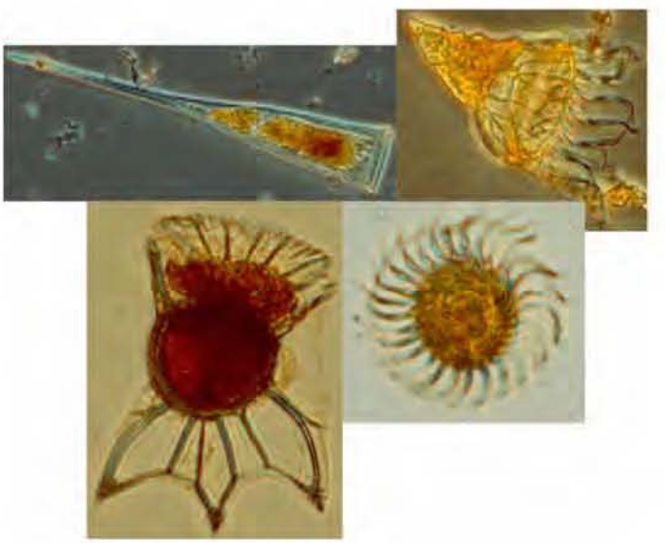
TESIS DOCTORAL

Planktonic community structure and variability in the Canary Islands waters: The role of microplankton

Claire Schmoker



UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA
Departamento de Biología

Septiembre 2010

Planktonic community structure and variability in the Canary Islands waters: The role of microplankton
Claire Schmoker
2010

D/D[a] Juan Luis Gómez Pinchetti SECRETARIO/A DEL DEPARTAMENTO DE Biología DE LA UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA,

CERTIFICA,

Que el Consejo de Doctores del Departamento en sesión extraordinaria tomó el acuerdo de dar el consentimiento para su tramitación, a la tesis doctoral titulada "*Planktonic community structure and variability in the Canary Islands waters: The role of microplankton"* presentada por el/la doctorando/a D/D[a] Claire Schmoker y dirigida por el Dr. Santiago Hernández-León.

Y para que así conste, y a efectos de lo previsto en el Artº 73.2 del Reglamento de Estudios de Doctorado de esta Universidad, firmo la presente en Las Palmas de Gran Canaria, a 8 de julio de 2010

UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA
Departamento de Biología

Las Palmas de Gran Canaria a 7 de julio de 2010

Programa de doctorado en Oceanografía

Bienio 2006-2008. Con Mención de Calidad de la ANECA.

Título de la Tesis:

Planktonic community structure and variability in the Canary Islands waters: The role of microplankton

(Estructura y variabilidad de la comunidad planctónica en aguas de las Islas Canarias: el papel del microplancton)

Tesis Doctoral presentada por Dª Claire Schmoker para obtener el grado de Doctor por la Universidad de Las Palmas de Gran Canaria

Dirigida por Dr. D. Santiago Hernández-León

El/la Director/a **El/la Doctorando/a**

AGRADECIMIENTOS / ACKNOWLEDGEMENTS

Esta tesis ha sido posible gracias a la financiación del Ministerio de Educación y Ciencia a través del proyecto CONAFRICA (CTM2004-02319/MAR) y al Gobierno de Canarias por una beca de postgrado para la realización de tesis doctorales, modalidad B.

En primer lugar, quisiera agradecer a mi director de tesis, Dr. Santiago Hernández León por haberme dado la oportunidad de hacer la tesis en su laboratorio bajo su supervisión, por la confianza en mi trabajo y por el apoyo durante estos años.

Estoy muy agradecida por los consejos, la ayuda, los ánimos recibidos de los Drs. Alicia Ojeda, Albert Calbet y Javier Arístegui durante toda la tesis. Gracias también a los Drs. Nandi Montero, Ted Packard y May Gómez.

I'm particually thankful to Dr. Benni Hansen and Dr. Peter Thor for giving me the opportunity to work with them in Roskilde. I will also thank Dr. Hans Henrik Jakobsen. That was a pleasure of working in Denmark. Thanks also to Guillaume, Zuzana, and Morten.

I would also like to thank Dr. Suzanne Strom for her hospitality in her lab at the Western Washington University. Thanks also to Dr. Jude Apple, Kelley Bright, Kerri Frederickson for your help with the experiments, with the cultures and all the life in the lab. Thank you also to Dr. Koslov for all these talks we had on microscopy, ciliates and Roscoff. Finally, thanks to Amanda, Jay, Ginny, Joyce, Emily for the social life!

Por supuesto, gracias a toda la gente del laboratorio B-201: Sébastien, Marta, Mayiya, Mar, Ico, Minerva, Ivan y Vero, Fede y Angela, Pascal, Gara, Inma, Alejandro, Yeray, Fede, Igor y Natalia, Valeria, Alicia. A la gente "fuera" del laboratorio: Paula, Mireya, Carolina, Patty, Evan, Manuela. A la gente del ICCM: Fernando, Tono, Leopoldo, Chano.

Merci à tous mes amis de Belgique et d'ailleurs: Isa, Phil, Séb, Chris, Syl, Muriel, Martine.

Pour terminer, merci à ma famille. À mes frères Benjamin et Aurélien. À Josiane et enfin à mes parents.

RESUMEN

La comunidad planctónica marina está compuesta por una gran variedad de organismos que explotan una amplia diversidad de fuentes de alimentación disponibles en su ambiente. Las interacciones tróficas, ya sean directas o indirectas, son importantes reguladores de la estructura de las comunidades planctónicas. En aguas oligotróficas, las pequeñas fracciones del plancton son uno de los mayores componentes de las redes tróficas. El bucle microbiano domina la mayor parte del año y el microplancton juega un papel central en estos sistemas. En este trabajo, hemos contribuido al estudio de la estructura de la comunidad planctónica y a su variabilidad temporal, utilizando un enfoque de "end-to-end", desde las bacterias hasta en el mesozooplancton haciendo especial énfasis en el microplancton. Nuestro trabajo muestra la importancia de los efectos "bottom-up" y "top-down" que regulan la estructura de las comunidades planctónicas. Hemos mostrado que los diferentes grupos del picoplancton autotrófico aparecen uno tras otro en función de la temperatura y disponibilidad de nutrientes. Sin embargo, también hemos visto que estos organismos están controlados por efectos "top-down". Efectivamente, sus principales depredadores son organismos nano- y microplanctónicos los cuales son presas del mesozooplancton. Por lo tanto, al verse el microplancton consumido por el mesozooplancton, se libera la presión de depredación sobre el picoplancton autótrofo y su biomasa puede aumentar. Hemos puesto en evidencia que el "bloom" de finales de invierno, característico de las aguas subtropicales que rodean las Islas Canarias, es un proceso más complejo de lo que se pensaba hasta ahora, estando compuesto de varios ciclos en la variación de las biomasas de los diferentes grupos de plancton, y siendo el aumento de la biomasa del picoplancton autotrófico el punto de partida, y terminando con el mesozooplancton. El presente trabajo demuestra la complejidad de las estructuras y funciones de los organismos planctónicos durante un ciclo anual y aporta evidencias de la necesidad de estudiar los flujos de carbono en los océanos con un enfoque de "end-to-end".

ABSTRACT

The marine zooplankton community is composed of a high variety of organisms with different feeding abilities, which exploit a wide diversity of food types available in the environment. Trophic interactions, direct or indirect, are important regulators of the structure of planktonic communities. In oligotrophic waters, small planktonic fractions play an important role and are one of the major components of the planktonic food web. Microbial loop dominates during most of the year and microplankton plays a fundamental role in these ecosystems. In this work, we contributed to increase the knowledge on the structure and temporal variability of the planktonic community, from bacteria to mesozooplankton. Our work highlights how “bottom-up” and “top-down” effects regulate planktonic community structure. We found that the different groups belonging to autotrophic picoplankton succeeded one to another in function of temperature and availability in nutrients. However, we also shown that these organisms were also controlled by higher trophic level organisms. Indeed, autotrophic picoplankton is mainly consumed by heteronano- and microzooplankton, being the latter organisms preyed by mesozooplankton. Therefore, when nano- and microplankton are eaten by mesozooplankton, autotrophs are free of predation pressure and their biomass can increase. We also found that the characteristic late winter bloom of subtropical waters was a more complex process than previously thought. Several cycles of the different planktonic organisms were observed. Each cycle started with the increase of autotrophic picoplankton biomass and was ended by mesozooplankton. The present work sheds light on the structure of planktonic organisms during the annual cycle and provides evidence of the need to afford the study of carbon flux in the ocean using an end-to-end approach.

PRESENTACIÓN DE LA TESIS

La presente tesis titulada *Estructura y variabilidad de la comunidad planctónica en las aguas de las Islas Canarias: el papel del microplancton* es una recopilación de seis trabajos realizados dentro de los proyectos de investigación ConAfrica (CTM2004-02319/MAR) y Lucifer (CTM2008-03538), dirigidos por el Dr. Santiago Hernández-León, Catedrático de la Facultad de Ciencias del Mar de la Universidad de Las Palmas de Gran Canaria.

Esta tesis está dividida en dos partes. La primera ha sido escrita integralmente en inglés y estructurada en Introducción, Objetivos, Contribuciones originales, Síntesis de Resultados y Discusión General, Conclusiones y Líneas Futuras de Investigación. Por lo tanto, el resumen y las conclusiones de esta tesis están escritas de acuerdo a la normativa para la obtención de la Mención europea del Título de Doctor (BOULPGC. Art. 1 Capítulo 4, a 5 de noviembre 2008).

La segunda parte de la tesis está escrita en español y, de este modo, contiene las 50 páginas en castellano requeridas por el Reglamento de Elaboración, Tribunal, Defensa y Evaluación de Tesis Doctorales de Universidad de Las Palmas de Gran Canaria (BOULPGC. Art. 2 Capítulo 1, a 5 de noviembre 2008). Además, sigue la estructura exigida por este Reglamento: Introducción, Objetivos, Planteamiento y Metodología, Resultados, Discusión General, Conclusiones y Futuras Líneas de Investigación.

THESIS PREVIEW

This thesis entitled *Planktonic community structure and variability in the Canary Islands waters: The role of microplankton* is a compilation of six works carried out in the frame on the research projects ConAfrica (CTM2004-02319/MAR) and Lucifer (CTM2008-03538) granted to Dr. Santiago Hernández-León, Professor at the Marine Science Faculty of the Universidad de Las Palmas de Gran Canaria.

This thesis starts with a general introduction on the description of the planktonic food web and particularly the temporal variability in the Canary Islands region. The original contributions are compiled in manuscript format. The thesis follows with a synthesis of results and a general discussion section and finishes with conclusions and suggestions of further research studies on the theme.

Finally, a summary in Spanish is included, containing more than fifty pages. This is a requirement from the PhD Thesis Regulations from the Universidad de Las Palmas de Gran Canaria (BOULPGC. Art. 2 Chapter 1, November 5th 2008). Besides, in order to obtain the *Doctor Europeus* Mention, Summary and Conclusions have been translated to English (BOULPGC. Art. 1 Chapter 4, November 5th 2008).

ÍNDICE / CONTENTS

Índice / Contents

INTRODUCTION

1. Plankton community

The term "plankton" (from Greek *planktos*, means "errant") was defined as the group of organisms, animals and plants drifting in the water which are unable to swim against ocean currents (Hensen 1887 in Dussart 1965). They are opposed to nekton, the community of actively swimming organisms such as large crustaceans, cephalopods, fishes, aquatic birds, and mammals. Traditionally, planktonic organisms have been assigned to one of the three compartmental groups: bacterioplankton, phytoplankton and zooplankton. Further, these groups were subdivided into trophic levels on the basis of taxonomic categories. However, this procedure was unfortunate regarding some protistan groups with different nutrition modes. All the flagellates were, thus, grouped within the algae and were considered as phytoplankton as well as the microplanktonic organisms (Dussart 1965). Sieburth et al. (1978) suggested that one approach to divide organisms into useful groups was to forget the taxonomic hierarchies and to split them when necessary into vernacular groupings on the basis of the level of organization (ultrastructure) and the mode of nutrition (Sierburth 1977). This author also suggested that new groupings or subdivisions should be made only when necessary and when the terms had been clearly defined. For example, for ecological purposes, the primary producers should be defined as those prokaryotic and eukaryotic organisms that contain chlorophyll. All phototrophic organisms including autotrophic, auxotrophic, and mixotrophic forms should be included within the phytoplankton. Conversely, all the chlorophyll-less microorganisms with osmotrophic or phagotrophic nutrition would be considered heterotrophic and excluded from the primary producers. These forms would be divided into bacterioplankton, mycoplankton, and protozooplankton on a basis of size, morphology, and ultrastructure.

Marine plankton comprises organisms, in a large size range, volumes and weights spanning eighteen orders of magnitude. Schütt (1892) made the first attempt to classify these on a size basis. He distinguished between micro-, meso- and macrozooplankton. In 1978, Sieburth et al. proposed a classification based on the organisms size splitting autotrophic organisms from the heterotrophic ones, and grouping planktonic organisms in 7 size ranges. This is the classification currently used by planktonologists and can be schematized separating autotrophic from heterotrophic organisms in the different size ranges from 0.2 to 20,000 µm (Fig. 1).

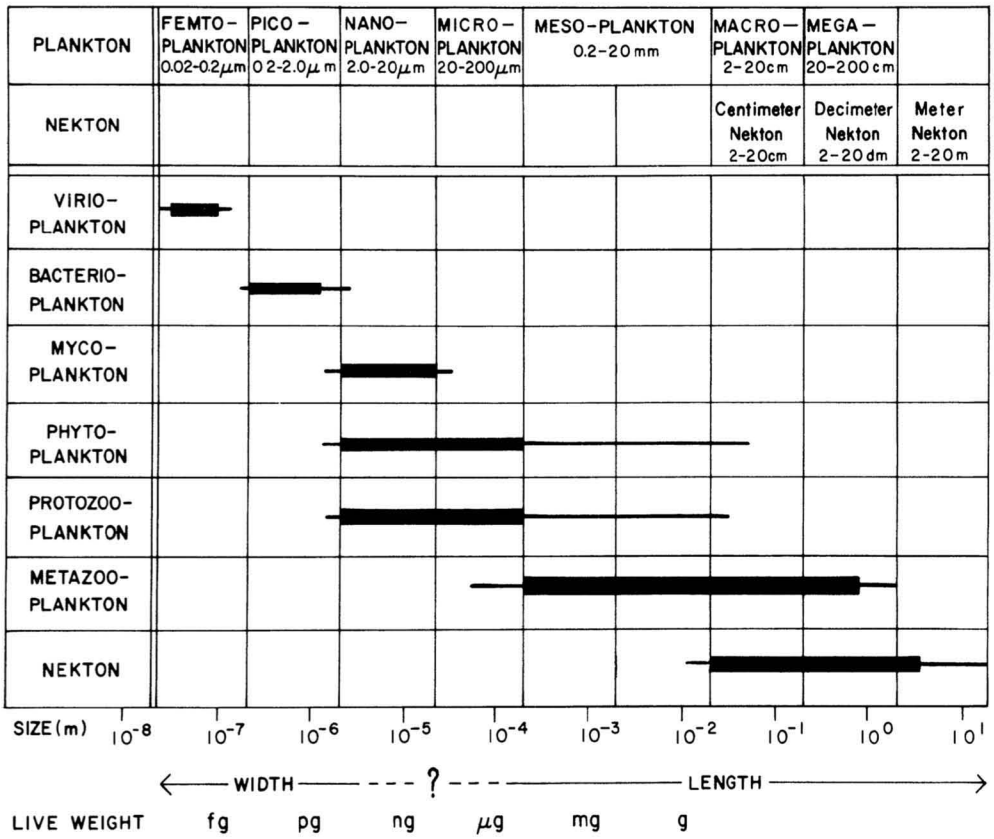


Fig. 1: Distribution of different taxonomic-trophic compartments of plankton in a spectrum of size fractions (From Sieburth et al. 1978)

Among all size classes described above, a wide range of nutritional modes and ways of feeding are normally found. Autotrophy is related to the only use of inorganic compounds for photosynthesis. Heterotrophy is, however, related to the feeding of organic compounds for metabolism, growth and reproduction. The classification of organisms in relation to size and nutritional modes illustrate the complexity of the community (Fig. 2).

