del mesozooplancton sobre el microzooplancton. En este trabajo también se ha estudiado el desarrollo de un bloom primaveral en una zona templada. Este evento anual, junto a las condiciones físicas y químicas, cambió drásticamente la comunidad fitoplanctónica, promoviendo también cambios en la comunidad del microzooplancton. Los resultados obtenidos en el fiordo de Roskilde (Dinamarca) durante el bloom primaveral mostraron un cambio en la comunidad de ciliados desde organismos mixotróficos a heterotróficos a

medida que avanzó el bloom y se agotaron los nutrientes. Esta variación en los ciliados conllevó un cambio en el fitoplancton, sugiriendo que estos organismos promovieron fluctuaciones rápidas en la comunidad de fitoplancton. Finalmente, los resultados obtenidos en el océano Atlántico tropical y subtropical mostraron que el microzooplancton dominó en el océano oligotrófico, mientras que en las zonas productivas se encontraron biomasas parecidas de micro- y mesozooplancton. El estudio también mostró un cambio de dinoflagelados en zonas oligotróficas a ciliados en sistemas productivos, implicando patrones diarios de pastaje más difusos cuando los ciliados dominaron la comunidad. Sin embargo, tanto en aguas oligotróficas como en productivas, el microzooplancton fue el principal consumidor de la producción primaria. Estos resultados proporcionan resultados novedosos sobre la dinámica del plancton que influyen en el escenario productivo del océano.

Preface

This thesis entitled *Microzooplankton in marine foodwebs: Top-down effects, bloom control, and grazing at ocean basin scale* was conducted under the supervision of Dr. Santiago Hernández-León at the Biological Oceanography Group belonging to the Instituto de Oceanografía y Cambio Global (IOCAG) of the Universidad de Las Palmas de Gran Canaria (ULPGC), within the Doctoral program in Oceanografía y Cambio Global. The thesis compiles three original studies, published or in peer-review journals (Journal Citations Reports), in the frame of projects LUCIFER (CTM2008-03538) and MAFIA (CTM2012-39587) granted to Dr. Santiago Hernández-León, as well as a research stay at the Institut for Bioscience in Aarhus University (Denmark) and the Institut de Ciències Marines of the Consejo Superior de Investigaciones Científicas (Barcelona). The thesis received financial support from Grant Programs as Formación de Personal Investigador from Ministerio de Economía y Competitivdad (MINECO, Spanish Gouvernment), Ayudas a la Formación de Personal Investigador en Formación from MINECO (Spanish Gouvernment), and Ayudas al Personal Investigador en Formación from the ULPGC.

The thesis is structured according to the regulation of Reglamento de Estudios de Doctorado from the ULPGC (BOULPGC, Chap. III, Art. 11 and 12, October 7th, 2016) in English language with a general introduction presenting the goals of the thesis, published studies and the justification of the thematic unit; then, the three scientific contributions following the conventional scientific paper format; finally, the main conclusions. The thesis also include a section written in Spanish language explaining the objectives developed and the main conclusions, in compliance with the regulations of Reglamento de Estudios de Doctorado from ULPGC (BOULPGC, Chap. III, Art. 10, October 7th, 2016).

Prefacio

La tesis doctoral titulada *El microzooplancton en las redes alimentarias marinas: Efectos top-down, control del bloom y pastaje a escala de cuenca oceánica*, se ha desarrollado bajo la supervisión del Dr. Santiago Hernández León, del Grupo de Oceanografía Biológica perteneciente al Instituto de Oceanografía y Cambio Global (IOCAG) de la Universidad de Las Palmas de Gran Canaria (ULPGC), dentro del Programa de Doctorado en Oceanografía y Cambio Global. La tesis está compuesta por una recopilación de tres trabajos originales, publicados o en proceso de revisión en revistas indexadas en el *Journal Citations Reports*, enmarcados dentro de los proyectos LUCIFER (CTM2008-03538) y MAFIA (CTM2012-39587) concedidos al Dr. Santiago Hernández León, así como de las estancias realizadas en el Institut for Bioscience en Aarhus University (Dinamarca) y el Institut de Ciències Marines del Consejo Superior de Investigaciones Científicas (Barcelona). La tesis ha recibido el apoyo financiero de los programas de Formación de Personal Investigador del Ministerio de Economía y Competitividad (MINECO, Gobierno de España), Ayudas a la Formación de Personal Investigador en Formación del MINECO (Gobierno de España), y Ayudas al Personal Investigador en Formación de la Universidad de Las Palmas de Gran Canaria.

La tesis se organiza según el Reglamento de Estudios de Doctorado de la ULPGC (BOULPGC, Cap. III, Art. 11 y 12, 7 de octubre de 2016) en lengua inglesa con una introducción general presentando los objetivos de la tesis, los trabajos publicados y la justificación de la unidad temática; a continuación las tres contribuciones científicas siguiendo el formato de artículo científico convencional; y por último, se sintetizan las principales conclusiones. La tesis también incluye una sección en lengua castellana explicando los objetivos desarrollados y las principales conclusiones, en cumplimiento de la normativa del Reglamento de Estudios de Doctorado de la ULPGC (BOULPGC, Cap. III, Art. 10, 7 de octubre de 2016).

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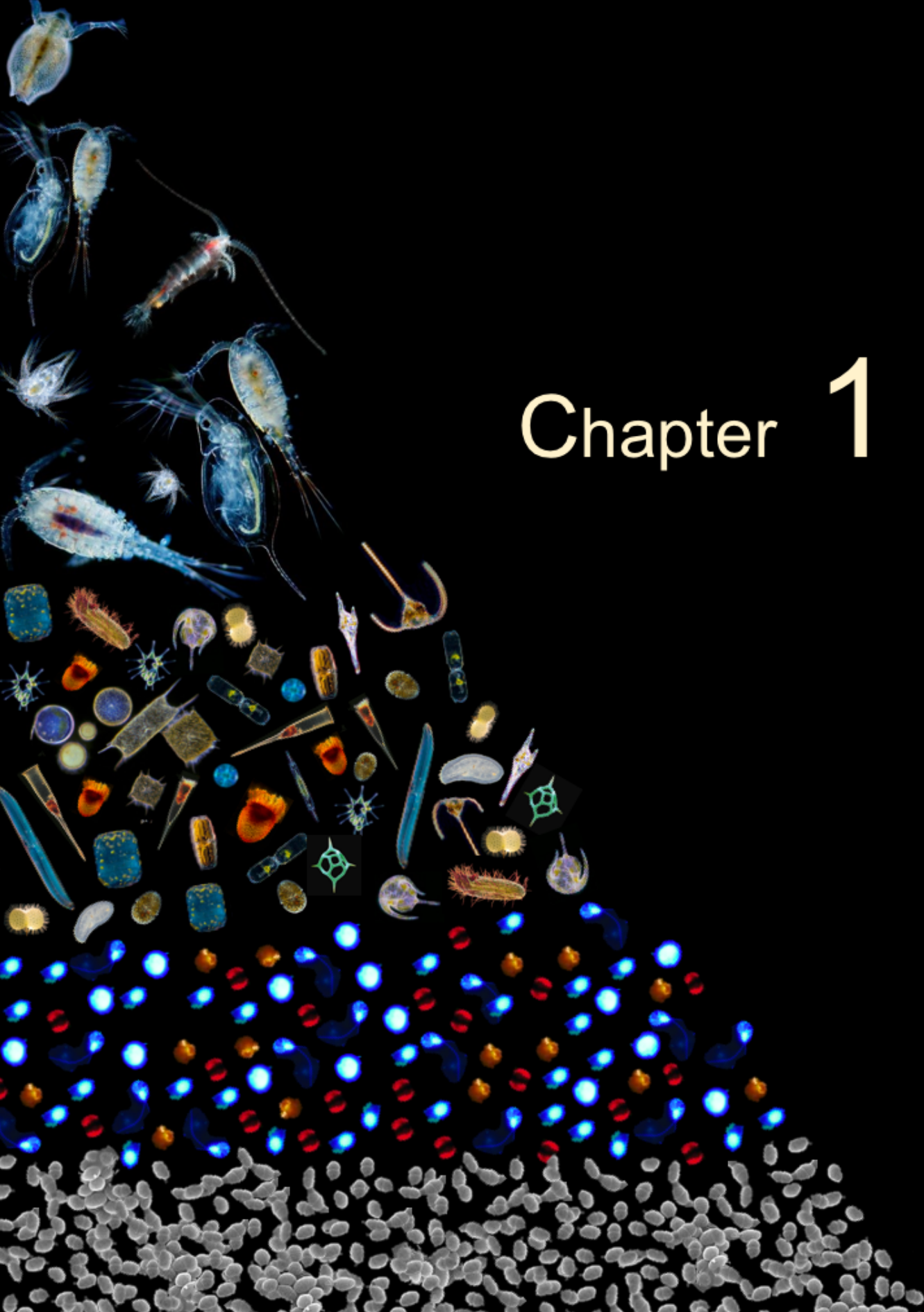

Chapter 1

Science knows no country, because knowledge belongs to humanity, and is the torch which illuminates the world.

Louis Pasteur

1 Introduction

CHAPTER

1.1 Microzooplankton background

Plankton is the basis of the marine trophic web and the study of its composition and processes taking place in these communities will contribute to the understanding of marine ecosystems (Fuhrman 2009). The term plankton was used to indicate all natural organic particles which float freely and involuntarily in the open ocean (Hensen 1887). In the marine environment, there is a wide variety of organisms that belong to different groups and can be classified according to their structural, functional or dimensional characteristics. Traditionally, marine plankton has been divided according to its trophic characteristics into phytoplankton (autotrophic organisms) and zooplankton (heterotrophic organisms), and more recently, it includes mixotrophs (Stoecker 1998, Sherr & Sherr 2002). Mixotrophic organisms are ubiquitous and combine autotrophic and heterotrophic feeding. The size range

of those organisms varies from pico- to mesoplankton and includes prokaryotes, single-celled eukaryotes, protists and zooplankton organisms (Stoecker et al. 2017). The mixotrophy in bacterioplankton, phytoplankton and microplankton is common in surface and warm waters, allowing organisms to survive during long periods of starvation (Swan et al. 2013). However, the most used classification is according to their size (Sieburth 1979): femtoplankton (< 0.2 µm), picoplankton (0.2-2 µm), nanoplankton (2-20 µm), microplankton (20-200 µm) and mesozooplankton (200-2000 µm) (Fig. 1.1). Protists, small metazoans and meroplankton belong to microzooplankton group, but the most common mixotrophic organisms are ciliates and dinoflagellates.

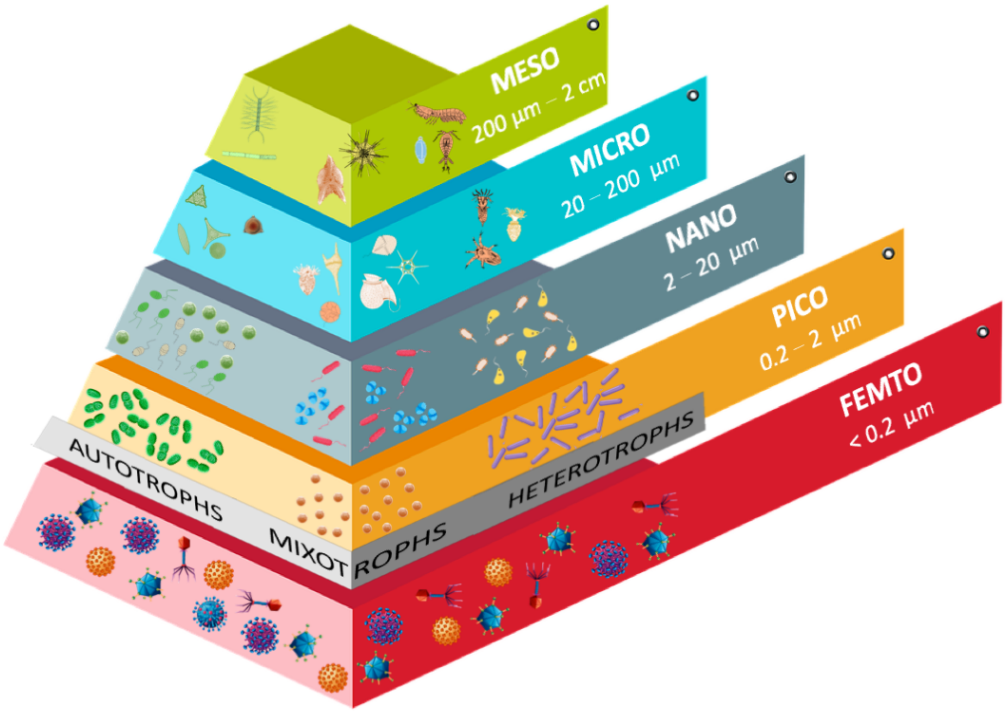

Figure 1.1 Plankton division according size (femto-, pico-, nano-, micro- and mesoplankton) and feeding behaviour (autotrophs, mixotrophs and heterotrophs).

From a perspective of trophic relationships, the categorization of "functional type" classifies organisms according their ecological (Gitay & Noble 1997) and physiological functions (Mitra et al. 2016): phagoheterotrophs (lacking phototrophic capacity); photoautotrophs (lacking

phagotrophic capacity); constitutive mixotrophs (phagotrophs with an inherent capacity for phototrophy); non-constitutive mixotrophs (phototrophic capacity acquired ingesting specific preys); and, general non-constitutive mixotrophs (phototrophic capacity acquired ingesting general non-specific preys) (Fig. 1.2).

A quarter of the global primary production occurs in the ocean (Field et al. 1998, Falkowski et al. 1998), thereby it is important to understand how organisms use carbon and how much is transferred to fish, respired and returned to atmosphere, sunk through organisms to meso- and bathypelagic zones, or sequestered in the ocean floor (Fig. 1.3). The interest of the carbon cycle has increased due to global warming, coastal eutrophication, and overfishing (Jackson et al. 2001; Pauly et al. 2003). In the ocean, two approaches exist to understand the functioning of marine trophic web: the "classic" trophic web and the microbial loop. The classic trophic web (Mills 1989), from a fishing perspective, establishes that phytoplankton (mainly diatoms and dinoflagellates) is consumed by planktonic metazoans (mostly copepods) and later by fish (Fenchel 1988; Mills 1989). However, this perspective ignored microorganisms such as bacteria which dominate the ocean in abundance, diversity and metabolic activity (Steel 1974; DeLong & Karl 2005). On the other side, microbial loop, taking into account bacteria, sustains that larger protists consumes small autotrophs and heterotrophs, while nourishing bacteria with their excretions. In this trophic web, microzooplankton acts as a link between primary producers and consumers (Pomeroy 1974; Azam et al. 1983; Sherr & Sherr 1988). In this frame, high trophic levels consume a small part of the organic matter produced by autotrophs, being the most recirculated by different trophic levels (Azam et al. 1983; Sherr et al. 1986).

The importance of microzooplankton lies as: (1) the main consumers of primary production, (2) its intermediary role between primary producers and mesozooplankton, and (3) as excretory organism (Gifford 1991; Calbet & Landry 2004; Calbet 2008). Microzooplankton are the main consumers of bacteria, small autotrophs, flagellates and even other protists (e.g. Campbell 1926, 1927; Sherr et al. 1986; Strom 1991; Hansen 1992; Sherr & Sherr 2003), and responsible for most remineralization of dissolved organic matter (Azam et al. 1983). In turn, copepods are the main consumers of microzooplankton due to their size and nutritional composition (Berggreen et al. 1988; Stoecker & Capuzzo 1990; Wickham 1995; Broglio et al. 2003), being

microzooplankton the intermediary organisms between mesozooplankton and phytoplankton.

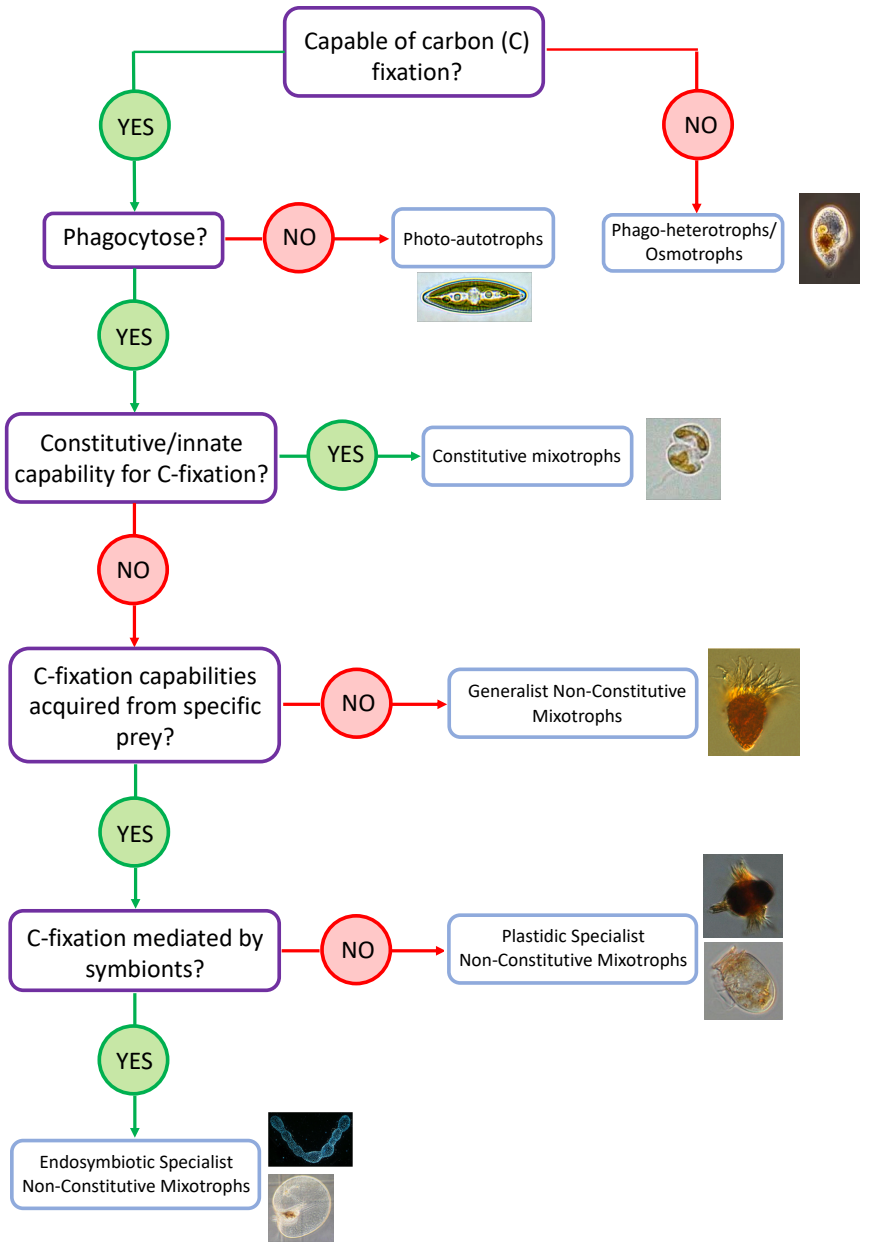

Figure 1.2 Functional protist classification, redrawn from Mitra et al. (2016).

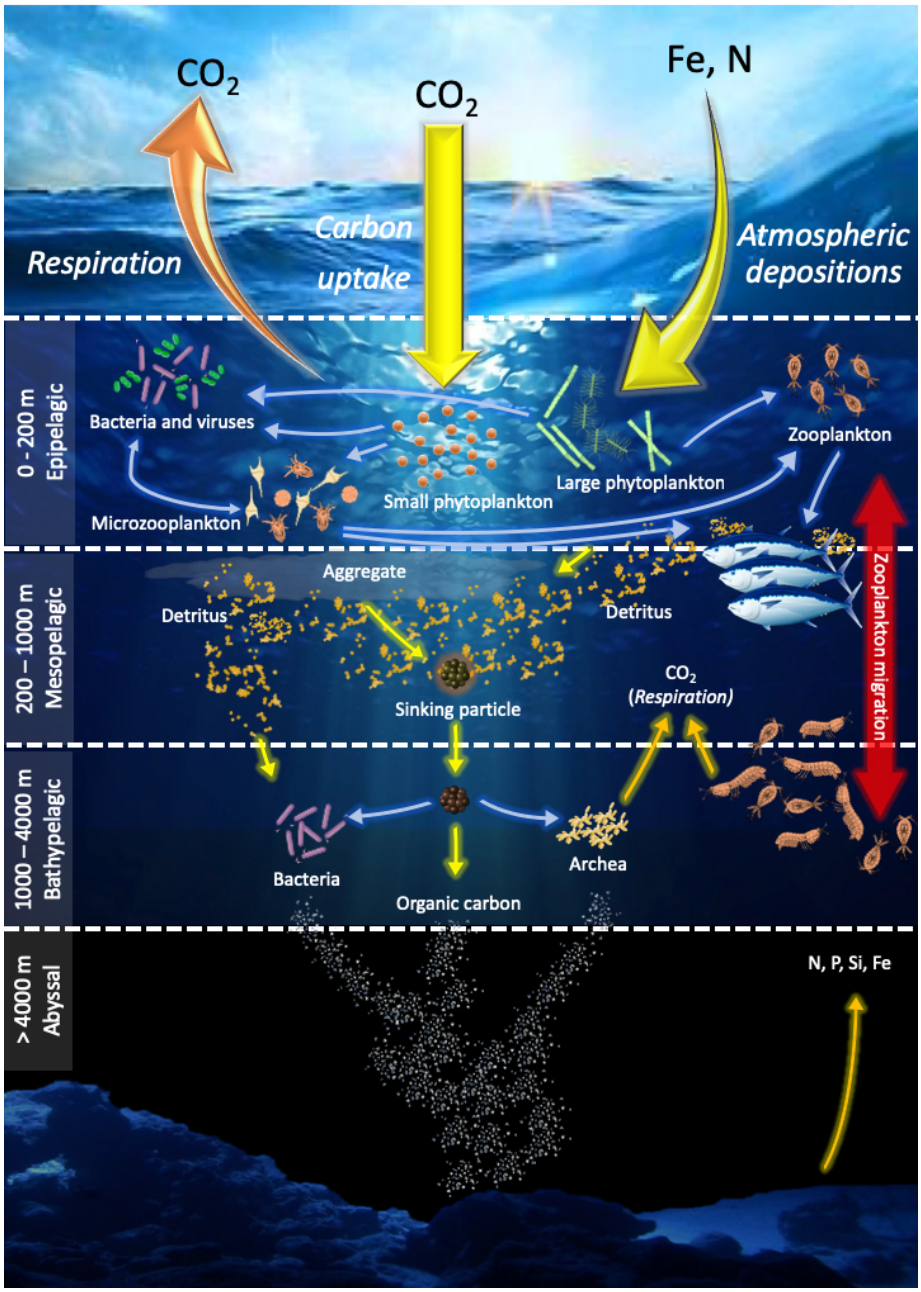

Figure 1.3 Scheme of the biological pump in the ocean: carbon and nutrients sinking (yellow rows), carbon and nutrients release (orange rows), and trophic interactions (blue rows). Modified from Oak Ridge National Laboratory.

Some studies have observed the control of mesozooplankton on microzooplankton, releasing primary producers from grazing pressure (Calbet & Landry 2004; Stibor et al. 2004a, b; Vadstein et al. 2004; Sherr & Sherr 2007). As a consequence of their feeding, microzooplankton excrete dissolved organic matter (e.g. Ward & Bronk 2001), and inorganic nutrients such as ammonium and phosphates (e.g. Dolan 1997), fertilizing the environment and promoting the growth of its potential preys (Dolan 1997). As a result of microzooplankton role in the trophic web, this group of organisms become the key stone in the microbial loop (Azam et al. 1983; Sherr & Sherr 2002).

Oligotrophic differ from productive areas in physical variables such as temperature and biological factors such as composition or abundance of the planktonic communities (Schmoker et al. 2016; Christaki et al. 2014; Billen et al. 1990). The warm and stratified waters of subtropical gyres are oligotrophic areas that cover approximately 40% of the planet's surface, and they are expanding 0.8-4.3 % y^{-1} as a result of global warming (Polovina et al. 2008). In these areas small cells dominate the plankton community and microzooplankton is more efficient than mesozooplankton to predate upon phytoplankton due to its similar size with phytoplankton, high growth rates, and high metabolism (Fenchel 1987; Sherr & Sherr 1994; Boëchat et al. 2007; Jones 2000), consuming more than 70% of primary production (Calbet & Landry 2004). In the other side, large cells such as diatoms dominate the autotrophic community in productive waters. Despite the composition and abundance of planktonic organisms vary from oligotrophic waters, microzooplankton also consume ca. 60 % of the primary production in these productive areas (Calbet & Landry 2004; Schmoker et al. 2016), while the impact of mesozooplankton is approximately 10% of the primary production consumed daily (Calbet 2001). In upwelling areas, characterized by swift changes in environmental conditions, microzooplankton own the ability to adapt at the same time scales as preys but not copepods, which require longer periods to develop (Calbet 2008; Hernández-León 2008; Schmoker et al. 2016). This fact explains the low impact of grazing by large metazoans (mainly copepods) on phytoplankton (Berggreen et al. 1988; Calbet 2008). However, mesozooplankton are important organisms restructuring the trophic webs (e.g. Gifford 1991; Gowen et al. 1999) and acting as a link between lower and high trophic levels (Cushing 1989).

Thus, phytoplankton growth and microzooplankton grazing rates are of paramount importance to study these communities. However, their knowledge is, at present, rather limited because of the difficulty to measure them. The dilution method (Landry & Hassett 1982) is the most widely used to estimate the microzooplankton grazing on phytoplankton in the ocean. This approach is based on three premises: (1) the presence or absence of other phytoplankton cells do not affect the phytoplankton growth; (2) the encounters between preys and grazers is proportional to the probability of the cells being consumed; (3) the growth of phytoplankton over time is exponential. The method consists of incubating bottles with different natural or whole seawater (WSW) dilutions increasing the filtered seawater volume, and therefore, decreasing the encounters between preys and grazers. The slope of the apparent growth (k) along dilutions is the mortality rate (m) of autotrophs as a result of grazing, and the net growth of phytoplankton (μ) is the intercept with y-axis. This methodology is rather difficult to carry out due to the different dilutions levels (4-5) and the large volume of water used, precluding to obtain high resolution data in oceanographic studies. Also, the non-linear responses observed in apparent growth rates, similar apparent growth rates in highly diluted treatments, and top-down effects are additional drawbacks of this methodology (Gallegos 1989; Calbet & Saiz 2013). A simplification of this method is the so-called "2-point method" which consists in the incubation of undiluted treatment (100% WSW) and a dilution of 33% WSW (Landry et al. 2009), or 37% WSW (Landry et al. 2011), or 10% WSW (Lawrence & Menden-Deuer 2012; Sherr et al. 2013) or 5% WSW (Strom & Frederickson 2008). The treatment of 100% WSW contains all organisms <200 μm, and represents the net growth rate of phytoplankton in presence of grazers (k, d^{-1}). The 5% WSW treatment is sufficiently diluted to assume as 0 the encounters between preys and grazers. The mortality by grazing is defined as:

$$g = \mu - k$$

At treatments of 5% WSW, the mortality by grazing is assumed to be 0 and, the intrinsic growth (μ, d^{-1}) is:

$$\mu = k$$

The photoaclimatization response of phytoplankton to experimental conditions of incubation, and the variations in daylight levels could result in negative or positive errors in growth rate estimation. To avoid these errors, negative growth rates are assumed as 0.01 d^{-1}, while negative grazing rates are assumed as 0 d^{-1} (Calbet & Landry 2004).

1.2 Objectives and outline

In this thesis, the study of the microzooplankton community has been approached from different perspectives: from trophic relationships and from the carbon and energy flux between microzooplankton and the high/low trophic levels. The specific goals are:

1. The effect of variations in abundance of mesozooplankton on natural planktonic communities (trigger a bottom-up or top-down control) and to determine the implications for the transfer of energy towards lower trophic levels. This objective is developed in *Chapter 2*.
2. The dynamics of the community of ciliates during a spring bloom in temperate waters, and the changes in abundance and functionality of organisms related to variations in nutrients, light, and temperature. This objective is addressed in *Chapter 3*.
3. Understand the relationships of communities from pico- to mesozooplankton, the impact of physical variables on organism distribution, and the trophic differences between oligotrophic and productive waters in the tropical and subtropical ocean. This objective is discussed in *Chapter 4*.

1.3 Rationale of the study

The use of fossil fuels is the main cause of atmospheric CO_2 emissions, which in turn together with other greenhouse gases, are responsible for the increase of global temperature (NOAA, 2015). Near 50% of emissions remain in the atmosphere, while the other 50% is sequestered by the ocean and terrestrial vegetation. The estimation of the anthropogenic CO_2 absorbed by

the ocean is nearly 70%, becoming the main sink of this gas (Siegenthaler and Sarmiento 1993). The sequestration of CO_2 by the ocean occurs through physical mechanisms (physical pump or solubility pump) and biological mechanisms (biological pump). The biological pump is the transfer of organic matter into the ocean through different processes such as physical mixing of particulate and dissolved organic matter, active flux by zooplankton and micronekton, and passive or gravitational flux, transferring carbon to the deep ocean (Volk & Hoffert 1985; Buesseler et al. 2007). First, phytoplankton capture CO_2 to carry out photosynthesis, transforming the inorganic carbon into particulate organic carbon (POC). Between 5 and 25% of primary production is transported from the euphotic zone to deeper layers, and only 3% of this primary production reaches the bathypelagic depths (De La Rocha and Passow 2007). The rest of primary production is remineralized in the superficial layers of the ocean. To understand the functioning of the biological pump and the carbon flow and its implications, it is important to understand the functioning of the plankton community, specially microzooplankton as key organisms between low and high trophic levels.

Chapter 2

I would like to reach the coast which gives the apples of the singing Hesperides, where the sovereign sea no longer grants route to sailors and fixes the limit of the sky that Atlas sustains! The springs distill ambrosia in the bridal chamber of the Zeus' palace, where a wonderful land feeds the happiness of the Gods.

Hippolytus, *Euripides*

2 CHAPTER

Effects of copepods on natural microplankton communities: do they exert top-down control?

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Annex IV

Abstract Top-down effects in the pelagic realm are quite well known in freshwater ecosystems. However, our knowledge of these effects in the ocean remains scant. It is known that copepods prefer to prey on ciliates and heterotrophic dinoflagellates, and their high or low abundances can change the structure of microplankton communities. Field studies in subtropical waters have shown parallel increases of mesozooplankton and phytoplankton without a lag, suggesting a top-down effect of mesozooplankton preying upon microzooplankton and releasing primary producers from predation. In the present work, we added copepods at increasing densities to natural plankton in 24 h experiments. A decrease in aloricated ciliates abundance of nearly 50% and increases in the abundances of picoeukaryotes, *Synechococcus*, *Prochlorococcus*, diatoms, and chlorophyll *a* were observed. No effect of

nutrient additions was observed in parallel grazing experiments. Thus, a top-down effect of copepods upon microzooplankton explains the observed changes in the abundance of the different phytoplankton groups. Copepods promote important changes down the food web, structuring the community by predation upon microzooplankton. There are biogeochemical consequences of zooplankton variability over short time scales in the ocean.

2.1 Introduction

Plankton can be divided according to the sizes of organisms (Sieburth et al. 1978) ranging from femto- (0.02-0.2 μm), through pico- (0.2-2 μm), nano- (2-20 μm), and micro- (20-200 μm) to mesozooplankton (0.2-20 mm). Therefore, important predator-prey interactions should be expected along the size gradient as feeding is roughly related to body size (Longhurst 1991). Among these interactions, the effects of mesozooplankton predators downward through the trophic web, and their effects in structuring the plankton communities, have scarcely been studied in comparison to the effects of the physical aspects of ocean ecosystems. Carpenter et al. (1985) defined the trophic cascade concept to describe the top-down effects from fish to phytoplankton in lakes. From that seminal paper to the present, numerous studies have described top-down effects occurring in freshwater systems. However, that is not the case for the oceanic environment, where the top-down control is substantially more difficult to observe.

Microzooplankton, mainly ciliates and dinoflagellates, act as an important link between primary producers and mesozooplankton, and are an important source of energy for copepods in the ocean (Burkill et al. 1993; Calbet and Landry 1999). In oligotrophic waters, copepods prefer to prey upon ciliates and dinoflagellates (Fessendem and Cowles 1994; Suzuki et al. 1999; Broglio et al. 2004; Calbet and Saiz 2005), probably because autotrophic production is low and mainly comes from small cells that are rarely consumed by copepods (Nival and Nival 1976; Berggreen et al. 1988; Dam et al. 1995; Calbet and Landry 1999). Calbet and Landry (2004) demonstrated the effect of microzooplankton control by mesozooplankton, and its release of primary production as a trophic cascade. Several earlier mesocosm studies observed copepods, specifically, as a keystone group structuring marine planktonic

communities by preying upon microzooplankton and allowing an increase of phytoplankton (Stibor et al. 2001; Vadstein et al. 2004). In these experiments, zooplankton concentration and nutrients were controlled (from controlling and replete to minimal and limiting, respectively), showing a top-down control by copepods preying upon microzooplankton and thus promoting an increase of primary producers. However, zooplankton effects on small and large phytoplankton varied in relative magnitude depending on other environmental conditions (Stibor et al. 2004).

The vertical distribution of epipelagic zooplankton in subtropical waters is layered (see Hernández-León et al. 1998), and their densities at the microscale are expected to be much higher than average values for the upper 100 or 200 m of the water column. In fact, thin layers of zooplankton in the ocean (McManus et al. 2003) extend for kilometres and persist for days, harbouring rather high densities. Benoit-Bird et al. (2013) showed >90% of zooplankton are located in layers thinner than 5 m. Using optical plankton counters, high density layers on the order of 4-5 individuals L^{-1} have been observed in warm waters (see Marcolin et al. 2015). Hence, high densities (>3 individuals L^{-1}) should be expected in the epipelagic zone, likely mesozooplankton moving to feed on microplankton layers. Copepod swarms hosting tens or even hundreds of copepods per litre have also been observed in subtropical waters (Ueda et al. 1983).

Previous studies have found that copepods prey selectively depending on environmental conditions, preferring larger particles or those most abundant (Frost 1972; Allan et al. 1977; Donaghay and Small 1979; DeMott 1989), and sometimes discriminating among algae depending on their nutritional quality (Libourel Houde and Roman 1987; Simpson and Raubenheimer 2012) or toxicity (Huntley 1982). Selective grazing is presumably based on choosing prey such that the cost of feeding is less than the benefit provided by the prey (Simpson and Raubenheimer 2012; Isari et al. 2013; Meunier et al. 2016).

In subtropical waters, Hernández-León (2009) observed a parallel increase in copepods and primary production during the late winter bloom without the expected lag about two weeks at 18.5 ºC for copepod development between them and the algae. He suggested that mesozooplankton consume microzooplankton during the bloom, consequently promoting the releasing the primary producers from grazing, and therefore inducing a top-down effect.

Schmoker et al. (2012) also observed a match between mesozooplankton and picoeukaryotes during the development of the late winter bloom in subtropical waters, suggesting a top-down control of microzooplankton by mesozooplankton. However, the effects of mesozooplankton and microzooplankton on the increase of picoplankton could be related to (1) the top-down effect produced by mesozooplankton feeding on microzooplankton, (2) fertilisation by the excretion (mainly ammonia) of micro- and mesozooplankton enhancing primary production, or (3) both.

In addition, several groups have reported a close relationship between mesozooplankton and chlorophyll *a* (Finenko et al. 2003) or primary production (Isla et al. 2004) in tropical and subtropical waters. This close relationship could be due to a bottom-up effect of the increase in primary production, or to top-down control by mesozooplankton on microzooplankton allowing more phytoplankton to survive and grow.

Thus, there are some indications that the top-down effect of mesozooplankton in the ocean should be of importance, explaining in part the transfer of energy and matter through the plankton community. In this study, we have addressed the hypothesis that an increase in mesozooplankton (especially copepods) in ocean ecosystems would decrease the microzooplankton and release autotrophs via release. The main objective of the study was to manipulate the effect of variations in mesozooplankton abundance on the natural plankton community at an oceanic site, and to determine the implications for the downward control of energy and matter transfers along the food web.

2.2 Materials and methods

2.2.1 Seawater sampling and experimental set up

Sampling of copepods and the natural plankton community for the experiments was carried out weekly on board the R. V. “Atlantic Explorer” from February to June of 2010 in oceanic waters to the north of Gran Canaria, Canary Islands (Fig. 2.1). Seawater samples were obtained at night to reduce stress on the plankton community prior to incubation, especially copepods and other light-sensitive plankton. Water was taken from the mixed layer at 20 m

depth with a 30 L Niskin bottle. Zooplankton was collected with a 100 µm mesh WP-2 net. Hauls were performed vertically from 100 m to surface at ~0.5 m s^{-1}. The organisms from the non-filtering cod-ends were gently transferred to a beaker and filtered through 2 mm mesh size net, and then a 500 µm mesh was used to concentrate copepods in a smaller volume of water with minimum stress. No species selection was done, but they were small calanoid copepods (mainly *Clausocalanus* spp. and *Paracalanus* spp.) typical of the sampling site.

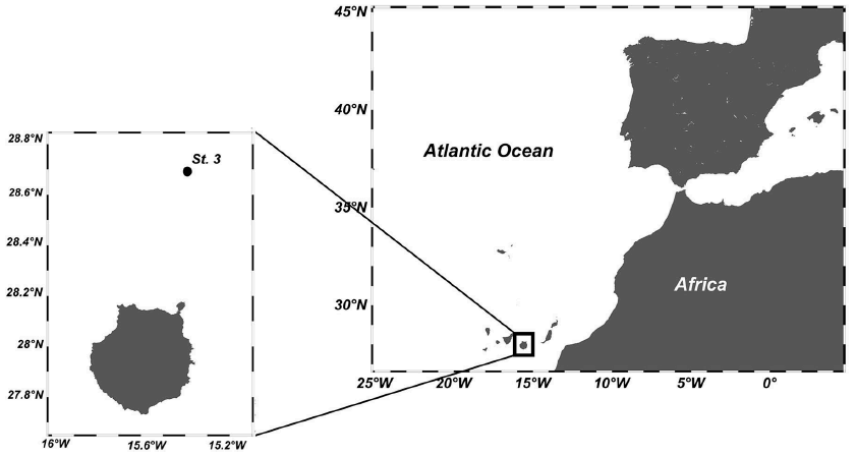


Figure 2.1 Location of the sampling station north of Gran Canaria Island, Canary Islands.

Seawater from the 30 L Niskin bottle was gently transferred to six Nalgene bottles (2.4 L) through a 200 µm filter to remove large copepods and other mesozooplankton. One bottle without copepods was used as a control, and the other bottles were incubated with approximately 1, 5, 10, 15, and 25 copepods each, simulating increasing copepod abundances from 0 to about 12 copepods per litre. An additional bottle was also incubated during for 15 minutes and then considered to represent the initial conditions, t = 0. The bottles were incubated on deck using a continuous seawater input system, thus maintaining nearly constant temperature. The incubator was covered with a net to avoid direct sunlight and simulate the solar radiation at 20 m depth. After 24 hours, the bottles were sampled for picoplankton, bacteria, microzooplankton, and chlorophyll *a*. Copepods were counted by filtering water in the bottle with 200 µm mesh. Only copepods alive at the end of the experiment were counted.

2.2.2 Phytoplankton analysis

Small photosynthetic eukaryotic cells (autotrophic picoeukaryotes), heterotrophic prokaryotes, and *Prochlorococcus* and *Synechococcus* types of cyanobacteria were counted by flow cytometry using a FACScalibur instrument (Becton and Dickinson). Samples of 1.6 mL of water from the Nalgene bottles were collected and fixed with 100 µl of paraformaldehyde, kept for 30 minutes at 4 ºC and then placed in liquid nitrogen. In the laboratory, samples were stored at -20 ºC until their analysis. The abundances of these organisms were converted to carbon-biomass using 1500 fgC $cell^{-1}$ for autotrophic picoeukaryotes (Zubkov et al. 1998), 17 fgC $cell^{-1}$ for heterotrophic prokaryotes (Bode et al. 2001), 29 fgC $cell^{-1}$ for *Prochlorococcus,* and 100 fgC $cell^{-1}$ for *Synechococcus* (Zubkov et al. 2000).

Samples of autotrophic and heterotrophic nanoflagellates were preserved following the procedure suggested by Haas (1982). Immediately after collection, the samples were fixed with glutaraldehyde (0.3% final concentration), placed into a filtration tower, fixed with diamidino-2-phenylindole for 5 minutes, filtered onto a 0.2 µm black polycarbonate membrane filter, and placed over a Whatman GF/C backing filter. The filter was mounted on a microscope slide with low-fluorescence paraffin oil. At least 300 cells, or 40 fields, were counted employing an epifluorescence Zeiss Axiovert 35 microscope under UV excitation at a magnification of x1000. The red fluorescence of chlorophyll under blue light (490/515 nm) allowed us to discriminate autotrophic (photosynthetic) from heterotrophic eukaryotes. Abundances of heterotrophs were converted to biomass assuming a mean volume of 10 μm^3 and a conversion factor of 220 fgC μm^{-3} (Børsheim and Bratbak 1987). For autotroph biomass we assumed a mean volume of 20 μm^3 $cell^{-1}$ and $0.433(BV)^{0.863}$ pgC $cell^{-1}$ (Verity et al. 1992).

Microplankton samples were collected in 500 mL amber bottles, fixed with acid Lugol's iodine (2% final concentration) and stored at room temperature in darkness until analysis. Aliquots of 100 mL were added to Utermöhl sedimentation chambers for 48 h, and we counted ciliates, tintinnids, dinoflagellates and diatoms settled on the entire chamber bottom. Ciliates and dinoflagellates were also classified according to size (larger and smaller than 15 µm). Microplankton abundances were converted to biomass using total biovolume data obtained from previous measurements in these waters (A. Ojeda, unpublished data). Biovolumes were converted to carbon using the

experimental factors for ciliates (Putt and Stoecker 1989), tintinnids (Verity and Langdon 1984), dinoflagellates (Mender-Deuer and Lessard 2000), and diatoms (Strathmann 1967).

Chlorophyll samples were also collected in 500 mL amber bottles, filtered through 47 mm Whatman GF/F filters and stored in liquid nitrogen. In the laboratory, samples were stored at -20 ºC until analysis. Pigments were extracted with 10 mL of cold acetone at 90% in darkness during 24 hours, then centrifuging at 0 ºC for 10 minutes at 2000 rpm. Chlorophyll *a* was measured fluorometrically in a Turner Designs bench fluorometer, previously calibrated with pure chlorophyll *a* (Yentsch and Menzel 1963).

In order to test the effect of nutrient fertilisation inside the incubation bottles due to ammonia excretion by microzooplankton and copepods, we measured phytoplankton growth rates using the dilution method of Landry and Hasset (1982) that provides simultaneous estimates of the rates of phytoplankton growth and grazing impact by microzooplankton. These dilution experiments were conducted during winter and spring of 2010 in parallel with the experiments described above at the same depth. Seawater was sampled 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 the dark while the experiment was performed. Fifteen 2.4 L bottles were used to obtain three replicates of 5 different dilution levels: 100, 75, 50, 25, and 10% of natural seawater. To avoid phytoplankton growth limitation, 1 mL of an NH_4Cl and Na_2HPO_4 solution was added to every container, producing final concentrations of 0.5 μM NH_4 and 0.03 μM PO_4. Four other bottles were filled with natural seawater. One was used to obtain initial conditions, and the others were incubated without added nutrients as controls. Algal growth (μ) and mortality from microzooplankton consumption (m) were determined, following Landry et al. (1995) for the different planktonic groups by linear regressions between net growth rate and dilution factor. In each dilution series, mortality rate (m) is the slope of the regression, and the y-intercept is the growth rate. We obtained a growth rate from the nutrient-amended series (μ_n), and then corrected it to obtain the intrinsic growth rate without nutrients.

Table 2.1 Sampling location was 28.69N and -15.38E, and sampling depth for microplankton was 20 m for all experiments.

CE	MGE	Date (2010)	MLD (m)	In situ conditions											
				T (°C)	Chl a (mg m^{-3})	PE (cell mL^{-1})	Syn (cell mL^{-1})	Pro (cells mL^{-1})	HB (cell mL^{-1})	AN (cell mL^{-1})	HN (cell mL^{-1})	Diat (cell mL^{-1})	Tin (cell mL^{-1})	Cil (cell mL^{-1})	Din (cell mL^{-1})
1		08 Feb	125	19.45	0.05	29	1678	3936		74	147	170	20	390	8360
2		23 Feb	110	19.63	0.06	149	1290	12380	63875	37	208	140	50	170	4410
3	D1	10 Mar	100	19.85	0.07	466	5820	29294	107503	74	74	380	30	560	6030
4	D2	17 Mar	110	19.46	0.07	216	1271	3412	116813	49	86	360	20	1140	14520
5		23 Mar	74	19.83	0.08	88	1448	10684	123758	601	282	180	40	1290	16390
6		09 Apr	121	19.51	0.14							340	30	900	16540
7		19 Apr	71	20.94	0.06	127	4102	31076	89458	110	356	410	0	720	15800
8		23 Apr	46	20.19	0.08	85	2671	36802	129747	25	674	260	10	750	27480
9		13 May	81	20.23	0.04							210	30	830	14090
10	D3	24 May	60	20.58	0.04	97	2911	6209	126123	184	233	200	10	430	8770
11	D4	02 Jun	49	20.66	0.05	117	2814	6309	108198	74	258	480	30	420	14170
12	D5	09 Jun	62	20.09	0.06	158	3710	22331	157301	49	466	380	60	990	16120
13		14 Jun	33	21.34	0.06	284	4762	17611	180914	0	135	610	30	980	11690

Copepod experiments (CE), microzooplankton grazing experiments (MGE), date and mixed layer depth (MLD) of water sampling for experiments. In situ conditions at the sampling depth for temperature (T), chlorophyll (Chl a), picoeukaryotes (PE), *Synechococcus* (Syn), *Prochlorococcus* (Pro), heterotrophic bacteria (HB), autotrophic nanoflagellates (AN), heterotrophic nanoflagellates (HN), diatoms (Dia), tintinnids (Tin), ciliates (Cil), and dinoflagellates (Din).

Picoplankton and bacteria, nanoplankton, microplankton and chlorophyll were sampled from each bottle. Finally, copepod ingestion rates on aloricated ciliates and copepod grazing rates on all planktonic groups were estimated using equations given by Frost (Frost 1972). Any negative grazing rate estimates were assumed to be zero.

2.2.3 Statistical analysis

In order to compare all experiments, abundance data from all planktonic groups were standardised as $\log_{10}$ (experimental treatment/control treatment). Positive values indicated an increase in abundance, negative values indicated a decrease and a value of 0 (ratio = 1) indicated no effect.

A total of 13 experiments were conducted during late winter and spring (Table 2.1). Each experiment showed different initial abundances as expected. A mixed effect model was also applied to data (Pinheiro et al. 2000; 2011; Zuur et al. 2009) in order to study these differences in abundance among the experiments:

$$y_{ij} = \beta_0 + \beta_{0i} + (\beta_1 + \beta_{1i})x_j + \epsilon_{ij}$$
$$\beta_{0i} \approx N(0, \sigma_0),\ \beta_{1i} \approx N(0, \sigma_1), \varepsilon_{ij} \approx N(0, \sigma)$$

where y_{ij} represents the abundance of different groups (picoeukaryotes, nanoflagellates…), x_j abundance of copepods, β_0 is the intercept of the linear regression, β_1 the slope of the linear regression, and σ_0 the difference in the slope respect to β_1. To study differences between slopes of all experiments, a probability test was used for comparing models with and without β_{1i} (slope of the linear regression), as described by Pinheiro et al. (2000, 2011) and Zuur et al. (2009). When this test is non-significant, no differences in slopes are found between the different experiments, and the model is simplified to:

$$y_{ij} = \beta_0 + \beta_{0i} + \beta_1 x_j + \varepsilon_{ij0}\ \beta_{0i} \approx N(0, \sigma_0), \varepsilon_{ij} \approx N(0, \sigma)$$

σ_1 representing the β_1 variability among different experiments for a particular group of organisms. When σ_1 is greater than β_1, the relationship between copepods and a particular group of organisms can be positive, negative or zero. Three parameters were studied in order to verify the model data: (1)

the slopes obtained for each group at different concentration of copepods, (2) the normality of the model residuals using the Shapiro-Wilk test, and (3) the study of variances at different concentrations of copepods using the Bartlett test (see annex II).

2.3 Results

Chlorophyll *a,* picoeukaryotes, *Synechococcus*, *Prochlorococcus*, diatoms and bacteria followed a similar pattern, showing positive and significant increases with copepod concentration (Figs. 2.2, 2.3 and 2.4, and Table 2.2). On average, picoeukaryotes doubled their abundances, while *Synechococcus*, *Prochlorococcus,* and diatoms increased by 50% (Figs. 2.2 and 2.3). Heterotrophic bacteria also showed a small but significant increase during the 24 hours incubation period (Fig. 2.4) and they were also positively correlated with copepod density (Table 2.2).

Neither autotrophic nor heterotrophic nanoflagellates showed a specific pattern related to copepod concentration (Fig. 2.5). The regression slope in both cases was non-significant (Table 2.2). Partial correlation analysis showed heterotrophic nanoflagellates positively correlated with smaller dinoflagellates ($r = 0.308$; $p < 0.05$) and negatively with chlorophyll *a* ($r = -0.373$; $p < 0.01$, see annex III). No relationship was observed between small, large, or total dinoflagellates, and copepod concentration (Table 2.2).

Both large and small aloricate ciliates significantly decreased with increasing copepod concentration (Figs. 2.7a and 2.7b). The slopes of linear regressions were highly significant for small, large and total ciliates (Table 2.2). At high copepod densities, the abundance of large and small ciliates decreased by 50%. Total, small, and large ciliates followed similar trends, and their decrease as copepod concentration increased was coupled with a rise in total autotrophic organisms (Fig. 2.8). However, the loricate ciliate (tintinnid) abundance increased with copepod concentration (Fig. 2.7c). This increase was on average, 40% from low to high copepod densities (Table 2.2). However, the mixed effect model showed that the relationship between tintinnids and copepods could be positive, negative, or null depending on the experiment ($\sigma_1 = 0.07$, $\beta_1 = 0.07$, $p = 0.003$).

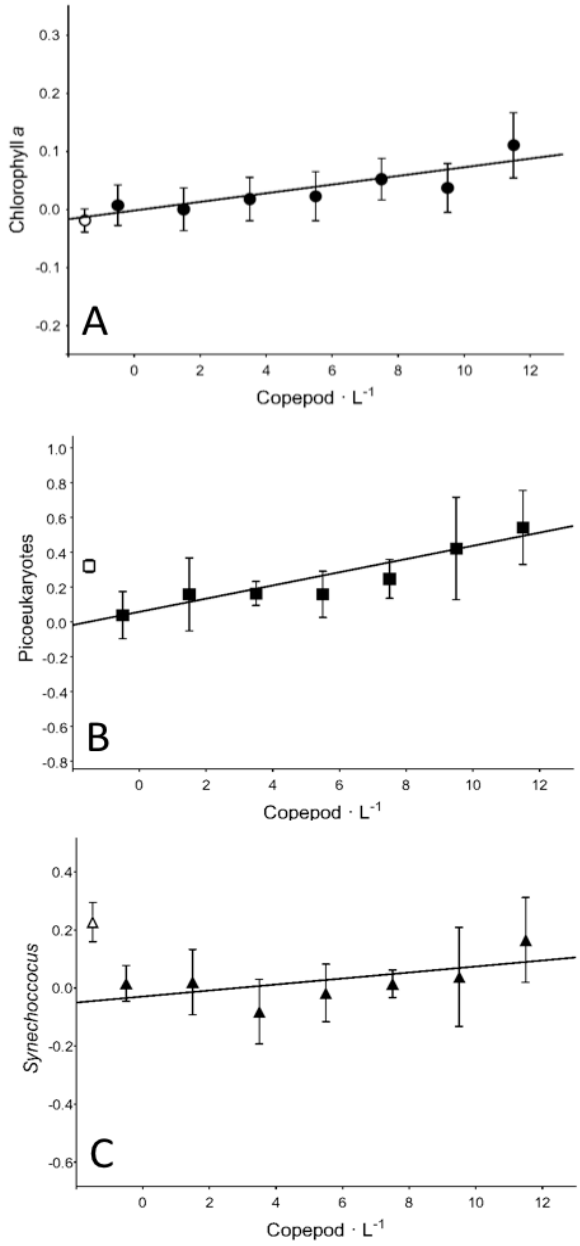


Figure 2.2 Variation in the standardized abundances for **a** Chlorophyll *a* (±SE), **b** picoeukaryotes (±SE), and **c** *Synechococcus* (±SE) in relation to copepod density. *White markers* show the mean values of all experiments for each variable at the initial time, and *black markers* show the mean values of all experiments for each variable at different copepod concentrations after 24 h of incubation.

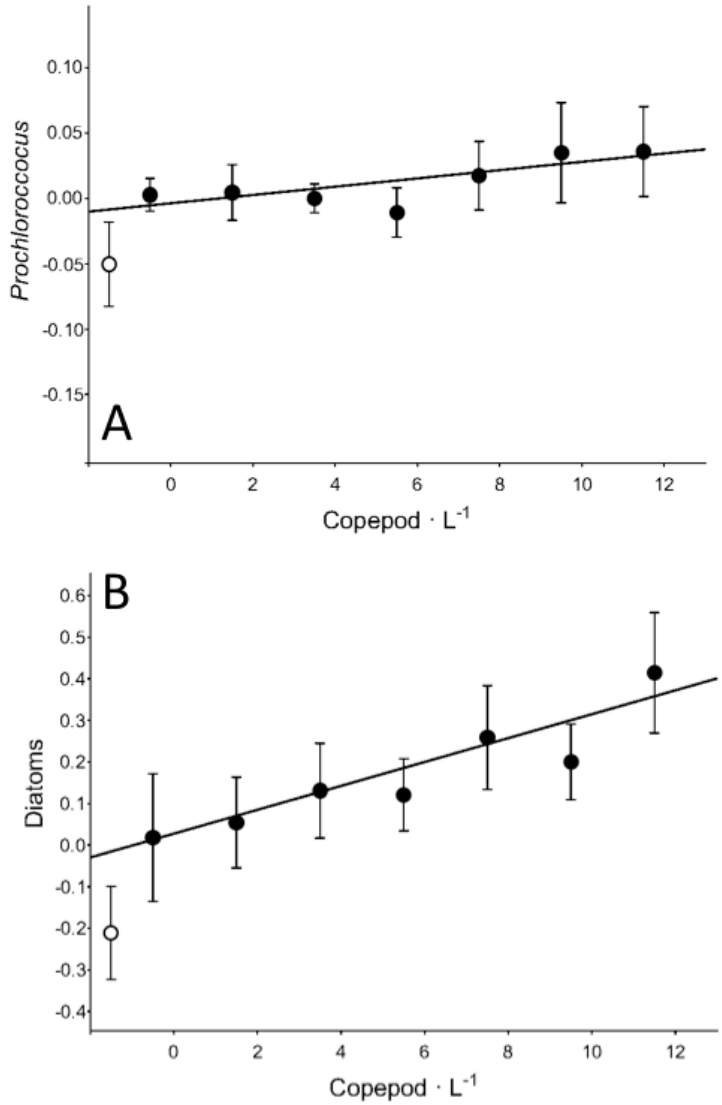


Figure 2.3 Variation in the standardized abundances for **a** *Prochlorococcus* (±SE), and **b** diatoms (±SE) in relation to copepod density. *White markers* show the mean values of all experiments for each variable at the initial time, and *black markers* show the mean values of all experiments for each variable at different copepod concentrations after 24 h of incubation.

Copepod ingestion rates of aloricate ciliates were quite variable but the highest values were achieved at low copepod concentrations (Fig. 2.9). The average ingestion rates for small ciliates was 0.07 ± 0.01 $\mu gC_{ciliate}$ copepod^{-1} day^{-1}, while for large ciliates the average was 0.13 ± 0.01 $\mu gC_{ciliate}$ copepod^{-1} day^{-1}. These values were within the range reviewed by Calbet and Saiz (2005).

For all experiments, the nutrient limitation index ($N_L = \mu_0/\mu_n$) was near 1 and no significant differences were found between average phytoplankton growth rates with and without nutrients ($p > 0.05$, $n = 15$). A higher average value of μ_0 was observed for *Prochlorococcus*, picoeukaryotes, diatoms, and dinoflagellates the bulk of autotrophs. In contrast, only two groups (*Synechococcus* and autotrophic nanoflagellates) showed a nutrient limitation index below 1 (growth rate higher in nutrient-enriched treatments, Fig. 2.10 and Table 2.3).

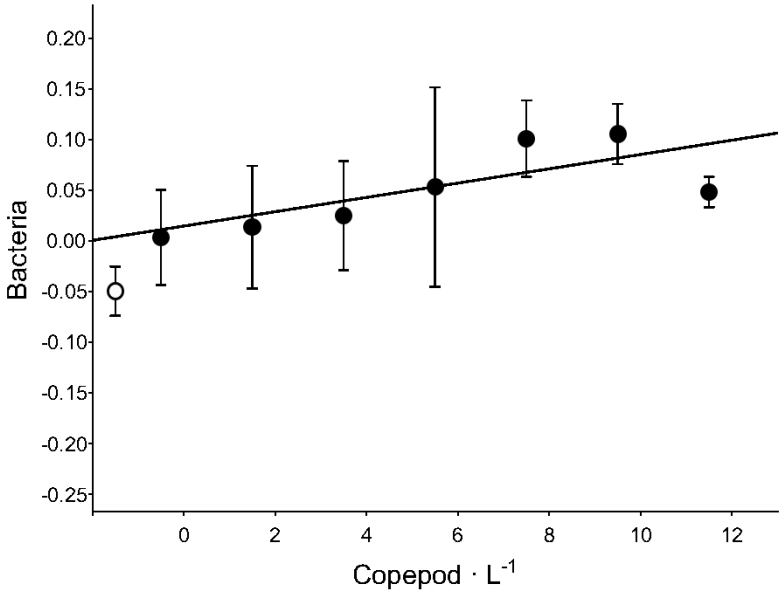


Figure 2.4 Variation in the standardized abundances for heterotrophic bacteria (± SE) in relation to copepod density. *White markers* show the mean values of all experiments for each variable at the initial time, and *black markers* show the mean values of all experiments for each variable at different copepod concentrations after 24 h of incubation.

Table 2.2 Summary of model parameters and their 95% confidence intervals.

Plankton group	β_0	β_1	σ_0	σ_1	σ
Chlorophyll *a*	0.01	**0.02***	**0.27*****		0.26
Synechococcus	−0.06	**0.02***	**0.38*****		0.32
Prochlorococcus	−0.09	**0.03***	**0.45*****		0.37
Picoeukaryotes	0.27	**0.05*****	**0.43*****		0.32
Heterotrophic bacteria	−0.03	0.03	**0.26*****	**0.06***	0.21
Dinoflagellates <15 µm	−0.18	−0.00	**0.42*****		0.29
Dinoflagellates >15 µm	−0.12	−0.02	**1.11*****		0.65
Total dinoflagellates	−0.12	−0.02	**01.11*****		0.65
Diatoms	0.01	**0.07****	**0.70*****		0.54
Tintinnids	0.28	**0.04***	**0.55*****		0.49
Ciliates <15 µm	−0.13	**−0.9***	0.73***	**0.11***	0.47
Ciliates >15 µm	0.01	**−0.05***	**0.88****		0.53
Total ciliates	−0.12	**−0.06***	**0.85*****	**0.07***	0.37
Autotrophic nanoflagellates	0.27	−0.03	**1.07*****		0.83
Heterotrophic nanoflagellates	0.10	0.01	**0.54****		0.43

β_0 and β_1 are the intercept and the slope of the linear regression of y on x, respectively, while σ is the deviation of points from the line. Bold numbers represent significant correlations at *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$

The average microzooplankton grazing rate upon total phytoplankton (Fig. 2.11) was 1.07 ± 0.89 d^{-1}, very close to the mean growth rate (0.81 ± 0.96 d^{-1}), although both rates showed high variability among experiments. *Synechococcus* and *Prochlorococcus* (0.38 ± 0.29 d^{-1} and 0.35±0.21 d^{-1} respectively) showed lowest rates, while autotrophic nanoflagellates, diatoms and picoeukaryotes supported highest grazing rates (2.18 ± 0.69 d^{-1}; 2.07 ± 0.10 d^{-1}; 0.84 ± 0.07 d^{-1} respectively). Copepod feeding rates (Fig. 2.12) for total phytoplankton, nanoflagellates, dinoflagellates and ciliates increased with copepod density. Diatoms and tintinnids showed negative feeding rates at all copepod densities, as did heterotrophic nanoflagellates and total

phytoplankton (chlorophyll *a*) at most copepod densities. At low copepod densities, ciliates <15 µm showed the highest copepod grazing rates. Conversely, dinoflagellates >15 µm supported the highest grazing rates when copepod density increased. When copepod density was between 6 and 10 copepods · L^{-1}, grazing rates upon autotrophic nanoflagellates showed maximum values.

Table 2.3 Nutrient limitation index (N_L) (Landry et al. 1995, 1998) calculated from phytoplankton growth rates (dilution experiments) without (μ_0) and adding nutrients (μ_N), $N_L = \mu_0 / \mu_n$ (±SD)

Phytoplankton group	μ_0	μ_n	N_L	*n*
Synechococcus	0.69 ± 0.06	0.84 ± 0.03	0.82 ± 0.10	2
Prochlorococcus	1.47 ± 0.79	1.02 ± 0.51	1.44 ± 1.50	3
Picoeukaryotes	0.54	0.33	1.64	1
Autotrophic nanoflagellates	1.54 ± 0.05	2.08 ± 0.13	0.74 ± 0.07	2
Diatoms	1.70 ± 0.24	1.32 ± 0.62	1.29 ± 0.79	2
Dinoflagellates	1.07 ± 0.91	0.87 ± 0.44	1.23 ± 1.67	3
Total phytoplankton	1.09 ± 1.17	1.28 ± 0.83	0.85 ± 1.47	2

Average values are given in day^{-1} (± standard deviation). Total phytoplankton stands for experiments which were estimated from chlorophyll *a*.

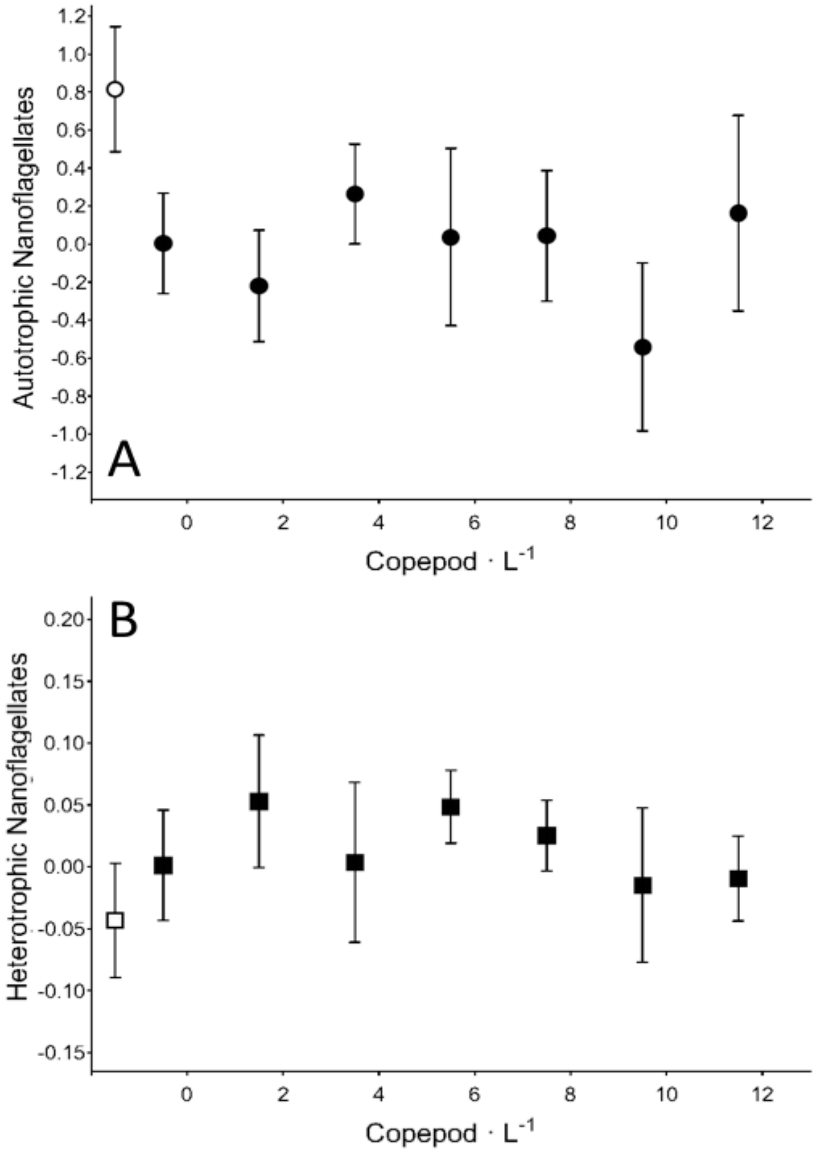


Figure 2.5 Variation in the standardized abundances for **a** autotrophic nanoflagellates (± SE) and **b** heterotrophic nanoflagellates in relation to copepod density. *White markers* show the mean values of all experiments for each variable at the initial time, and *black markers* show the mean values of all experiments for each variable at different copepod concentrations after 24 h of incubation.

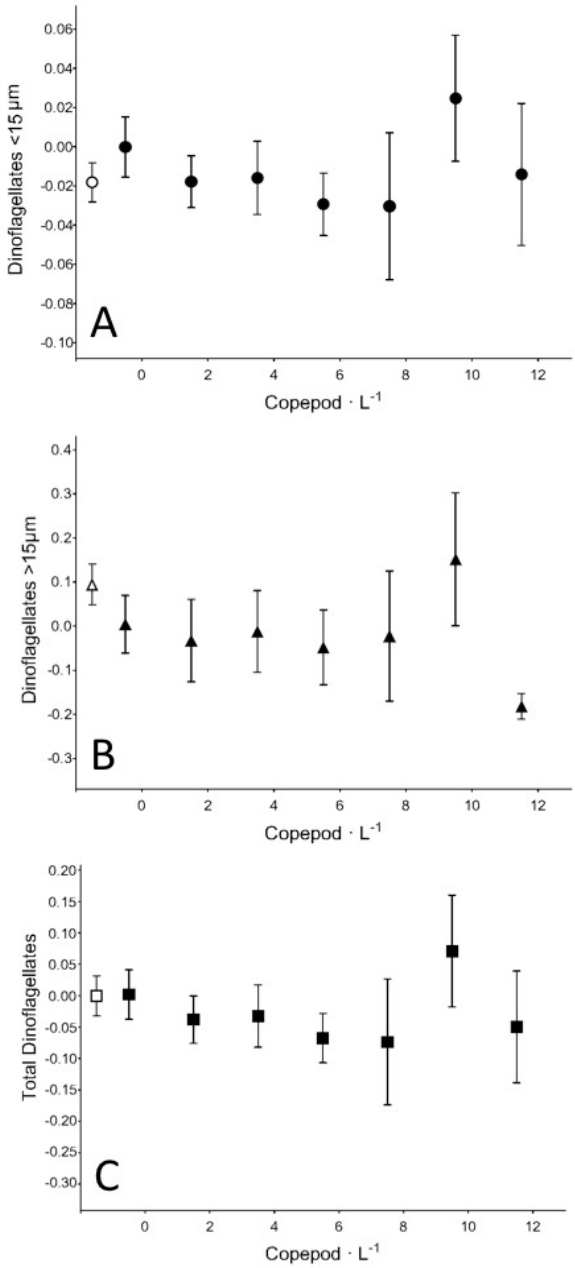


Figure 2.6 Variation in the standardized abundances for **a** dinoflagellates <15 µm (± SE), **b** dinoflagellates >15 µm (± SE), and **c** total dinoflagellates (± SE) in relation to copepod density. *White markers* show the mean values of all experiments for each variable at the initial time, and *black markers* show the mean values of all experiments for each variable at different copepod concentrations after 24 h of incubation.