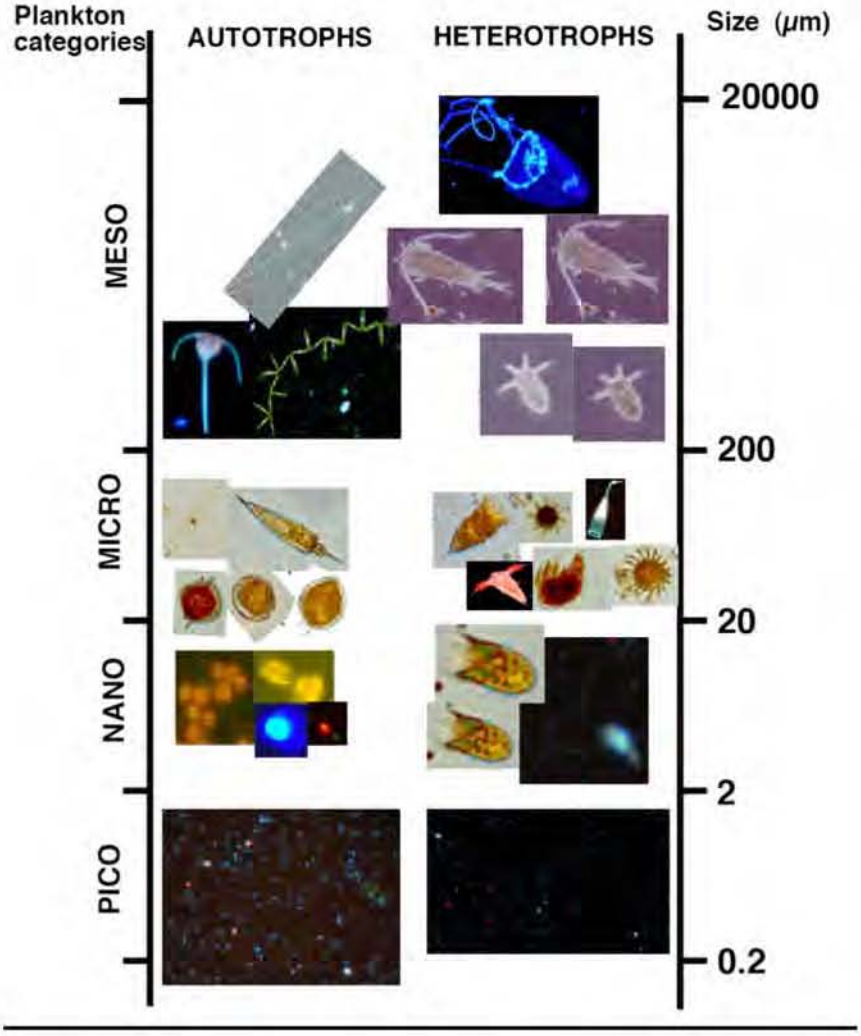


Fig. 2: Plankton categories (Redrawn from A. Calbet, photos from C. Schmoker and M. Espino)

Sieburth et al. (1978) defined the bacterioplankton compartment as that one composed of unattached unicellular bacteria, which could be selectively filtered with 0.1 and 1.0 μm-porosity. The size fractions occupied by bacterioplankton (0.2-2 μm)

and virioplankton (0.02-0.2 µm) were called picoplankton and femtoplankton. In this thesis, we will use the term bacterioplankton exclusively to refer to heterotrophic prokaryotes with size <2 µm. Heterotrophic bacteria are generally separated on the basis on their genetic material, low or high DNA content.

Phytoplankton refers to photosynthetic cells that drift with currents in aquatic systems (Falkowski et al. 2004). Their photosynthetic products constitute the build up of organic matter to fuel the pelagic food web (Chisholm 1992). They are the basis of the planktonic food web, fixing dissolved inorganic carbon to produce organic matter, and determining the levels of dissolved inorganic carbon in surface waters and the exchange of carbon between the ocean and the atmosphere.

Autotrophic picoplankton (size range 0.2-2 µm) includes eukaryotes and prokaryotes, differentiated by their nuclear structure. Photosynthetic eukaryotic picoplankton is composed by a high taxonomical diversity of algae (Vaulot et al. 2008, Worden and Not 2008), while prokaryotic picoplankton is mainly composed by cyanobacteria *Synechococcus* and *Prochlorococcus*. Autotrophic nanoplankton (size range 2-20 µm) is mainly composed by flagellates. Microphytoplankton (size range 20-200 µm) mainly contains autotrophic dinoflagellates, few ciliates but also a large number of diatom species.

Zooplankton are heterotrophic organisms and according to their food preferences, they are classified as herbivorous, detritivorous, carnivorous or omnivorous. The main components of nanozooplankton (2-20 µm) are heterotrophic nanoflagellates feeding on bacterio- and picoplankton. Microzooplankton (20-200 µm) are mainly composed by ciliates and heterotrophic dinoflagellates. They also include foraminiferans as well as some metazoans, such as copepod nauplii, copepodites and meroplanktonic larvae. Protozoans have a rapid cellular division rate enabling them to keep pace with

phytoplankton. They react immediately to algal growth and take advantage of the food supply offered.

Mesozooplankton includes organisms from 200 to 20,000 µm. It is composed by copepods, small hydromedusae, ctenophores, chaetognaths, appendicularians, doliolids, fish eggs and larvae. It comprises also older stages of crustacean plankton and meroplanktonic larvae. Copepods are the dominant mesozooplankton group, typically comprising around 80% of mesozooplankton biomass in the oceans (Verity and Smetacek 1996 in Halvorsen et al. 2001).

Mixotrophy is the combination of auto- and heterotrophy nutrition mode. If either autotrophy or heterotrophy, alone can support cell functions, the nutrition mode is termed optional mixotrophy. If both are required, it is called obligatory mixotrophy (Taylor 1987). It is quite commonly found in marine planktonic community. Many ciliates and dinoflagellates can be mixotroph. But we also find nanoflagellates presenting this feeding behavior.

2. Marine planktonic webs

All the groups forming the planktonic community are linked one to another by an interplay between preys and predators influenced by sizes, ecological preferences, top-down and bottom-up effects (Zöllner et al. 2009). The classical food chain was described as a linear relation between the photosynthetic organisms from the bigger fraction, diatoms and dinoflagellates, and copepods as their main grazers, transferring the photosynthetic carbon to higher trophic levels (Steele 1974). Nevertheless, this perception of the planktonic webs did not give their true role to bacteria, protozoan and viruses. Bacteria were thought to be rare and were assigned to a quasi-exclusive decomposer role. In 1974, the traditional vision of the planktonic food web started to

change with the work of Pomeroy (1974) who gave a bigger importance to the small planktonic fractions, showing that pico- and nanoplankton where the dominating fractions of epipelagic phytoplankton in subtropical oceans (Platt et al. 1983). The microbial loop theory showed the existence of more trophic levels between primary producers and consumers (Azam et al. 1983). The theory was based on the particle-size model of Sheldon (1972) in which organisms are supposed to feed on particles one order of magnitude smaller than themselves. In that sense, bacteria utilize the dissolved organic matter (DOM) released by phytoplankton and other organisms. Heterotrophic bacteria and autotrophic picoplankton (cyanobacteria in the size range of 0.3 to 1 µm) are controlled primarily by heterotrophic flagellates (3 to 10 µm). Microzooplankton, at its turn, eats the flagellates. In the microbial loop theory exposed by Azam et al. (1983), DOM is, thus, restored through the circuit bacteria-flagellate-microzooplankton to the main food web chain and other trophic levels (Fig. 3).

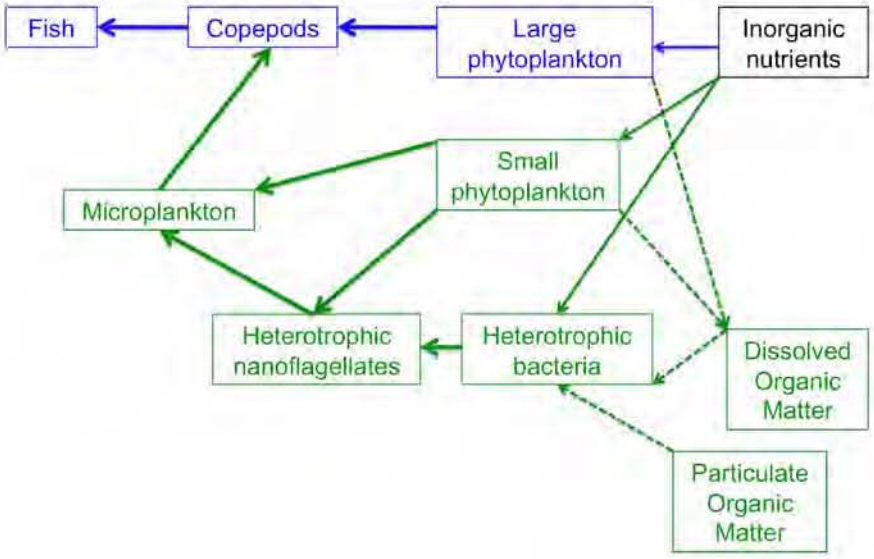


Fig. 3: Simplified diagram of the ocean's food web showing the 'microbial loop' (green) and the 'classical food web' (blue). Major fluxes of carbon are marked by continuous lines (Redrawn from Pomeroy et al. 2007)

3. Microplankton role in the marine trophic web

As above mentioned, microplankton comprises organisms in the size range of 20-200 µm (Fig. 1). It includes a large variety of organisms, which can be autotrophic, heterotrophic as well as mixotrophic. Aloricate ciliates and heterotrophic dinoflagellates represent the larger part of microplankon in pelagic systems. Hence, we will develop the next aspects on these two dominant groups of microplankton.

Aloricate ciliates oligotrichs (e.g. *Strombidium*, *Tontonia*, *Laboea*) and choreotrichs (*Strobilidium*, *Strombidinopsis*) tend to dominate the ciliate abundance, ranging from ten to thousand cells per liter. They are considered to play an important role in microbial food webs (e.g. Sanders and Wickham 1993, Weisse 2006). Tintinnid choreotrichs typically comprise a smaller fraction of the total ciliate abundance, ranging up to 25% of the abundance of their aloricate relatives (e.g. Lynn et al. 1991). In assemblages of heterotrophic dinoflagellates in marine systems, athecate dinoflagellates are typically more abundant (in both number and biomass) than armoured dinoflagellates, which have cellulosic thecal plates.

In the Canary Islands waters, total microplanktonic biomass is dominated by aloricate ciliates (average size range 20-40 µm). Small athecate dinoflagellates (average size range 15-20 µm) dominate in total cell abundances. However, these organisms being <20 µm should be considered as nano-dinoflagellates (Fig. 4).

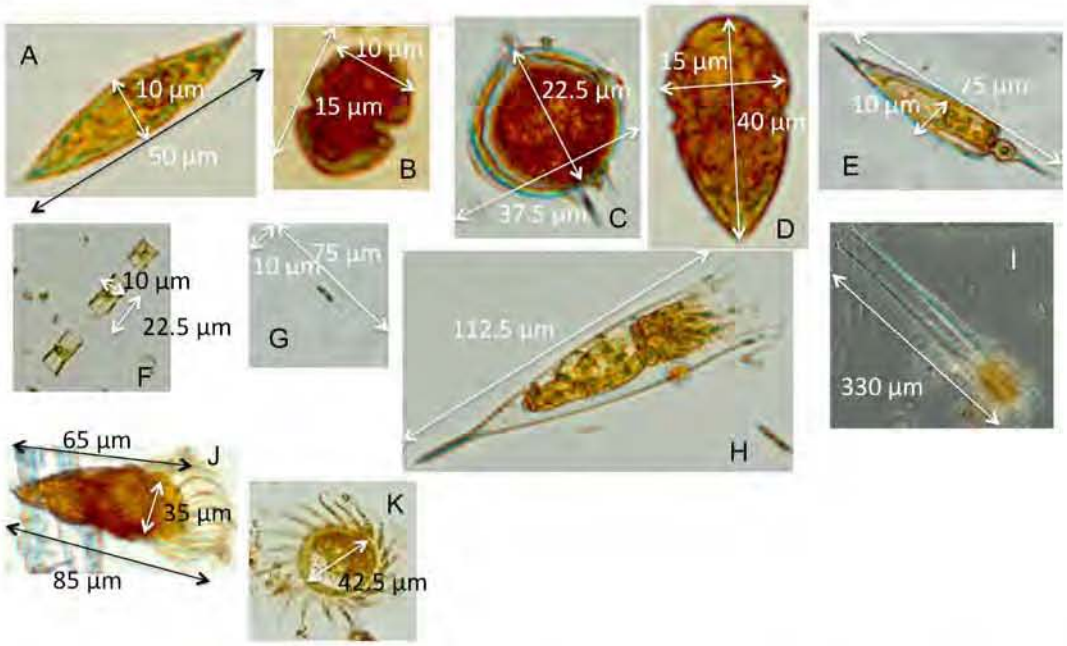


Fig. 4: Representative microplankton from the Canary Islands waters. Dinoflagellates: (A) *Amphidinium sphenoides*, (B) *Scrippsiella sp.*, (C) *Protoperidinium sp.*, (D) *Gymnodium sp.*, (E) *Oxytum scolopax*. Diatoms: (F) *Chaetoceros af. laciniosus*. (G) *Cylindrotheca closterium*. Tintinnids: (H) *Dadayiella*, (I) *Eutintinnid*. Unidentified aloricate ciliates: (J) and (K) (Photos by C. Schmoker)

Because they are important grazers in planktonic food webs, in recent years, interest in the feeding biology of oligotrichs and choreotrichs has increased considerably (Sanders and Wickham 1993). They can consume significant proportions of the primary production, up to 25% (Capriulo and Carpenter 1983, Verity 1985) and over 30% of the bacterial standing stock (Sime-Ngando et al. 1999). Tintinnids and oligotrichs generally consume food particles that are less than 20 µm in diameter (Rassoulzadegan et al. 1988). Furthermore, their excretion of phosphorus and ammonia may fuel over 15% of the net primary production (Verity 1984, Dolan 1997).