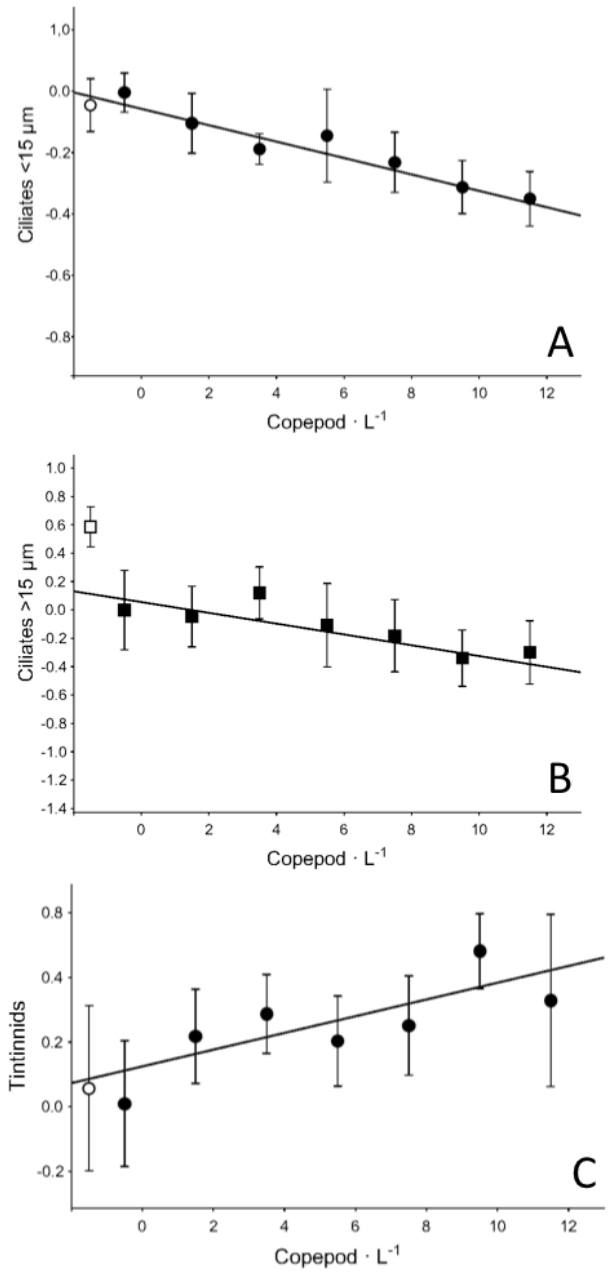


Figure 2.7 Variation in the standardized abundances for **a** ciliates <15 µm (± SE), **b** ciliates >15 µm (± SE), and **c** tintinnids (± SE) in relation to copepod density. *White markers* show the mean values of all experiments for each variable at the initial time, and *black markers* show the mean values of all experiments for each variable at different copepod concentrations after 24 h of incubation.

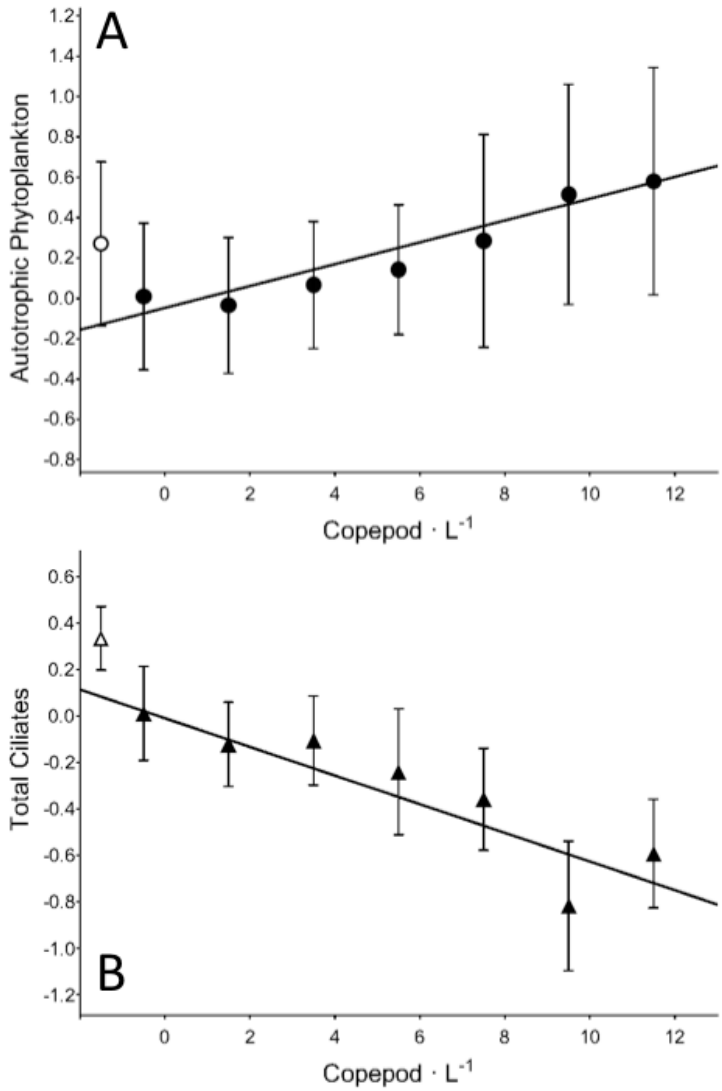


Figure 2.8 Variation in the standardized abundances for **a** autotrophic phytoplankton (± SE) and **b** total ciliates (± SE) in relation to copepod density. *White markers* show the mean values of all experiments for each variable at the initial time, and *black markers* show the mean values of all experiments for each variable at different copepod concentrations after 24 h of incubation.

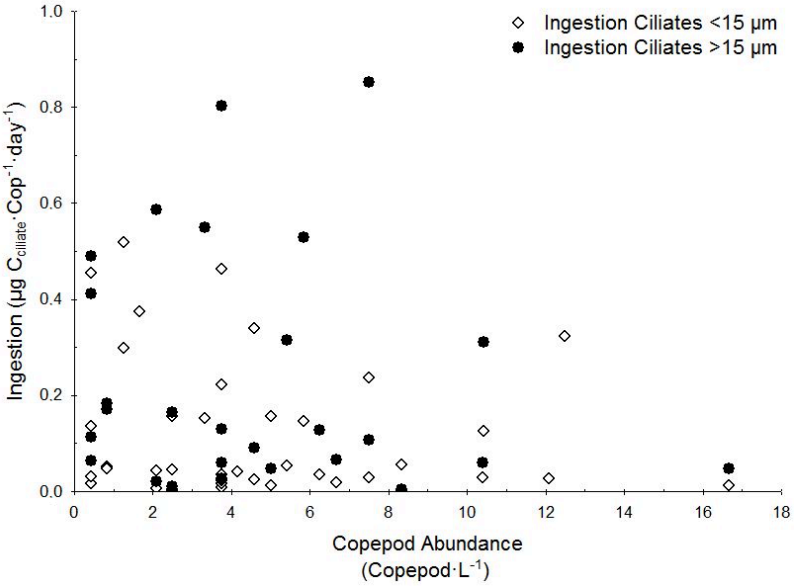


Figure 2.9 Ingestion rates of ciliates in relation to copepod abundance. *Black dots* show the average values for ciliates >15 µm and *white dots* show ciliates <15 µm.

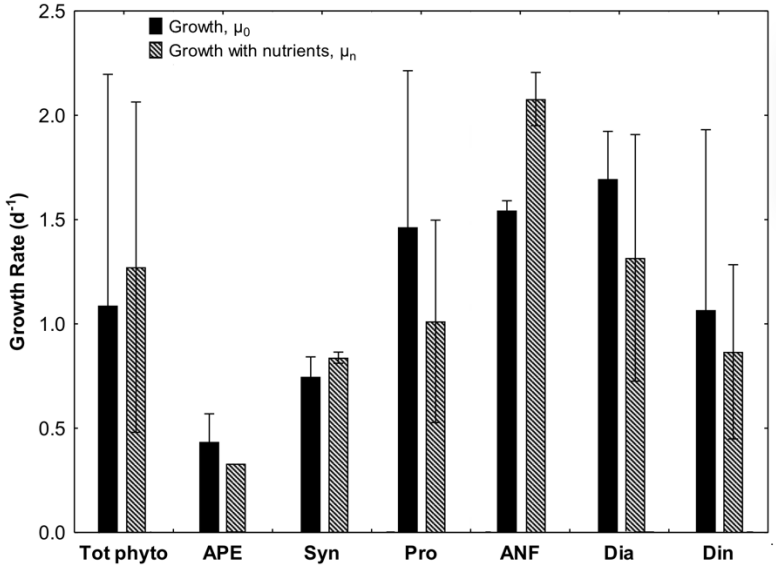


Figure 2.10 Average growth rates (day^{-1}) for different phytoplankton groups obtained from dilution experiments with and without adding nutrients. Tot. phyto, total phytoplankton estimated from Chlorophyll *a*; APE, autotrophic picoeukaryotes; Syn, *Synechococcus*; Pro, *Prochlorococcus*; ANF, autotrophic nanoflagellates; Dia, diatoms; Din, dinoflagellates.

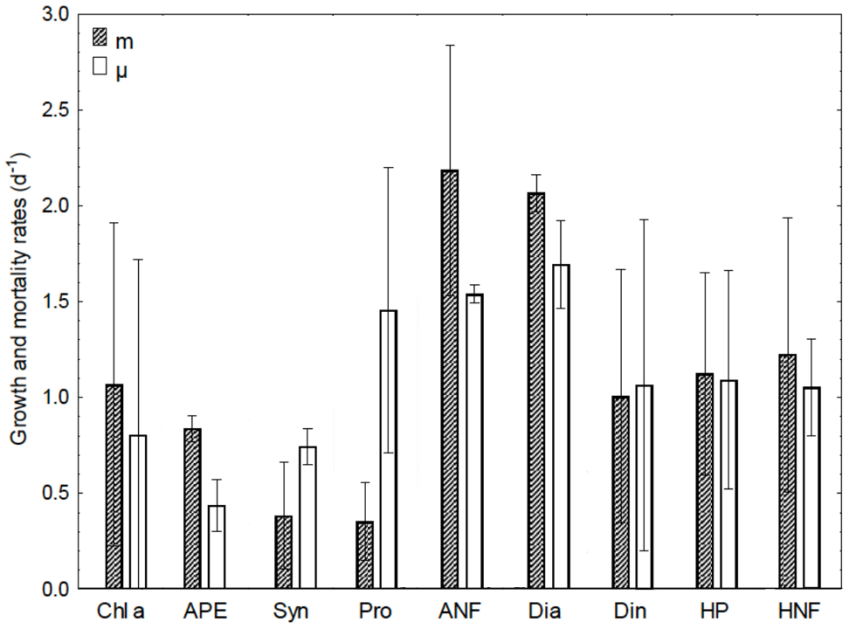


Figure 2.11 Mean rates (day^{-1}, ± SD) of growth and mortality (m) of total phytoplankton (Chl *a*), autotrophic picoeukaryotes (APE), *Synechococcus* (Syn), *Prochlorococcus* (Pro), autotrophic nanoflagellates (ANF), diatoms (Dia), dinoflagellates (Din), heterotrophic bacteria (HP), and heterotrophic nanoflagellates (HNF).

2.4 Discussion

The main finding of this study was the increase in autotrophic organisms abundance at increasing copepod concentration (Figs. 2.2 and 2.3) matching the decrease in abundance of aloricated ciliates (Figs. 2.7a and 2.7b). The latter organisms are by far the most abundant grazers in these subtropical waters (Schmoker et al. 2012), explaining the release of phytoplankton from grazing with increasing copepod densities. Chlorophyll *a*, *Synechococcus*, *Prochlorococcus,* picoeukaryotes and diatoms showed a similar pattern in our experiments, increasing with copepod abundance (Figs. 2.2 and 2.3), and displaying significant positive correlations as shown in the mixed effects model (see annex III). Picoeukaryotes achieved the most significant correlation, since they are one of the main preys for microzooplankton (Fig. 2.11), matching the parallel increase of mesozooplankton and those autotrophs

observed in field studies around the Canary Islands (Schmoker et al. 2012). Moreover, all autotrophs showed an increase corresponding to decreasing abundances of aloricated ciliates, which was the effect of increasing copepod densities. Copepods feeding rates upon ciliates confirm this fact (Fig. 2.12). These results also support previous studies carried out in mesocosms (Vadstein et al. 2004; Stibor et al. 2004) where a negative correlation between ciliates and copepods and a positive correlation between copepods and phytoplankton biomass were found. In those experiments, picoplankton was also released from grazing pressure.

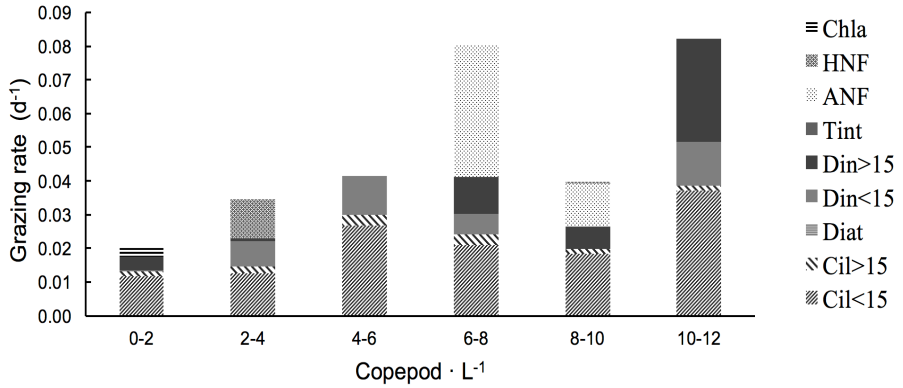


Figure 2.12 Mean of copepod grazing (day^{-1}) of total phytoplankton (Chl *a*), heterotrophic nanoflagellates (HNF), autotrophic nanoflagellates (ANF), tintinnids (Tint), dinoflagellates >15 µm (Din >15), dinoflagellates <15 µm (Din <15), diatoms (Diat), ciliates >15 µm (Cil >15), ciliates <15 µm (Cil <15).

However, no clear effect of copepod addition upon dinoflagellates was observed, at least at very high copepod densities (>8 copepods L^{-1}). Copepods prefer to prey on ciliates, as observed at low densities. However, ciliates alone are not sufficient to support copepod growth, thereby increasing the grazing rate upon dinoflagellates. The lack of a pattern may be related to (1) the fact that copepods prefer to prey upon ciliates and this causes a decrease in predation pressure on dinoflagellates; (2) microzooplankton prey on dinoflagellates, so when its abundance decreases due to copepod feeding, dinoflagellates are released from predation pressure and increase their abundance; (3) the slightly higher abundance of fecal pellets at high copepod

densities, increases dinoflagellate grazing on them (Poulsen et al. 2011), or (4) a combination of the three mechanisms. Smaller dinoflagellates also prey upon nanoflagellates and could enter into competition with ciliates (Strom 1991; BjØrnsen and Kuparinen 1991; Hansen 1992; Nakamura et al. 1992; Nakamura et al. 1995), which would explain that grazing rates on heterotrophic nanoflagellates increases when the abundance of dinoflagellates is large. The importance of the size spectrum in these organisms seems to preclude any conclusion about the effect of copepod density.

Another interesting result was the variable tendency of tintinnids as copepods increased. The general increasing pattern in Fig. 2.7c was not observed in all experiments as deduced from the mixed effect model (see Results). The pattern in experiments showing the increasing tendency and a feeding rate of zero (Fig. 2.12) may be due to (1) the unpalatability of the lorica for copepods, (2) the increasing phytoplankton abundance at increasing copepod densities, (3) the absence of predators, or (4) a mix of these factors. Tintinnids are known to feed on small cells such as bacteria (Campbell 1926, 1927; Hollibaugh et al. 1980), phytoflagellates (Gold 1968, 1969, 1973), small diatoms (Campbell 1926, 1927), small dinoflagellates (Beers and Stewart 1967), and small tintinnids (Blackbourn 1974). The top-down effect on ciliates favouring small cells could also promote an advantage for those organisms. However, this observation needs further research.

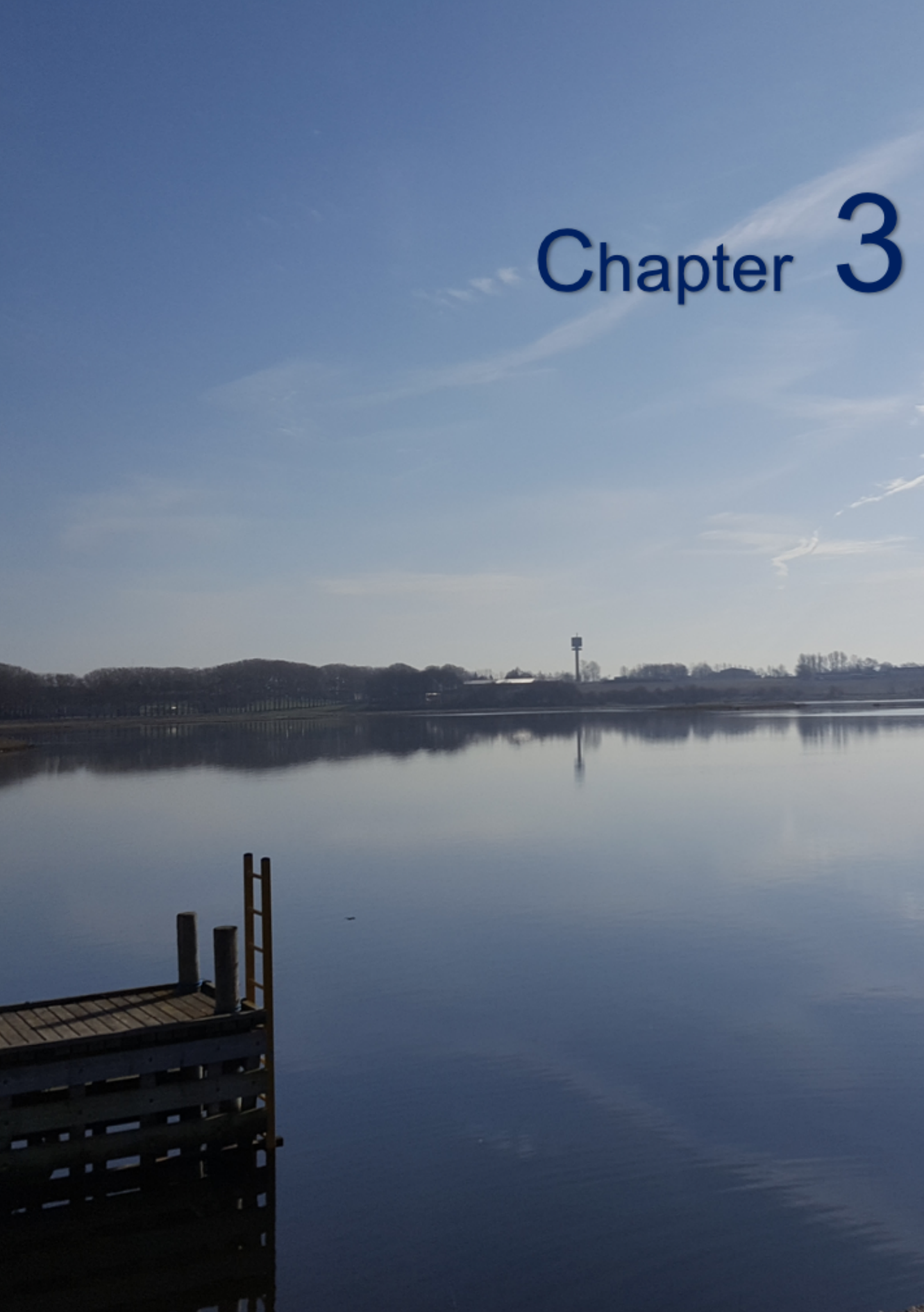
The microzooplankton consumption was closely related to the growth of its prey, both for autotrophic and heterotrophic organisms. Autotrophic nanoflagellates, diatoms, and picoeukaryotes are the favourite prey for ciliates, so they increase their abundance when copepods remove ciliates. However, nanoflagellates follow another trend, probably because dinoflagellates and tintinnids continue to prey upon them. As in previous studies, copepods predate selectively on ciliates and dinoflagellates instead of diatoms. This may be because they are more abundant, at least in the case of ciliates. Diatoms are more abundant than dinoflagellates, yet grazing rates are higher on dinoflagellates. Previous studies have suggested that dinoflagellates have a higher nutritional value than diatoms (Stoecker and Capuzzo 1990; Wickham 1995; Broglio et al. 2003), so their preference for dinoflagellates may be due to this fact. Another reason may be related to the exudation of toxins by diatoms, inhibiting predation on them (Malej and Harris 1993).

The increased copepod density in our experiments could also regenerate nitrogen by their excreting ammonia. Our parallel experiments to test the influence of ammonium inside the experimental bottles adding nutrients showed no or little nutrient limitation (Fig. 2.10 and Table 2.3). These results could be due to the fact that most incubations were performed during the so-called late winter bloom in subtropical waters (see Hernández-León 2009). During this period of the year, the slight atmospheric cooling promotes the erosion of the thermocline, allowing nutrients to reach the euphotic zone. Besides, this is the season of increased dust deposition in the Canary Current System. Dust is known to increase nutrient load and productivity (Wells et al. 1999; Andreae et al. 2003; Behrenfeld et al. 2006). Moreover, taking into account the equations given by Ikeda (1985) and the results of Hernández-León and Torres (1997) for the Canary Islands, our maximum copepod density in the experiments (10-12 ind·L^{-1}) could not produce values higher than the 0.5 μM added to the nutrient treatment, considered as saturating for phytoplankton growth (Cullen et al. 1992). Therefore, the growth experienced by phytoplankton can be explained mainly as a result of the disappearance of grazers from the system and not to the fertilisation by ammonium promoted by copepods.

To our view, the most important contribution of this work is to show the effect of mesozooplankton on primary producers, explaining, at least in part, the parallel increase of mesozooplankton and primary production observed in these subtropical waters (Hernández-León 2009; Schmoker et al. 2012; Torreblanca et al. in prep.). In this sense, the important consumption of diel vertical migrants (DVMs) on epipelagic mesozooplankton described by Hernández-León et al. (2010) and references therein) not only promotes an enhanced active flux to the mesopelagic zone, but also modifies the structure of the plankton community in the euphotic zone. The DVMs remain in the dark at deep layers of the ocean during the day but at night they migrate to shallower layers to feed. During the dark phase of the lunar cycle they reach the upper 100 m of the water column, while during the illuminated phase they do not reach this layer in order to avoid predators. The presence of DVMs in the upper layers of the ocean during the dark phase of the lunar cycle predating upon epipelagic (non-migrant) mesozooplankton, promotes the increase in microzooplankton as they are released from predation, potentially increasing respiration and recycling organic matter in the euphotic layer. By contrast, the absence of DVMs during the illuminated phase of the lunar cycle promotes a

higher mesozooplankton crop preying upon microzooplankton as suggested by Hernández-León (2009) and Schmoker et al. (2012). Thus, these top-down effects seem of importance to study the functioning of the biological pump in the warm oligotrophic ocean, supporting the conceptual model given by Hernández-León (2009) of two contrasted scenarios in oligotrophic waters depending on the presence or absence DVMs.

In summary, autotrophic organisms raised with increasing copepod concentration match the decrease in abundance of aloricated ciliates. Thus, a top-down effect was unveiled in these experiments using natural samples in the oceanic waters as no effect of regenerated nutrients was observed (Löder et al. 2011). Copepods promote important changes down the food web structuring the community with the effect of predation upon microzooplankton. This effect could have biogeochemical consequences because of the open ocean variability of zooplankton at short time scales. Finally, these results also caution about the use of relationships between chlorophyll and zooplankton biomass.


Chapter 3

How wonderful that we have met with a paradox.
Now we have some hope of making progress.

Niels Bohr

3 Shift in ciliate community during the spring phytoplankton bloom in a temperate fjord

CHAPTER

L. Armengol, H.H. Jakobsen, L. Haraguchi, S. Hernández-León

Abstract Spring blooms in temperate coastal ecosystems are remarkable events in the annual cycle where the pelagic phytoplankton community undergoes large changes. However, the underlying changes in the protist grazer community and the internal interactions between their prey remains poorly understood. Here, we investigate how the nutrient regime promotes changes in picoeukaryotes, phytoflagellates, large phytoplankton, mixotrophic and heterotrophic ciliates. We used pulse shape flow cytometry, FlowCAM, and classical dilution experiments to address phytoplankton growth and grazing upon these organisms. Our estimated grazing rates showed that ciliates having mixotrophic nutrition were feeding at similar rates as ciliates with obligate heterotrophic nutrition. We found a decrease of mixotrophic ciliates and an increase in heterotrophic ciliates when nutrients became depleted. This

change in the ciliate community with a different nutrition mode was followed by a shift from large to smaller phytoplankton. This suggests that protozoans coincide with swift changes in phytoplankton group composition along the bloom. However, the extent to which changes were promoted by grazing or cascading effects remain unclear.

3.1 Introduction

The phytoplankton spring bloom is a dramatic event during the annual cycle in temperate latitudes, where phytoplankton growth exceeds losses (Cushing 1959; Sommer et al. 1986). In fjords, the onset of the bloom is driven by the increase in irradiance exceeding a threshold level sufficient to allow phytoplankton to take advantage of nutrients accumulated during winter (Cushing 1959; Sommer et al. 1986). Phytoplankton blooms develop because a disruption of chemical or physical conditions unbalancing the relationships between nutrients, phytoplankton and grazers, that ultimately opens a loophole for the success autotrophic phytoplankton (Strom 2001, Bakun & Broad 2003, Irigoien et al. 2005, Sherr & Sherr 2009). As the bloom ages, a protistan grazing community develops within days followed by large zooplankton grazers developing at scales from weeks to months (Kiørboe & Nielsen 1994; Nielsen & Kiørboe 1994) ultimately controlling the bloom.

In the "classic" view, transfer of matter during spring blooms in meso- or eutrophic systems dictates that large phytoplankton feeds a short linear zooplankton-fish food chain, with a high transfer efficiency (Cushing 1989). By contrast, at low nutrients levels the classical food chain is transformed into a complex microbial web. In this scenario, microzooplankton, mainly ciliates, are the main grazers upon small phytoplankton cells, favoured in these environments, promoting an important recycling and a low transfer efficiency to higher trophic levels (Azam et al. 1983; Fenchel 1988). Knowledge about the impact of microzooplankton upon phytoplankton in both scenarios is of paramount importance to study the fate of primary production in the marine environment (Banse 1992). In this sense, physical, chemical, and biological conditions change during the development of the bloom. Temperature and light normally increase, nutrients are consumed or the balance between the

different elements change, promoting new scenarios for phytoplankton and microzooplankton.

In Roskilde Fjord (RF, Denmark), a shallow temperate estuary, the spring bloom normally develops from early March to early April heterotrophic (Staehr et al. 2017). During this period the plankton community becomes net autotrophic whereas during the remaining part of the year the community is net heterotrophic (Staehr et al. 2017). This is the effect of a high concentration of nitrogen during winter and spring that become depleted after the bloom. Phosphorous is present for most of the time although temporal phosphorous imbalances occur during short intervals in which nitrogen appears at quite high concentrations. During the dynamic change from nutrient repleted to nutrient depleted or imbalanced, the ciliate community display a large shift in species composition (Haraguchi et al. 2018).

Here, we explore how the protist community undergo a swift change in species composition and functionality at the same scale as the nutrients, light and temperature changes. To answer this, we used dilution experiments (Landry and Hassett 1982) combined with *in vivo* analysis of the phytoplankton community by pulse shape flow and microzooplankton by FlowCAM. This allowed for a detailed study of growth in the different phytoplankton groups and species, and differential grazing by ciliates with different trophic modes.

3.2 Materials and methods

Water for experiments and plankton monitoring were collected from surface waters at Risø pier (55.69º N, 12.08 E, Fig. 3.1) in the inner part of the RF during March 2017 (Table 3.1). This sampling site is close to a monitoring station for nutrients and phytoplankton followed since early 90´s at a high frequency (20 times per year). We used the monitoring nutrient data to describe the overall annual pattern of the study site.

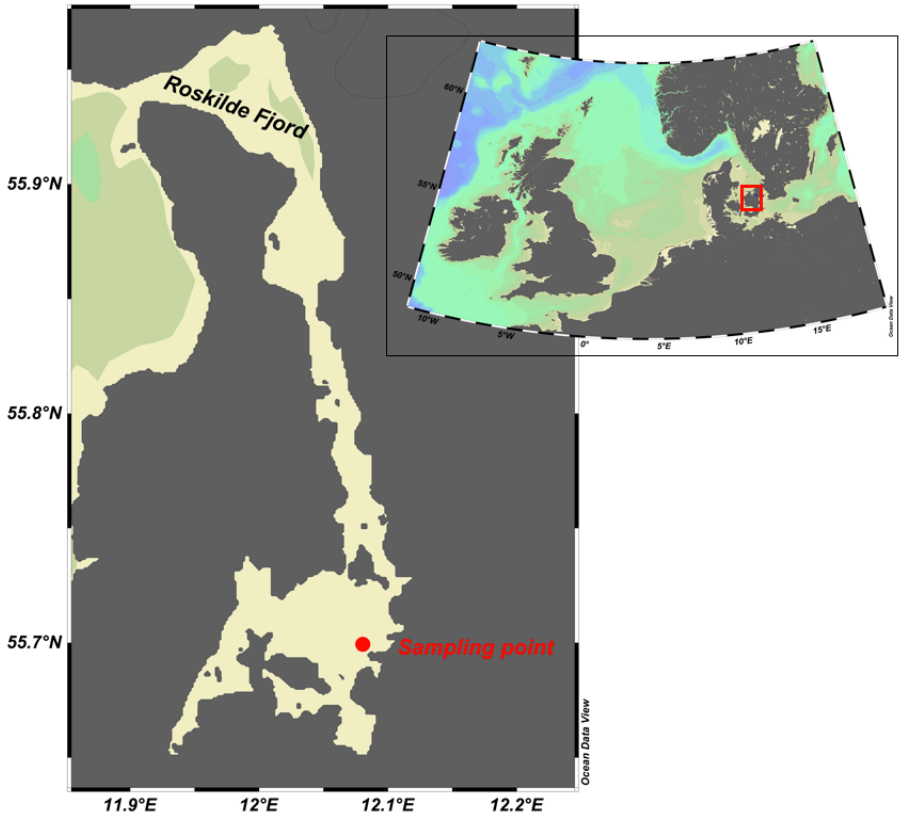


Figure 3.1 Roskilde Fjord (Denmark) and location of the monitoring station at the Pier Station. Water for dilution experiments was obtained from this pier.

3.2.1 Physical parameters

Water temperature and salinity were measured daily using a YSI Pro30 instrument. Rain, wind direction, and speed were obtained from Weather Underground Network (https://www.wunderground.com/) averaging IROSKILD4 and EKRK meteorological stations. Cloud cover was obtained from the World Weather Online (https://www.worldweatheronline.com/) averaging Roskilde and Jyllinge meteorological stations. Daylight hours were obtained from the website http://staging.timeanddate.com/.

3.2.2 Nutrients

Samples from the monitoring program and from our study site were processed accordingly to (Kaas & Markager 1998) following the recommendation given by Strickland and Parsons (1972). Inorganic nutrients such as nitrite (NO_2^-), nitrate (NO_3^-), ammonium (NH_4^+), orthophosphate (PO_4^{3-}), dissolved inorganic phosphate (DIP) and dissolved inorganic silicate (Si) were collected in 30 ml brown glass bottles, first filled with ultra-pure water before sampling, and stored frozen in 30 ml acid-washed plastic bottles. Collected samples were analysed on a San ++ Continuous Flow Analyser (Skalar Analytical B.V. Breda. NL) as previously described by Grasshoff (1976) and Kaas & Markager (1998). Detection limits were 0.04, 0.1, 0.3, 0.06 and 0.2 µmol L^{-1} for NO_2^-, NO_3^-, NH_4^+, PO_4^{3-} and Si, respectively. Total nitrogen and phosphorous (TN and TP) were determined on fully digested samples adding oxidants followed by autoclaving. The digested samples were analysed as outlined above for phosphorus and nitrogen species. Detection limits for TN and TP were 1.0 µM for nitrogen and 0.1 µM for phosphorous. Dissolved inorganic nitrogen (DIN) concentrations were calculated as the sum of $NO_2^- + NO_3^- + NH_4^+$.

3.2.3 Growth and grazing

Microzooplankton growth and grazing rates were obtained from dilution experiments using the 2-point method (Worden & Binder 2003, Landry et al. 2008, Strom & Fredrickson 2008). Net phytoplankton growth rate (k, d^{-1}) was obtained from 100% whole seawater (WSW) treatment under the assumptions that net phytoplankton growth rate in the 5% WSW treatment was equal to phytoplankton intrinsic growth rate (μ) (Strom and Fredrickson 2008). Net growth rate was calculated for each treatment as:

$$k = \ln \frac{P_f}{P_0} \cdot t^{-1} \qquad \text{Eq. 1}$$

where P_f and P_0 are the final and initial concentration of either chlorophyll *a* (Chla) or plankton cells counted by the pulse shape flow cytometer. From these two estimates, microzooplankton grazing rate (g, d^{-1}) was calculated as:

$$g = \mu - k \qquad \text{Eq. 2}$$

Table 3.1 Initial conditions for dilution experiments. Values (mean ± SD)

Experiment	Date (2017)	Wind Speed ($m\ s^{-1}$)	Rain (mm)	Temperature (ºC)	PAR* (mol phot. $m^{-2}\ d^{-1}$)	Salinity (PSU)	PO_4^{3-} (µM)	DIN (µM)	TN (µM)
1	06-Mar	10.3 ENE	10.25	4.0 ± 0.4	5.3	15.2	1.1	19.9	67.2
2	09-Mar	7 SW	7	4.4 ± 0.4	9.8	14.2	0.9	25.83	77.5
3	13-Mar	6 S	6	4.7 ± 0.4	10.9	15	0.4	14.5	67.8
4	16-Mar	10.3 W	10.25	6.8 ± 0.6	15.7	14.4	0.1	5.7	64.6
5	20-Mar	7.4 W	7.38	6.4 ± 0.4	14.7	14.4	0.1	5.6	60.7
6	23-Mar	3.8 NE	3.75	7.2 ± 0.6	27.9	14.3	0.1	3.0	47.1

* Light was measured as global irradiance multiplied by 2.1 assuming 45% of irradiance was within the PAR range.

Samples with negative values of grazing were set to 0 d^{-1} whereas negative values for phytoplankton growth were set to 0.01 d^{-1} (Calbet and Landry 2004). Primary production consumed by heterotrophic grazers were estimated according to Calbet and Landry (2004) as:

$$\% PP = \frac{g}{\mu} \cdot 100 \qquad \text{Eq. 3}$$

3.2.4 Phytoplankton analysis

Chlorophyll a

Chla samples were collected in 320 mL dark glass bottles, filtered through 25 mm Whatman GF/F filters, and stored at -20ºC in darkness until analysis by a three point calibrated Turner AU10 fluorometer accordingly to Strickland & Parsons (1972), using ethanol as extracting solvent (Riemann & Ernst, 1982).

Flowcytometry

A CytoBuoy™ pulse shape flowcytometer (CytoBuoy b.v. Woerden, NL) was used to count and size phytoplankton. Aliquots from each treatment were taken at the beginning and at the end of the experiment and analysed according to (Haraguchi et al. 2017). Samples were analysed in triplicate using the high sensitivity red fluorescence sensor (FLR-hs) at a trigger level of 30 mV to obtain only cells with Chla content. Flow rates of the instrument were set at 14.27 µL s^{-1} and stop conditions were 5000 µL analysed for the 5% WSW treatment, and 500µL for the 100% WSW treatment. Data was manually gated using EasyClus (CytoBuoy b.v. Woerden. NL) and divided into autotrophic picoplankton

(picoEUK), autotrophic nanoflagellates (NF) and small, medium and large cryptophytes (SC, MC, LC).

Cell concentrations were calculated as the average of each triplicate bottle experiments. The CytoBuoy pulse shape flow cytometer was calibrated as in (Haraguchi et al. 2017), using a model II regression of the integrated forward scatter against particles of known volume. The volume was, in turn, converted into cell-specific carbon following the generic protist carbon-to-volume relationship (Menden-Deuer & Lessard 2000).

Microzooplankton analysis

Ciliates samples were analysed using a colour FlowCAM IV (www.fluidimaging.com/). In this application, we used a 10X objective analysing samples in auto-imaging mode equipped with a FC100 flow cell. A final volume of 10 mL was analysed during each run. Because the high magnification compromises analysis speed in favour of image quality, we could only analyse two samples per day from the 100% WSW treatment. Thus, we analysed the initial and final concentration of ciliates of one of the 100% WSW treatment, for one of the replicates. The ciliate concentration was calculated as the mean of these two numbers. After data acquisition, the FlowCAM data was sorted manually into morphotypes and nutritional mode was assigned on the basis of cell coloration and food vacuole characteristics. Ciliates were assumed to have mixotrophic nutrition if vacuoles, often densely packed and coloured green or reddish, were in the periphery of cell, whereas ciliates appearing colourless or having large separate green-brown vacuoles in their middle or posterior cell end, were assumed to be obligate heterotrophic (Haraguchi et al. 2018). We identified the species *Mesodinium rubrum* (Annex V, plate 1), *Balanion comatun* (Annex V, plate 2), and other five organisms identified to genera as *Urotrichia* sp. (Annex V, plate 4), and *Strombidium* spp. (Annex V, plate 3, 5, 7 and 8). Two large oligotrich ciliates were also identified to genera (Annex V, plate 6 and plate 7). The ciliate *Mesodinium rubrum* is mixotrophic and we also assigned mixotrophic nutrition to three of the Strombidids spp. (Annex V, plate 3, 5 and 8) based on their coloration (see methods). Non-identified ciliates were summarized into a group termed "undetermined". Carbon biomass of ciliates was calculated based on area-based diameter volume (ABD) (Jakobsen & Carstensen 2011) and converted into cell-specific carbon following the generic protist carbon-to-volume relationship (Menden-Deuer & Lessard 2000).

3.2.5 Statistics

Statistics analysis were conducted using "Statistica" software. A Wilcoxon test was performed to study the significant differences between nutrient-enriched and non-nutrient treatment. Kruskal-Wallis and Median Test were conducted to compare phytoplankton biomass through six experiments. Spearman Rank correlation coefficients were applied to study the relationships between biomass of organisms, nutrient concentration, and rates of growth and mortality.

3.3 Results

Study site

The annual cycle of the long-term data showed nitrogen and phosphorous decreasing during spring, remained low during summer, and increasing through fall and winter (Fig. 3.2). Chla was low until February and peaked in March, decreasing through summer followed by a quite small increase in August (Fig 3.2). During this study, light hours and water temperature increased over days (Table 3.1). A low-pressure front passed affecting the study area from 16th to 20th March, resulting in an increase in wind speed and rainfall (Table 3.1).

Nutrients and chlorophyll a

Nutrient concentrations decreased from 19.9 μM on 6th to 3.0 μM on 23th of March for dissolved inorganic nitrogen (DIN) and from 1.1 to 0.1 μmol L^{-1} for phosphate (Fig. 3.3; Table 3.1). Chla concentration increased from 3.78 μg L^{-1} on the 6th to 5.4 μg L^{-1} on the 16th March, reaching the maximum value, and thereafter decreasing until the end of the study on 23th March (Fig. 3.3).

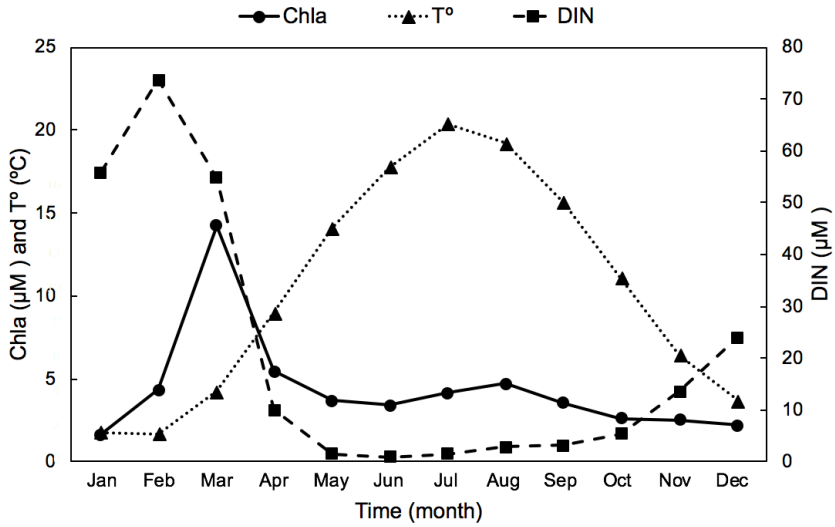


Figure 3.2 Chlorophyll *a* (Chla, µM), temperature (T°, ºC) and Dissolved Inorganic Nitrogen (DIN, µM) obtained from the Danish monitoring program from the nearby monitoring station 60. Data are monthly averages for the 2006–2016 period.

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Autotrophic phytoplankton community

Biomass of picoEUK oscillated throughout the study reaching the maximum value on 20th March (1.6 µgC L^{-1}; Fig. 3.4a), and their abundance scaled with DIP ($\rho = 0.89$; $p < 0.05$, Spearman Rank correlation test; Table 3.2 and 3.3). By contrast, NF increased their biomass from 1.5 to 13.8 µgC L^{-1} in parallel with nutrient depletion ($\rho = -0.89$ $p < 0.05$ for DIP and $\rho = -0.94$; $p < 0.05$ for DIN, Spearman Rank correlation test; Fig. 3.4a; Table 3.2 and 3.3), and they were negatively correlated with mixotrophic ciliates ($\rho = -0.89$; $p < 0.05$, Spearman Rank correlation test; Fig. 3.5; Table 3.3). By far, cryptophytes dominated the phytoplankton biomass community during the study. However, their biomass decreased by ca. 56% during 20th March (Fig. 3.4b), coinciding with the decrease in mixotrophic ciliates and the increase in heterotrophic ciliates. SC reached the maximum (64.9 µgC L^{-1}) during the heterotrophic ciliate dominance. MC dominated the planktonic community over time, except during 20th March, where its biomass was ca. 88 µgC L^{-1}. Finally, LC decreased their biomass over time from 10.8 to 2.4 µgC L^{-1} coinciding with phosphate depletion ($\rho = 0.89$; $p < 0.05$; Spearman Rank correlation test; Table 3.3) and high grazing rates.

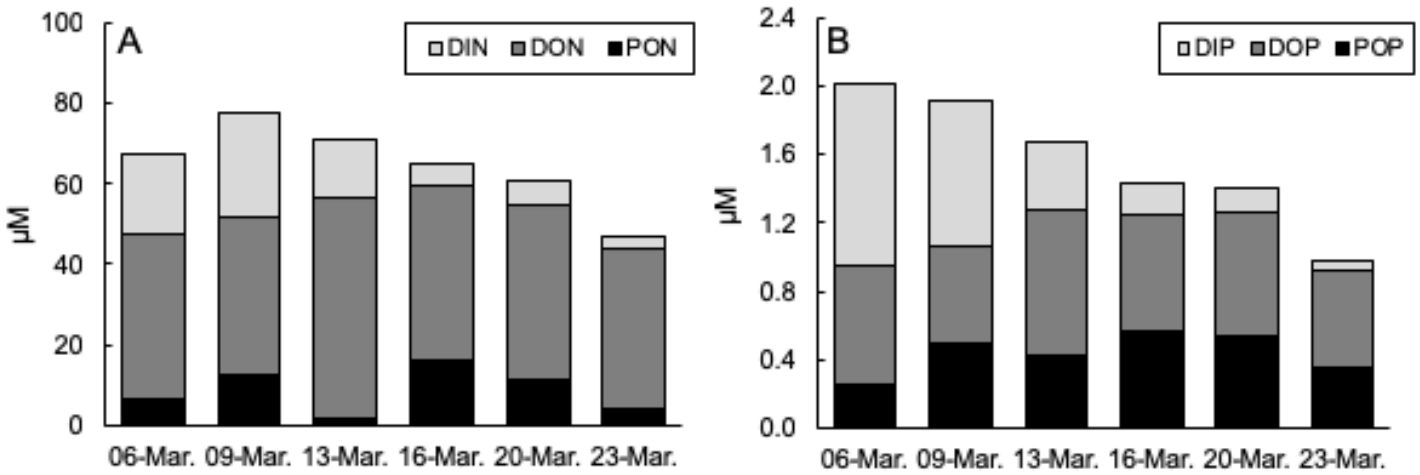

Figure 3.3 Water concentration (µM) of **a** dissolved inorganic nitrogen (DIN), dissolved organic nitrogen (DON) and particulate organic nitrogen (PON); and **b** dissolved inorganic phosphorus (DIP), dissolved organic phosphorus (DOP) and particulate organic phosphorus (POP) during study period.

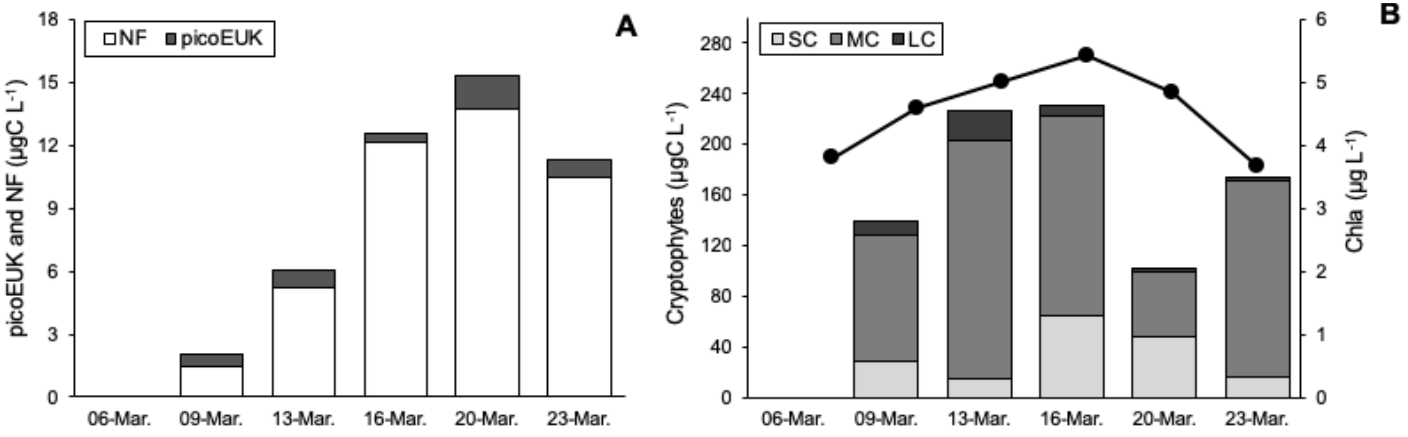

Figure 3.4 Biomass (µgC L^{-1}) of **a** autotrophic picoeukaryotes (picoEUK) and nanoflagellates (NF); **b** small cryptophytes (SC), medium cryptophytes (MC), large cryptophytes (LC) and chlorophyll *a* (Chla) concentration (µg L^{-1}) (dots) at fjord during study period.

Table 3.2 Correlation of a) Dissolved Inorganic Nitrogen (DIN) and b) Dissolved Inorganic Phosphates (DIP) in μM with autotrophic picoeukaryotes (picoEUK), nanoflagellates (NF), small cryptophytes (SC), medium cryptophytes (MC) and large cryptophytes (LC).

		Slope (μ d^{-1} M^{-1})	*p*-value	N
picoEUK	DIN	0.06	0.1	6
	DIP	0.83	0.35	6
NF	DIN	0.02	0.28	6
	DIP	0.32	0.37	6
SC	DIN	0.03	0.11	6
	DIP	0.44	0.25	6
MC	DIN	0.00	0.95	6
	DIP	0.01	0.92	6
LC	DIN	0.04	0.44	6
	DIP	0.84	0.1	6

Ciliates

Ciliate biomass of each identified group during the six dilution experiments is shown in Figure 3.5. Ciliate biomass in the fjord decreased from 32.81 μgC L^{-1} to 10.46 μgC L^{-1} during the study period. Mixotrophic ciliates dominated during March 9th and 13th showing a significant positive relationship with total nitrogen and significant negative relationship with NF ($\rho = 0.83$, $p < 0.05$ for TN; and $\rho = -0.89$, $p < 0.05$ for TN; Spearman Rank correlation test; Table 3.3). Heterotrophic ciliates peaked during 16th March and no significant scaling was observed with nutrients or phytoplankton (Figs. 3.3, 3.4 and 3.5).

Growth and grazing

Phytoplankton growth in nutrient enriched and non-enriched treatments showed no significant differences ($p = 0.99$, Mann-Whitney Rank Sum Test). Our experiments showed high values for both growth and grazing based on Chla (Fig. 3.6). Microzooplankton grazing based on Chla was higher than phytoplankton growth for all experiments except during 6th March (Fig. 3.6). Furthermore, Chla growth and grazing rates increased progressively and

reached maximum values on 16th March, (k=3.6 ± 0.04 d^{-1} and g=5. 8 ± 0.3 mean ± SE).

Specific growth rates calculated for picoEUK, NF, and small, medium and large sized cryptophytes tended to decrease with nutrient depletion, except during 23th March that showed higher growth rates than preceding experiments (Fig. 3.7a). Growth of picoEUK decreased with nutrient depletion (Fig. 3.7a). LC almost disappeared since 16th March, and their growth rates determined by the dilution method was undetectable in these experiments (Fig. 3.7a).

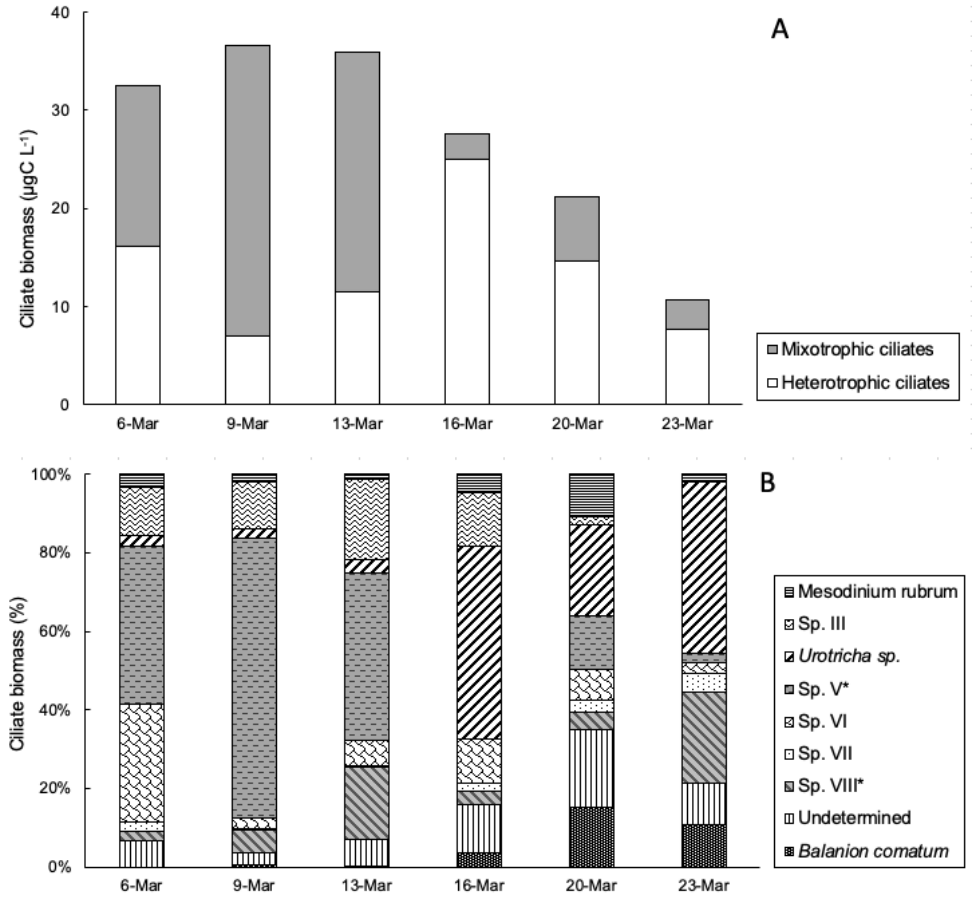

Figure 3.5 **a** Biomass (µg C L^{-1}) of mixotrophic (grey bars) and heterotrophic (white bars) ciliates; **b** proportion (%) of biomass ciliate species during the study period. *Asterisk denote mixotrophic nutrition assigned.

Ciliate grazing upon different phytoplankton groups

Grazing rates varied over phytoplankton groups and changed with composition of ciliates (Fig. 3.7b). Grazing upon picoEUK increased at high ciliate biomass (Fig. 3.5 and 3.7b) from 6th to 20th March. At high concentration of mixotrophic ciliates, grazing rates on SC (9th March) and LC (6th and 13th March) increased. On the other hand, the dominance of heterotrophic ciliates increased grazing rates on NF (from 20th to 23th March), SC (from 16th to 20th March) and MC (from 16th to 23th March) (Fig. 3.7b).

Phytoplankton growth was completely controlled by microzooplankton grazers (% PP) based on Chla dilution experiments (Fig. 3.8a), despite the minimum ciliate biomass (10.5 μgC L^{-1}) during the 23th March sampling. The specific consumption of each phytoplankton group by microzooplankton remained below 80% from 6th to 13th and on 23th March (Fig. 3.8b). However, microzooplankton grazers consumed all production of picoEUK, SC on 16th march, and MC on 20th March (Fig. 3.8b).

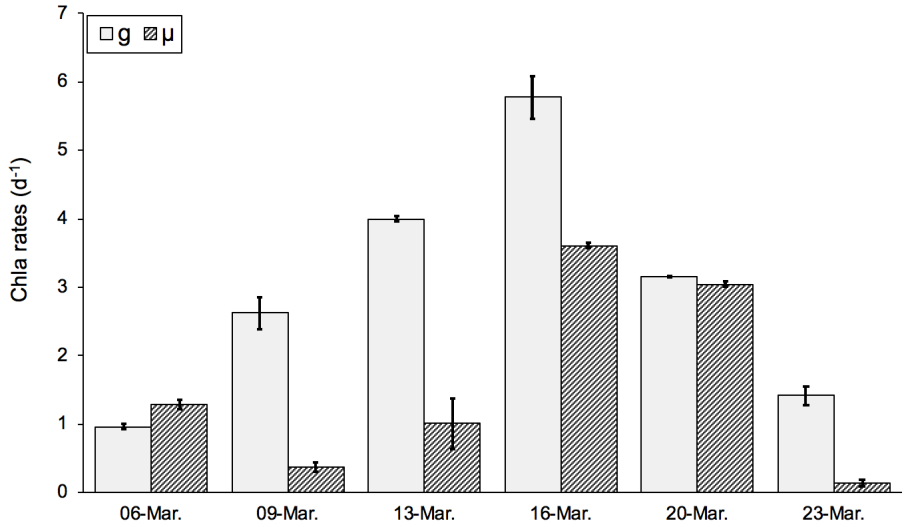

Figure 3.6 **a** Biomass (μg C L^{-1}) of mixotrophic (grey bars) and heterotrophic (white bars) ciliates; and **b** Proportion (%) of biomass ciliate species during the study period. *Asterisk denote mixotrophic nutrition assigned.

Table 3.3 Spearman correlations coefficients between variables: Total Nitrogen (TN), Total Phosphate (TP), Dissolved Inorganic Nitrogen (DIN), Particulated Organic Phosphate (POP), Dissolved Inorganic Phosphate (DIP), Dissolved Organic Phosphate (DOP), autotrophic picoeukaryotes (picoEUK), nanoflagellates (NF), small cryptophytes (LC), chlorophyll a (Chl a), Heterotrophic Ciliates (H. Cil), Total Ciliates (T. Cil), Mixotrophic Ciliates (M. Cil). Bold numbers represent significant correlations at $p < 0.05$.

	TN	TP	DIN	DON	PON	POP	DIP	DOP	APE	NF	SC	MC	LC	Chla
picoEUK	0.71	**0.89**	0.77	-0.03	-0.26	-0.54	**0.89**	0.17	1.00					
NF	**-0.89**	**-0.89**	**-0.94**	0.20	-0.09	0.20	**-0.89**	-0.17	-0.66					
SC	-0.14	-0.26	-0.09	-0.54	**0.83**	0.60	-0.26	-0.61	-0.43	0.26				
MC	-0.14	0.26	0.09	-0.54	-0.03	-0.60	0.26	-0.61	0.43	0.09	0.14			
LC	0.71	**0.89**	0.77	-0.03	-0.26	-0.54	**0.89**	0.17	1.00	-0.66	-0.43	0.43		
Chla	0.26	0.03	0.09	0.66	0.37	0.77	0.03	0.67	-0.03	0.03	0.14	-0.54	-0.03	
H. Cil	-0.26	0.09	-0.14	0.49	0.31	0.26	0.09	0.41	0.09	0.26	0.03	0.20	0.09	0.49
T. Cil	1.00	**0.83**	**0.94**	-0.09	0.09	-0.03	**0.83**	0.23	0.71	**-0.89**	-0.14	-0.14	0.71	0.26
M. Cil	**0.83**	0.66	0.77	-0.14	-0.26	-0.31	0.66	0.17	0.54	**-0.89**	-0.43	-0.26	0.54	-0.14

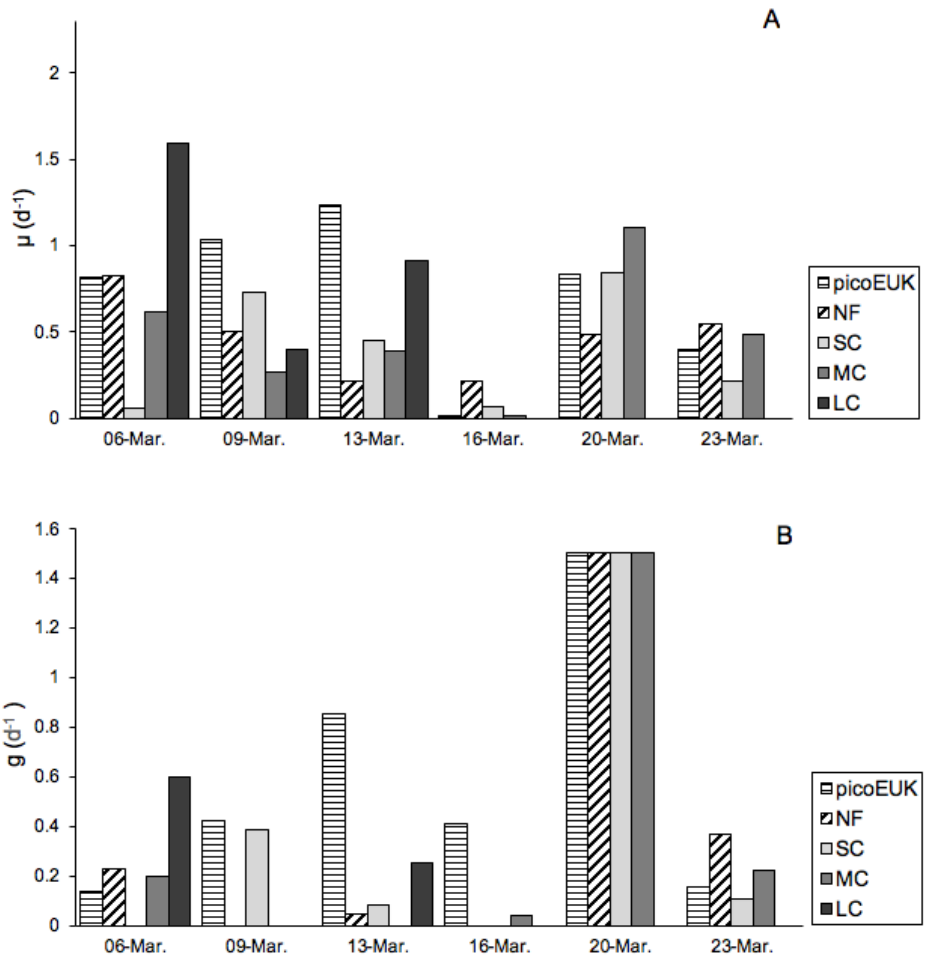

Figure 3.7 Autotrophic picoeukaryotes (picoEUK), autotrophic nanoflagellates (NF), small cryptophytes (SC), medium cryptophytes (MC) and large cryptophytes (LC) rates (d^{-1}) of **a** growth and **b** microzooplankton grazing during the study period.

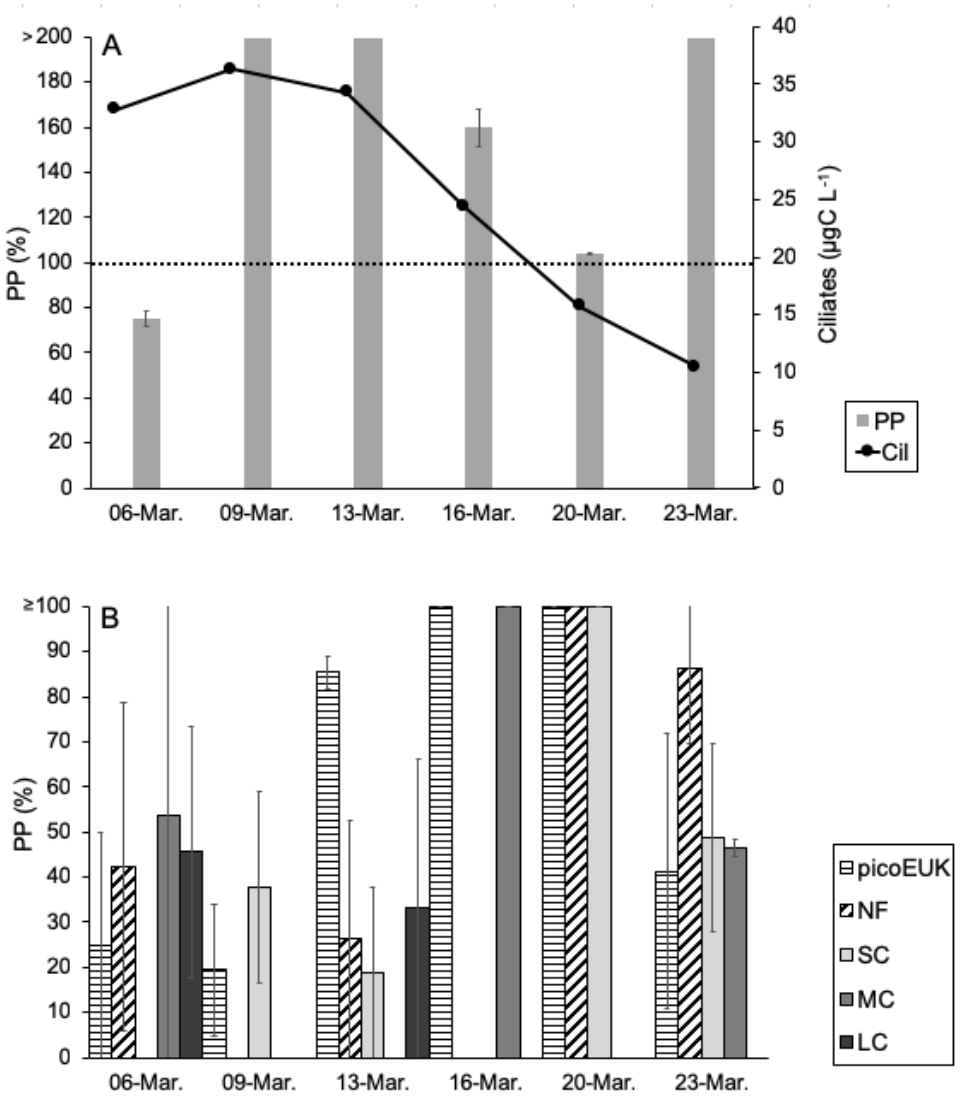

Figure 3.8 Primary production consumed by microzooplankton (%, PP ±SE) based on **a** chlorophyll *a* (bars, left-hand *y*-axis) and biomass of ciliates (µgC L^{-1}) (dots, right-hand *y*-axis); and **b** autotrophic picoeukaryotes (picoEUK), autotrophic nanoflagellates (AN), small cryptophytes (SC), medium cryptophytes (MC) and large cryptophytes (LC) during the study period.

3.4 Discussion

The spring bloom in RF is normally build up during February and decrease during March consuming nutrients (Fig. 3.2). The relative high DIN levels observed during our study were not close to nitrogen limitation whereas DIP was limiting by the end of March. Moreover, Chla did not develop into a typical spring bloom of ≈15 µg Chla L^{-1} (Fig. 3.2), but remained around ≈5 µg Chla L^{-1} at similar nutrient concentrations (Fig. 3.4). Thus, this could be explained as the effect of the high values of grazing measured during this study (Fig. 3.6), controlling primary producers and limiting the bloom.

Mixotrophic protists combine strategies of autotrophic and heterotrophic nutrition, and their classification depends on their ability for obtaining and/or fixing carbon (Mitra et al. 2016). Mixotrophy allow organisms to growth and resist to starvation in low food conditions (Stoecker 1998). However, previous studies observed that mixotrophic ciliates rarely dominate the ciliate community (Dolan 1992) although energy costs, such as the investment in photosynthetic and heterotrophic cellular apparatus, do not appear severe (Dolan and Pérez 2000). We found that during the early spring bloom, ciliates assigned as mixotrophs dominated the protozoan community at high nutrient concentrations concurrently to moderate-high grazing rates estimated. This observation suggests that mixotrophs also ingested preys but at lower rates than obligate heterotrophic ciliates. Comparable results were found in laboratory studies with chloroplast retaining ciliates since mixotrophic ciliates behaved as heterotrophs at high concentrations (Jakobsen & Strom 2004; Schoener & McManus 2012).

Nutrient depletion during the bloom (Fig. 3.3) promoted a new prey field in which heterotrophic ciliates developed successfully as of 16th March (Fig. 3.5). This change in the ciliate community in addition to the decrease in nutrients, shaped the planktonic community throughout the spring bloom. This is an unexpected observation because mixotrophic nutrition is in general assumed to be favoured by nutrient limitation and/or at low prey concentration (see review Jones 2000; Stoecker et al. 2017). In this scenario, different ways to obtain energy and optimise exploitation of resources are of importance to understand the trophic dynamics in the pelagic microbial food web (e.g. Stoecker 1998, Mitra et al. 2016). In this sense, Haraguchi et al. (2018), working in the same fjord, applied rates derived from the literature in relation to cell sizes (Hansen et al. 1997), and they found ciliates with chloroplast

acquired from their prey to be food limited for most of the study period (from February 2016 to November 2017). The development of the ciliate community was on a scale of days, similar to the one observed in mesocosms experiments (Calbet et al. 2014). Although physical perturbations cannot be excluded, the ciliate community in fact has the potential to develop at high rates, something observed in our measured values of autotrophic growth. During the first half of the bloom (from 6th to 16th March), high nutrient concentrations allowed high growth rates, promoting large prey availability for grazers (Fig. 3.4).

The use of flow cytometry for phytoplankton analysis allowed us to follow growth and grazing mortality of the main phytoplankton groups during the spring bloom. To our view, the change in the ciliate community from mixotrophic to heterotrophic nutrition promoted a mismatch between grazers and their potential prey *sensu* (Sherr & Sherr 2009). Cryptophytes decreased parallel to mixotrophic ciliates due to nutrient exhaustion. MC dominated by far over other cryptophytes (large and small-sized) and, in our view, this should be related to their lower dependence upon phosphate and/or nitrate concentration (Table 3.3), or perhaps their ability to survive from osmotrophy (Gervais 1997). Here, we also speculate that the high concentration of MC are fingerprints of their mixotrophic behaviour as observed by previous studies (Marshall & Laybourn-Parry 2002, Hammer & Pitchford 2006, Czypionka et al. 2011, Yoo et al. 2017).

The nutrient exhaustion and the decrease in cryptophytes and mixotrophic ciliates promoted a small increase in SC, NF and picoEUK, also observed in growth. Those increments also coincided with the increase in heterotrophic ciliates and grazing upon all these groups (Figure 3.7). There was a switch from large to smaller phytoplankton which coincided with the increase in heterotrophic ciliates. Thus, the community evolved quite rapidly to a post-bloom scenario. Heterotrophic ciliates preyed upon medium cryptophytes which could be mixotrophic as stated above, also exerting a top-down control over NFs, releasing their biomass (Fig. 3.4a). These interactions between predators and preys were similar to the results obtained by Strom & Morello (1998) and Sherr & Sherr (2009). However, this cascading effect will remain for further studies in the fjord

In summary, nutrient depletion influenced negatively the growth and composition of planktonic organisms as expected. At high nutrient concentrations phytoplankton, mainly MC, increased similarly to mixotrophic

ciliates, although we also measured relatively high grazing rates, suggesting that mixotrophs also ingested preys at similar rates as obligate heterotrophic ciliates. The succession from mixotrophic to heterotrophic ciliates was a consequence of the nutrient depletion and prey availability (high grazing rates), and it was followed by a quite rapid switch from large to smaller phytoplankton. This fact suggests that ciliates promotes swift changes in phytoplankton composition along the bloom. However, whether the changes were also promoted by cascading effects will remain.

Chapter 4

El mar és com un desert d'aigua,
no té camins ni té senyals;
El mar és un desert d'onades,
una lluita sorda i constant;
és el mar la nostra terra ferma
on vivim arrelats en el vent.

Mar i Cel, *Dagoll Dagom*

4 CHAPTER

Planktonic food web structure and trophic transfer efficiency in oligotrophic and upwelling waters of the tropical and subtropical Atlantic Ocean

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A. Rodríguez-Santos, S. Hernández-León

Submitted

Abstract Oligotrophic and productive areas of the ocean differ in the plankton community composition and biomass transfer efficiency from lower to higher trophic levels. Few field studies comprise examples of both trophic systems, providing a detailed description of the plankton groups and trophic interactions. Here, we describe the plankton community from picoplankton to mesozooplankton, including biomasses and microzooplankton diel grazing rates on phytoplankton on a latitudinal transect along the tropical and subtropical Atlantic Ocean. Prochlorococcus dominated autotrophic community at surface and mixed layer in oligotrophic stations, and were replaced by phototrophic picoeukaryotes and Synechococcus in productive waters.