Spirotrichs are considered to be upstream filter feeders that "select" particles primarily on the basis of the structural nature of the oral apparatus (Fenchel 1980a, 1980b). Furthermore, a positive relation exists between the size of the ciliate and the average size of the prey (Bernard and Rassoulzadegan 1990). In addition to heterotrophy, some spirotrichs exhibit varying degrees of mixotrophy, either by

sequestering chloroplasts from their prey or by harbouring symbiotic *Chlorella* species (Sanders 1991). Retention of prey chloroplasts is common in oligotrichs (Stoecker et al. 1989) and in dinoflagellates (Taylor 1987). These chloroplasts remain functional for several days in *Laboea* and *Strombidium*, enabling the ciliate to fix carbon during this time (Stoecker et al. 1988). Mixotrophy may serve a variety of functions for the grazers, including providing fixed carbon during periods when preys are not abundant (Dolan and Pérez 2000).

Dinoflagellates are one of the dozen groups of predominantly unicellular, or so, of eukaryotic, flagellate organisms that possess both photosynthetic and non-photosynthetic members. Because both photosynthetic and non-photosynthetic are known, because they can swim and because many have cell walls, they have been claimed by both botanists and zoologists, each emphasizing the 'plant' or 'animal' features. Botanists group them with the 'algae' and zoologists with the 'protozoa'. However, Lessard and Swift (1985, 1986) showed that about half of dinoflagellate species in marine plankton did not have chloroplasts and consume other plankton cells. They are common in marine pelagic systems and have a potentially important role as herbivores (Lessard 1991, Hansen 1992, Jeong 1999, Jeong et al. 2004).

In addition to their role as herbivores in marine systems, aloricate ciliates and heterotrophic dinoflagellates in the microzooplankton serve as quantitatively and qualitatively significant food resource for mesozooplankton (Stoecker and Capuzzo 1990, Kleppel 1993, Suzuki et al. 1999), serving as links to higher trophic levels (Sanders and Wickham 1993). They are consumed by rotifers, copepods, cladocerans, barnacle nauplii, euphasiids, larval and post-larval ctenophores, nematod worms, freswater oligochaetes, oysters and larval fish (e.g. Sanders and Wickham 1993, Nagano et al. 2000). They are even consumed by other protists, both ciliates and dinoflagellates (Dolan 1991). Due to their cell size and biochemical composition,

microzooplankton along with phytoplankton can enhance copepod fecundity (Kleppel 1993, Bonnet and Carlotti 2001, Castellani et al. 2005).

Microzooplankton is the main consumer of phytoplanktonic primary production (Calbet and Landry 2004, Irigoien et al. 2005). In oceanic, coastal, polar, tropical and temperate regions, microzooplankton consumes between 59 and 74 % of primary production (Calbet and Landry 2004). Their high growth rates, comparable to those exhibited by their preys, along with high feeding rates, confer them the capability to cope with phytoplankton production. Based on size-dependent trophic web structure, their impact upon phytoplankton is thought to be more important in oligotrophic systems, where growth of dominating small cells is sustained by regenerated nutrients, than in non-steady situations such as blooms, where large cells become more important. However, there are evidences that challenge this view and support the importance of microzooplankton even during bloom situations dominated by large cells. Field studies carried out during upwelling conditions in the Oregon coast (Neuer and Cowles 1994) and off the North Pacific West coast (Strom et al. 2001) reported significant impact of microzooplankton upon diatom dominated blooms, with evidences suggesting that large dinoflagellates (>20 µm) were using large diatoms as a food source (Sherr and Sherr 2007 and references therein).

Many experiments based on the dilution method (Landry and Hasset 1982) were carried out in various regions of the world ocean. This method is the only one, which provides simultaneous estimates of both rates of phytoplankton growth (µ) and grazing impact (m) by microzooplankton (e.g. Burkill et al. 1995, First et al. 2007). Impact of microzooplankton on primary production is thus important and to our view, it was interesting to make a compilation of data from dilution experiments data in relation to latitude.

However, this approach also has some methodological constraints. Results can be sometimes uninterpretable or the plot of apparent growth against the dilution factors unable to yield a significant regression (Murrell and Hollibaugh 1998, Landry et al. 1995). The regression analysis is commonly done by using a small number of values (12-15 bottles) which makes the slight slopes or low grazing rates difficult to detect and can give non-significant results. Moreover, grazing pressure could not be linearly related to dilution factor. This is because consumption rates of microzooplankton could vary in the different dilution treatments (Gallegos 1989, Evans and Paranjape 1992) and because grazer concentration could be not related linearly to dilution factor throughout time (Dolan et al. 2000). The combination of grazer mortalities in diluted water with phytoplankton growth in undiluted treatments could result in an over-estimation of grazing rate (Gallegos 1989).

4. The Canary Current System

Depending of the marine system considered, the classical food web or the microbial loop dominates. In productive and cold waters, the classical food web dominates. These are generally coastal upwelling systems where the surface waters are enriched by deep cold water rich in nutrients which can support relatively high productivity and large plankton standing stocks (Hutchings et al. 1995, Wilkerson et al. 2000). These systems are characterized by the presence of large cell-sized phytoplankton (Hutchings et al. 1995). Classical herbivorous food web controls organic matter and energy fluxes. Also, this food web is more linear and offers fewer shortcuts for local nutrient regeneration.

Conversely, the oligotrophic areas generally present a quasi-permanent strong stratification, as do the subtropical gyres, which are dominated by the microbial

components and particularly by picophytoplankton cells. In the subtropical gyres, nutrient availability is low and the phytoplankton community dominated by small cells grows actively on nutrient rapidly recycled by grazing and bacterial activity. Food web structures are more complex, with multiple trophic levels that offer several feedback loops to recycle essential nutrients. This would be the paradigm of a conservative system where the leakage of matter out of the euphotic zone is minimal. However, these situations are the two ends of a wide range of states where, for example, the microbial loop could be running during the whole year. In some ecosystems like the subtropical waters off the Canary Islands, during short periods of nutrient availability, the microbial loop and the classical food web are connected (Thingstad and Sakshaug, 1990).

The Archipelago of the Canary Islands, located between 28-29°N and 10-20ºW, approximately (Fig. 5), is crossed by the Canary Current, the extension of the Azores Current (Stramma 1984).

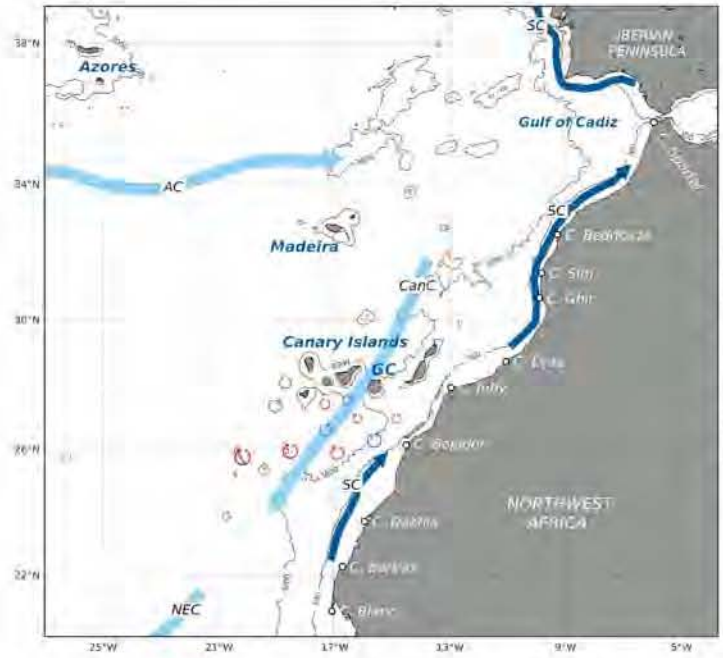


Fig. 5: Schematic map of the Canary Basin showing the main currents (light blue: surface currents; dark blue: slope currents) and mesoscale eddies (blue: cyclones; red: anticyclones) south of the Canary Islands. AC: Azores Current; CanC: Canary Current; NEC: North Equatorial Current; SC: Slope Current (Redrawn by E. Mason from Arístegui et al. 2009)

This region is called the Coastal Transition Zone and the islands span the transition between the cold and productive waters of the coastal African upwelling and the more unproductive oceanic waters of the subtropical gyre (Barton et al. 1998).

Waters around the Canary Islands are oligotrophic and characterized by a late winter bloom. Due to this oligotrophy during an important period of the year, picoplanktonic cells dominate the total abundance and primary production (Zubkov et al. 2000a). During most of the year, the strong heating of surface waters causes a quasi-permanent thermocline, which restrains the pumping of nutrients to upper layers and limits phytoplankton growth. However, during winter months, the surface waters cool off, the thermocline is eroded and the nutrients become available (de León and Braun 1973, Braun 1989) promoting an increase in primary production and chlorophyll (Menzel and Ryhter 1961). In April-May, the thermocline starts to reform, leading to the normal situation of a surface euphotic zone with reduced inorganic nutrients. Unicellular picoplanktonic prokaryotes and eukaryotes play a fundamental role in these waters (Azam et al. 1983, Sherr and Sherr 2007) as their small size associated to the reduced diffusion boundary layer and large surface area per unit volume are an advantage to uptake nutrients (Raven 1998).

This subtropical region combines, thus, both types of extreme ecosystems. It is then important and interesting to characterize planktonic populations during the whole year and to study possible shifts in the planktonic composition from one period to another. In this region, microzooplankton is mainly preying on picoplankton, provoking a high predation pressure on their biomass but also being limited by their presence or absence. Knowing their temporal variability and seasonal patterns is also important to characterize microzooplankton during the two contrasting periods of the year. Although the late winter bloom is now well known and described, only few studies (Arístegui et al. 2001, Arístegui and Montero 2005, Hernández-León et al. 2004, Tsai et al. 2008)

have covered the temporal variability of small planktonic communities in subtropical waters.

THESIS OUTLINE

This thesis was centered on three main objectives:

1. The assessment of the global impact of microzooplankton grazing on primary production and the latitudinal variability of microalgal growth. To complete this first objective (**Chapter I**), we reviewed all existing data, to our knowledge, in the literature of grazing and growth rates obtained by dilution experiments. We compared the estimations of global primary production in the ocean with the phytoplanktonic growth rates obtained with the dilution technique, and studied the latitudinal variation in phytoplankton growth and mortality rates by microzooplankton.

2. The reaction of a heterotrophic dinoflagellate to a simulated bloom period was evaluated in a laboratory experiment, giving for the first time a complete energy budget for a heterotrophic dinoflagellate. To reach this second goal (**Chapter II**), we performed an experimental study with the heterotrophic dinoflagellate *Gyrodinium dominans*. We compared grazing, growth, and respiration rates of the dinoflagellate growing under steady (constant food concentration) and non-steady (one single pulse of food) conditions.

3. The description of the planktonic community structure in the Canary Island waters in an end-to-end approach by (1) evaluating the planktonic composition around Gran Canaria Island (**Chapters III** and **IV**), (2) evaluating the relationships existing between planktonic communities, in particular for the Subtropical Oceanic waters (**Chapter V**), and (3) estimating micro- and mesozooplankton grazing rates in these warm, oligotrophic region of the ocean (**Chapter VI**). To reach the third objective we carried out an intensive sampling of more than two years, around Gran Canaria Island. This sampling allowed to describe the different components of the pelagic food web and to compare warm and cold years in order to study the effect of stratification on development and fate of these communities.

1. Dilution method

The database used in this work (Chapter I) consisted of a set of 1267 corresponding values (Annexe 1), including the whole dataset of the 788 experiments analysed by Calbet and Landry (2004) plus those studies published since 2004.

The dilution technique has been broadly applied in aquatic ecosystems to estimate phytoplankton growth and microzooplankton grazing impact rates on phytoplankton. The method is based on three assumptions (Landry and Hasset, 1982):

a) Growth of individual phytoplankton is not directly affected by the presence or absence of other phytoplankton *per se*. The implication of this assumption is that a reduction in the density of cells in natural seawater will not cause direct changes in the growth rate of the remaining cells.

b) The probability of a phytoplankton cell to be consumed is a direct function of the rate of encounter of consumers with prey cells. This implies that consumers are not food-satiated at natural prey densities and that the number of prey ingested by a given consumer is linearly related to prey density.

c) Changes in the density of phytoplankton (P) over time can be represented appropriately by the exponential equation:

$$P_t = P_0 e^{(\mu - m)t} \qquad (1)$$

where μ and m are the instantaneous coefficients of population growth and grazing mortality, respectively. Phytoplankton growth and microzooplankton grazing rates are determined as the y-intercept and the slope of the regression line, respectively, of a series of dilutions (Fig. 1).

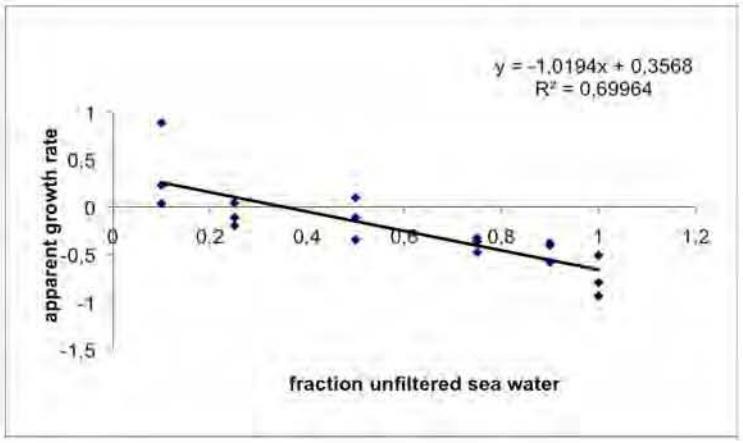


Fig. 1: Standard dilution plot between the apparent growth rate and the fraction of undiluted water

2. Primary production

Global primary production ($gC \cdot m^{-2} \cdot d^{-1}$) and chlorophyll *a* ($g \cdot m^{-2}$) values were obtained from the estimation done by Longhurst (1995) based on satellite radiometer data for all the oceans. Variation of primary production and chlorophyll *a* across the major latitudinal bands (only accounting oceanic areas) were represented.