Depth-integrated biomass of microzooplankton was higher than mesozooplankton in oligotrophic stations, and showing similar biomasses in productive waters. Microzooplankton community switched the dinoflagellates dominance in oligotrophic waters to ciliates in upwelling region. In oligotrophic areas, microzooplankton consumed ca. 80% of the production, whereas in the upwelling they consumed ca. ~66%. The differences in microzooplankton and phytoplankton community explain the microzooplankton diel feeding rhythms observed: higher grazing rates during daylight in oligotrophic areas and diffuse grazing patterns in productive waters. Oligotrophic areas were more efficient recycling and using nutrients, while the biomass transfer from microzooplankton to mesozooplankton appeared less efficient than in productive waters.

4.1 Introduction

On a global basis, microzooplankton (μZ) daily graze between 60 and 75% of the primary production (PP), whereas mesozooplankton (MZ) consume from 12 to 35% (Hernández-León & Ikeda 2005). Therefore, the combined impact of both groups account, on average, for ca. 3/4 of the total PP (Schmoker et al. 2013). Given this important role of zooplankton in organic matter turnover, and to fully understand and model the ocean carbon cycle, the rate processes between producers and consumers and their biomass and community structure should be assessed at the ocean basin scale (Calbet et al. 1996). However, the trophic relationships between consumers and producers are highly variable and difficult to parameterize. For instance, some authors found either a bottom-up linkage, top-down control or a slight coupling between the different planktonic food web levels at different regions of the ocean (Falkowski et al. 1998; Aebischer et al. 1990; Irigoien et al. 2004; Shurin et al. 2002). This is expected given the complexity and variability observed in systems of different trophic status (Polis, G. A. & Strong 1996; Weitz et al. 2015; Legendre et al. 1995).

Oligotrophic food webs are substantially different to those from productive areas (Schmoker et al. 2016; Christaki et al. 2014; Billet et al.

1990). However, general ecological rules should apply irrespectively of the ecosystem under study (e.g., metabolic theory, Q_{10} concept, etc.). Thus, interconnecting the dynamics of diverse trophic areas is a challenge, and the identification of these key processes influencing the dynamics of the marine food web has important implications to understand the role of these organisms in the fate of carbon in the ocean. Numerous studies have addressed trophic relationships between planktonic organisms in the ocean. However, few studies cover a wide spectrum of ecological scenarios (Calbet & Landry 1999; Sommer et al. 2002; Polovina et al. 2008).

The warm and stratified subtropical gyres are oligotrophic areas covering approximately 40% of the planetary surface, and they are expanding 0.8-4.3%·y^{-1} (Polovina et al. 2008). Because of their large area, oligotrophic gyres have an important relevance in the contribution of PP and carbon export from the euphotic zone at the global scale[18]. Small cells predominate in these waters, and μZ are more effective than MZ to prey upon phytoplankton, as a result of their similar size with phytoplankton, high growth rates and high metabolism (Fenchel 1987; Sherr & Sherr 1994; Boëchat 2007; Jones 2000). Growth rates based on chlorophyll *a* (Chla) reported in the literature range from 0.1 to 2 d^{-1} in these systems, probably due to the different phytoplankton responses to nutrient inputs and temperature (Laws et al. 1987; Goericke & Welschmeyer 1998; Marañón et al. 2000; Quevedo & Anadón 2001; Marañón 2005). Likewise, μZ consume up to 70% of the PP in tropical and subtropical systems, being the major grazers (Calbet & Landry 2004). Unlike oligotrophic areas, diatoms (Dia) dominate the autotrophic community in more productive systems, being likely dinoflagellates (Din) the potential microbial grazers there (Calbet 2008). Even in these rich waters, μZ are the major grazers, consuming ca. 60% of the PP (Schmoker et al. 2013; (Calbet & Landry 2004). Additionally, MZ have been reported as large consumers of μZ in oligotrophic environments and, with less impact, in upwelling systems (Saiz & Calbet 2011). Therefore, the relationship between these two important groups of organisms (i.e. μZ and MZ) influences the energy and carbon flow throughout the food web (Calbet 2008).

In this work, we covered a wide range of different scenarios in the tropical and subtropical regions, from oligotrophic to productive areas. We aimed to understand the trophic relationships from pico- to MZ at the basin scale from 13ºS to 25ºN in the Atlantic Ocean. Physico-chemical (temperature, salinity, oxygen and inorganic nutrients) and biological variables (micro- and

MZ biomass and μZ grazing) were studied along environments as different as the subtropical gyre and the African upwelling system.

4.2 Materials and methods

4.2.1 Sampling and hydrographic measurements

Sampling took place from 5th to 29th April, 2015 on board the R.V. *Hespérides* from Salvador da Bahia (Brazil) to Canary Islands (Spain). Twelve stations were sampled between 13ºS-25ºN (Fig. 4.1, Table 4.1), and at each station two casts were conducted using a General Oceanics rosette equipped with 24 L PVC Niskin bottles and Seabird 911-plus CTD equipped with a Seapoint Chlorophyll Fluorometer and a Seabird-43 Dissolved Oxygen Sensor. The first cast was carried out down to 3500 m depth during night, and the second cast was carried out from the surface to 200 m depth during daylight hours. Vertical distribution of the photosynthetically active irradiance (PAR, 400-700 nm) was measured using a radiometer Biospherical/Licor installed in the rosette sampler. Water samples to calibrate dissolved oxygen sensor were collected with Niskin bottles along all the water column.

4.2.2 Nutrients and oxygen

Inorganic nutrients were sampled from hydrographic bottles with polyethylene tubes and stored frozen (-20ºC) until their analysis in the laboratory. Samples were analysed with a QuAAtro 39-SEAL Analytical AutoAnalyzer following the protocol by Armstrong et al. (1967). On board oxygen calibration was carried out with the potentiometric end-point Winkler method (Moreno-Ostos 2010).

Table 4.1 Location of the studied stations and initial conditions for microzooplankton grazing experiments.

Station	Latitude	Longitude	Depth (m)	Temperature (° C)	Salinity	Dissolved O_2 (µmol Kg^{-1})
1	-13.12	-34.05	5	28.54	37.02	236.17
2	-9.96	-31.79	5	28.79	36.66	222.73
			20	28.43	36.66	156.31
			135	21.74	36.65	157.71
3	-6.51	-30.22	5	28.74	36.35	184.32
			20	28.6	36.35	155.02
			95	23.95	36.46	177.18
4	-3.03	-28.46	5	29.39	35.75	314.15
			20	28.65	36.01	159.68
			65	22.24	36.20	123.94
5	0.25	-26.70	5	28.45	35.78	255.37
			20	28.10	35.96	159.48
			65	22.64	36.42	146.71
6	3.73	-25.32	5	28.14	35.89	252.51
			20	27.86	35.91	158.43
			46	18.65	35.78	112.55
7	7.30	-23.93	5	25.73	35.73	199.51
			20	25.04	35.75	166.03
			41	21.26	36.00	145.73
8	10.87	-22.65	5	24.13	35.73	210.41
			29	23.72	35.76	169.47
			49	21.18	35.72	160.18
9	14.44	-21.36	5	22.09	35.90	173.51
			30	21.92	35.90	172.38
10	18.04	-20.22	5	20.10	36.00	177.00
			20	20.07	35.99	173.88
11	21.63	-18.76	5	17.98	35.91	163.43
			15	17.69	35.90	205.50
12	25.24	-17.38	5	19.32	36.64	181.81

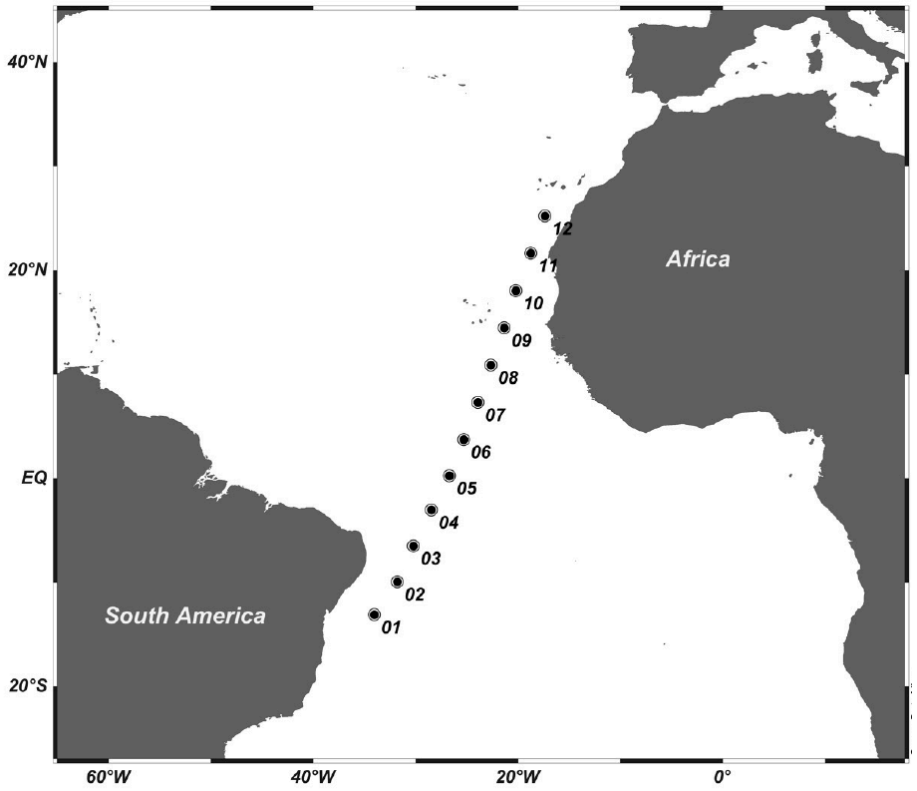


Figure 4.1 Map of the study area across the Atlantic Ocean.

4.2.3 Chlorophyll *a* and picoplankton

Chla samples were taken at 5 levels from the surface to 200 m depth in order to calibrate the fluorescence sensor installed in the rosette. Samples of 500 mL were collected from the Niskin bottles, filtered through 25 mm Whatman GF/F filters and stored frozen until their analysis. In the laboratory, pigments were extracted in cold acetone (90%) for 24 h and analysed using an AU TurnerDesigns bench fluorometer previously calibrated with pure Chla (Sigma Aldrich) according to Yentsch & Menzel (1963) and acidified following Welschmeyer (1994). Chla concentration was converted to carbon assuming a C:Chl of 50 (Harris 1986).

In order to better define the upwelling stations, PP data were obtained from the Ocean Productivity website using the VGPM model following Behrenfeldand Falkowski[35] (http://www.science.oregonstate.edu/ocean.productivity/index.php).

Picoplankton samples were taken from the initial conditions of the 100% whole seawater (WSW) treatments of grazing experiments (see "Microzooplankton grazing experiments"). PE, Syn and *Proch* were counted by flow cytometry using FACScalibur cytometer (Gasol & Del Giorgio 2000). Abundance was converted to biomass using the carbon conversion factor of 1500 fgC cell^{-1} for PE (Zubkov et al. 1998), 29 fgC cell^{-1} for *Proch* and 100 fgC cell^{-1} for *Syn* (Zubkov et al. 2000).

4.2.4 Micro- and mesozooplankton stock measurements

Microplankton samples were collected directly from the Niskin bottle during the daylight cast at 5 m depth (surface), mixed layer (20-30 m) and Chla maximum depth (Table 4.1). Samples of 500 mL were preserved in alkaline Lugol's solution until their analysis in the laboratory. An aliquot of 100 mL of each sample was allowed to settle using sedimentation chambers (Uthermöhl 1958) and analysed on an inverted Olympus IX83 microscope equipped with a motorized focus drive. The microscope was controlled by CellSens software using the automated image acquisition at 200x magnification. More than 25% of total sample area (minimum of 300 organisms counted) was imaged using the functions of Multiple Image Aligning (MIA) and Z-stack. MIA takes pictures of an area and the Z-stack gets images in the Z plane. Identification and counting of organisms was carried out manually from the digital image. Main microplankton groups were identified: Dia, Din, tintinnids and Cil. Din, considered all as μZ, and Cil were counted as <20 μm, 20-40 μm y >40 μm in order to convert abundance to biomass more accurately.

MZ samples were collected during daylight hours at each station with a Multiple Opening and Closing Net and Environmental Sensing System (MOCNESS) equipped with a 200 μm mesh net at 0-50, 50-100 and 100-200 m depth intervals. Oblique trawls were conducted at a towing speed of ca. 3 knots, measuring the volume of water filtered using a calibrated electronic flowmeter. MZ biomass was directly obtained on board through image processing using the software ZooImage 1, version 1.2-1(Garijo 2016) and using a conversion factor from Uye (Uye 1982).

4.2.5 Microzooplankton grazing experiments

To estimate μZ grazing upon phytoplankton, dilution experiments were carried out using the 2-treatments method (Strom & Frederickson 2008) based on the seawater dilution technique (Landry et al. 1984; Landry & HAsset 1982). Briefly, seawater in two treatments consisting in 100 and 5% whole seawater (WSW) was incubated for 24h to obtain the net growth rate of phytoplankton. The 100% WSW treatment is used to measure the net growth rate of phytoplankton (k), while the intrinsic growth rate (μ) is measured from the 5% WSW treatment. μZ grazing rate (g) was obtained from $g = \mu - k$. Negative values of μ were converted to 0.001 $d^{-1,}$ while negative values of g were converted to 0 d^{-1} (Calbet & Landry 2004).

Water for experiments was collected at the surface (5 m depth), mixed layer (20 m) and at the chlorophyll maximum (CM) during the daylight cast (Table 4.1). Vertical PAR distribution was measured prior to incubation and light profiles were simulated on board incubator using a set of neutral density and blue plastic filters (Marañón et al. 2000). Temperature was controlled using a series of Titan 2000 coolers. Each experiment was carried out in triplicate using 3.4 L Tedlar® bags during 24 h. The 100% WSW was gently screened with a 200 μm mesh net to avoid MZ, while the filtered seawater was gravity-filtered through 0.2 μm Whatman® Polycap filter. Experiments were run with added nutrients at saturating concentrations in all stations. Nutrient concentration were obtained from Chla concentration observed by Marañón et al. (Marañón et al. 2000) and converted first to C (Harris 1986) and then to N and P using the Redfield ratio (final nutrient concentrations were: 2-6 μM of NH_4Cl and 0.1-0.5 μM of Na_2HPO_4). Chla and picoplankton were sampled at t=0 h (initial conditions) and t = 24 h from each treatment (see methods of analysis above).

The impact of μZ grazing on phytoplankton production was estimated using the ratio g:μ for Chla, PE, *Syn* and *Prochloroccocus* (Calbet & Landry 2004). It should be noted that we added nutrients to the bottles in order to warrant a critical assumption of the dilution method (phytoplankton growth rates should be independent from the dilution level) (Landry et al. 1984). Thus, we obtained potential growth rates of phytoplankton.

4.2.6 Diel phytoplankton growth and mortality

In order to study the daily phytoplankton growth and mortality, grazing experiments were carried out using surface waters (5 m depth) of Chla and picoplankton at t = 0 h (near dusk), t = 12 h (early in the morning) and t = 24 h (near dusk). This depth was selected because the signature of the diel rhythm should be stronger at more illuminated layers, and organisms at the surface are less photosensitive than those inhabiting deeper layers. In this sense, natural variations in light such as clouds or waves, as well as manipulation have a lower impact on surface organisms than most light sensitive organisms.

4.2.7 Statistical analysis

Principal component analysis (PCA) was used to reduce the dimensionality of physical and biological variables, and the generalized additive modelling (GAM) was used to explore the dependence between biological and physical parameters (R Project software). Kendall Rank correlation coefficients were used to study the relationships between biomass, mortality rates, and environmental variables. Kendall Rank is preferable to Spearman test because of its robustness and efficiency in the study of populations with scarcely or tied data. For statistical comparisons, a t-test was used for data with a normal distribution and a Wilcoxon-Mann-Whitney for data with no normal distribution. To study the normality of data, a Shapiro-Wilk test was performed. We carried out the Wilcoxon test to investigate differences between growth and mortality during the day and night (Statistica software).

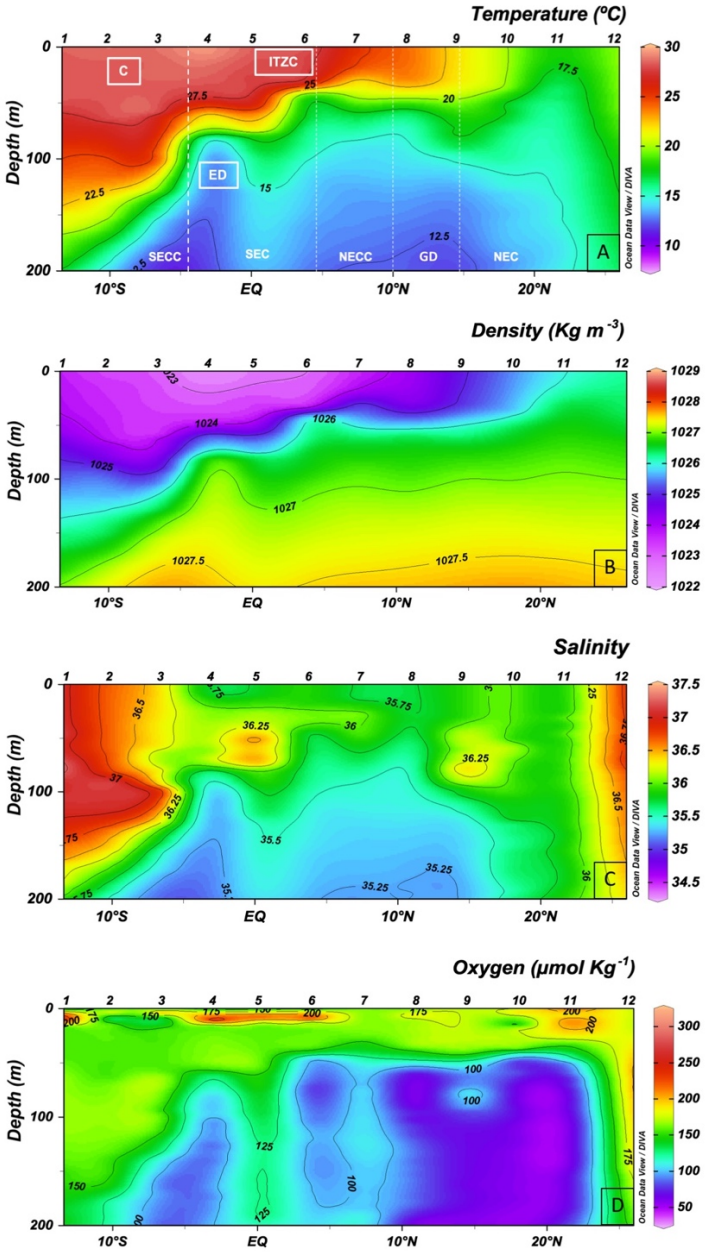


Figure 4.2 Vertical section (0-200 m) of **a** temperature (ºC), water currents (South Equatorial Counter Current (SECC), South Equatorial Current (SEC), North Equatorial Counter Current (NECC), Guinea Dome (GD). North Equatorial Current (NEC)) and physical processes (Convergence (C), Equatorial divergence (ED), Intertropical Convergence Zone (ITCZ)); **b** density (Kg m^{-3}); **c** salinity; and **d** dissolved oxygen (µmol Kg-1) along transect in the Atlantic basin, based on CTD data. Biogeochemical areas are indicated at the top of panels.

4.3 Results

4.3.1 Hydrological structure

We observed a sharp temperature and density gradient along the transect as expected (Fig. 4.2). A convergence of the South Equatorial Counter Current (SECC) (Reid 1959) showed a deeper thermocline and high salinity (stations 1 to 3), while the Equatorial Divergence within the South Equatorial Current (SEC) promoted a shallower thermocline and a decrease in dissolved oxygen concentration (station 4) (Fig. 4.2). The Intertropical Convergence Zone (ITCZ) showed a slightly deepest thermocline as well as high oxygen concentration (between stations 5 and 6). At station 8, the North Equatorial Current (NEC) decreased the temperature northward of 10ºN and showed an oxygen minimum zone (OMZ). Station 9 showed typical features of the Guinea Dome, characterized for anticlinal thermal and saline structure. The upwelling off Cape Blanc originated cold temperatures and less stratified waters (stations 10 and 11), while the Canary Current showed waters with high salinity and oxygen concentration (station 12, Fig. 4.2).

4.3.2 Nutrients distribution

The inorganic nutrient concentrations were higher below the thermocline throughout the transect, as expected (Fig. 4.3). Nitrite showed highest values at the Equatorial Divergence, at mid-ocean upwelling below 50 m depth, at Guinea Dome and on surface at the Cape Blanc upwelling (Fig. 4.3a). Nitrates, phosphates and silicates showed a core around 50 m depth in the Equatorial Divergence, while from the mid-ocean upwelling (station 6) to the Cape Blanc upwelling its concentration increased in the mixed layer. The Guinea Dome was an exception because nutrients decreased in the upper layers (Fig. 4.3b, c, d). Ammonium was slightly higher near the thermocline but showing an important increase (>2 μmol L^{-1}) near the Guinea Dome (stations 8 and 9, Fig. 4.3e).

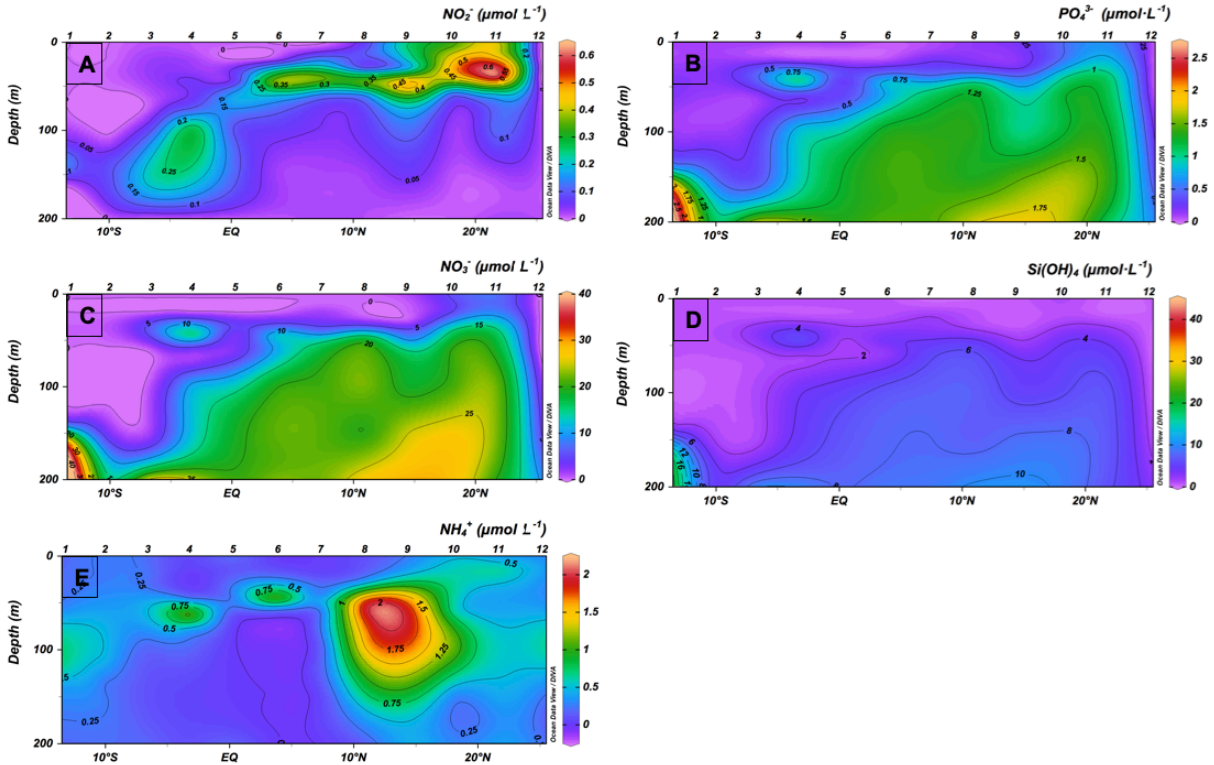


Figure 4.3 Vertical section (0-200 m) of **a** nitrites, **b** phosphates, **c** nitrates, **d** silicates and **e** ammonia (µmol L^{-1}).

4.3.3 Phytoplankton community

Along the transect, the Chla maximum (CM) followed the base of the thermocline (Fig. 4.4), being deeper in the warmest and oligotrophic areas (stations 1-3), and shallower in the coldest and upwelling influenced areas (stations 10 and 11). The CM showed the highest values at the mid-ocean equatorial upwelling and Cape Blanc upwelling. On the contrary, the lowest Chla values were observed at surface and at the ML in the oligotrophic area (Fig. 4.4, 4.6a). The Kendall Rank correlation test showed a negative correlation between Chla and temperature ($\tau = -0.74$; $p < 0.001$) and positive with nutrient concentration ($\tau = 0.69$, $p < 0.001$ for NO_3+NO_2; and $\tau = 0.57$, $p < 0.001$ for phosphates).

Physical factors, such as temperature and nutrient concentration, as well as MZ biomass explained 85.2 % of the variance in the distribution of Chla (PCA and GAM tests, Table 4.2). The signature of the Guinea Dome and Northwest African upwelling were also conspicuous on the satellite data showing rather high values of PP (Fig. 4.5).

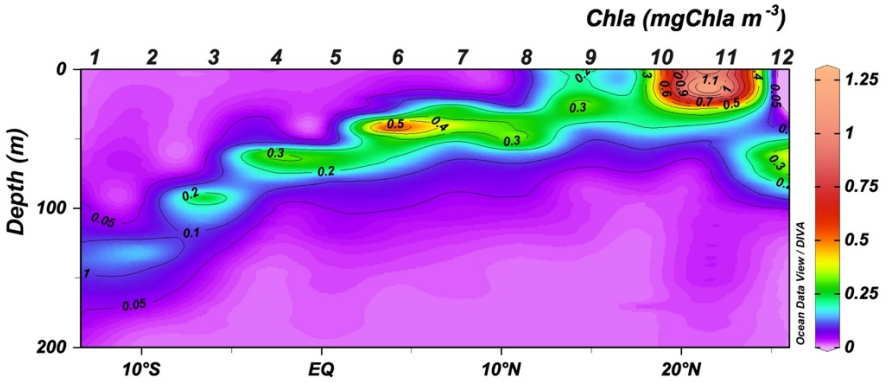


Figure 4.4 Vertical section (0-200 m) of Chlorophyll *a* (mg Chla m^{-3}).

The biomass of phototrophic picoplankton based on cytometry data increased from oligotrophic to upwelling regions (Fig. 4.6), with a further decrease at the Cape Blanc upwelling (station 11), where Chla showed maximum concentrations (Fig. 4.4).

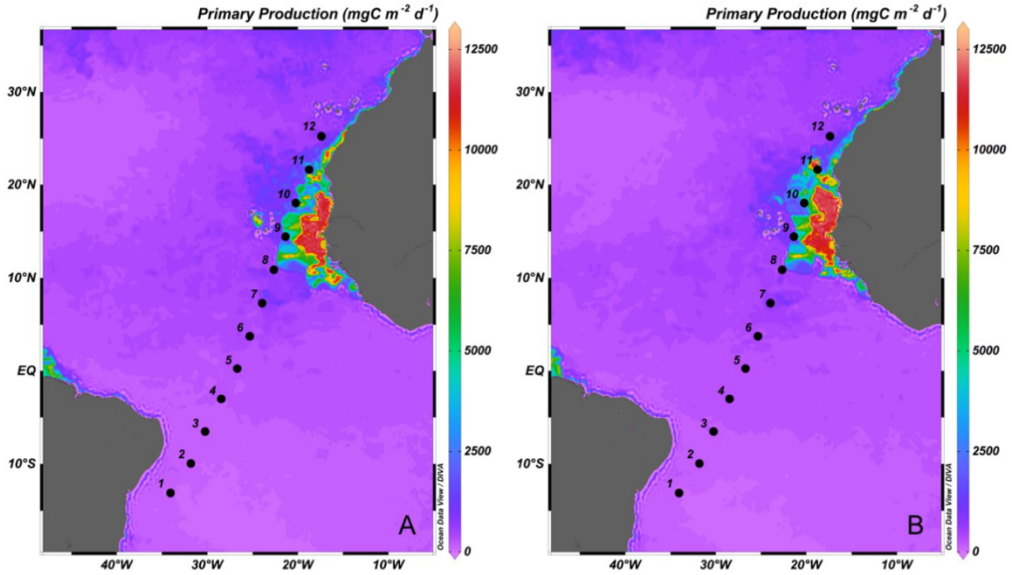


Figure 4.5 Surface maps of Primary Production (mgC m^{-2} d^{-1}) from satellite data during 15-22 April **a** and 23-30 April **b**.

Proch dominated at surface and ML in the most oligotrophic and warmest stations (τ = 0.40, p<0.01 *Prochlorococcus* (*Prochl*) with temperature; Kendall Rank correlation test) and was replaced by *Synechococcus* (*Syn*) in more productive waters (τ = -0.28, p<0.05 with temperature; Kendall Rank correlation test). Picoeukaryotes (PE) dominated the autotrophic community at low temperature and high nutrient availability stations such as in the Guinea Dome and Cape Blanc upwelling zone, as well as at the CM throughout all stations (τ = -0.64, p<0.01 for temperature; and τ = 0.61, p<0.001 for NO_3+NO_2; Kendall Rank correlation test) (Fig. 4.6). 81% of *Proch* and 64.1 % of PE biomass variability was explained by temperature, nutrients, µZ and MZ; whereas *Syn* distribution was determined by 75.2 % by temperature, µZ and MZ (PCA and GAM tests, Table 4.2).

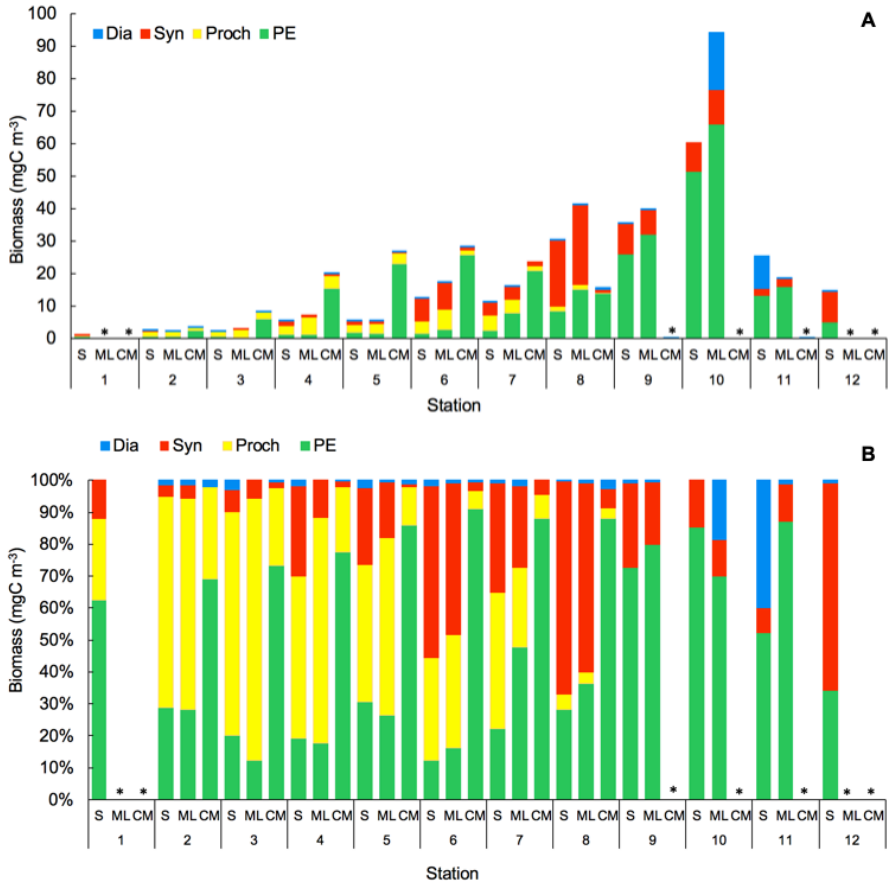


Figure 4.6 **a** Proportion of biomass (%) and **b** Biomass (mgC m^{-3}) of Cyanobacteria (*Synechococcus*, Syn; *Prochlorochoccus*, Proch; and autotrophic picoeukaryotes, PE) at the surface layer (5 m depth, S), mixed layer between 20-30 m depth, ML) and chlorophyll *a* maximum (CM). * = no data available.

4.3.4 Micro- and mesozooplankton community

The oligotrophic stations and mid-ocean upwelling showed the highest μZ biomass (mean 20.61 ± 3.49 SE mgC m^{-3}), and its importance decreased along the transect (Fig. 4.7a, b, c) towards lower temperature (τ = -0.38, p<0.01; Kendall Rank correlation test). Chla, PE, *Syn* and MZ explained 85 % of μZ biomass variability (PCA and GAM tests, Table 4.2). Dinoflagellate (Din) biomass dominated microzooplankton in the warmest and stratified waters, comprising 60-80% of total μZ biomass (τ = 0.29, p<0.05 with

temperature; Kendall Rank correlation test). From mid-ocean upwelling, Din dominance became irregular decreasing its abundance and increasing that of the naked ciliates (Cil) (Fig. 4.7b). This change in micro-grazers dominance was especially evident in upwelling stations were temperature sharply decreased (τ = -0.40, p<0.01; Kendall Rank correlation test between naked ciliates and temperature). Tintinnids contributed <5% of the total μZ biomass in all stations (Fig. 4.7b).

Table 4.2 Principal Component Analysis (PCA) and Generalized Additive Model (GAM) for groups of organisms using biological and physical variables as effects; n = 28.