3. Sampling

The sampling was carried out between January 2005 to June 2007 around Gran Canaria Island. We sampled during three periods. The first one between January 2005 to October 2005 and involved 6 fixed stations. The second period was from October 2005 to June 2006 and involved 5 stations. Finally, the last period was a compilation of sampling period from January 2005 to June 2007, but only taking into account one station, the one near the city of Las Palmas de Gran Canaria. The general procedure followed to analyze the planktonic different components was:

- Chlorophyll was measured fluorometrically on a Turner Designs bench fluorometer 10A, previously calibrated with pure chlorophyll *a* (Sigma-Aldrich Inc., St. Louis, MO, USA; Yentsch and Menzel 1963). Depth profiles of *in situ* fluorescence were converted to chlorophyll from a fluorescence versus chlorophyll relationship.

- Heterotrophic prokaryotes (HP), small photosynthetic eukaryotic cells (APE), *Prochlorococcus* (Pro) and *Synechococcus* (Syn) type cyanobacteria, were counted by flow cytometry using a FACScalibur instrument (Becton and Dickinson).

- Autotrophic (ANF) and heterotrophic (HNF) nanoflagellates 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.

- Microplankton organisms were counted and biomass estimated by (1) in a first time using a FlowCAM and (2) in a second time using the Utermöhl light microscopy technique.

- Zooplankton was caught (1) in oblique hauls using a Bongo net of 40 cm diameter and samples were fractionated with a 1 mm mesh and both size fractions were used to quantify mesozooplankton biomass as dry weight, following Lovegrove (1966) method, and (2) using a 100 µm-mesh WP-2 net (UNESCO 1968). Two vertical hauls were made and one of the samples was fractionated into size categories of 100-200, 200-500, 500-1000 and > 1000 µm by passing through sieves of mesh size of 100, 200, 500 and 1000 µm. Size-fractionated samples were then frozen in liquid nitrogen for later analysis. The second sample was preserved in formaldehyde (4%) and divided in the laboratory with a Folsom plankton splitter for dry weight measurements. The samples frozen in liquid nitrogen were homogenized, and subsamples were taken for

protein and gut fluorescence analysis. Biomass of each size fraction was also determined as protein content using the method of Lowry et al. (1951).

CHAPTER I

Global impact of microzooplankton grazing on primary production and latitudinal variability of algal growth

Claire Schmoker, Santiago Hernández-León

Abstract

Global primary production was reviewed and compared with phytoplankton growth and mortality by grazing rates obtained with the dilution technique. Latitudinal distributions of algal growth, mortality and microzooplankton respiration were also reviewed. The proportion of primary production grazed by microzooplankton was assessed by the dilution experiment data and was quite constant along latitudes. The primary production grazed was also estimated from respiration rates, assuming that all the food ingested was respired. We found that grazing was also constant along latitudes. Our results show that grazing rates of microzooplankton estimated by the dilution technique are probably not underestimated but that in the natural environment, microzooplankton should live under low food concentration leading to low metabolic conditions.

Introduction

The biomass and growth of phytoplankton are key parameters in order to study the primary productivity of the oceans. Growth of algal cells in natural sea water can be measured by several techniques; e.g. ^{14}C tracer technique, measurement of the global sea-surface chlorophyll field by satellite imagery, or the microzooplankton growth rate with the dilution method. Each technique has its advantages and limitations. However, simultaneous estimations of phytoplankton growth and mortality by microzooplankton grazing rates are restricted to the dilution method. A comparative study of those methods is needed to evaluate the use of growth and grazing results from the dilution method when investigating food webs in oceanic waters.

Microplanktonic community (20-200 µm organisms) includes autotrophs, heterotrophs, as well as mixotrophs and constitutes a significant portion of total

zooplankton biomass in aquatic environments (Sherr and Sherr 2000). The microplanktonic community is an important regulator of bacterial and phytoplankton productions (Fenchel 1982, McManus and Fuhrmann 1988) as well as a significant remineralization pathway in the euphotic zone (Goldman et al. 1987, Sherr and Sherr 2000). On the other hand, microscopic examinations of consumer gut contents, feeding structures and faecal material reveal that a large number of invertebrate and fish larvae consume microzooplankton (Stoecker and Capuzzo 1990). This positions microzooplankton as a trophic intermediate between the small bacteria, nanoplankton and the larger meso- and macrozooplankton (Gifford 1988).

In both eutrophic and oligotrophic pelagic ecosystems, microzooplankton is responsible of the major consumption of primary production (Sherr and Sherr 2002, Calbet and Landry 2004). Many experiments based on the dilution method (Landry and Hasset 1982) were carried out in various regions of the world ocean and provided simultaneous estimates of both rates of phytoplankton growth (μ) and grazing impact (m) by microzooplankton (e.g. Burkill et al. 1995, First et al. 2007). However, this approach also has methodological constrains. Results can sometimes be uninterpretable or the plot of apparent growth against the dilution factors cannot yield a significant regression (Landry et al. 1995, Murrell and Hollibaugh 1998). The regression analysis is done commonly by using a small number of values (12-15 bottles) which makes the slight slopes, or low grazing rates, difficult to detect and can give non-significant results. Moreover, grazing pressure could not be linearly related to dilution factor. This is because consumption rates of microzooplankton can differ in the different dilution treatments (Gallegos 1989, Evans and Paranjape 1992) and because grazer concentration could be non-linear to the dilution factor throughout time (Dolan et al. 2000). The combination of grazer mortalities in diluted water with phytoplankton growth in undiluted treatments could result in an over-estimation of the grazing rate (Gallegos 1989).

Nevertheless, dilution experiment results from the last two decades showed that grazing by microzooplankton on phytoplankton was quite invariant, ranging between 59 and 74% of phytoplankton primary production across systems despite its seasonality, trophic status, latitude or salinity (Calbet and Landry 2004).

Metabolism represents the sum of two fundamental and complementary processes: growth and respiration. While the amount of studies on marine productivity (including growth rates for autotrophs and heterotrophs) is high, studies on microzooplankton respiration are scarce (Verity 1985, Stoecker and Michaels 1991). However, respiration is the key index for energy consumption at a given time and place (Pomeroy and Johannes, 1968), and respiration estimations are essential for making comparisons with other whole-system processes, such as integrated primary production.

The main goal of the present work was to review and compare the estimations of global primary production in the ocean with the phytoplanktonic growth rates obtained by the dilution technique. Secondly, we studied the latitudinal variation in phytoplankton growth rates and mortality by microzooplankton grazing rates. The results showed that most of the global primary production in the ocean seemed to be grazed by microzooplankton, varying from a moderate proportion in polar zones to a high percentage in equatorial areas.

Material and methods

Primary production

Global primary production ($gC \cdot m^{-2} \cdot d^{-1}$) and chlorophyll *a* (Chl *a*) ($g \cdot m^{-2}$) values were obtained from the estimation done by Longhurst (1995) based on satellite radiometer data for all the oceans. The variability of primary production and Chl *a* across the major latitudinal bands (only accounting oceanic areas) were represented.

Phytoplankton carbon content is difficult to assess in the oceans but was necessary to our further calculations. Instead of carbon content, Chl *a* concentration is routinely measured and also commonly used as proxy of phytoplanktonic biomass. As a result, a carbon-to-chlorophyll ratio (C:Chl) is used to convert measured Chl *a* to phytoplankton carbon (e.g. Marañon et al. 2007, Pérez et al. 2007). The problem with using such conversion factor is the variable relationship between Chl *a* concentration and phytoplankton carbon content. In pure cultures of phytoplankton, C:Chl can vary between 10 and 100 (Cullen 1982, Geider 1993). In the open ocean, C:Chl values higher than 100 have been frequently reported in the surface zone (Buck et al. 1996). In this work, we used a C:Chl factor of 50 (Harris 1986).

Dilution method

The dilution technique has been broadly applied in aquatic ecosystems to estimate phytoplankton growth rates and microzooplankton grazing impact on phytoplankton. The method is based on three assumptions (Landry and Hasset 1982): (1) Growth of individual phytoplankton is not directly affected by the presence or absence of other phytoplankton *per se*. The implication of this assumption is that a reduction in the density of cells in natural seawater will not cause direct change in the growth rate of the remaining cells. (2) The probability of a phytoplankton cell to be consumed is a direct function of the rate of encounter of consumers with prey cells. This implies that consumers are not food-satiated at natural prey densities and that the number of prey ingested by a given consumer is linearly related to prey density. (3) Changes in the density of phytoplankton (P) over time can be represented appropriately by the exponential equation $P_t = P_0 e^{(\mu-m)t}$ where μ and m are instantaneous coefficients of population growth and grazing mortality, respectively and t is the incubation time. Phytoplankton growth and microzooplankton grazing rates are determined as the y-intercept and the slope of the regression lines, respectively, of a series of dilutions.

The database used in this work consisted of a set of 1267 corresponding values (Table 1 in Annexe 1), including the whole dataset of the 788 experiments analyzed by Calbet and Landry (2004) plus those studies published since 2004. Negative growth rate estimates were corrected to 0.01. Negative rate estimates for microzooplankton grazing were equalled to zero. Average values and error plots were used to investigate possible relationships between phytoplankton growth and mortality rates and the latitudinal variation. The m:μ ratio was taken as a reasonable estimation of the proportion of primary production grazed by microzooplankton. To reduce the impact of large ratios from the individual experiments and to get a more normal distribution of the data, we transformed the ratios to their arctangent values. The percentage of primary production grazed by microzooplankton was calculated as the invert function, tangent(x) (Calbet and Landry 2004).

Ciliate respiration rates

Assuming a Q_{10} = 2.5 (Caron et al. 1990), we calculated the theoretical ciliate respiration at different temperatures T_2 (Ikeda 1985) following the equation of Van't Hoff $Q_{10} = (R_2/R_1)^{10/(T1-T2)}$ where R_1 is the respiration rate of the ciliate *Tetrahymena* at different respiration rates (R_1 from 0.02 to 0.12 nl $O_2 \cdot cell^{-1} \cdot h^{-1}$; Finlay et al. 1983) at 20°C (T_1) and R_2 is the ciliate respiration rate at different temperatures T_2. Ciliate respiration rate R_2 in d^{-1} was calculated by converting body volume to carbon units using the factor experimentally derived for Lugol's-fixed marine oligotrichs from Putt and Stoecker (1989). We calculated the primary production grazed (%PP = m'/μ') by ciliates assuming that all the food ingested was respired (R = m') and calculating phytoplankton growth rates (μ') from primary production and phytoplanktonic biomass data estimated by Longhurst (1995). Finally, assuming a growth net efficiency of 0.2 (NGE as μ/m = 0.2; Schmoker et al. subm.) and assimilation efficiency of 0.76 (AE as A/I = 0.76 for a median or low food level), we estimated the ingestion (I = R = m) from

the respiration. In that way, we could calculate in a third way, the percentage of primary production grazed by microzooplankton.

Results

The variation of global primary production obtained from the estimation done by Longhurst (1995) across the latitudinal bands (only accounting oceanic areas) showed high values in the polar zones and low to moderate values in the equatorial, subtropical and temperate areas. Phytoplanktonic biomass latitudinal distribution followed the same pattern (Fig. 1).

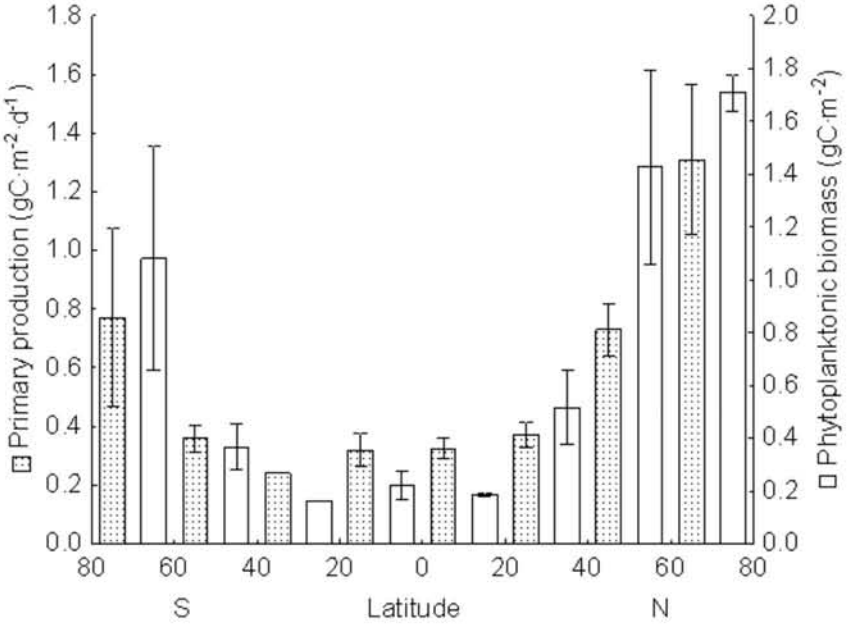


Fig. 1: Average values and error plots of primary production and phytoplanktonic biomass by latitude ranges. Primary production values are from literature. Phytoplanktonic biomass is obtained by multiplying chlorophyll *a* literature values by a C:Chl *a* ratio of 50

Most of the individual dilution experiments were done in the northern hemisphere (from latitudes between 20°N and 60°N, Fig. 2).

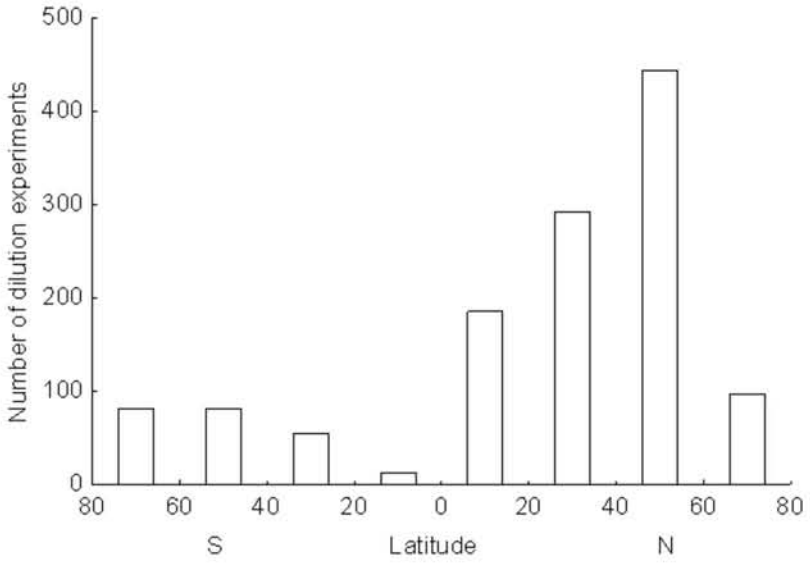


Fig. 2: Number of data from dilution experiments by latitude ranges

Phytoplankton growth and mortality rates among coastal, estuarine and oceanic zones were significantly different (One-way ANOVA, $p < 0.001$). However, as the objective of this work was to study the latitudinal variation, growth and mortality rates were studied as a function of latitude, without taking into account the different zones. Phytoplankton growth rates were lowest in polar zones and highest in the temperate zone of the northern hemisphere (Fig. 3A). Phytoplankton mortality rates showed a clear latitudinal variation with higher values at low latitudes decreasing towards both polar zones (Fig. 3B).

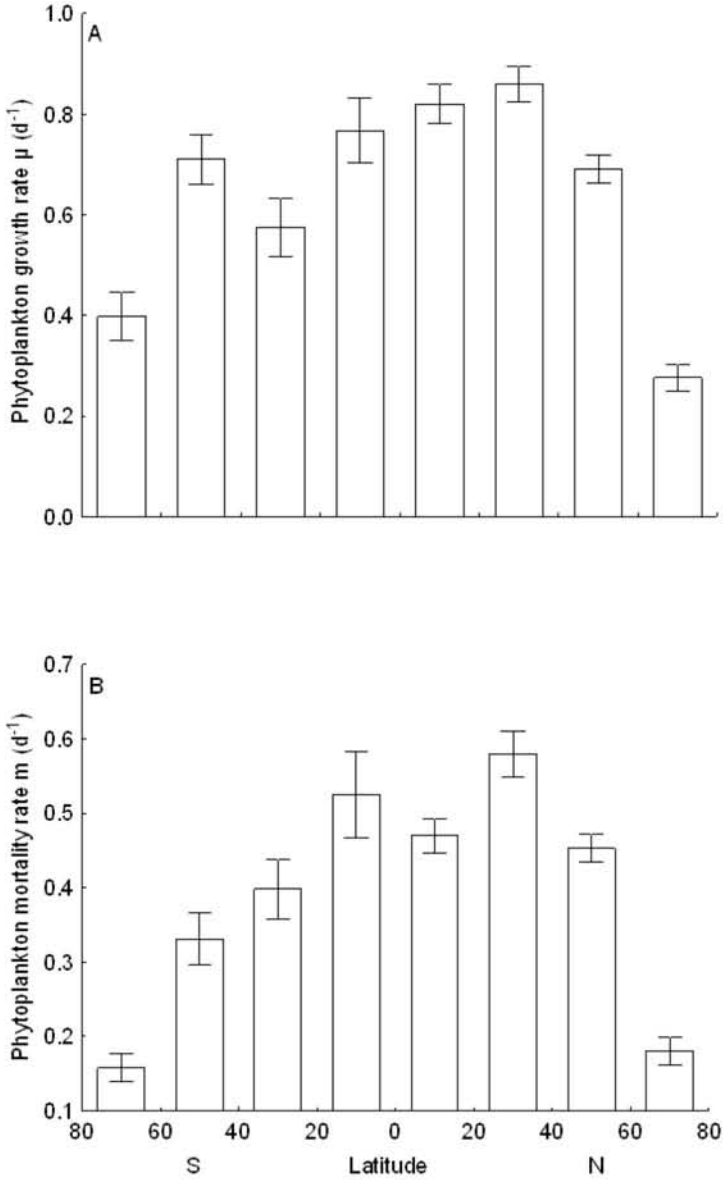


Fig. 3: Average values and error plots of (A) phytoplankton growth μ and of (B) phytoplankton mortality m rates by microzooplankton grazing from dilution experiments by latitude ranges

The percentage of primary production grazed (m:μ ratio) varied from 41 ± 9% to 68 ± 1% throughout the different latitudinal bands. Higher values occurred in tropical and subtropical zones while lowest values were found at high latitudes (Fig. 4A). Primary production grazed by microzooplankton assessed by dilution experiments in the oceanic zone was compared to the global estimation by Longhurst (1995). We observed that half of the total primary production was grazed in the different latitudinal zones of the southern hemisphere. However, this proportion increased with increasing latitudes and in northern temperate and polar zones, 75 to 88% of primary production

was grazed by microzooplankton (Fig. 4B). The same pattern was observed when we used the grazing rates estimated from assimilation efficiency and the primary production grazed varying from 61 to 92 ± 6% (Fig. 4C).

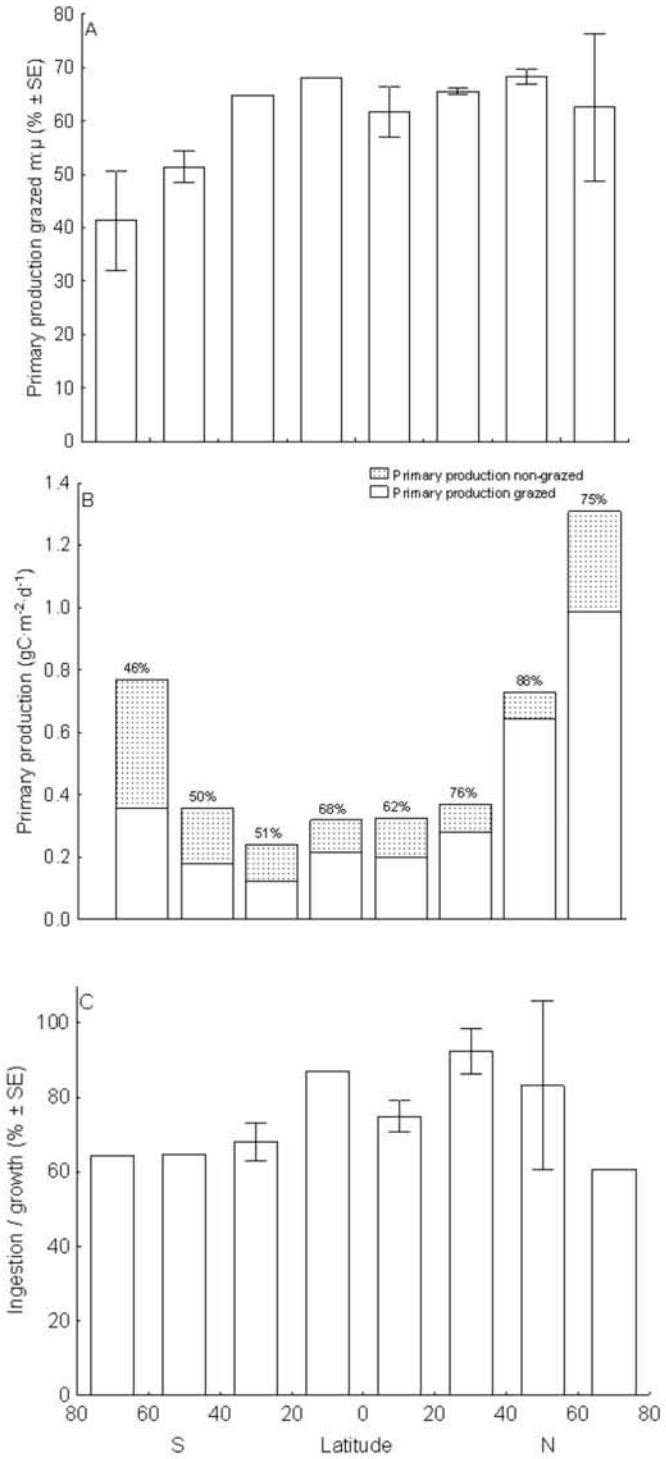


Fig. 4: Average values and error plots of (A) primary production grazed by microzooplankton as m:μ ratio from dilution experiment results, (B) primary production grazed and non-grazed by microzooplankton by latitude ranges and, (C) primary production grazed by microzooplankton assuming that grazing is the sum of assimilation and defecation by latitude ranges

As expected, higher respiration rates for tropical microzooplankton and lower rates in polar areas were observed (Fig. 5A). The proportion of phytoplankton mortality by grazing estimated from ciliate respiration rates varied from 37% to 56 ± 4% of ciliate respiration rates. They were quite constant along latitudes (Fig. 5B).

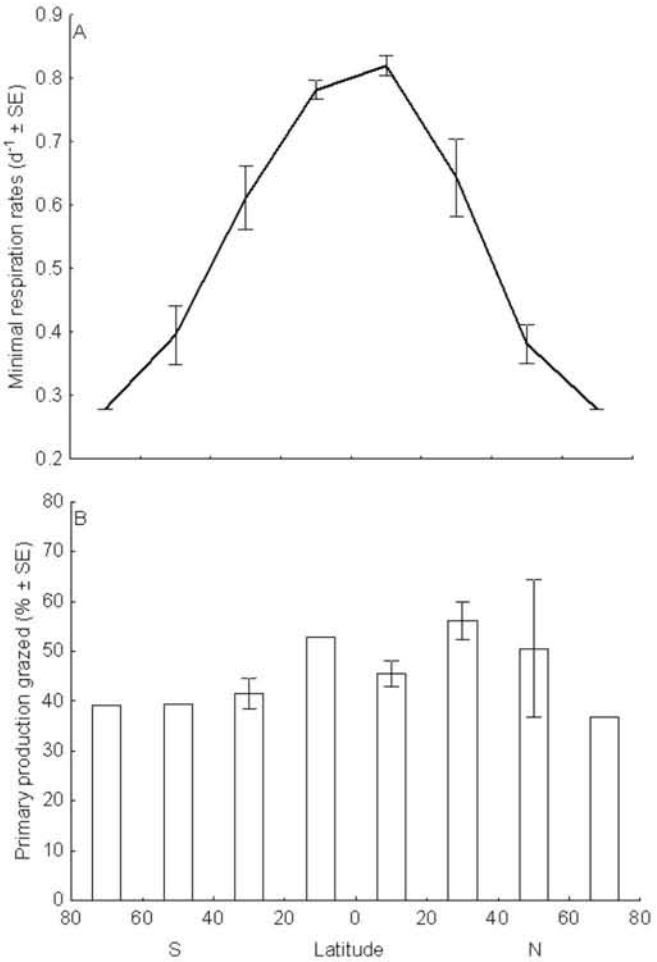


Fig. 5: Average values and plots of (A) theoretical ciliate respiration rates and, (B) primary production grazed by microzooplankton assuming that all the food ingested is respired (R = m')

Discussion

The latitudinal description of planktonic communities in the overall ocean gives information on the species tolerance to temperature and light as well as on different interactions between the ecological groups and the control of herbivores over primary producers. Nutrient supply has a direct influence on phytoplankton but its effect is secondary on zooplankton distribution.

Polar and subpolar regions are well known to be highly productive and to have high phytoplankton biomass caused by mixing of cold deep waters enriched with nutrients and surface waters during winter. Primary production was estimated at 0.77 ± 0.32 $gC \cdot m^{-2} \cdot d^{-1}$ in the Southern Ocean and at 1.31 ± 0.27 $gC \cdot m^{-2} \cdot d^{-1}$ in the northern polar region. North Atlantic and Pacific regions were also productive and phytoplankton biomass reached values of 1.42 ± 0.39 $gC \cdot m^{-2}$. Warm waters and low nutrient availability characterize tropical waters. Subtropical gyres are considered as the most oligotrophic areas of the oceans and showed the lowest values of primary production and phytoplankton biomass.

It is interesting to note that phytoplankton mortality rates obtained with the dilution method vary inversely to global primary production obtained from the estimation of Longhurst (1995). Results showed that microzooplankton grazing in polar regions is lower than in other regions of the oceans as also shown by Calbet and Landry (2004). Thus, this fact allows phytoplankton biomass and primary production to remain high. The low grazing could be due to lower microzooplankton abundance at high latitudes (Taniguchi 1984 in Zeitzschel 1990).

The percentage of primary production grazed by microzooplankton was rather constant along latitudes. Our estimations varied in the same ranges as those given by Calbet and Landry (2004). Hence, addition of new dilution experiments data does not change the m:μ ratio, even if some experiments were performed in estuarine zones and in the Mediterranean Sea. During the absence of new inputs of nutrients into the euphotic zone as it is the case in low productive areas, losses should be close to or slightly higher than production. This is congruent with results obtained comparing primary production grazed by microzooplankton assessed by dilution experiments and the global primary production estimated by Longhurst (1995). In this estimation, half of the primary production was grazed by microzooplankton in the Southern Ocean area,

while two third of the primary production was grazed by microzooplankton in the subtropical gyres. This observation could be explained by lower importance of mesozooplankton in these areas (Hernández-León and Ikeda 2005) promoting high microzooplankton abundance. Because microzooplankton has a rapid growth and high specific ingestion, they might be able to control phytoplankton-standing stocks since subtropical gyres show low values in zooplankton biomass. However, dilution experiments were mainly performed in the northern area of the oceans. The apparent disproportion in the availability of data between southern and northern hemispheres could explain the apparent disagreement between results of both polar zones.

From an energetic perspective, respiration represents the portion of energy contained in the ingested food, which is not egested or used for growth. Fenchel and Finlay (1983) showed that metabolic and growth rates of protozoa vary proportionally. These authors also showed that there is a positive correlation between protozoan respiration and cell size, thus, a larger individual biomass of organisms will consume more oxygen per unit of time. On the other hand, all biological processes are affected by temperature. Verity (1985) also showed that respiration rates of tintinnids increased with increasing temperature as well as Rassoulzadegan (1982) did it for the naked-ciliate *Lohmanniella spiralis*. Higher respiration rates for tropical microzooplankton are due to a combined effect of warm water temperatures and smaller body size of individuals, which are both parameters increasing the respiration rates. Similarly, low rates in polar areas are the combined result of cold temperatures and large body sizes.

An interesting result of the present work is the constant values of the percentage of primary production grazed by microzooplankton estimated by dilution experiments. This feature has been thought doubtfully by some authors (e.g. Dolan and McKeon 2005). Using a minimal respiration rate (in starvation) of an organism similar in size to the most frequent microzooplankton, we also found a low latitudinal variability of primary production grazed by microplankton. Grazing rates of cultured microplanktonic

organisms are much higher than field rates (Hansen 1992, Strom 1991; see Chapter II). Some dinoflagellates can double their biovolume when food is available in high concentration (Strom 1991). Our results show that grazing rates of microzooplankton estimated by the dilution technique are probably not underestimated but that in natural environment, microzooplankton should live under low food concentration leading to low metabolic conditions. Microzooplankton is rather plastic and is quite efficiently adapted to exploit patches of food, but this seems to be rather the exception to the rule.

Nevertheless, the reliability of the analysis depends on several parameters. First, the dataset size of the estimation of global primary production is greater than that of dilution experiments. Secondly, global primary production values correspond to overall year data on vertical profiles grouped by province as described and defined in Longhurst (1995), while dilution data were obtained for a punctual date, to a given depth and at a precise location. The robustness of the analysis depends then on the precision of the dataset and on the calculations made with them. Considering phytoplanktonic biomass latitudinal variation, we assume a constant C:Chl conversion factor for all the latitudinal ranges, which could imply an underestimation of phytoplanktonic biomass in polar zones and in productive areas.