Model	PCA (variance, %)	GAM		
		Residual *Df*	F-value	Deviance explained
Chlorophyll a	85.2 %			71.2 %
Terms:				
[+]Temperature			-3.19**	
[+]NO_3			1.6	
[+]Mesozooplankton			2.3**	
PE	64.1 %			99.3 %
Terms:				
Temperature		7.65	22.74***	
NO_3		2.64	9.99**	
Mesozooplankton		8.98	16.8***	
Synechococcus	75.2 %			95.8 %
Terms:				
NO_3		2.46	37.64***	
Dinoflagellates		6.24	3.23*	
Mesozooplankton		5.79	19.89***	
Prochlorococcus	81 %			81.5 %
Terms:				
Temperature		7.52	3.96**	
NO_3		3.08	1.19	
Microzooplankton		1.00	2.01	
Microzooplankton	85 %			70.6 %
Terms:				
Temperature		2.06	9.37**	
Chlorophyll *a*		3.84	4.68**	
Mesozooplankton		1.00	0.003	
Mesozooplankton	85.2 %			85.4 %
Terms:				
Temperature		3.02	4.39*	
Chlorophyll *a*		2.50	6.98**	
Microzooplankton		6.74	1.70	

[+]Linear adjust and t-value instead F-value
Significance level: * p < 0.1; ** p < 0.01; *** p <0.001

MZ biomass increased along the transect (Fig. 4.7c) with temperature decrease (τ = -0.38, p<0.01; Kendall Rank correlation test), showing the lowest MZ biomass at oligotrophic region (mean 4.89 ± 1.64 SE mgC m^{-3}) (Fig. 4.7c). In size terms, the organisms of the MZ with a size >1000 µm dominated the MZ community in all stations, although in the stations 8 and 9 increased the biomass of organisms sized between 500 and 1000 µm.

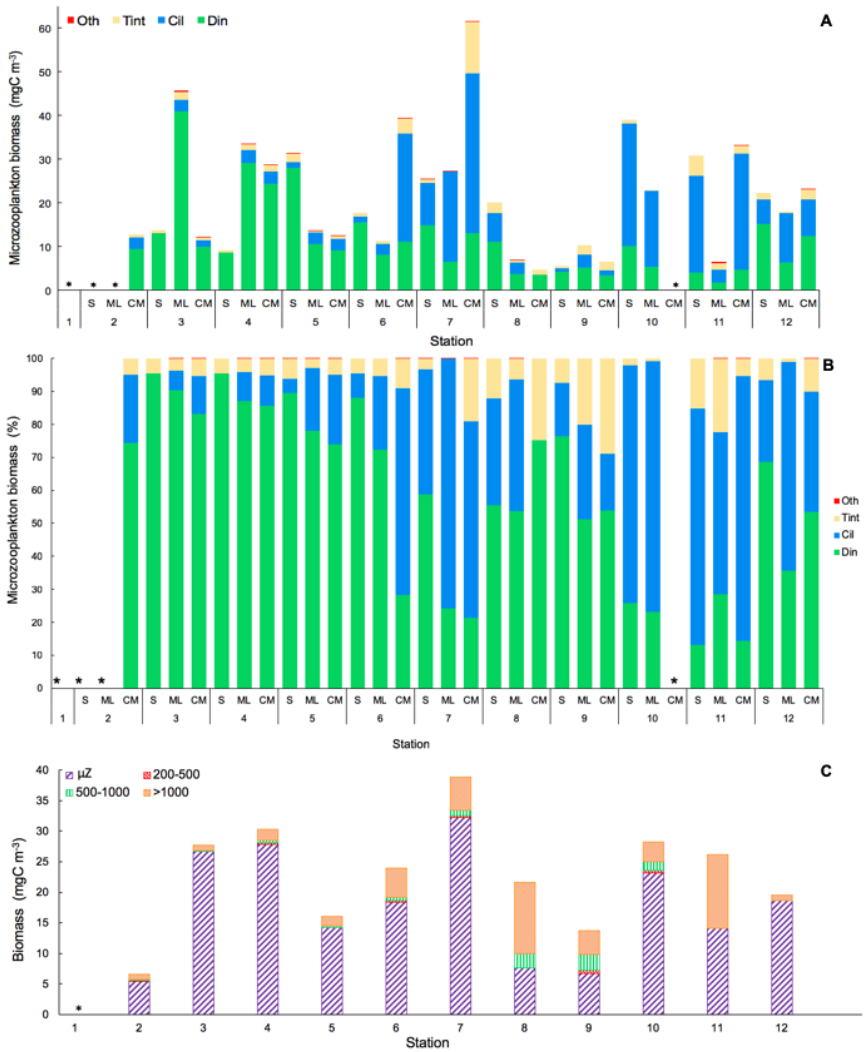


Figure 4.7 Biomass of dinoflagellates (Din), ciliates (Cil), tintinnids (Tint), and others microzooplankton group (Oth) **a** in mgC m^{-3} and **b** in %; **c** Integrated biomass (mgC m^{-3}) in the water column of microzooplankton (µZ) and different mesozooplankton size-fraction: 200-500 µm (200-500), 500-1000 µm (500-1000) and >1000 µm (>1000). * = no data.

Table 4.3 Phytoplankton growth (μ) and microzooplankton grazing (g) rates (d^{-1}) for total chlorophyll *a* (Chla), picoeukaryotes (PE), *Synechococcus* (Syn) and *Prochlorococcus* (Proch) from seawater dilution experiments at surface (5 m), mixed layer (20m) and chlorophyll maximum (CM). Negative growth and grazing rates were converted to 0.001 and 0, respectively. Note *Proch* were not present at stations 9 to 12. Values (mean ± SE), n.s (non-significant p-value).

Station	Depth (m)	Growth (d^{-1})				Grazing (d^{-1})			
		μ_{Chla}	μ_{PE}	μ_{Syn}	μ_{Proch}	g_{Chla}	g_{PE}	g_{Syn}	g_{Proch}
1	5	0.155 ± 0.00	0.073 ± 0.03	0.013 ± 0.01	0.031 ± 0.000	0.177 ± 0.034	0.186 ± 0.014	0.298 ± 0.123	0.114 ± 0.043
2	5	0.119 ± 0.048	0.047 ± 0.027	0.073 ± 0.006	0.071 ± 0.008	0.098 ± 0.005	0.071 ± 0.024	0.265 ± 0.02	0.001 ± 0.024
	20	0.666 ±0.025	0.069 ± 0.03	0.783 ± 0.039	0.001	0.682 ± 0.009	0.086 ± 0.005	0.705 ± 0.027	0.055 ± 0.01
	135 (CM)	0.285 ± 0.041	0.048 ± 0.011	0.001	0.024 ± 0.013	0.179 ± 0.029	0.134 ± 0.008	0	0.047 ± 0.004
3	5	0.502 ± 0.015	0.05 ± 0.031	0.106 ± 0.023	0.952 ± 0.047	0.421 ± 0.013	0.046 ± 0.022	0.18 ± 0.022	0.83 ± 0.031
	20	1.26 ± 0.013	0.318 ± 0.052	0.259 ± 0.059	0.673 ± 0.065	0.769 ± 0.007	0.325 ± 0.006	0.247 ± 0.019	0.718 ± 0.027
	95 (CM)	0.229 ± 0.021	0.252 ± 0.055	0.05 ± 0.017	0.073 ± 0.01	0.167 ± 0.034	0.197 ± 0.019	0.12 ± 0.007	0.057 ± 0.014
4	5	0.400 ± 0.021	0.642 ± 0.104	0.221 ± 0.048	0.074 ± 0.017	0.023 ± 0.051	0.547 ± 0.033	0.249 ± 0.016	0.148 ± 0.022
	20	0.706 ± 0.077	0.045 ± 0.018	0.064 ± 0.005	0.001	0.647 ± 0.021	0.071 ± 0.045	0.081 ± 0.01	0
	65 (CM)	0.077 ± 0.008	0.495 ± 0.05	0.113 ± 0.024	0.527 ± 0.027	0.114 ± 0.027	0.416 ± 0.039	0.092 ± 0.01	0.515 ± 0.01
5	5	0.226 ± 0.048	0.032 ± 0.007	0.396 ± 0.099	0.039 ± 0.023	0	0.058 ± 0.019	0.322 ± 0.041	0.093 ± 0.022
	20	0.061 ± 0.010	0.019 ± 0.009	0.535 ± 0.037	0.001	0.095 ± 0.003	0.062 ± 0.014	0.483 ± 0.012	0.582 ± 0.085
	65 (CM)	0.182 ± 0.018	0.341 ± 0.04	0.397 ± 0.043	0.077 ± 0.012	0.148 ± 0.009	0.303 ± 0.01	0.345 ± 0.027	0.077 ± 0.011
6	5	0.051 ± 0.024	0.642 ± 0.007	0.653 ± 0.067	0.063 ± 0.022	0.081 ± 0.006	0.283 ± 0.009	0.331 ± 0.095	0.093 ± 0.007
	20	0.187 ± 0.024	0.568 ± 0.043	0.408 ± 0.029	0.001	0.161 ± 0.020	0.232 ± 0.027	0.056 ± 0.36	0.544 ± 0.037
	46 (CM)	0.241 ± 0.018	0.225 ± 0.018	0.129 ± 0.008	0.237 ± 0.014	0.205 ± 0.005	0.022 ± 0.023	0.113 ± 0.007	0.249 ± 0.012

Station	Depth (m)	Growth (d^{-1})				Grazing (d^{-1})			
		μ_{Chla}	μ_{PE}	μ_{Syn}	μ_{Proch}	g_{Chla}	g_{PE}	g_{Syn}	g_{Proch}
7	5	0.408 ± 0.036	0.027 ± 0.014	0.81 ± 0.093	0.409 ± 0.115	0.162 ± 0.015	0	0.183 ± 0.038	0.392 ± 0.011
	20	0.228 ± 0.056	0.593 ± 0.044	0.549 ± 0.025	0.09 ± 0.023	0.153 ± 0.006	0.538 ± 0.014	0.449 ± 0.032	0.593 ± 0.025
	41 (CM)	0.256 ± 0.011	0.169 ± 0.034	0.334 ± 0.049	0.132 ± 0.009	0.181 ± 0.020	0.221 ± 0.015	0.306 ± 0.024	0.178 ± 0.007
8	5	0.426 ± 0.038	0.475 ± 0.079	1.016 ± 0.111	0.24 ± 0.027	0.316 ± 0.017	0.239 ± 0.014	0.658 ± 0.063	0.309 ± 0.011
	29	0.112 ± 0.026	0.47 ± 0.015	0.181 ± 0.047	0.073 ± 0.016	0.144 ± 0.026	0.224 ± 0.075	0.134 ± 0.019	0.493 ± 0.013
	49 (CM)	0.142 ± 0.026	0.265 ± 0.022	0.336 ± 0.029	0.003 ± 0.001	0 ± 0.037	0.294 ± 0.004	0.242 ± 0.025	0.096 ± 0.013
9	5	0.356 ± 0.052	0.939 ± 0.007	1.238 ± 0.037		0.082 ± 0.067	0.621 ± 0.032	0.56 ± 0.022	
	30 (CM)	0.198 ± 0.016	0.313 ± 0.049	0.11 ± 0.031		0.209 ± 0.010	0.29 ± 0.01	0.098 ± 0.012	
10	5	0.159 ± 0.006	0.609 ± 0.023	0.711 ± 0.066		0.040 ± 0.011	0.398 ± 0.036	0.426 ± 0.041	
	20	0.170 ± 0.013	0.737 ± 0.054	0.868 ± 0.064		0.110 ± 0.021	0.68 ± 0.026	0.725 ± 0.011	
11	5	0.273 ± 0.008	0.329 ± 0.047	0.523 ± 0.016		0.250 ± 0.007	0.298 ± 0.044	0.182 ± 0.021	
	15 (CM)	0.076 ± 0.026	0.315 ± 0.047	0.516 ± 0.013		0	0.311 ± 0.005	0.497 ± 0.01	
12	5	0.442 ± 0.080	0.043 ± 0.009	0.173 ± 0.025		0	0.079 ± 0.019	0.115 ± 0.029	

4.3.5 Microzooplankton grazing

Potential phytoplankton growth rates based on Chla (μ_{Chla}) at the ML were higher in the oligotrophic stations within SECC and Equatorial Divergence than other oligotrophic stations (Fig. 4.8a; Table 4.3). However, the growth rates of the different groups of autotrophs differed from those based on Chla (Fig. 4.8, Table 4.3) showing significant differences between oligotrophic and productive areas ($p < 0.001$; Wilcoxon-Mann-Whitney test). PE and *Syn* potential growth rates (μ_{PE}, μ_{Syn}) showed slightly higher values at surface and ML in productive areas (mean 0.52 ± 0.08 SE d^{-1} and 0.65 ± 0.14 SE d^{-1} for PE and *Syn* respectively), and lowest rates (mean 0.23 ± 0.07 SE d^{-1} for PE and 0.36 ± 0.07 SE d^{-1} for *Syn*) in oligotrophic stations ($p < 0.001$; Wilcoxon-Mann-Whitney test for PE and t-test for *Syn*) (Fig. 4.8b, c). Potential growth rates of *Proch* (μ_{Pro}) were lower than other picoplankton organisms at all stations except station 3 (Fig. 4.8d). At the CM, potential growth rates for autotrophic picoplankton and Chla showed non-significant differences between oligotrophic and productive areas (t-test for μ_{Chla} and μ_{Syn}; Wilcoxon-Mann-Whitney test for μ_{PE} and μ_{Pro}; Fig. 4.8, Table 4.3).

At surface and ML, μZ grazing rates on phytoplankton based on Chla (g_{Chla}) showed the highest rates at SECC and Equatorial Divergence (stations from 1 to 4) (Fig. 4.9a; Table 4.3). Also, at surface and ML, grazing rates on PE (g_{PE}) and *Syn* (g_{Syn}) were significantly lower in oligotrophic stations (0.19 ± 0.05 SE for PE and 0.28 ± 0.05 SE for *Syn*) than in productive stations (0.38 ± 0.06 SE for PE and 0.41 ± 0.09 SE for *Syn*) ($p < 0.001$ Wilcoxon-Mann-Whitney for PE; and $p < 0.01$ t-test for *Syn*) (Fig. 4.9b, c; Table 3). The μZ grazing rates on *Proch* (g_{Proch}) were higher at the surface and ML in stations with a shallower thermocline (stations 5 to 8; Fig. 4.9d; Table 4.3). At CM, μZ grazing rates of Chla, PE, *Syn* and *Proch* showed a non-significant difference between oligotrophic and productive regions (Wilcoxon-Mann-Whitney test for Chla, *Syn* and *Proch*; t-test for PE) (Fig. 4.9, Table 4.3). Overall, μZ grazing rates for all organisms were lower at the CM than in the upper layers (Fig. 4.9, Table 4.3).

The ratio of grazing rates to phytoplankton growth (g/μ) provided an estimation of the proportion of the potential PP consumed by microbial grazers (%PP). Based on Chla the $\%PP_{Chla}$ showed non-significant differences (t-test) from oligotrophic to upwelling areas at the surface and ML (Fig. 4.10a). In the same water column range, the impact upon PE ($\%PP_{PE}$) and *Syn* ($\%PP_{Syn}$) were

higher in the oligotrophic areas (134.75 ± 25.03 SE for PE; 108.01 ± 19.04 SE for *Syn*) than in the upwelling (79.15 ± 7.28 SE for PE; 69.01 ± 8.63 SE for *Syn*) ($p < 0.05$ for PE and $p < 0.01$ for *Syn* Wilcoxon-Mann-Whitney test), while the impact of grazers on *Proch* ($\%PP_{Proch}$) increased at surface as the thermocline shallowed except in the equatorial regions (Wilcoxon-Mann-Whitney test) (Fig. 4.10b, c, d).

4.3.6 Diel growth and grazing rates

No clear pattern of diel growth and grazing were observed based on total Chla (Fig. 4.11a). However, a more detailed study of different groups of plankton showed different daily patterns. PE and *Syn* displayed a clear rhythmicity on both growth and grazing, with higher rates during the day, vanishing this pattern in upwelling waters (Fig. 4.11b, c). *Proch* showed higher growth and grazing rates during night in the most oligotrophic and stratified areas (stations 1 and 2), but the rhythm was the opposite in the Equatorial Divergence (Stations 4 and 5, Fig. 4.11d, Table 4. 4).

4.3.7 Trophic transfer efficiency

The ratio between the biomass of upper and lower trophic levels can be used as a proxy of the trophic transfer efficiency within the food web. Thus, the ratio of Chla:(NO_2+NO_3) showed that each µM of N sustained, on average, 22.9 µg C of phytoplankton (± 16.86 SE) in oligotrophic regions, and 2.6 µg C of phytoplankton (± 0.74 SE) in productive regions. The ratio between biomass of µZ:(NO_2+NO_3) showed that each µmol of N supported 27.9 µg C of µZ (± 12.98 SE) in oligotrophic zones, whereas for productive areas decreased to 2.9 (± 0.68 SE) µg C of µZ. For MZ, the ratio between their biomass and NO_2+NO_3 resulted in lower values than µZ at oligotrophic stations (mean 5.8 µg of MZ ± 1.26 SE), while at productive stations showed higher values than µZ (mean 14.2 µgC of MZ ± 7.83 SE). The carbon transferred from phytoplankton to µZ (µZ biomass:phytoplankton biomass) averaged 3.9 ± 0.68 SE in oligotrophic stations, and decreased to 0.70 ± 0.39 SE in productive stations. Using the same quotient for MZ, in oligotrophic areas the ratios were slightly lower (mean 0.92 ± 0.44 SE) than in productive areas (mean 1.28 ± 0.33 SE). MZ biomass supported by µZ biomass averaged

0.15 ± 0.03 SE in oligotrophic areas, and 1.44 ± 0.33 SE in the upwelling region.

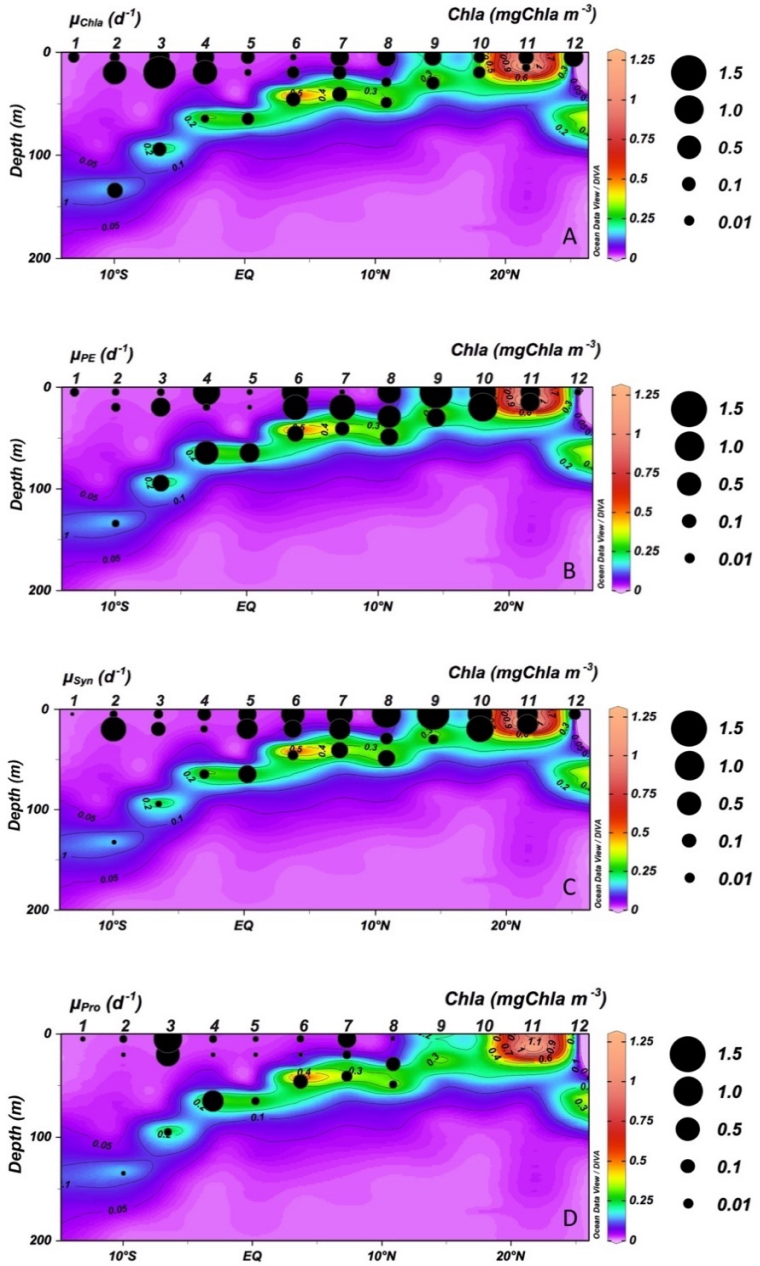


Figure 4.8 Vertical section (0-200 m) of Chlorophyll a (mg Chla m^{-3}) and potential growth rates (μ, d^{-1}) for **a** Chlorophyll a (μ_{Chla}), **b** autotrophic picoeukaryotes (μ_{PE}), **c** *Synechococcus* (μ_{Syn}) and **d** *Prochlorococcus* (μ_{Pro}).

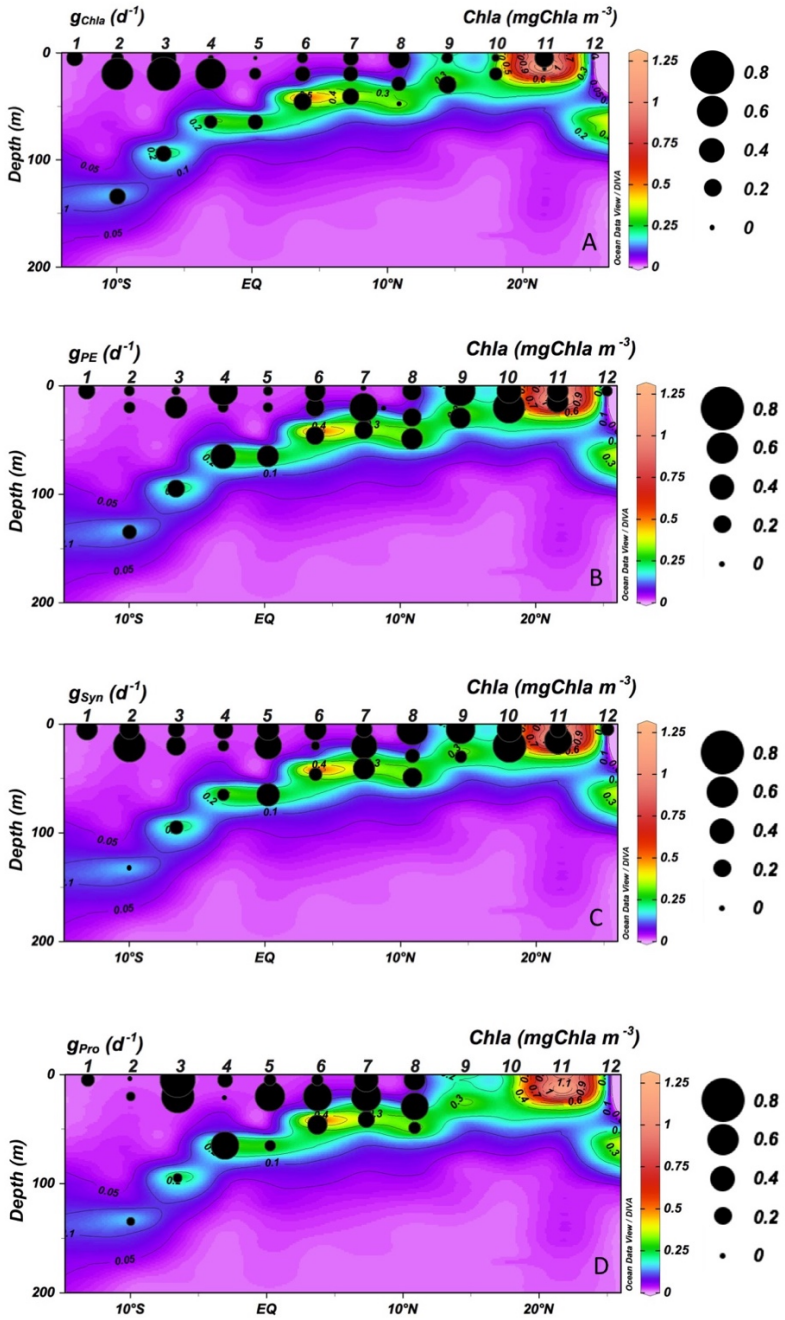


Figure 4.9 Vertical section (0-200 m) of Chlorophyll a (mg Chla m^{-3}) and microzooplankton grazing rates (g, d^{-1}) for **a** Chlorophyll a (g_{Chla}), **b** autotrophic picoeukaryotes (g_{PE}), **c** *Synechococcus* (g_{Syn}) and **d** *Prochlorococcus* (g_{Pro}).

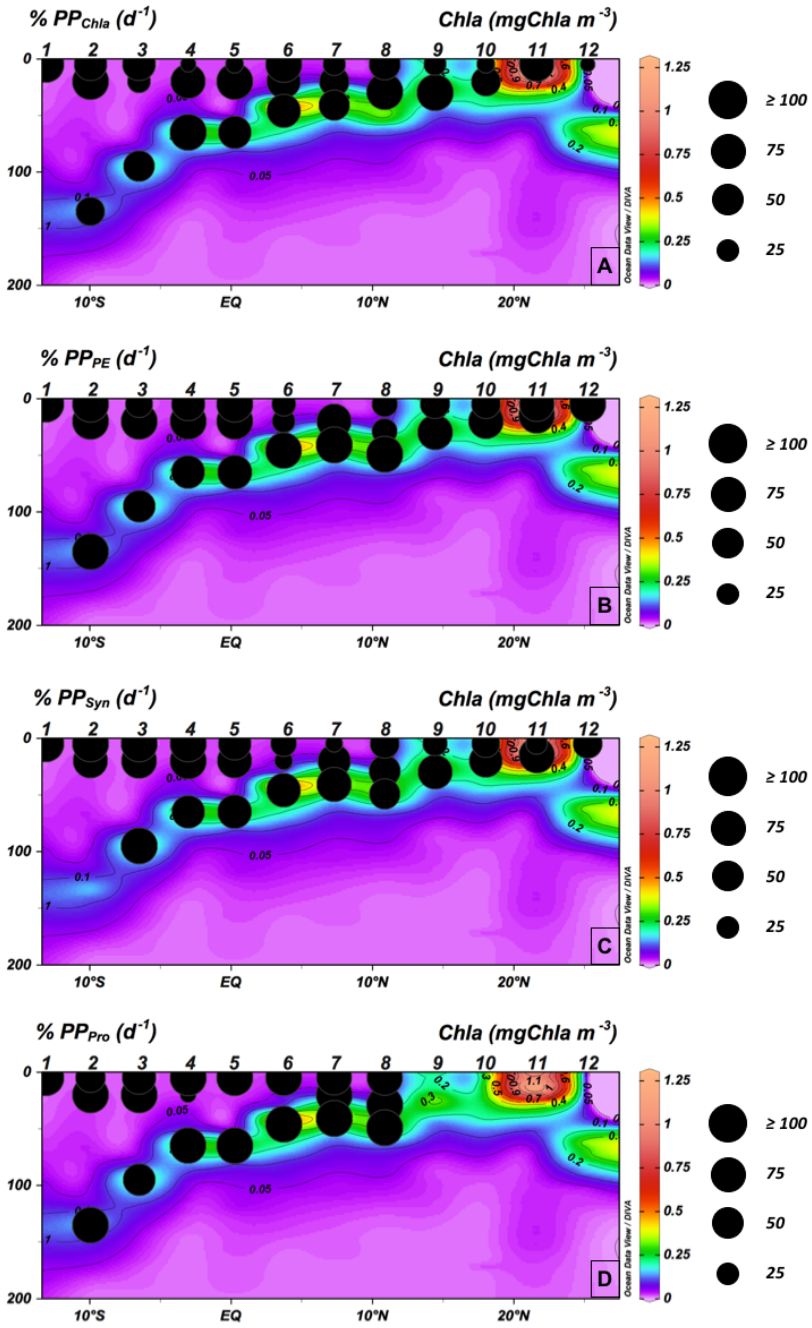


Figure 4.10 Vertical section (0-200 m) of Chlorophyll a (mg Chla m^{-3}) and microzooplankton grazing rates on potential phytoplankton production (% PP) for **a** Chlorophyll a (% PP$_{Chla}$), **b** picoeukaryotes (% PP$_{PE}$), **c** *Synechococcus* (% PP$_{Syn}$) and **d** *Prochlorococcus* (% PP$_{Pro}$).

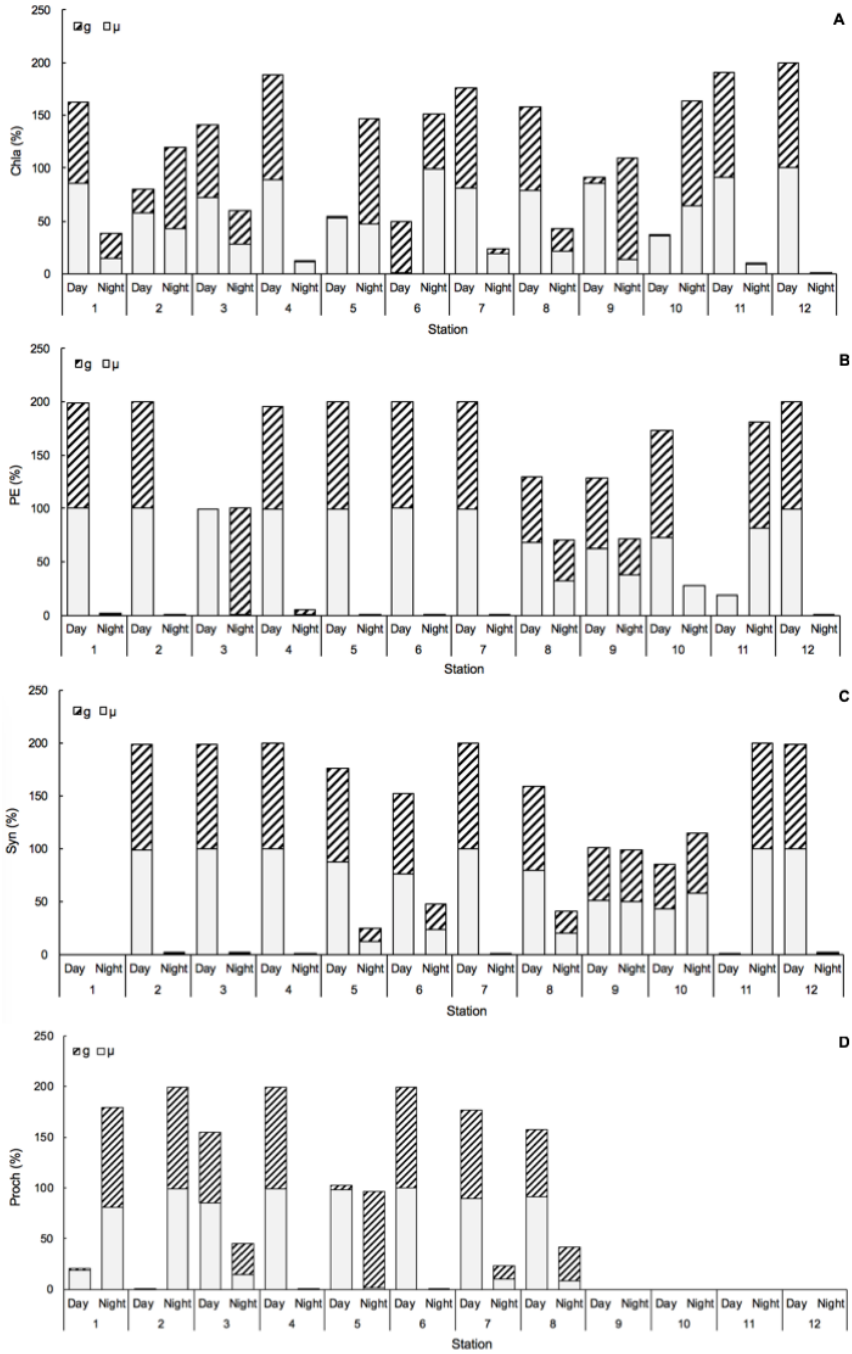


Figure 4.11 Mean (±SE) of phytoplankton potential growth (μ) and microzooplankton grazing (g) rates (h-1) during day and night for **a** Chlorophyll a, **b** picoeukaryotes, **c** *Synechococcus* and **d** *Prochlorococcus* during daylight and night hours at each station.

Table 4.4 Phytoplankton growth (μ) and microzooplankton grazing (g) rates (d^{-1}) for total chlorophyll *a* (Chla), picoeukaryotes (PE), *Synechococcus* (Syn) and *Prochlorococcus* (Proch) from superficial waters dilution experiments (5 m) during daylight and night hours. Negative growth and grazing rates were converted to 0.001 and 0 respectively. Note *Proch* were not present from station 9 to 12. Values (mean ± SE), n.s (non-significant p-value).