In summary, comparison of global primary production in the oceans and the phytoplankton growth rate obtained by the dilution technique made in this study provides coherent results. More productive areas are observed at high latitudes, which are also the zones of lowest phytoplankton growth and microzooplankton grazing. Subtropical gyre regions are the less productive regions but also the areas where grazing by microzooplankton is higher. The combination of both processes promotes that most of the primary production is grazed by microzooplankton in these regions. The constancy of values of control by microzooplankton on primary production is replicable using different data sets and different approaches (e.g. respiration).

Therefore, those constant values should not be a criticism to the dilution method. Finally, microzooplankton probably lives under low metabolic conditions in the whole ocean suggesting that their success highly depends on their encounter rates with food patches.

Acknowledgements

This study was funded by the projects "ConAfrica" (CTM2004-02319) and "Lucifer" (CTM2008-03538) from the Ministry of Science and Innovation of Spain and from PhD fellowship from the Government of Canary Islands, Spain to C.S.

References

Buck KR, Chavez FP, Campbell L (1996) Basin-wide distributions of living carbon components and the inverted trophic pyramid of the central gyre of the North Atlantic Ocean, summer 1993. Aquat Microb Ecol 10: 283-298

Burkill PH, Edwards ES, Sleigh MA (1995) Microzooplankton and their role in controlling phytoplankton growth in the marginal ice zone of the Bellingshausen Sea. Deep-Sea Res II 42: 1277-1290

Calbet A, Landry MR (2004) Phytoplankton growth, microzooplankton grazing, and carbon cycling in marine systems. Limnol Oceanogr 49: 51-57

Cullen JJ (1982) The deep chlorophyll maximum: comparing vertical profiles of chlorophyll *a*. Can J Fish Aquat Sci 39: 791-803

Dolan JR, Gallegos CL, Moigis A (2000) Dilution effects on microzooplankton in dilution grazing experiments. Mar Ecol Prog Ser 200: 127-139

Evans GT, Paranjape MA (1992) Precision of estimates of phytoplankton growth and microzooplankton grazing when the functional response grazers may be nonlinear. Mar Ecol Prog Ser 80: 285-290

Fenchel TM (1982) The ecology of heterotrophic microflagellates. IV. Quantitive occurrence and importance as bacterial consumers. Mar Ecol Prog Ser 9: 35-42

Fenchel T, Finlay BJ (1983) Respiration Rates in Heterotrophic, Free-living Protozoa. Microb Ecol 9: 99-122

Fileman E, Burkill P (2001) The herbivorous impact of microzooplankton during two short-term Lagrangian experiments off the NW coast of Galicia in summer 1998. Prog Oceanogr 51: 361-383

First MR, Lavtentyev PJ, Jochem FJ (2007) Patterns of microzooplankton growth in dilution experiments across a trophic gradient: Implications for herbivory studies. Mar Biol 151: 1929-1940

Gallegos CL (1989) Microzooplancton grazing on phytoplankton in the Rhode River, Maryland: nonlinear feeding kinetics. Mar Ecol Prog Ser 57: 23-33

Gallegos CL, Vant WN (1996) An incubation procedure for estimating carbon-to-chlorophyll ratios and growth-irradiance relationships of estuarine phytoplankton. Mar Ecol Prog Ser 138: 275-291

Geider RJ (1993) Quantitative phytoplankton physiology: implications for primary production and phytoplankton growth. ICES Marine Science Symposium 197: 52-62

Gifford DJ (1988) Impact of grazing by microzooplankton in the Northwest Arm of Halifax Harbour, Nova Scotia. Mar Ecol Prog Ser 47: 249-258

Goldman JC, Caron DA, Andersen OK (1987) Nutrient cycling in a microflagellate food chain. I. Nitrogen dynamics. Mar Ecol Prog Ser 38: 75-87

Hansen PJ (1992) Prey size selection, feeding rates and growth dynamics of heterotrophic dinoflagellates with special emphasis on *Gyrodinium spirale*. Mar Biol 114: 327-334

Heinbokel JF (1978a) Studies on the functional role of tintinnids in the Southern California Bight. 1. Grazing and growth rates in laboratory cultures. Mar Biol 47: 177-189

Heinbokel JF (1978b) Studies on the functional role of tintinnids in the Southern California Bight. 2. Grazing rates of field populations. Mar Biol 47: 191-197

Hernández-León S, Ikeda T (2005) A global assessment of mesozooplankton respiration in the ocean. J Plankton Res 27: 153-158

Ikeda T (1985) Metabolic rates of epipelagic marine zooplankton as a function of body mass and temperature. Mar Biol 85: 1-11

Landry MR, Hasset RP (1982) Estimating the Grazing Impact of Marine Microzooplankton. Mar Biol 67: 283-288

Landry MR, Constantinou J, Kirshtein J (1995) Microzooplankton grazing in the central equatorial Pacific during February and August, 1992. Deep-Sea Res II 42: 657-671

Latasa M, Morán XAG, Scharek R, Estrada M (2007) Estimating the carbon flux through main phytoplankton groups in the northwestern Mediterranean. Limnol Oceanogr 50: 1447-1458

Lessard EJ, Murrel MC (1998) Microzooplankton herbivory and phytoplankton growth in the northwestern Sargasso Sea. Aquat Microb Ecol 16: 173-188

Longhurst A (1995) An estimate of global primary production in the ocean from satellite radiometer data. J Plankton Res 17: 1245-1271

Marañón E, Pérez V, Fernández E, Anadón R, Bode A, González N, Huskin I, Isla A, Morán XAG, Mouriño B, Quevedo M, Robienson C, Serret P, Teira E, Varela MM, Woodward EMS, Zubkov MV (2007) Planktonic budget in the eastern subtropical North Atlantic. Aquat Microb Ecol 48: 261-275

McManus GB, Fuhrmann JA (1988) Control of marine bacterioplankton populations: measurement and significance of grazing. Hydrobiologia 159: 51-62

Murrell MC, Hollibaugh JT (1998) Microzooplankton grazing in the northern San Francisco Bay measured by the dilution method. Aquat Microb Ecol 15: 53-63

Pérez V, Fernández E, Marañón E, Morán XAG, Zubkov MV (2007) Vertical distribution of phytoplankton biomass, production and growth in the Atlantic subtropical gyres. Deep-sea Res I 53: 1616-1634

Pomeroy LR, Johannes RE (1968) Occurrence and respiration of ultraplankton in the upper 500 meters of the ocean. Deep-sea Res 15: 381 - 391

Putt M, Stoecker DK (1989) An experimentally determined carbon-volume ratio for marine oligotrichous ciliates from estuarine and coastal waters. Limnol Oceanogr 34: 1097-1103

Rassoulzadegan F (1982) Dependance of grazing rate, gross growth efficiency, and food size range on temperature in a pelagic oligotrichous ciliate *Lohmanniella spiralis* Leed., fed on naturally occurring particulate matter. Ann Inst Oceanogr Paris 58: 177-184

Redden AM, Sanderson BG, Rissik D (2002) Extending the analysis of the dilution method to obtain the phytoplankton concentration at which microzooplankton grazing becomes satured. Mar Ecol Prog Ser 226: 27-33

Sherr EB, Sherr BF (2000) Marine microbes-an overview In Kirchmann DL (ed), Microbial Ecology of the Oceans, John Wiley A & Sons, Inc, Publication, New York, pp. 13-46

Sherr EB, Sherr BF (2002). Significance of predation by protests in aquatic microbial food webs. Antoine Leewenhoek Int J Gen Mol Microbiol 81: 293-308

Stelfox-Widdicombe CE, Edwards ES, Burkill PH, Sleigh MA (2004) Microzooplankton grazing activity in the temperate and sub-tropical NE Atlantic: summer 1996. Mar Ecol Prog Ser 208: 1-12

Stoecker DK, Capuzzo JM (1990) Predation on protozoa: its importance to zooplankton. J Plankton Res 12: 891-908

Stoecker DK, Michaels E (1991) Respiration, photosynthetis and carbon metabolism in planktonic ciliates. Mar Biol 108: 441-447

Stoecker DK, Gifford DJ, Putt M (1994) Preservation of marine planktonic ciliates: losses and cell shrinkage during fixation. Mar Ecol Prog Ser 110: 293-299

Strom SL (1991) Growth and grazing rates of the herbivorous dinoflagellate *Gymnodinium sp*. from the open subarctic Pacific Ocean. Mar Ecol Prog Ser 78: 103-113

Taniguchi K (1984) Radiolaria: sinking population, standing stock, and production rate. Mar Micropaleontol 8: 171-181

Taylor AH, Geider RJ, Gilbert FJH (1997) Seasonal and latitudinal dependencies of phytoplankton carbon-to-chlorophyll *a* ratios: Results of a modelling study. Mar Ecol Prog Ser 152: 51-66

Verity PG (1982) Effects of temperature, irradiance, and day length on the marine diatom *Leptocylindrus danicus Cleve*. 4. Growth. J Exp Mar Biol Ecol 60: 209-222

Verity PG (1985) Grazing, Respiration, Excretion, and Growth Rates of Tintinnids. Limnol Oceanogr 30: 1268-1282

Zeitzschel B (1990) Zoogeography of Marine Protozoa: An overview Emphasizing Distribution of Planktonic Forms. In Capriulo GM (ed) Ecology of Marine Protozoa. Oxford University Press, New-York, pp. 139-186

CHAPTER II

Feeding, growth and metabolism of a marine heterotrophic dinoflagellate, *Gyrodinium dominans*, in steady and non-steady state conditions

Claire Schmoker, Peter Thor, Santiago Hernández-León, Benni Winding Hansen

Abstract

Heterotrophic dinoflagellates are ubiquitous marine protozoans, which constitute an often significant component of the phagotrophic protists in the microplankton size class. Rates of grazing, growth, and respiration were studied in the heterotrophic dinoflagellate *Gyrodinium dominans* growing under steady state (constant food concentration) and non-steady state (one single pulse of food) conditions. Maximal specific growth rates of non-steady state *G. dominans* were similar to those of *G. dominans* acclimated to a constant food level, 0.01-0.06 h^{-1}. Thus, our results support the hypothesis that *G. dominans* growth responds quickly to changes in prey abundance. Specific respiration rates of non-steady state organisms were constant over time at 0.08 ± 0.01 h^{-1} and de-coupled from growth rates suggesting that non-steady state conditions are energy demanding.

Introduction

It is now well recognized that microzooplankton, of which ciliates and heterotrophic dinoflagellates constitute the bulk biomass, are the major consumers of pelagic primary production (Calbet & Landry 2004). The microplanktonic community is an important regulator of both bacterial and phytoplankton production (Fenchel 1982, McManus & Fuhrman 1988), and they have an important role in the remineralization process in the euphotic zone (Goldman et al. 1985, Sherr & Sherr 2000). Moreover, microscopic examinations of consumer gut contents, feeding structures and faecal material reveal that a large number of invertebrate and fish larvae consume microzooplankton (Stoecker & Capuzzo 1990). Thus, microzooplankton acts as a trophic intermediate between small bacteria, nanoplankton and the larger meso- and macrozooplankton (Gifford 1988). While ecological information about heterotrophic dinoflagellates is now increasing (e.g. Hansen 1991, Strom 1991, Hansen 1992, Nakamura et al. 1992,

Jacobson & Anderson 1993, Strom & Buskey 1993, Verity et al. 1993), studies of this group in relation to abundance, succession, grazing and growth are still sparse in comparison to ciliates, which have been studied extensively (e.g. Verity 1985, Bernard & Rassoulzadegan 1990, Verity 1991). However, the abundance and biomass of heterotrophic dinoflagellates are comparable to or in some case exceed these of ciliates in many planktonic ecosystems (Hansen 1991, Lessard 1991), and several studies emphasize the ecological importance of heterotrophic dinoflagellates as microheterotrophic grazers (Lessard & Swift 1985, Hansen 1991, Strom 1991, Bjørnsen & Kuparinen 1991, Sherr & Sherr 2008). However, there is still very little quantitative information on the ecological function of heterotrophic dinoflagellates in plankton communities. These organisms are raptorial feeders capable of feeding on prey items of their own size (Jacobson & Anderson 1986, Hansen 1992). Small heterotrophic dinoflagellates (5-20 µm) feed mainly on nanoflagellates and thereby compete with planktonic ciliates for preys (Strom 1991, Bjørnsen & Kuparinen 1991, Hansen 1992, Nakamura et al. 1992, Nakamura et al. 1995). However, small dinoflagellates are within the prey size range of their ciliate competitors. Thus, while small dinoflagellates may be competitors with ciliates for food, they are also potential prey for the ciliates. In addition to their role as herbivores in marine systems, heterotrophic dinoflagellates serve as a quantitatively and qualitatively significant food resource for mesozooplankton (Stoecker & Capuzzo 1990, Kleppel 1993, Suzuki et al. 1999, Levinsen et al. 2000, Vincent & Hartmann 2001, Liu et al. 2005, Leising et al. 2005a, Leising et al. 2005b, Olson et al. 2006).

Due to their quantitative importance, knowledge of feeding, growth and metabolism, is of importance in order to understand the functioning of heterotrophic dinoflagellates and their role in the flow of energy in the pelagic ecosystem. A substantial amount of literature about feeding and growth of this group of microzooplankton exists (e.g. Strom 1991, Hansen 1992, Jeong et al. 2005). However, there is a particular lack in our

knowledge of the metabolism of this community (Verity 1985, Stoecker & Michaels 1991). Respiration is considered the key index for energy consumption by consumers (Pomeroy & Johannes 1968) and its estimation is essential for making comparisons with other whole-system processes, such as integrated primary production. Verity (1985) studied grazing, growth, excretion and respiration on two tintinnid ciliate species. Grazing and growth rates have been studied in *Gyrodinium* but unfortunately these did not include respiration rates (Hansen 1992, Nakamura et al. 1992, Nakamura et al. 1995).

Contrary to earlier belief, the pelagic realm is not homogenous (Andersen & Sørensen 1986, Owen 1989, Franks 1995) and steady state conditions are rarely found in nature and, if ever, only in short time slots. However, most laboratory experiments are carried out under steady state conditions. Microzooplankton grazers experience variations in prey abundances constantly and their metabolism may shift and adapt accordingly. In the present study we compared grazing, growth and respiration rates in *Gyrodinium dominans* growing under steady state (constant food concentration) and non-steady state (one single pulse of food) conditions. The results show that specific growth rates of non-steady state and steady state organisms were similar. Moreover, even if food availability is fluctuating over time, respiration rates were rather constant over time.

Materials and methods

Culture of algae and heterotrophic dinoflagellates

A stock culture of exponentially growing *Gyrodinium dominans* was kept on a plankton wheel (1 rpm rotation speed) at high concentration levels (3000 cell ml^{-1}) at 17ºC in a 24-hour light period. The organisms were fed every 24h with excess

Rhodomonas salina, which were maintained in exponential growth on B1 media (Hansen 1992), also at 17ºC in a 24-hour light period and at gentle oxygenization.

Abundances and sizes of both *G. dominans* and *R. salina* were measured using an electronic particle counter (Coulter Multisizer M3, USA). Cell volumes were calculated from the particle diameter values output from the particle counter assuming a spherical cell shape. The carbon contents were calculated applying the equation M = 0.295V + 0.850, where M is mass in pgC and V is volume in μm^3. The parameters of this equation were calculated as averages from several different studies on the carbon content of microorganisms (Strathmann 1967, Moal et al. 1987, Verity et al. 1992, Montagnes et al. 1994). Biomasses of *G. dominans* and *R. salina* were determined by multiplying abundances given by the particle counter by cell carbon content calculated as described above.

Steady state experiments

To obtain steady state energetics, *G. dominans* were acclimated for 24 hours to 5 different *R. salina* concentrations prior to incubations. The five *R. salina* concentrations were 2500, 5000, 10000, 20000, and 40000 cells ml^{-1}. The organisms were then incubated for 48 hours at these concentrations in four bottles (500 ml) holding *G. dominans* and four control bottles (500 ml) holding *R. salina* only. Initial concentration of *G. dominans* was 470 ± 205 cells ml^{-1}. Prey and predator abundances and cell sizes were measured as above after 24 and 48h.

Specific growth rates of *G. dominans* were calculated as:

$$\mu = \frac{M_2 - M_1}{\overline{M} \cdot t},$$

where M_1 and M_2 are biomasses of *G. dominans* at the beginning and at the end of each sampling interval, respectively, $\overline{M}$ is the average *G. dominans* biomass during the sampling interval and t is the sampling time interval (h).

Specific grazing rates were calculated as:

$$I = \frac{-k_{din} + \mu_{control}}{\overline{M} \cdot t},$$

where k_{din} is calculated as the difference in *R. salina* biomass (µgC l^{-1}) in bottles containing *G. dominans* grazers before and after the incubation period and $\mu_{control}$ is the difference in *R. salina* biomass before and after the incubation period in control bottles without the heterotrophic dinoflagellates. Gross growth efficiency was calculated as μ/I.

Specific grazing and growth rates were fitted to a modified Hollings type II functional response model:

$$R = R_0 + R_{\max}\left(\frac{C_p}{K_m + C_p}\right),$$

where R is growth or grazing rate, R_0 is growth rate at zero prey concentration (only used in the growth functional response), R_{max} = maximum specific growth or grazing rate; C_p = prey concentration; K_m = prey concentration at $R_{max}/2$.

Non-steady state experiments

G. dominans were deprived of prey 24 hours prior to the 3 non-steady state experiments. Experiments started with the addition of a known concentration of *R. salina*. Three different initial prey and predator concentrations were used (Table 1).

Table 1: Non-steady state experiments with the heterotrophic dinoflagellate *Gyrodinium dominans*. Average values (± SD) of initial abundances (A) (cells l^{-1}) and biomasses (B) (µgC l^{-1}) of the food algae *Rhodomonas salina* and *Gyrodinium dominans* in experiments 1-3

	A		B	
	R. salina	*G. dominans*	*R. salina*	*G. dominans*
Experiment 1	33565 ± 3634	2775 ± 362	1309 ± 142	608 ± 41
Experiment 2	41676 ± 281	2427 ± 527	1625 ± 11	599 ± 148
Experiment 3	40354 ± 660	1367 ± 163	1574 ± 26	232 ± 20

Initial prey:predator abundance ratio was set at 12:1 in experiment 1, 18:1 in experiment 2 and 30:1 in experiment 3. Initial *G. dominans* concentrations were higher than what is observed in the field (Nakamura et al. 1992, Nakamura et al. 1995) but concentrations were chosen to facilitate reliable subsequent measurements of respiration. Four experimental bottles (500 ml) and 4 control bottles (500 ml) containing only prey (in order to measure prey growth) were prepared and kept on the plankton wheel for 48 hours. Measurements were taken every 2 hours during the first 24 hours and every 4 to 6 hours for the remainder of the experiments. Each bottle was sampled 3 times and average concentrations and cell sizes calculated. For comparison with steady state conditions in *G. dominans*, specific growth rates (µgC µgC^{-1} h^{-1}) were calculated as averages over 6 to 8 hours.

Respiration rates of *G. dominans* were measured at every sampling event in experiments 2 and 3. Oxygen consumption was monitored continuously in a 750 µl sample enclosed in a chamber in which an oxygen microelectrode (OX 25) was injected (Unisense A/S, Denmark). Oxygen concentrations were collected by a computer every 30 s during 2 hours. Specific respiration rates (R, µgC µgC^{-1} h^{-1}) were computed from the slope of the decrease in oxygen concentration in the chamber during the incubation:

$$R = \frac{12V(\alpha - \alpha_{control})}{\overline{M}},$$

where α is the slope of the decrease in oxygen tension (µM O_2 h^{-1}) in chambers with *G. dominans*, $\alpha_{control}$ is the slope of the decrease in control chambers holding only *R. salina*, V is volume of incubation chamber, 12 is a conversion factor from µM O_2 to µgC assuming a respiratory quotient of one, and $\overline{M}$ is the average carbon biomass of *G. dominans* during the 2-hour incubation period. Two hour periods were sufficiently short to avoid significant population changes during measurements but long enough to obtain a significant reduction in the oxygen concentration signal.

Results

Steady state experiments

Specific grazing rates of steady state *G. dominans* increased significantly with increasing algal concentrations (1-factor ANOVA: $F_{5,23} = 31.0$, $p < 0.001$) (Fig. 1A). The Holling type II functional response model fitted the relationship between grazing rate and prey concentration ($F_{1,23} = 27.9$, $p < 0.001$). Maximum grazing rate was 0.25 ± 0.02 µgC µgC^{-1} h^{-1} and the half saturation constant (K_m) was 48.3 ± 27.9 µgC l^{-1}. Specific growth rates increased significantly (1-factor ANOVA: $F_{5,23} = 7.64$, $p < 0.001$). The modified type II functional response model fitted the relationship between growth and prey concentration ($F_{2,23} = 11.5$, $p < 0.001$) and maximum growth rate was 0.05 ± 0.02 h^{-1}, whereas K_m was 715 ± 570 µgC l^{-1} (Fig. 1B). Gross growth efficiencies (GGEs) calculated on the 24-48 hour time interval ranged from 0.03 to 0.20, being the highest values at the highest prey concentrations (1400 µgC l^{-1}) (Fig. 1C). Specific grazing, growth rate and GGE calculated on the 0-24 hour time interval showed similar patterns (data not shown).

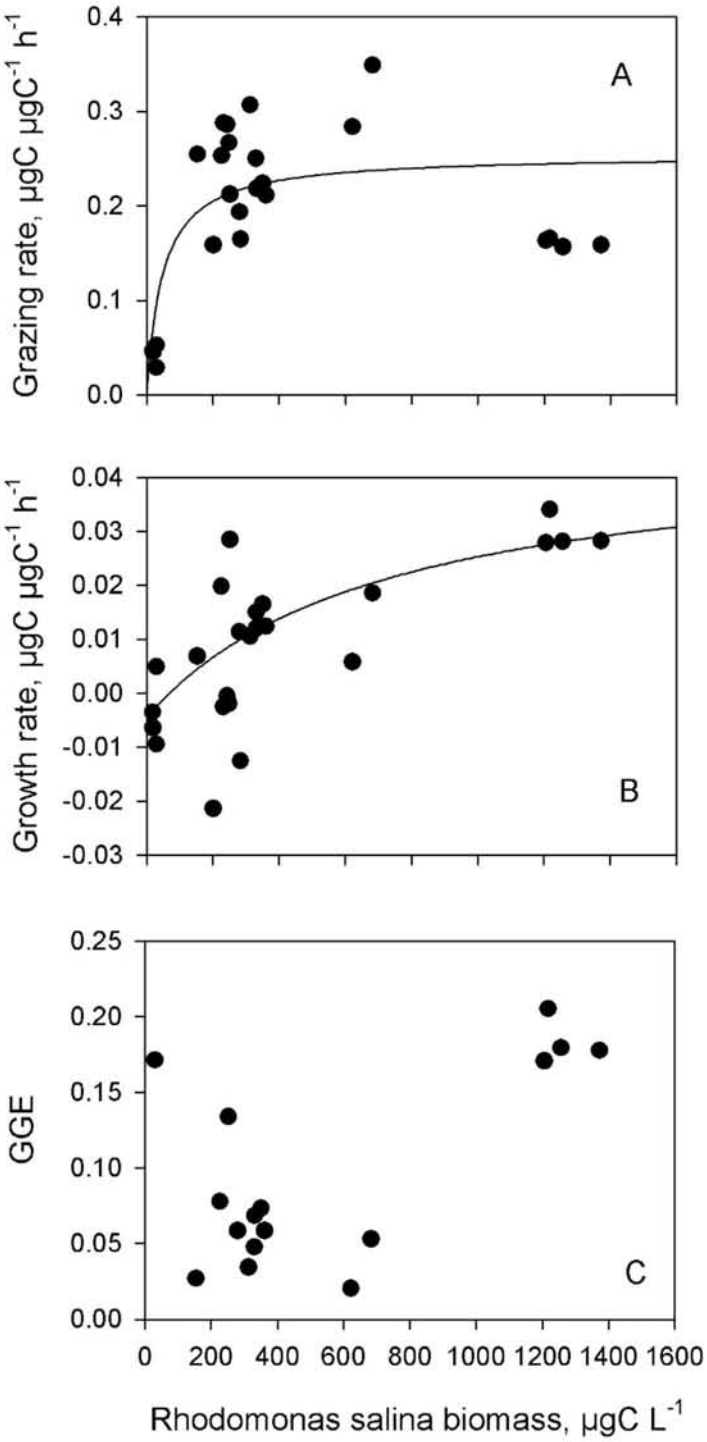


Fig. 1: Steady state *Gyrodinium dominans*. (A) Specific grazing rates on *Rhodomonas salina*. The line depicts the Holling type II functional response model fitted to the data. (B) Specific growth rate, and (C) Gross growth efficiency (GGE) calculated as specific growth rate/specific grazing rate

Non-steady state experiments

Abundance and biomass of *Rhodomonas salina* prey decreased during the incubation period in all 3 experiments (Fig. 2) so that *Gyrodinium dominans* experienced a pulse of prey with concentrations above 200 µgC l^{-1} for 16 hours in experiment 2, 28 hours in experiment 1, and 37 hours in experiment 3.

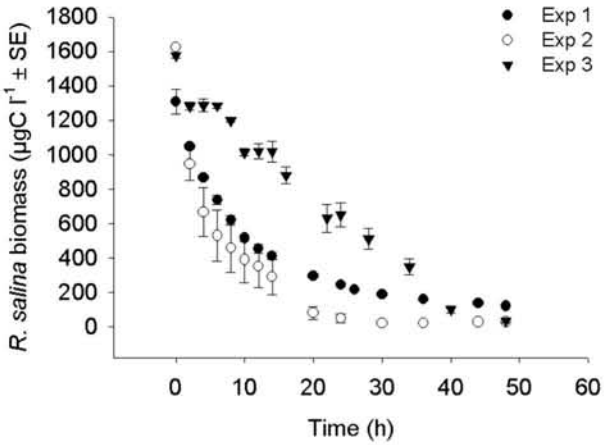


Fig. 2: Non-steady state experiments (exp 1-3). Time series of average values (±SE) of *Rhodomonas salina* abundance

After an initial decrease during the first 8-10 hours, abundances of *G. dominans* increased significantly in all three experiments (1-factor ANOVA: Exp 1: $F_{14,59} = 14.1$, $p < 0.001$, Exp 2: $F_{13,41} = 8.75$, $p < 0.001$, Exp 3: $F_{14,58} = 181$, $p < 0.001$) (Fig. 3).

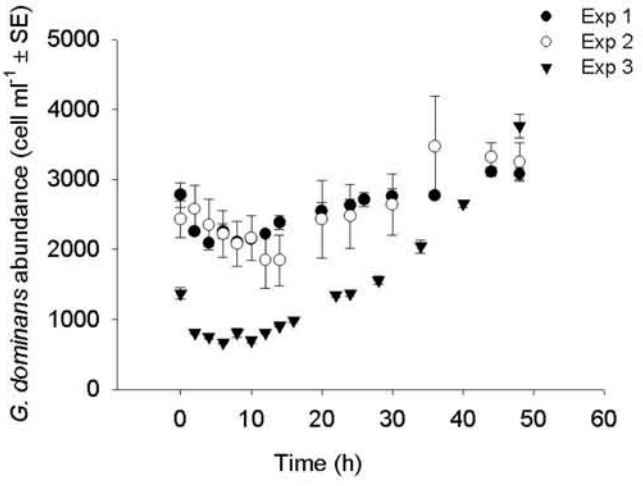


Fig. 3: Non-steady state experiments (exp 1-3). Time series of average values (±SE) of *Gyrodinium dominans* abundance

Concurrently, cell volume increased significantly during the first 8-10 hours (increase: 197% ± 67% [mean ± SD]) and returned to the initial volume towards the end when *R. salina* concentrations felt below 200 µgC l^{-1} (1-factor repeated measures ANOVA: Exp 1: $F_{14,59} = 38.2$, $p < 0.001$, Exp 2: $F_{13,55} = 18.8$, $p < 0.001$, Exp 3: $F_{14,59} = 13.2$, $p < 0.001$) (Fig. 4). Cell volumes increased with specific grazing rates calculated on 8-hour periods in experiments 1 and 2 and were correlated significantly in experiment 1 (linear regression: $r^2 = 0.382$, $p < 0.01$).

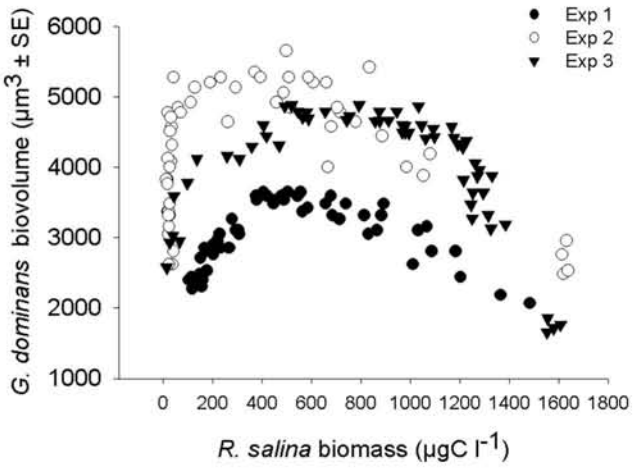


Fig. 4: Non-steady state experiments (exp 1-3). Average values (±SE) of *Gyrodinium dominans* biovolume as a function of *Rhodomonas salina* biomass

Prey:predator abundance ratios decreased directly from the beginning in experiments 1 and 2 whereas in experiment 3 it remained high during the 8 first hours and then decreased towards the end of the incubation period (Fig. 5). These differences gave rise to different and significant biomass increases among the three experiments (1- factor ANOVA among slopes from linear regressions on biomass versus time: $F_{2,43} = 46.26$, $p < 0.001$).