Station	Time	Growth (h^{-1})				Grazing (h^{-1})			
		μ_{Chla}	μ_{PE}	μ_{Syn}	μ_{Proch}	g_{Chla}	g_{PE}	g_{Syn}	g_{Proch}
1	Day	n.s	0.037 ± 0.004		0.002 ± 0.002	n.s	0.23 ± 0.001		0.000 ± 0.001
	Night	n.s	0.001		0.010 ± 0.011	n.s	0.001		0.015 ± 0.002
2	Day	0.006 ± 0.005	0.049 ± 0.021	0.010 ± 0.001	0.001	0.002 ± 0.002	0.074 ± 0.007	0.033 ± 0.004	0.000 ± 0.014
	Night	0.004 ± 0.001	0.001	0.001	0.024 ± 0.011	0.006 ± 0.002	0.001	0.001	0.023 ± 0.006
3	Day	0.029 ± 0.000	0.028 ± 0.005	0.017 ± 0.001	0.059 ± 0.003	0.015 ± 0.001	0.001	0.006 ± 0.002	0.037 ± 0.007
	Night	0.011 ± 0.000	0.001	0.001	0.010 ± 0.002	0.007 ± 0.000	0.005 ± 0.004	0.005 ± 0.001	0.016 ± 0.002
4	Day	0.030 ± 0.003	0.052 ± 0.014	0.031 ± 0.001	0.022 ± 0.006	0.013 ± 0.018	0.043 ± 0.004	0.019 ± 0.003	0.016 ± 0.003
	Night	0.004 ± 0.001	0.001	0.001	0.001	0	0.002 ± 0.001	0.001 ± 0.001	0.001
5	Day	0.010 ± 0.003	0.029 ± 0.007	0.025 ± 0.009	0.005 ± 0.001	0	0.026 ± 0.001	0.008 ± 0.004	0.000 ± 0.002
	Night	0.009 ± 0.005	0.001	0.003 ± 0.001	0.001	0.014 ± 0.004	0.001	0.009 ± 0.001	0.004 ± 0.002
6	Day	0.001	0.056 ± 0.001	0.032 ± 0.007	0.038 ± 0.004	0.003 ± 0.003	0.087 ± 0.001	0.017 ± 0.001	0.030 ± 0.003
	Night	0.010 ± 0.000	0.001	0.010 ± 0.000	0.001	0.004 ± 0.002	0.001	0.005 ± 0.004	0.001
7	Day	0.027 ± 0.001	0.017 ± 0.008	0.076 ± 0.003	0.026 ± 0.005	0.013 ± 0.002	0.013 ± 0.001	0.014 ± 0.003	0.024 ± 0.005
	Night	0.007 ± 0.002	0.001	0.001	0.003 ± ± 0.001	0.001 ± 0.002	0.001	0.000 ± 0.004	0.003 ± 0.003

Station	Time	Growth (h^{-1})				Grazing (h^{-1})			
		μ_{Chla}	μ_{PE}	μ_{Syn}	μ_{Proch}	g_{Chla}	g_{PE}	g_{Syn}	g_{Proch}
8	Day	0.028 ± 0.004	0.019 ± 0.007	0.053 ± 0.008	0.021 ± 0.007	0.021 ± 0.003	0.008 ± 0.002	0.044 ± 0.002	0.012 ± 0.000
	Night	0.008 ± 0.001	0.009 ± 0.004	0.014 ± 0.002	0.002 ± 0.003	0.005 ± 0.001	0.005 ± 0.001	0.004 ± 0.002	0.006 ± 0.000
9	Day	0.026 ± 0.005	0.034 ± 0.002	0.034 ± 0.007		0	0.025 ± 0.001	0.030 ± 0.001	
	Night	0.004 ± 0.000	0.021 ± 0.002	0.034 ± 0.003		0.002 ± 0.000	0.013 ± 0.001	0.008 ± 0.001	
10	Day	0.005 ± 0.001	0.028 ± 0.004	0.016 ± 0.005		0	0.046 ± 0.001	0.027 ± 0.003	
	Night	0.009 ± 0.001	0.011 ± 0.002	0.021 ± 0.001		0.015 ± 0.002	0.001	0.003 ± 0.002	
11	Day	0.021 ± 0.001	0.003 ± 0.002	0.001		0.019 ± 0.001	0.001	0.001	
	Night	0.002 ± 0.000	0.012 ± 0.003	0.026 ± 0.001		0	0.014 ± 0.001	0.015 ± 0.001	
12	Day	0.047 ± 0.004	0.021 ± 0.008	0.017 ± 0.003		0.018 ± 0.002	0.013 ± 0.003	0.019 ± 0.001	
	Night	0.0001	0.001	0.001		0	0.001	0.001	

4.4 Discussion

The main finding of this study was the close relationship between the distribution and trophic relationships of the planktonic organisms with the physical variables. Strongly stratified areas showed higher PP consumption by μZ, in contrast to the more productive areas, which displayed a lower PP control by these organisms. Another striking result was the daylight grazing pattern of μZ. In poor areas, characterized by a clear dominance of Din, the highest grazing rates were observed during daylight hours. By contrast, in productive areas dominated by Cil and higher MZ biomass, no clear daylight grazing pattern was observed.

During the study, we crossed different oceanic areas with distinctive trophic characteristics. The SECC generates a convergence zone (Reid 1959) with a deep thermocline, while the Equatorial Divergence within SECC promoted a shallow thermocline. Both areas were characterized by highly stratified water due to high temperature and salinity, and low availability of nutrients associated with low Chla (Marañón et al. 1999; Planas et al. 1999), showing the typical structure of the oligotrophic subtropical gyre. The SEC provides oxygen to ITCZ increasing the dissolved oxygen concentration (Stramma et al. 2005; Stramma et al. 2005). This convergence zone is a consequence of the equatorward dynamics as a result of the soft wind and non-significant Ekman transport (Peterson & Stramma 1991; Colling 2001). The Trade Winds push the NECC westwards but landmasses block the flow to the west basin developping a mid-ocean equatorial upwelling (Cromwell 1953; Stommel. To the East, the deflection of the NEC generates the Guinea Dome, a structure with a cyclonic circulation and weak horizontal mixing between the dome and surrounding water (Outdot 1989). The CM was found below the thermocline and above the nutricline, being shallower northward near the upwelling, as expected. The effect of the nutrient through the diffusive flow of the thermocline, and the growth limitations due to the lack of light at depth determined the depth and size of the CM (Longhurst, A. R. & Harrison 1989).

Low nutrient concentration and high temperature resulted in plankton communities dominated by *Proch* (Morán et al. 2010; Karl et al. 2001) coincident with previous studies (Zubkov et al. 1998). *Proch* dominated in the oligotrophic and warmest waters, whereas *Syn* and PE showed higher biomass in colder and nutrient richer waters (Fig. 4.6). Differences in their cell structure and physiology may explain this zonation. *Proch* and *Syn* have different sizes

and light-harvesting antenna systems, and the first one is unable to use nitrates, whereas *Syn* uses them as a main source of N (for a review, see DuRand et al. 2001; Moore et al. 2001; Moore et al. 2008; Partensky et al. 1999). Moreover, *Proch* uptakes phosphate in nutrient-depleted zones as a result of phosphate specific acquisition genes, which gives them an advantage in oligotrophic areas (Partensky et al. 1999; Martiny et al. 2006; Zubkov et al. 2007). These features promoted their dominance at surface and ML in the South Atlantic gyre and Equatorial Divergence. Higher PE biomass occurred in areas with relatively high concentration of nutrients as at the CM and upwelling regions, in accordance to observations by Tarran et al. (2006). As expected, Dia contributed largely in the upwelling station although they did not dominate the community, as also observed by Marañón et al. (2000).

Prokaryotes are more efficient than protists assimilating nutrients at low concentrations due to their higher cell surface-to-volume ratio (Chisholm 1992), thus dominate the uptake of nitrates and phosphates in oligotrophic areas (Hartmann et al. 2011). However, the biomass and distribution of phytoplankton do not solely depend on nutrient availability or temperature, and grazing is also an important factor shaping the autotrophic communities (Burkepile & Hay 2006; Pascal & Fleeger 2013). Our results are similar to those found by Calbet and Landry (2004), showing that at surface and ML of the oligotrophic ocean μZ consumed approximately 78% of primary production, whereas in upwelling areas this consumption was slightly lower (~66%, Fig. 4.10). High grazing rates upon PE at productive stations could be the consequence of their higher abundance, or the preference on them by Cil, which dominated the μZ community in those areas (Jonsson 1986; Irigoien et al. 2005; Aberle et al. 2007). *Syn* consumption was similar throughout the basin, indicating that Din, which dominated μZ community in oligotrophic regions (Fig. 4.7c), and Cil consume them indistinctly (e.g. refs Fenchel 1987; Sherr & Sherr 1988; Sanders & Wickham 1993; Christaki et al. 1999). Moreover, the specific grazing on Chla and upon each autotrophic group was lower in the CM than in the surface and ML (Fig. 4.9). As hypothesized by Landry et al.(2009), low grazing rates in areas with high availability of prey, as the CM, could be a consequence of the low concentration of μZ.

In the Northwest African upwelling, each μM of N supported much less MZ than in the oligotrophic Atlantic. This result is not surprising because oligotrophic food webs are known to recycle nutrients more efficiently, allowing for a proportional higher biomass of μZ than very productive ones

(mallin 1994; Vargas et al. 2007; Ward & Follows 2016). The increase in μZ biomass at the warmer and oligotrophic stations did not suppose an increase in the biomass transfer upwards to MZ, since in oligotrophic environments the carbon of μZ that supported MZ is smaller than in the upwelling areas. These results evidence the bottom-up control of μZ in oligotrophic areas, and suggest a closer link between MZ and μZ in upwelling regions. At warm oligotrophic regions where prey have lower size and are less numerous, Din dominated the microplankton community (Fig. 4.7c), and copepods could show a low preference to predate upon them (Huntley et al. 1986; Löder et al. 2011). The prey selection (Saiz & Calbet 2007; Saiz & Calbet 2011; Boersma et al. 2015) and the preference of copepods for Cil (e.g. Armengol et al. 2017) could explain the increase of MZ and the decrease of μZ, mostly dominated by Cil, in the upwelling areas. Phototrophic organisms grew faster at higher nutrient availability developing defensive mechanisms to avoid grazing, as a result, the %PP consumed by μZ was lower in the upwelling region. Moreover, the increase of MZ biomass in upwelling environments could exert a control upon the μZ community. More predation upon μZ release PE and *Syn* increasing their biomass (e.g. Armengol et al. 2017). A fingerprint of this cascade effect was the positive correlation between MZ biomass with picoautotroph cells. μZ, which dominated in the oligotrophic areas, show a low efficiency to consume *Proch* (Fig. 4.9d, 4.10d) (Stelfox-Widdicombe et al. 2000; Karayanni et al. 2005). However, μZ grazing rates on *Proch* increased in the stations of ITZC and mid-ocean upwelling, coinciding with the PE increase (Fig. 4.7b), which has been documented to be efficient mixotrophs (Zubkov et al. 2007; Unrein et al. 2007; Hartmann et al. 2013). Therefore, high grazing rates on *Proch* in those areas may be due to a cascade effect where MZ (which increased their biomass) consume μZ (decreasing their biomass) (Fig. 4.7a), releasing PE from grazing pressure and increasing their biomass, which in turn increases *Proch* consumption ($\tau = -0.27$, $p < 0.05$; Kendall Rank correlation test between biomass of *Proch* and PE).

The CM in oligotrophic areas is formed as a result of photoaclimation of the cells and/or an increase in phytoplankton growth due to the nutrients diffusion through the thermocline. Previous studies found lower growth rates in this environment than in the mixed layer, suggesting a low turnover of the phytoplankton community (Marañón et al. 2000; Cullen 1982; Goericke 1990). In this study, we found higher growth rates based on Chla than others studies in the same area (e.g. Marañón 2000). These discrepancies on

phytoplankton growth in oligotrophic areas may be due to natural variability of physical process and biological rates (Laws et al. 1987; Letelier et al. 1996), or in our case, may be result of an overestimation of phytoplankton production due to our assessment of the potential growth of autotrophic organisms. Worden and Binder (2003) found non-significant differences between growth rates with and without nutrient addition treatments in oligotrophic areas, pointing that growth rates respond to nutrient enrichment at time scales greater than 24 hours, or a lack of nutrient limitation due to fast recycling. If this were fulfilled in our study, we should consider the estimated potential growth rates at oligotrophic stations similar to the real rates. Conversely, growth rates based on Chla (Fig. 4.8a) at surface layers in the productive stations (the mid-ocean upwelling, Guinea Dome and Northwest African upwelling) were similar to those obtained by Marañón et al. (2000) where, as in this study, the nitracline showed a similar depth.

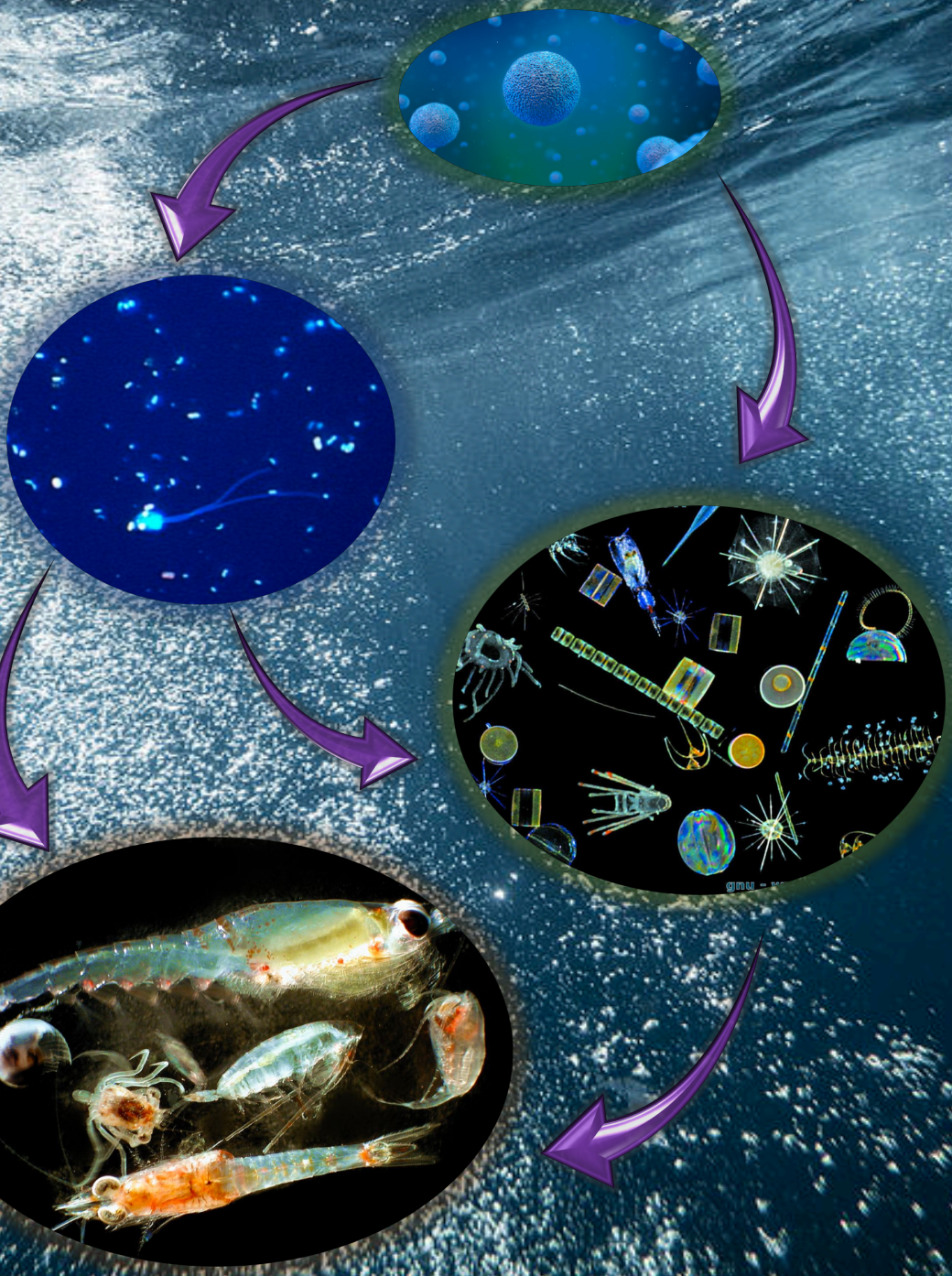
Previous studies showed daily variations of phytoplankton in oligotrophic areas more important than seasonal or annual scales (Siegel et al. 1989; Gardner et al. 1995; Claustre et al. 2002). Certainly, cloud cover, sinking, advection, and turbulence transporting cells between darkness and full sunlight (MacIntyre et al. 2000) modify the intensity of light experienced by cells in the ocean and may have important consequences on phytoplankton growth. On a general basis, light controls cell cycles in many phytoplankters either directly or by adjusting the biological clock (Edmunds & Adams 1981; Sweeney & Borgese 1989). For picoplankton, cell division begins near dusk, starting the process *Syn*, followed by *Proch,* and finally PE (Jacquet et al. 2001; Ribalet et al. 2015). Conversely, cell-biomass increase occurs during daylight hours (Ribalet et al. 2015; Diamond et al. 2015; Dimier et al. 2009), as we observed in oligotrophic stations (Fig. 4.11).

Diel cycles of growth have also been identified for μZ species, such as *Gymnodinium* sp. (Skovgaard 1998) or *Coxiella* sp. (Strom 2001), showing higher growth rates during day-light with a few exceptions (Jakobsen & Strom 2004). Likewise, specific protozoan grazing activity seem to occur mostly during the day (Strom 2001; Jakobsen & Strom 2004; Arias et al. 2017; Ng et al. 2017). The reasons for this rhythm could be endogenous circadian cycles, light-aided digestion, and diel variations on phytoplankton stoichiometry (Strom 2001; Jakobsen & Strom 2004; Arias et al. 2017; Ng et al. 2017). Recently, Arias et al. (2017) suggested that the diel rhythms in μZ were inverse to those of their consumers in order to avoid being more conspicuous during

grazing and, therefore, being more prone to predation (i.e., copepods Calbet & Landry 1999; Burkill et al. 1993; Calbet & Saiz 2005). Arias et al. (2017) also found that diel rhythms of feeding were modulated by the hunger and satiation; only satiated protozoans showed full amplitude diel feeding rhythms. A similar response to food availability was observed in copepods as well (Calbet & Landry 1999). However, opposite to expected, diel feeding rhythms in upwelling areas were fuzzy compared with areas with low availability of food. We propose two alternative hypotheses to explain this. On the one side, species adapted to low food environments may found their satiation thresholds at lower concentrations than those adapted to richer environments. On the other side, given the specificity of the diel feeding response (Jakobsen & Strom 2004; Arias et al. 2017), it is possible to explain the variations in diel feeding behaviour by changes in the composition of the μZ community. Backing up this hypothesis, we found oligotrophic areas being dominated by Din (usually showing more evident diel grazing rhythms than Cil), whereas the μZ of more productive waters, mostly dominated by Cil, seems to be highly species-specific on their diel behaviours (Jakobsen & Strom 2004).

In summary, across the tropical and subtropical Atlantic Ocean, we found a close relationship between physico-chemical variables and the distribution of planktonic organisms. These changes in distribution and species composition drive at their turn the trophic relationships within plankton, consolidating the paradigms of a more complex and efficient in nutrient recycling microbial food web in the oligotrophic ocean compared with a "classic" and shorter one in the more productive areas.

Chapter 5



Cal no abandonar mai ni la tasca
ni l'esperança.
Pompeu Fabra

5 Conclusions and Future Research

CHAPTER

5.1 Conclusions

Attending to the results reported throughout this thesis, the main conclusions arises from all studies are:

1. The consumption of epipelagic mesozooplankton modifies the structure of the planktonic community in the photic zone. Variability in the abundance of copepods promote important changes in lower trophic levels and restructure the community:
    a. The increase in the concentration of copepods promotes a decrease in the abundance of ciliates, releasing autotrophs from grazing pressure and increasing their concentration. This top-down effect may partially explain the parallel increase in mesozooplankton, chlorophyll, and primary production observed in the subtropical ocean.

b. Copepods predate upon ciliates but the inherent cascade effect within the microzooplankton community make it difficult to discern the effect of copepods upon dinoflagellates.

2. The typical decrease in nutrients during the spring bloom in temperate zones entails changes in the planktonic community. The nutrient depletion and the decrease of preys promotes a succession in the ciliate community, changing from mixotrophic at the beginning of the bloom to heterotrophic at the end. The dominance of mixotrophic ciliates results in lower grazing rates compared to the later heterotrophic ciliates increase, and in turn, promotes a match-mismatch between consumers ad their potential preys.

3. Organisms distribution and abundance are a function of the physico-chemical variables. Changes in distribution and composition of the planktonic community have a strong impact on trophic relationships, consolidating the paradigms by which microbial loop predominate in oligotrophic waters, recycling nutrients efficiently, while the "classic" trophic web dominates in productive waters.
    a. As a consequence of the physiological differences, *Prochlorococcus* and dinoflagellates dominate in oligotrophic areas; while *Synechococcus*, autotrophic picoeukaryotes, diatoms and ciliates dominate the most productive regions.
    b. The consumption of primary production by microzooplankton at the surface and mixed layers is larger in oligotrophic waters than in productive waters. Likewise, grazing rates in the deep chlorophyll maximum are lower compared to those at the surface and in the mixed layer, probably as a result of the lower concentration of microzooplankton in these layers.
    c. The increase of microzooplankton in oligotrophic zones, the lack of a mesozooplankton increase there, and the poor transfer of biomass towards the higher trophic levels are hints of the bottom-up control from microzooplankton to mesozooplankton. Otherwise, mesozooplankton could

promote a top-down control in productive areas. The cascade effect could release autotrophs from microzooplankton grazing pressure.

d. Microzooplankton grazing occur during the day in the oligotrophic ocean, while they become more diffuse in the productive zones.

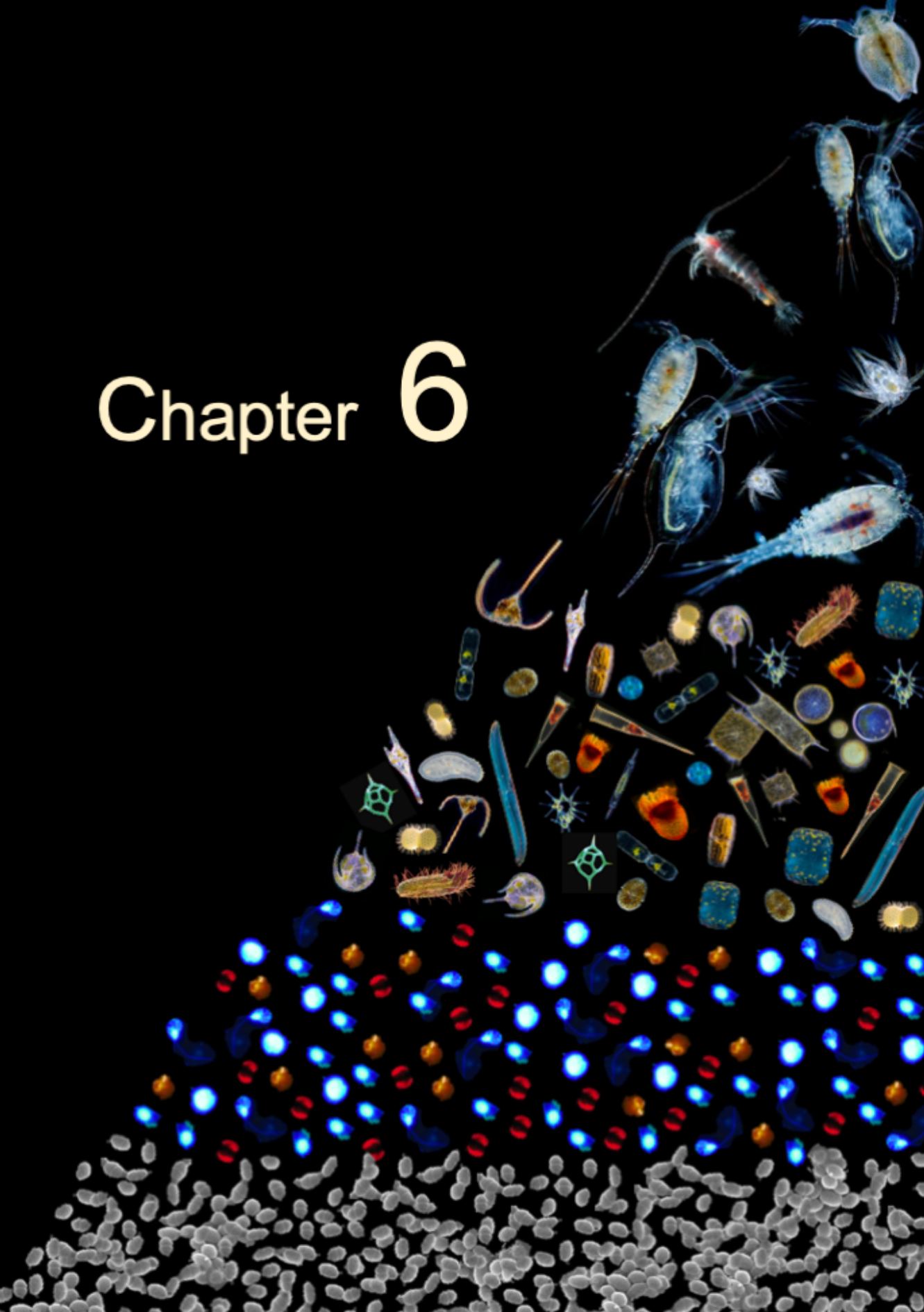
5.2 Future Research

This thesis studied the role of microzooplankton within the trophic community, gathering information about how the microzooplankton community develops in different physical, chemical and biological scenarios. Results reported throughout this study highlighted the need to address new research objectives to be solved in the future:

1. Mixotrophy is common in low trophic levels enhancing the need to carry out parallel grazing experiments to study the grazing of heterotrophic and mixotrophic microzooplankton, discerning between top-down effects from the direct consequence of consumption. In this sense, genetic studies could help to discern the type of prey consumed by a specific fraction of consumers, and show their preferences.

2. In order to follow our study at ocean basin scales in the tropical and subtropical ocean, a similar research is now underway to know the large-scale variability of microzooplankton and grazing rates from subtropical to temperate latitudes. These results, jointly with our previous research in polar areas, will provide a complete picture of the role of this community in the oceans.

Chapter 6



La ecología demanda que miremos a la naturaleza una y otra vez con ojos de niño, y no hay nada más opuesto a los ojos de un niño que un pedante.

Ramón Margalef

6 Resumen

CHAPTER

6.1 Objetivos y esquema de la tesis

En esta tesis, se ha estudiado la comunidad de microzooplancton desde distintas perspectivas como son las relaciones tróficas y, el flujo de carbono y energía entre el microzooplancton y los niveles tróficos superiores/inferiores. Los objetivos específicos son:

1. El efecto de las variaciones en la abundancia del mesozooplankton sobre comunidades planctónicas naturales (si se desencadena un control buttom-up o top-down) y determinar las implicaciones en la transferencia y energía hacia los niveles tróficos inferiores. Este objetivo se desarrolla en el *Capítulo 2*.
2. La dinámica de la comunidad de ciliados durante el bloom primaveral en aguas templadas, y los cambios en la abundancia y funcionalidad

de los organismos a la misma escala que cambian los nutrientes, luz y temperatura. Este objetivo se aborda en el *Capítulo 3*.

3. Entender las relaciones y comunidades tróficas que se establecen en el plancton desde el pico- hasta el mesozooplancton, el impacto de las variables físicas sobre los organismos y las diferencias en los roles tróficos entre las zonas productivas y oligotróficas del océano. Este objetivo se trata en el *Capítulo 4*.

6.2 Justificación del estudio

El uso de los combustibles fósiles es una de las principales causas de emisión de CO_2 hacia la atmosfera que, a su vez junto con otros gases de efecto invernadero, son responsables del aumento de la temperatura global del planeta (NOAA, 2015). Alrededor del 50% de las emisiones permanecen en la atmósfera, y el otro 50% es secuestrado por el océano y la vegetación terrestre. Se estima que el océano absorbe cerca del 70% del CO_2 antropogénico, convirtiéndose en el principal sumidero de este gas (Siegenthaler and Sarmiento 1993). El secuestro de CO_2 por parte del océano se lleva a cabo por mecanismos físicos (bomba física o de solubilidad) y biológicos (bomba biológica). La bomba biológica es el transporte de materia orgánica en el océano a través de diferentes procesos como mezcla física de materia orgánica particulada y disuelta hacia las capas profundas del océano, el flujo activo por parte del zooplancton y micronecton, y el flujo pasivo o gravitacional de materia orgánica (Volk & Hoffert 1985; Buesseler et al. 2007). Primeramente, el fitoplancton captura el CO_2 para llevar a cabo la fotosíntesis y lo convierte en carbono orgánico particulado (POC, particulated organic carbon). Entre el 5 y el 25% de la producción primaria se transporta desde la capa eufótica a capas más profundas, y solo el 3% de la producción primaria llega a las profundidades batipelágicas (De La Rocha y Passow, 2007). El resto de la producción primaria es remineralizada en las capas superficiales del océano por los organismos. Para comprender el funcionamiento de la bomba biológica, el flujo de carbono y sus implicaciones, es importante entender el funcionamiento de los ecosistemas en el océano, especialmente el microzooplancton ya que son los organismos clave entre los niveles tróficos superiores e inferiores.

6.3 Antecedentes

El plancton es la base de la cadena trófica marina y el estudio de su composición y de los procesos que tienen lugar en estas comunidades es de suma importancia para conocimiento de los ecosistemas (Fuhrman, 2009). El término plancton fue empleado para indicar todas las partículas orgánicas naturales que flotan libre e involuntariamente en aguas abiertas (Hensen 1887). En el medio marino, hay una amplia variedad de organismos que pertenecen a distintos grupos y pueden ser clasificados según sus características estructurales, funcionales o dimensionales. Tradicionalmente el plancton marino se ha dividido según las características tróficas en fitoplancton (organismos autótrofos) y zooplancton (organismos heterótrofos), y recientemente se han incluido a los organismos mixotróficos. Los organismos mixotróficos son ubicuos y combinan la nutrición autótrofa y heterótrofa. El tamaño de estos organismos varía desde el pico- al mesoplancton, e incluye procariotas, eucariotas unicelulares, protistas y organismos zooplanctónicos (Stoecker 1998, Sherr & Sherr 2002). Sin embargo, la forma más utilizada para clasificar los organismos planctónicos es según su tamaño (Sieburth 1979): femtoplancton (< 0.2μm), picoplankton (0.2-2 μm), nanoplankton (2-20 μm), microplankton (20-200 μm) y mesozooplankton (> 200 μm) (Fig. 6.1). Los protistas, pequeños metazoos y meroplancton pertenecen al grupo de microzooplancton, siendo los ciliados y dinoflagelados los organismos mixotróficos más comunes.

Desde la perspectiva de las relaciones tróficas, la categorización según el "tipo funcional", clasifica los organismos según sus funciones ecológicas (Gitay and Noble 1997) y fisiológicas (Mitra et al. 2016): fagoheterótrofos (carecen de capacidad fototrófica); fotoautótrofos (carecen de capacidad fagotrófica); mixotrofos constitutivos (fagotrófos con capacidad inherente para la fototrofía); mixotrofos no-constitutivos (capacidad fagotrófica adquirida ingiriendo presas específicas); y, mixotrofos no-constitutivos generales (capacidad fototrófica adquirida ingiriendo presas generales no específicas) (Fig. 6.2).

Un cuarto de la producción primaria global ocurre en el océano (Field et al. 1998; Falkowski et al. 1998) por lo que es importante entender como utilizan los organismos ese carbono y cuánto es transferido a los peces, respirado y devuelto a la atmosfera, hundido a través de los organismos a las zonas meso- y batipelágicas, o secuestrado en el suelo oceánico (Fig. 6.3).

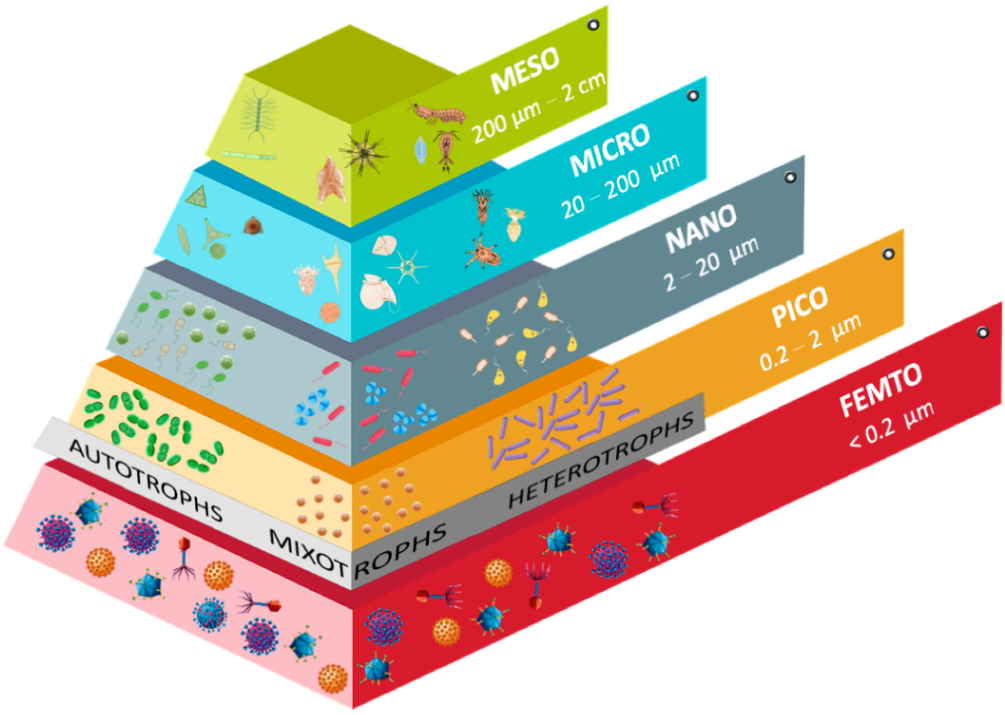


Figura 6.1 División del plancton según su tamaño (femto-, pico-, nano-, micro- and mesoplankton) y comportamiento nutricional (autótrofos, micotróficos y heterotróficos).

El creciente interés por el estudio del ciclo del carbono se debe principalmente al calentamiento global, la eutrofización costera y la sobrepesca (Jackson et al. 2001; Pauly et al. 2003). En el océano existen dos aproximaciones para entender el funcionamiento de las redes tróficas marinas: la cadena trófica "clásica" y el bucle microbiano. La red trófica clásica (Mills 1989), estudiada desde una perspectiva pesquera, establece que los metazoos planctónicos (compuestos en su mayoría por copépodos) consume el fitoplancton (principalmente diatomeas y dinoflagelados), y por último, los peces consumen los metazoos (Fenchel 1988; Mills 1989). Sin embargo, esta perspectiva ignora los microorganismos como las bacterias, que dominan en abundancia, diversidad y actividad metabólica en el océano (Steel 1974; DeLong & Karl 2005). El bucle microbiano, que tiene en cuenta a las bacterias, sostiene que los grandes protistas consumen a los pequeños autótrofos y heterótrofos, a la vez que alimentan a las bacterias con sus propias excreciones. En esta red trófica, el microzooplancton actúa de intermediario entre los productores primarios y los consumidores (Pomeroy 1974; Azam et al. 1983;

Sherr & Sherr 1988). En este marco, los niveles tróficos superiores consumen una pequeña parte de la materia orgánica producida por los autótrofos, siendo la mayor parte recirculada por los distintos niveles tróficos (Azam et al. 1983; Sherr et al. 1986).

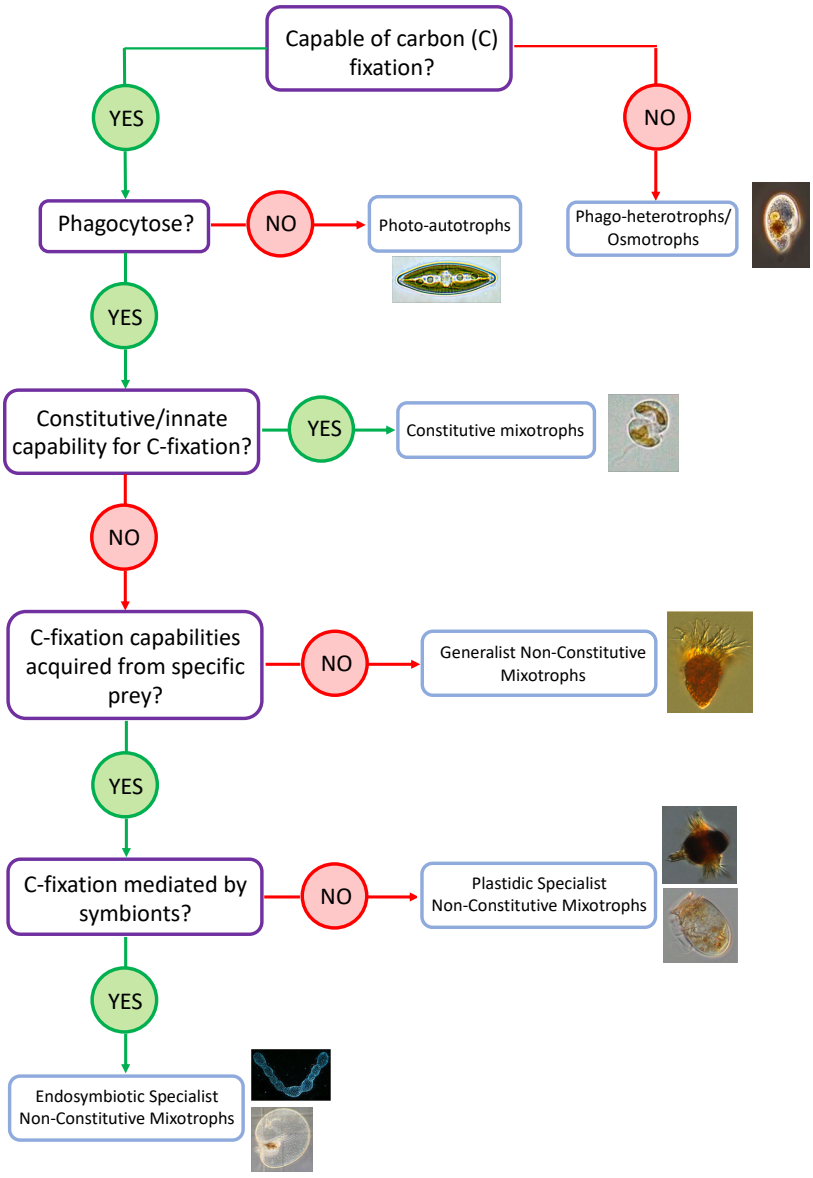


Figure 6.2 Clasificación funcional de los protistas, modificación del esquema original de Mitra et al. (2016).

Por tanto, la importancia del microzooplancton se basa en: (1) ser los principales consumidores de la producción primaria, (2) su rol de intermediario entre los productores primarios y el mesozooplancton, y (3) como organismos excretores (Gifford 1991; Calbet & Landry 2004; Calbet 2008). El microzooplancton es el principal consumidor de bacterias, pequeños autótrofos, flagelados e incluso otros protistas (ej. e.g. Campbell 1926, 1927; Sherr et al. 1986; Strom 1991; Hansen 1992; Sherr & Sherr 2003), y es responsable de una importante parte de la materia orgánica disuelta (Azam et al. 1983). A su vez, los copépodos son los principales consumidores de microzooplancton debido a su talla y composición nutricional (Berggreen et al. 1988; Stoecker & Capuzzo 1990; Wickham 1995; Broglio et al. 2003), siendo los organismos del microzooplancton intermediarios entre el mesozooplancton y el fitoplancton. Se ha observado en algunos estudios que el control del mesozooplancton sobre el microzooplancton, libera a los productores primarios de la presión de pastaje (Calbet & Landry 2004; Stibor et al. 2004a, b; Vadstein et al. 2004; Sherr & Sherr 2007). Como consecuencia del proceso de alimentación, el microzooplancton libera nutrientes, como amonio y fosfatos, y materia orgánica disuelta (p.e., Dolan 1997), fertilizando el medio y promoviendo el crecimiento de sus potenciales presas (Dolan 1997). Como resultado del rol del microzooplancton en la cadena trófica, este grupo de organismos es la piedra angular del bucle microbiano (Azam et al. 1983; Sherr & Sherr 2002).

Las zonas oligotróficas son distintas a las zonas productivas tanto en factores físicos como la temperatura, y biológicos como la composición y abundancia de las comunidades planctónicas (Schmoker et al. 2016; Christaki et al. 2014; Billen et al. 1990). Las zonas cálidas y estratificadas de los giros subtropicales son áreas oligotróficas que cubren aproximadamente el 40% de la superficie del planeta, y se expanden a razón de 0.8-4.3 % año^{-1} como consecuencia del calentamiento global (Polovina et al. 2008). En estas zonas dominan las células pequeñas y el microzooplancton es más efectivo que el mesozooplancton en el consumo de fitoplancton debido a su tamaño similar al fitoplancton, las altas tasas de crecimiento y su alto metabolismo (Fenchel 1987; Sherr & Sherr 1994; Boëchat et al. 2007; Jones 2000), consumiendo más del 70% de la producción primaria (Calbet & Landry 2004).

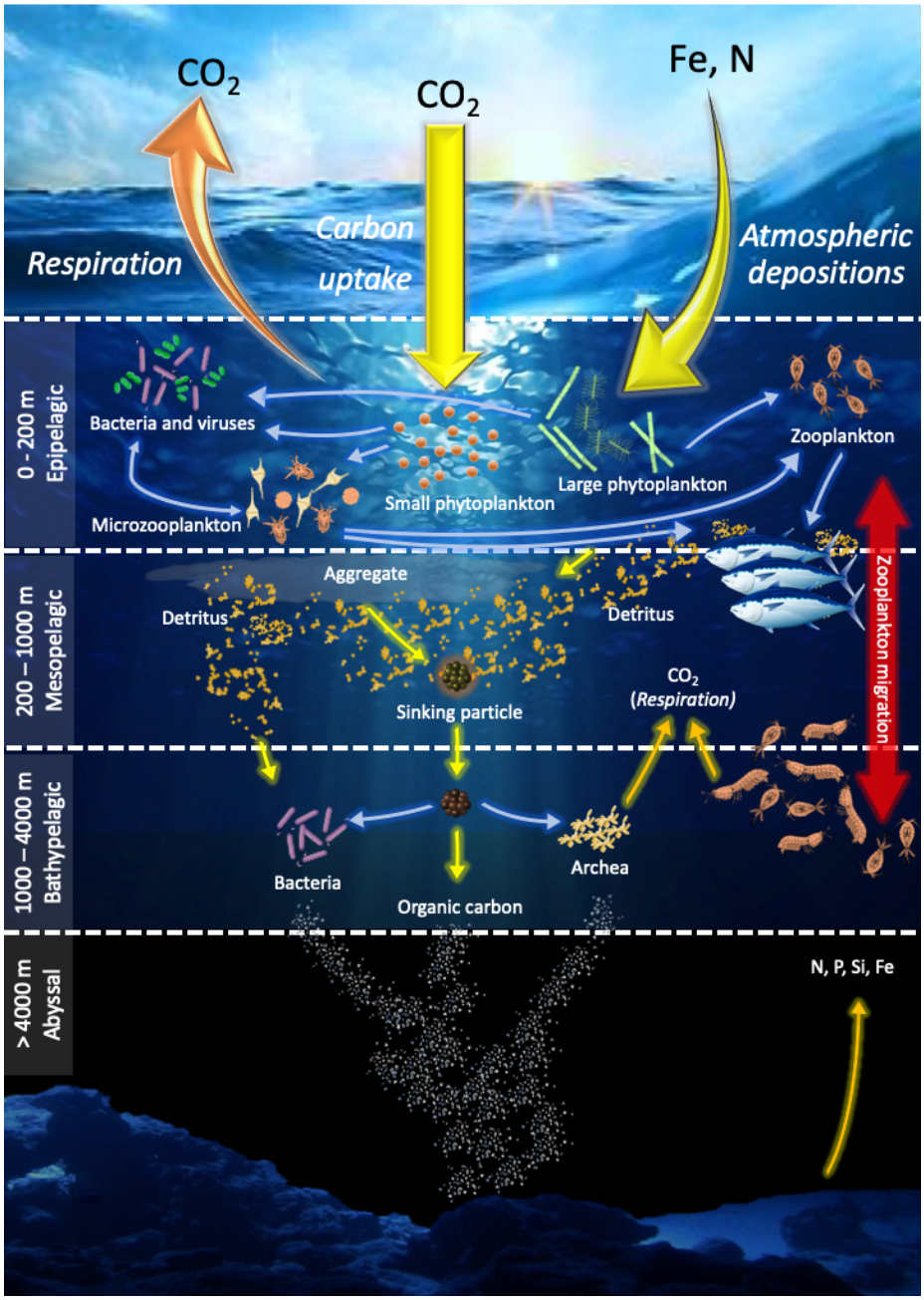


Figure 6.3 Esquema de la bomba biológica en el océano: hundimiento de carbono y nutrientes (flechas amarillas), liberación de nutrientes y carbono (flechas naranjas), interacciones tróficas (flechas azules). Modificación de Oak Ridge National Laboratory.

Por el contrario, en las zonas más productivas dominan las células grandes como las diatomeas. A pesar de que la composición y abundancia de los organismos planctónicos es distinta a las zonas oligotróficas, el microzooplancton consume casi el 60% de la producción primaria en las aguas productivas (Calbet & Landry 2004; Schmoker et al. 2016), mientras que el mesozooplancton consume aproximadamente el 10% de la producción primaria diaria (Calbet 2001).

En las zonas de afloramiento, caracterizadas por una rápida variabilidad de las condiciones ambientales, el microzooplancton se adapta a los cambios en las mismas escalas de tiempo que las presas pero no sucede lo mismo con los copépodos, ya que precisan de periodos de tiempo más largos para desarrollarse (Calbet 2008; Hernández-León 2008; Schmoker et al. 2016). Este hecho explica el bajo impacto por grazing de los grandes metazoos (principalmente copépodos) sobre el fitoplancton (Berggreen et al. 1988; Calbet 2008). Sin embargo, el mesozooplancton puede tener la capacidad de estructurar las comunidades pelágicas (ej. Gifford 1991; Gowen et al. 1999) y actúan de intermediarios entre los niveles tróficos superiores e inferiores (Cushing 1989).

Por otro lado, el crecimiento del fitoplancton y las tasas de pastaje del microzooplancton son de suma importancia para estudiar el papel de estas comunidades. Sin embargo, su conocimiento es, en la actualidad, bastante limitado debido a la dificultad para medirlos. El método de las diluciones (Landry and Hassett 1982) es el más extendido para la estimación del pastaje del microzooplancton sobre el fitoplancton en el océano, y se base en tres premisas: (1) el crecimiento del fitoplancton no se ve afectado por la presencia o ausencia de otras células fitoplanctónicas; (2) el encuentro entre predadores y presas es proporcional a la probabilidad de las células de ser consumidas; (3) el crecimiento del fitoplancton a lo largo del tiempo es exponencial. Este método consiste en incubar botellas con distintas diluciones de agua de mar natural (WSW, whole seawater) donde se va incrementando la cantidad de agua de mar filtrada, y por lo tanto, disminuyendo la tasa de encuentro entre depredadores y presas. La pendiente del crecimiento aparente (k) a las distintas diluciones es la tasa de mortalidad (m) de los autótrofos debido al pastaje, y el crecimiento neto del fitoplancton (μ) es la intercepción con el eje y. Esta metodología es difícil de llevar a cabo debido a los distintos niveles de dilución (4-5) y el gran volumen de agua necesario que imposibilitan obtener datos de alta resolución en los estudios oceanográficos. Asimismo, las respuestas no

lineales observadas en las tasas de crecimiento aparente, tasas de crecimiento aparente parecidas en los tratamientos altamente diluidos, y los efectos top-down son otros inconvenientes de esta metodología (Gallegos 1989; Calbet & Saiz 2013). Una simplificación es el denominado método de los dos puntos (2-point) donde se incuban únicamente los tratamientos sin diluir (100 % WSW) y una dilución del 33% (Landry et al. 2009), o del 37% (Landry et al. 2011), o del 10% (Lawrence & Menden-Deuer 2012; Sherr et al. 2013) o del 5 % (Strom & Frederickson 2008). El tratamiento del 100 % WSW contiene todos los organismos <200 μm, y representa la tasa de crecimiento neta del fitoplancton en presencia de herbívoros (k, d^{-1}). El tratamiento del 5 % WSW es suficientemente diluido para asumir que los encuentros entre depredadores y presas es nulo. La mortalidad por pastaje (d^{-1}) se define como:

$$g = \mu - k$$

En los tratamientos de 5 % WSW donde se asume que no hay mortalidad por pastaje, se puede definir el crecimiento intrínseco (μ, d^{-1}) como:

$$\mu = k$$

La respuesta a la fotoaclimatación del fitoplancton a las condiciones incubación, y las variaciones en los niveles de luz de un día a otro puede dar lugar a errores negativos o positivos en la estimación de las tasas de crecimiento. Para evitar estos errores, el crecimiento negativo se asume como 0.01 d^{-1}, mientras que las tasas de pastaje negativas se asumen como 0 d^{-1} (Calbet & Landry 2004).

6.4 Conclusiones

A partir de los resultados obtenidos en los trabajos de esta tesis, las principales conclusiones que se extraen son:

1. El consumo de microzooplancton por parte del mesozooplancton epipelágico modifica la estructura de la comunidad planctónica en la zona fótica. Las variaciones en la abundancia de copépodos

promueven importantes cambios en los niveles tróficos inferiores y reestructuran la comunidad:

a. El aumento en la concentración de copépodos promueve una disminución en la abundancia de ciliados, por lo que se libera a los autótrofos de la presión de pastaje y aumentan su concentración. Este efecto top-down puede explicar parcialmente el aumento paralelo de mesozooplancton, clorofila y producción primaria observado en el océano subtropical.
b. Los copépodos depredan sobre los ciliados pero el efecto en cascada inherente dentro de la comunidad de microzooplancton dificulta discernir los efectos de los copépodos sobre los dinoflagelados.

2. La disminución típica de nutrientes durante el bloom primaveral en zonas temperadas conlleva cambios en la comunidad planctónica. El agotamiento de nutrientes y la disminución de presas origina una sucesión en la comunidad de ciliados, cambiando de mixotrófica al inicio del bloom a heterotrófica al final del bloom. El dominio de ciliados mixotróficos origina tasas de pastaje más bajas comparadas con la de los ciliados heterótroficos, y a su vez, promueve un desacople entre los consumidores y sus potenciales presas.

3. La distribución y abundancia de organismos están en función de las variables físico-químicas. Los cambios en la distribución y composición de la comunidad planctónica tienen un fuerte impacto en las relaciones tróficas, consolidando el paradigmas por el cual en las aguas oligotróficas predomina el bucle microbiano, reciclando los nutrientes eficientemente, mientras que en aguas productivas domina la cadena trófica "clásica".
    a. Debido a las diferencias fisiológicas, en zonas oligotróficas domina *Prochlorococcus* y dinoflagelados, mientras que en las regiones más productivas dominan *Synechococcus*, picoeucariotas autótrofos, diatomeas y ciliados.
    b. El consumo de producción primaria por parte del microzooplancton en superficie y en la capa de mezcla es mayor en aguas oligotróficas que en aguas productivas.

Asimismo, las tasas de pastaje en el máximo profundo de clorofila son menores que en superficie y en la capa de mezcla, probablemente como resultado de la baja concentración de microzooplancton en estas capas.

c. El aumento de microzooplancton en las zonas oligotróficas, la ausencia de mesozooplancton y la baja transferencia de biomasa hacia los niveles tróficos superiores son indicios del control bottom-up del microzooplancton hacia el mesozooplancton. Por el contrario, el mesozooplancton podría promover un control top-down en los sistemas productivos. El efecto en cascada podría liberar a los autótrofos de la presión de pastaje del microzooplancton.
d. El pastaje del microzooplancton ocurre durante el día en el océano oligotrófico, mientras que estos patrones se vuelven más difusos en las zonas productivas.

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List of Acronyms

%PP	Primary production consumed by heterotrophic grazers
$\%PP_{Chla}$	Primary Production based on chlorophyll *a*
$\%PP_{PE}$	Primary Production based on autotrophic picoeukaryotes
$\%PP_{Proch}$	Primary Production based on *Prochl*orococcus
$\%PP_{Syn}$	Primary Production based on *Syn*echococcus
C	Carbon
Chla	Chlorophyll *a*
Cil	Naked ciliates
CM	Chlorophyll *a* maximum
Dia	Diatoms
DIN	Dissolved Inorganic Nitrogen
Din	Dinoflagellates
DIP	Dissolved Inorganic Phosphate
g	Mortality by grazing rate
GAM	Generalized Additive Modelling
g_{Chla}	Grazing based on chlorophyll a
g_{PE}	Grazing based on autotrophic picoeukaryotes
g_{Proch}	Grazing based on Prochlorococcus
g_{Syn}	Grazing rate based on Synechococcus
ITCZ	Intertropical Convergence Zone
k	Net phytoplankton growth rate
LC	Large cryptophytes

MC	Medium cryptophytes
ML	Mixed Layer
MZ	Mesozooplankton
N	Nitrogen
NF	Autotrophic nanoflagellates
NH_4^+	Ammonium
NO_2^-	Nitrite
NO_3^-	Nitrate
OMZ	Oxygen minimum zone
P	Phosphorous
PAR	Photosynthetically active irradiance
PCA	Principal Component Analysis
PE	Picoeukaryotes
picoEUK	Autotrophic picoplankton
PO_4^{3-}	Ortophosphate
PP	Primary production
Prochl	*Prochlorococcus*
SC	Small cryptophytes
SEC	South Equatorial Current
SECC	South Equatorial Counter Current
Si	Dissolved Inorganic Silicate
Syn	*Synechococcus*
TN	Total Nitrogen
TP	Total Phosphorous
WSW	Whole Seawater
μ	Intrinsic growth rate
μ_{Chla}	Growth based on chlorophyll *a*
μ_{PE}	Growth based on autotrophic picoeukaryotes
μ_{Pro}	Growth based on *Prochl*orococcus
μ_{Syn}	Growth rate based on *Synechococcus*
μZ	Microzooplankton

Annex I

P-values for common slope test, normality test (Shapiro-Wilk test) and homoscedasticity test (Bartlett test).

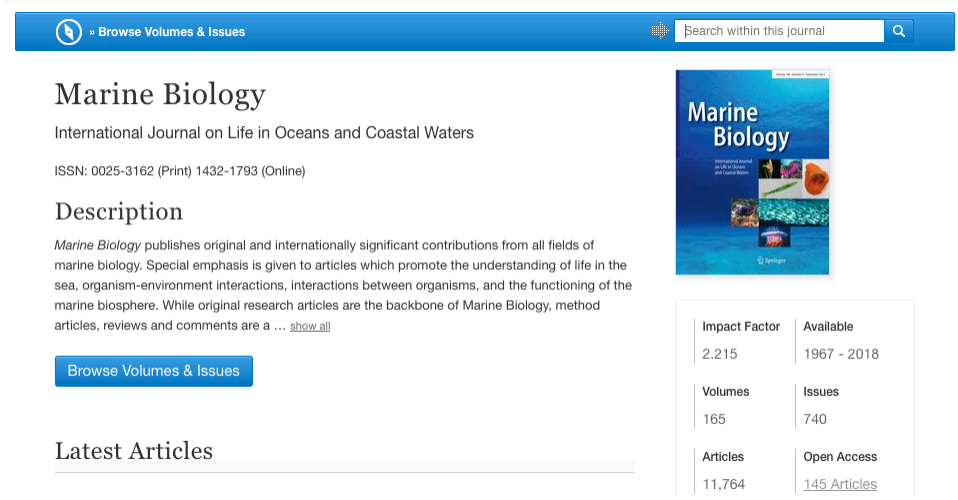
Organism	Common Slope test	Shapiro-Wilk test	Bartlett test
Chlorophyll *a*	0.66	0.47	0.11
Synechoccocus	0.71	0.52	0.19
Prochloroccocus	0.31	0.24	0.19
Picoeukaryotes	0.78	0.28	0.06
Heterotrophic bacteria	0.02	0.17	0.50
Dinoflagellates <15µm	0.48	0.67	0.62
Dinoflagellates >15µm	0.45	0.84	0.06
Total Dinoflagellates	0.45	0.84	0.06
Diatoms	0.25	0.68	0.52
Tintinnids	0.49	0.41	0.06
Ciliates <15µm	0.06	0.92	0.08
Ciliates >15µm	0.20	0.58	0.06
Total Ciliates	0.38	0.48	0.13
Autotrophic Nanoflagellates	0.10	0.37	0.84
Heterotrophic Nanoflagellates	0.21	0.34	0.58

Partial correlations coefficients (r) between variables; in each case the intermediate effect of the logarithm of the copepods concentration were subtracted. Variables are: chlorophyll a (Chl a), picoeukaryotes (Pico), Synechoccocus (Syn), Prochloroccocus (Pro), heterotrophic, bacteria (HB), dinoflagellates < 15µm (Din < 15µm), dinoflagellates (Din > 15µm), total dinoflagellates (Tot Din), diatoms, tintinnids (Tint), ciliates < 15µm (Cil < 15µm), ciliates > 15µm (Cil > 15µm), total ciliates (Tot Cil), autotrophic nanoflagellates (AN), heterotrophic nanoflagellates (HN). Bold numbers represent significant correlations at *p < 0,05, **p < 0,01 and ***p < 0,001.

Organism	Chl *a*	Syn	Pro	Pico	HB	Din<15	Din>15	Tot Din	Diatoms	Tint	Cil<15	Cil>15	Tot Cil	AN
Syn	**0.09****													
Pro	**0.31****	**0.81*****												
Pico	**0.02*****	**-0.192*****	-0.01											
HB	**-0.09*****	0.212	0.15	0.09										
Din<15	0.04	0.10	-0.01	0.17	0.16									
Din>15	-0.15	0.24	0.02	-0.16	**0.40****	**0.50*****								
Tot Din	-0.15	0.24	0.02	-0.16	**0.40****	**0.50*****	**0.70*****							
Diatom	0.20	-0.21	-0.25	0.19	0.06	**0.29****	-0.02	-0.22						
Tint	**-0.03***	-0.09	**-0.36***	**-0.41***	0.07	**0.02***	**0.30***	**0.30***	0.07					
Cil<15	-0.04	0.00	0.09	**-0.14***	0.18	**0.57*****	**0.33***	**0.33***	0.22	-0.13				
Cil>15	**-0.33***	-0.26	**-0.03***	-0.14	**0.43****	**0.45****	**0.29***	**0.29***	**-0.40****	**-0.04***	**0.48*****			
Tot Cil	-0.17	-0.20	-0.03	-0.19	**0.31***	**0.47*****	**0.37****	**0.37****	**0.19***	-0.06	**0.74*****	**0.95*****		
AN	-0.25	0.14	0.14	0.12	0.04	**-0.35***	-0.03	-0.03	**-0.25***	-0.01	-0.04	**-0.40***	-0.02	
HN	-0.05***	0.20	0.20	0.13	0.17	**0.006***	**-0.05***	-0.05	**0.30***	-0.11	0.29	0.04	0.06	**0.36***

Annex III

Armengol, L., Franchy, G., Ojeda, A., Santana-del Pino, Á., & Hernández-León, S (2017) Effects of copepods on natural microplankton communities: do they exert top-down control?. *Marine Biology*, *164*(6), 136.



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ORIGINAL PAPER

Effects of copepods on natural microplankton communities: do they exert top-down control?

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Abstract Top-down effects in the pelagic realm are quite well known in freshwater ecosystems. However, our knowledge of these effects in the ocean remains scant. It is known that copepods prefer to prey on ciliates and heterotrophic dinoflagellates, and their high or low abundances can change the structure of microplankton communities. Field studies in subtropical waters have shown parallel increases of mesozooplankton and phytoplankton without a lag, suggesting a top-down effect of mesozooplankton preying upon microzooplankton and releasing primary producers from predation. In the present work, we added copepods at increasing densities to natural plankton in 24 h experiments. A decrease in aloricated ciliates abundance of nearly 50% and increases in the abundances of picoeukaryotes, *Synechococcus*, *Prochlorococcus*, diatoms, and chlorophyll *a* were observed. No effect of nutrient additions was observed in parallel grazing experiments. Thus, a top-down effect of copepods upon microzooplankton explains the observed changes in the abundance of the different phytoplankton groups. Copepods promote important changes down the food web, structuring the community by predation upon microzooplankton. There are biogeochemical consequences of zooplankton variability over short time scales in the ocean.

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Introduction

Plankton can be divided according to the sizes of organisms (Sieburth et al. 1978) ranging from femto- (0.02–0.2 µm), through pico- (0.2–2 µm), nano- (2–20 µm), and micro- (20–200 µm) to mesozooplankton (0.2–20 mm). Therefore, important predator–prey interactions should be expected along the size gradient as feeding is roughly related to body size (Longhurst 1991). Among these interactions, the effects of mesozooplankton predators downward through the trophic web, and their effects in structuring the plankton communities, have scarcely been studied in comparison to the effects of the physical aspects of ocean ecosystems. Carpenter et al. (1985) defined the trophic cascade concept to describe the top-down effects from fish to phytoplankton in lakes. From that seminal paper to the present, numerous studies have described top-down effects occurring in freshwater systems. However, that is not the case for the oceanic environment, where the top-down control is substantially more difficult to observe.

Microzooplankton, mainly ciliates and dinoflagellates, act as an important link between primary producers and mesozooplankton and are an important source of energy for copepods in the ocean (Burkill et al. 1993; Calbet and Landry 1999). In oligotrophic waters, copepods prefer to prey upon ciliates and dinoflagellates (Fessenden and Cowles 1994; Suzuki 1999; Broglio et al. 2004; Calbet and Saiz 2005), probably because autotrophic production is low and mainly comes from small cells that are rarely consumed

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Annex IV

Plates of ciliates from Roskilde Fjord (Denmark)

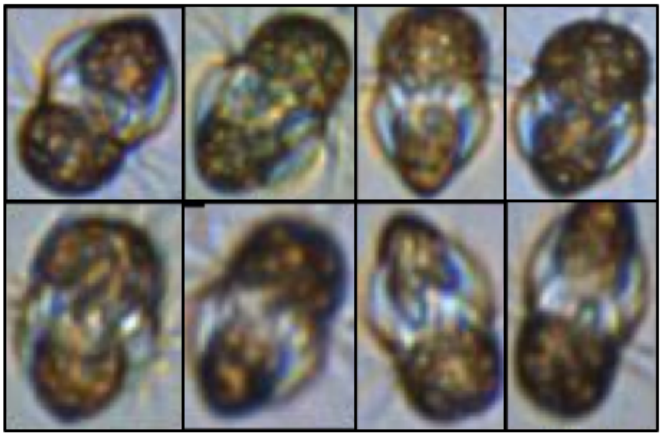

Plate 1. *Mesodinium rubrum*

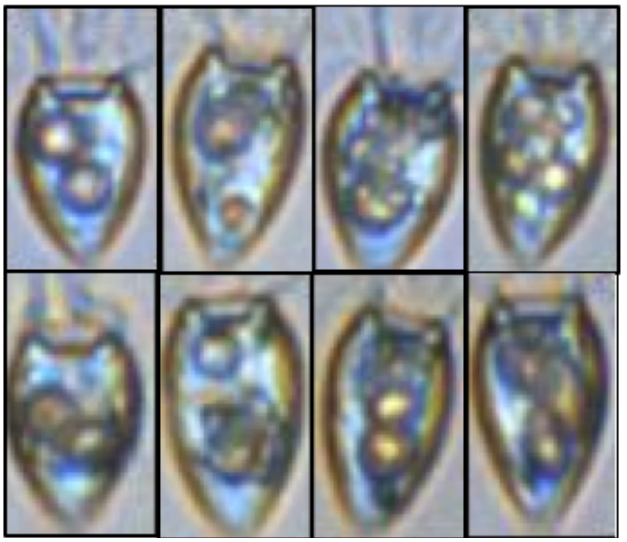

Plate 2. *Balanion comatum*

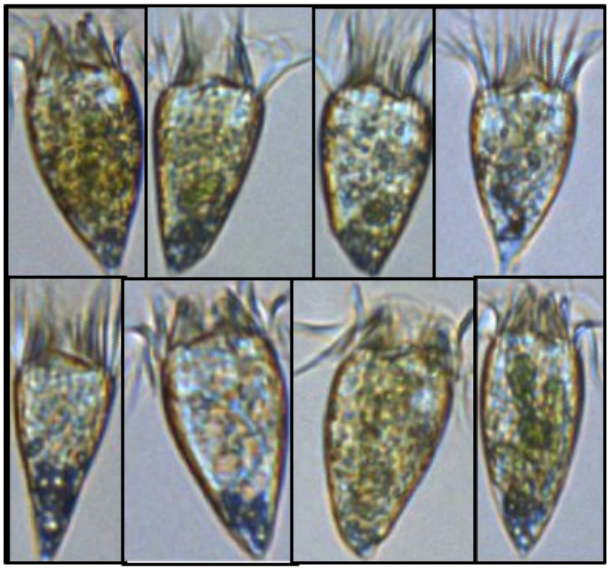

Plate 3. Heterotrophic ciliate SpIII.

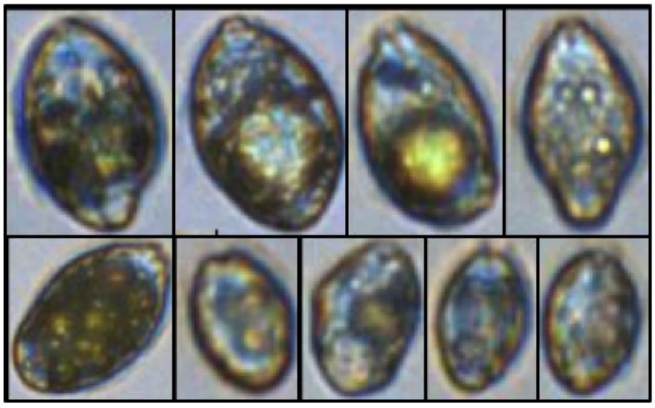

Plate 4. *Urotrichia* sp.

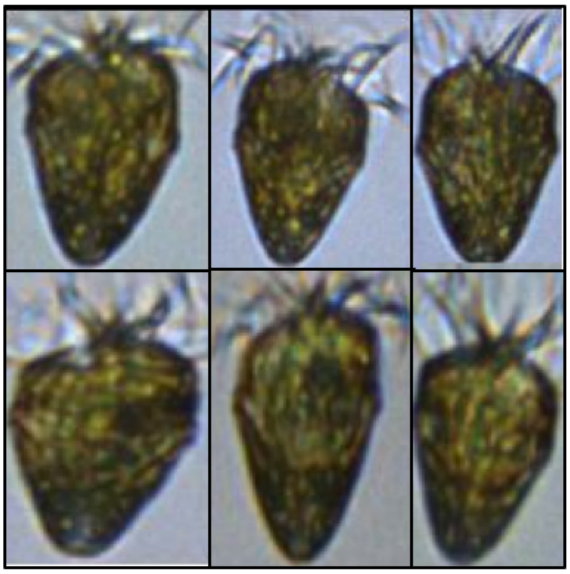

Plate 5. Mixotrophic ciliate SpV

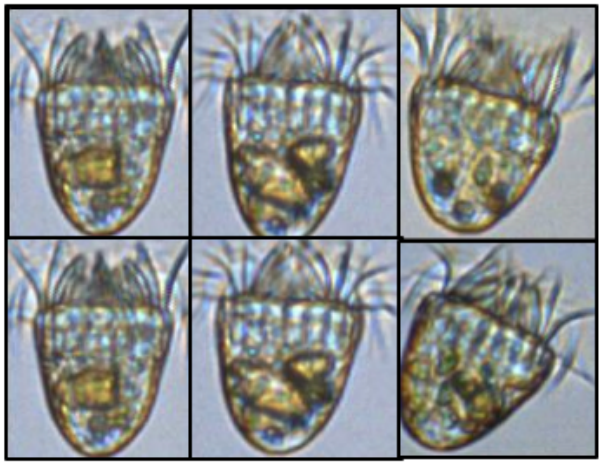

Plate 6. Heterotrophic ciliate SpVI

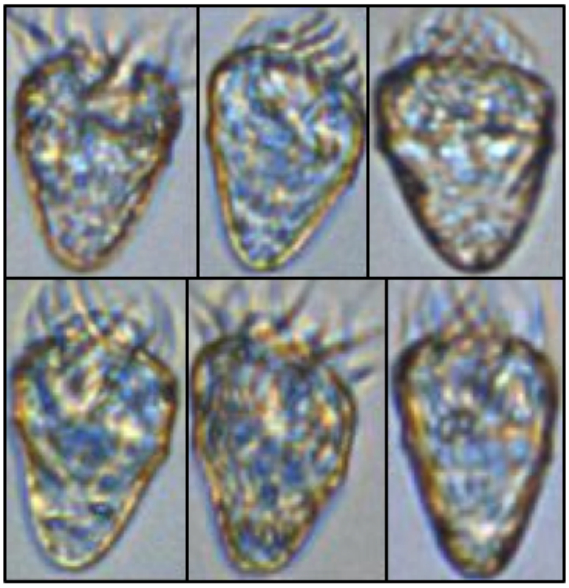

Plate 7. Heterotrophic ciliate SpVII

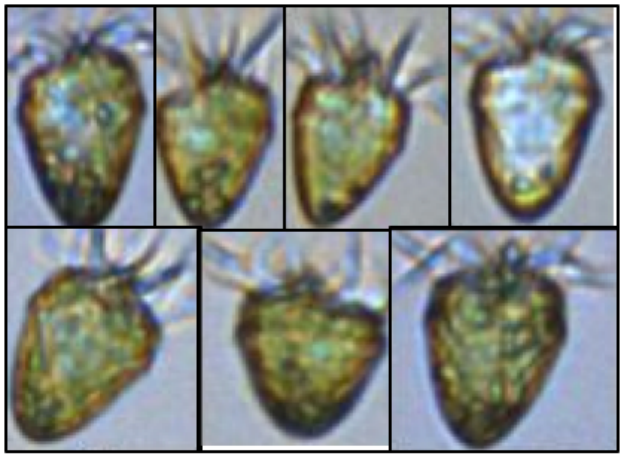

Plate 8. Mixotrophic ciliate SpIIX

Trabajos Relacionados

A continuación se detallan otros trabajos que se desprenden del trabajo de investigación desarrollado durante los años de formación. Estos trabajos no se han presentado en esta tesis, y está prevista su publicación en revistas científicas con índice JCR.

1. Diel patterns of microzooplankton grazing on phytoplankton in subtropical waters
2. Phytoplankton and mesozooplankton distribution in the Northwest African Upwelling
3. Impact of acidification and nutrient availability on microzooplankton grazing and phytoplankton growth during mesocosm experiments
4. Trophic and planktonic food web structure along the subtropical and temperate Atlantic ocean during the spring bloom.