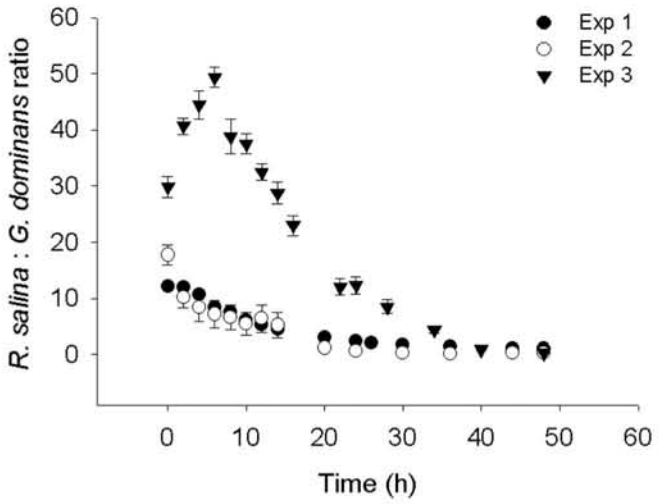


Fig. 5: Non-steady state experiments (exp 1-3). Time series of average values (±SE) of prey:predator abundance ratio

Specific growth rates varied from ~ -0.01 to ~ 0.04 h^{-1} in experiment 1, from ~ -0.03 to ~ 0.06 h^{-1} in experiment 2 and from ~ -0.04 to ~ 0.06 h^{-1} in experiment 3. (Fig.6). To test for differences in the relationships between growth rates and prey concentrations between steady state and non-steady state *G. dominans*, we compared calculated functional responses for non-steady state *G. dominans* growth with the functional responses of steady state *G. dominans* (Fig. 6). This test showed no significant differences in functional responses among *G. dominans* in the 5 incubations (3 non-steady state experiments and two periods in the steady state experiment; 0-24h and 24-48 h) (1-factor ANOVA on R_0, R_{max}, and K_m, $p > 0.85$).

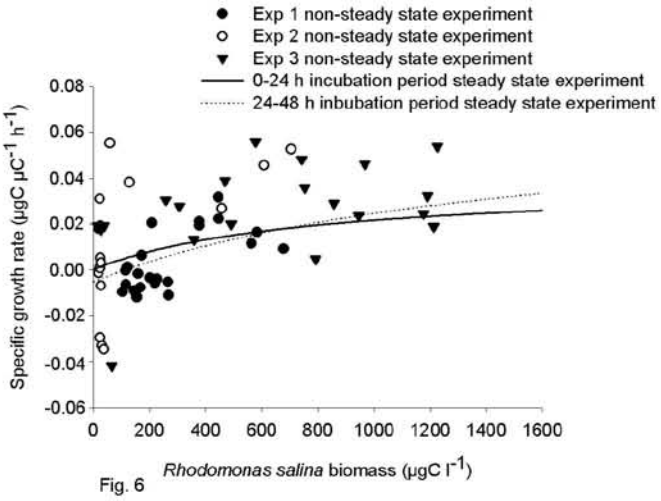


Fig. 6: Comparison of steady and non-steady state experiments on *Gyrodinium dominans* of specific growth rate as a function of *Rhodomonas salina* biomass

Specific respiration rates showed no pattern throughout the incubation periods but exhibited an average of 0.08 ± 0.01 h^{-1} for experiments 2 and 3 (Fig 7; linear regressions, slopes: $p > 0.05$ not shown on figure). In order to assess a preliminary energetic budget of *G. dominans*, we grouped the data in relation to food concentration: low (18 to 39 $\mu gC \cdot l^{-1}$), medium (469 to 656 $\mu gC \cdot l^{-1}$) and high values (874 to 1314 $\mu gC \cdot l^{-1}$, see Table 2). While growth and respiration rates were low and similar, ingestion rates increased more than 3-fold at high food levels. GGE was the highest at medium level of food whereas assimilation efficiency increased at low food levels.

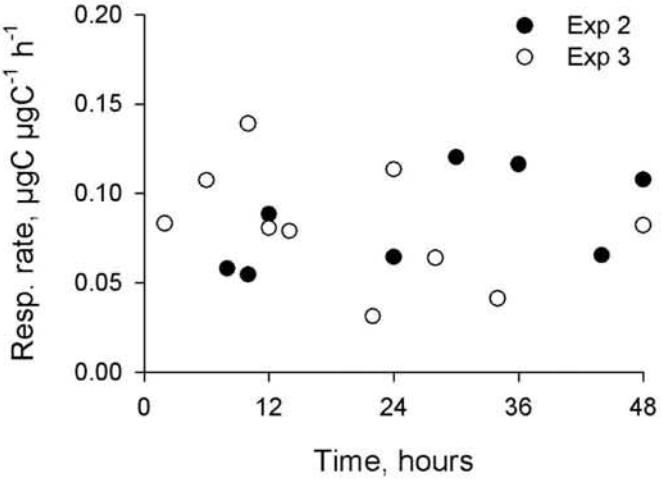


Fig. 7: Non-steady state experiments (exp 2-3). Specific respiration rate during the incubation period

Table 2: Energetic estimations of non-steady state of the heterotrophic dinoflagellate *Gyrodinium dominans* in experiment 2 and 3. B stands for the food algae *Rhodomonas salina* biomass (µgC l^{-1}), µ for specific growth rate (h^{-1}), R for specific respiration rate (h^{-1}), I for specific ingestion rate (h^{-1}), GGE (%) for gross growth efficiency, A for specific assimilation rate (h^{-1}), F for specific defecation rate (h^{-1}), and AE (%) for assimilation efficiency. GGE was calculated as µ/I*100, A as µ+R, F as I-A and AE as A/I*100

R. salina	B	µ	R	I	GGE (%)	A	F	AE (%)
High	1146 ± 237	0.03 ± 0.02	0.08 ± 0.00	0.42 ± 0.01	6 ± 6	0.11 ± 0.02	0.32 ± 0.04	25 ± 6
Medium	650 ± 160	0.03 ± 0.03	0.06 ± 0.03	0.12 ± 0.06	30 ± 13	0.08 ± 0.02	0.04 ± 0.05	76 ± 24
Low	24 ± 11	0.01 ± 0.01	0.10 ± 0.03	0.14 ± 0.02	17 ± 24	0.10 ± 0.02	0.04 ± 0.04	77 ± 27

Discussion

In natural heterogeneous pelagic environments protists are adapted to fluctuations in food availability by developing complex life cycles and by regulating their metabolism. Our main finding during these experiments is that we did not detect any differences in the patterns of specific growth rates of *Gyrodinium dominans* in steady and non-steady state conditions. This observation allows us to suggest that *G. dominans* growth responds quickly to changes in prey abundance. Heterotrophic dinoflagellates, as observed in our experiments, can track increases in phytoplankton biomass during bloom situations and may be able to control phytoplankton biomass

during e.g. the spring bloom far better than mesozooplankton grazers (e.g. Hansen 1991).

Specific growth rates of the heterotrophic dinoflagellate previously adapted to a constant algal concentration showed the typical logarithmic growth as a function of prey biomass. The maximal growth μ_{max} of 0.05 µgC µgC^{-1} h^{-1} is in accordance with data from the literature for this species or other heterotrophic dinoflagellates feeding on *R. salina* (Strom 1991, Hansen 1992, Jakobsen & Hansen 1997). At food concentrations below 200 µgC l^{-1}, specific growth rates of *G. dominans* became negative, suggesting that the energy needed for the organism maintenance is higher than the energy obtained by feeding. This is also observed in the relatively low GGE values reported here.

Non-steady state *G. dominans* experienced significant cell volume increases at the onset of feeding early in the incubations. Cell volumes were ~1.5 times lower during the first hours of the incubation period, when *R. salina* concentrations were maximal, than their maximal volume reached after 10 hours. In the present work, we found a significant relationship between predator cell volume and grazing rate. Accordingly, Strom (1991) observed a positive relationship between *Gymnodinium* cell volume and total phytoplankton ingestion and suggested that cell-volume changes in that organism may reflect the amount of food contained within grazer vacuoles at any given time. Therefore, these results support that average number of cells contained in a grazer should increase with food concentration until digestion.

In our time-course experiments, non-steady state *G. dominans* cell volume firstly increased. Secondly, below a given prey concentration, cell volume decreased concurrently with the increase in cell abundances. Logically, this may be a result of cell division. It seems that the onset of cell division may relate to some specific prey concentration since we observed decreased average cell volumes when *R. salina*

concentrations decreased below 400 µgC l^{-1} in all 3 experiments. *Gyrodinium* spp. has the ability to adopt a swarmer strategy when they experience starvation. They produce small and fast swimming individuals at low prey abundances (Hansen 1992). Moreover, at the highest food levels, ingestion rate was the highest while the mean growth rate was higher at medium food level than at the beginning and at the end of the experiments. These results also support the suggestion that *G. dominans* responds quickly to changes in prey abundance.

The GGE ratio is the fraction of prey carbon consumed converted to body mass and it gives an estimation of the success of an organism in converting ration to biomass. In a review on gross growth efficiency of different taxonomic groups from protozoan to metazoan, Straile (1997) concluded that mean and median values for all taxa scatter between 20 and 30%. In their review on zooplankton (scaling within the 2 – 2,000 µm body size range) grazing and growth, Hansen et al. (1997) arrive at 33 ± 3%. GGEs determined here for *G. dominans* are within the range of values reported for other heterotrophic protozoans (Caron & Goldman 1990). In ciliates, GGEs generally decrease with increasing algal concentration (Verity 1985, Jonsson 1986, Strom 1991). This means that success of an organism transferring phytoplankton carbon is higher when food is scarce. However, in our experiments with the heterotrophic dinoflagellate, we did not find this pattern.

Respiration in aquatic organisms gives a baseline of consumption in the marine environment. In heterotrophic organisms, excluding temperature and size, the level of feeding affects respiration rates. The so-called “specific dynamic action” (SDA) is the result of an elevated energy demand for the integrated physical and physiological process of feeding (Jobling 1983, Kiørboe et al. 1985, Brown & Cameron 1991). Accordingly, respiration rate is tightly coupled to both ingestion rate and growth rate in marine pelagic copepods (Thor 2000, Thor 2002, Thor et al. 2002). However, in our study, respiration rates of non-steady state *G. dominans* were not significantly coupled

to specific growth rates and remained fairly constant during the experiments. Moreover, specific respiration rates seemed to be higher than specific growth rates. We suggest that non-steady state life is quite energy demanding for metabolism because of the increased physiological investment during acclimatization activity. A striking result of our study is the high assimilation efficiency at the lower food levels (Table 2). This is similar to the findings of high efficiencies at low ingestion rates in copepods of (Gaudy 1974, Thor & Wendt 2010). In fact, these organisms show lower evacuation rates and high gut passage times at low food concentrations (Dagg and Walser 1987). This behavior allows better assimilation at low food and therefore an optimization of the low energy available.

In summary, we found that the heterotrophic dinoflagellate *Gyrodinium dominans* is able to obtain similar growth rates in steady state and in non-steady state experiments, suggesting that growth responds quickly to changes in prey abundance. We also gave an estimation of respiration rates of a heterotrophic dinoflagellate, which jointly with grazing and growth rate estimations allowed us to construct a preliminary metabolic budget for *G. dominans*.

Acknowledgments

The authors would like to thank H.H. Jakobsen for providing *G. dominans* cultures and support on experimental designs. This study was funded from the projects “ConAfrica” (CTM2004-02319) and “Lucifer” (CTM2008-03538) from the Ministry of Science and Innovation of Spain, from PhD fellowship to C. Schmoker from the Government of Canary Islands, Spain, from The Swedish Research Council for Environment, Agricultural sciences, and Spatial Planning to P. Thor (grant no. 2004-2484), and from The Danish National Science Research Council (grant no. 272-07-0485) to B.W. Hansen.

References

Andersen P, Sørensen HM (1986) Population dynamics and trophic coupling in pelagic microorganisms in eutrophic coastal waters. Mar Ecol Prog Ser 33: 99-109

Bernard C, Rassoulzadegan F (1990) Bacteria or microflagellates as a major food source for marine ciliates: possible implications for the microzooplankton. Mar Ecol Prog Ser 64: 147-155

Bjørnsen PK, Kuparinen J (1991) Growth and herbivory by heterotrophic dinoflagellates in the southern ocean, studied by microcosm experiments. Mar Biol 109: 397-405

Brown CR, Cameron JN (1991) The relationship between specific dynamic action (SDA) and protein synthesis rates in the Channel catfish. Physiol Zool 64: 298-309

Calbet A, Landry MR (2004) Phytoplankton growth, microzooplankton grazing, and carbon cycling in marine systems. Limnol Oceanogr 49: 51-57

Caron DA, Goldman JC, Fenchel T (1990) Protozoan respiration and metabolism. In: Capriulo GM (ed) Ecology of Marine Protozoa, Oxford University Press, pp. 307-322

Dagg MJ, Walser WEJr (1987) Ingestion, gut passage, and eggestion by the Copepod *Neocalanus plumchrus* in the laboratory and in the Subarctic Pacific Ocean. Limnol Oceanogr 32: 178-188

Fenchel T (1982) Ecology of heterotrophic microflagellates. IV. Quantitative occurrence and importance as bacterial consumers. Mar Ecol Prog Ser 9: 35-42

Franks PJS (1995) Thin layers of phytoplankton: a model of formation by near-interstitial wave shear. Deep-Sea Res 42: 75-91

Gaudy R (1974) Feeding four species of pelagic copepods under experimental conditions. Mar Biol 25: 125-141

Gifford DJ (1988) Impact of grazing by microzooplankton in the Northwest Arm of Halifax Harbor, Nova-Scotia. Mar Ecol Prog Ser 47: 249-258

Goldman JC, Caron DA, Andersen OK, Dennett MR (1985) Nutrient cycling in a microflagellate food chain: I. Nitrogen dynamics. Mar Ecol Prog Ser 24: 231-242

Hansen PJ (1991) Quatitative importance and trophic role of heterotrophic dinoflagellates in a coastal pelagial food web. Mar Ecol Prog Ser 73: 253-261

Hansen PJ (1992) Prey size selection, feeding rates and growth dynamics of heterotrophic dinoflagellates with special emphasis on *Gyrodinium spirale*. Mar Biol 114: 327-334

Hansen PJ, Bjørnsen PK, Hansen BW (1997) Zooplankton grazing and growth: scaling within the 2-2,000-µm body size range. Limnol Oceanogr 42: 687-704

Jacobson DM, Anderson DM (1986) Thecate heterotrophic dinoflagellates: feeding behaviour and mechanisms. J Phycol 22: 249-258

Jacobson DM, Anderson DM (1993) Growth and grazing rates of *Protoperidium hirobis abe*, a thecate heterotrophic dinoflagellate. J Plankton Res 15: 723-736

Jakobsen HH, Hansen PJ (1997) Prey size selection, grazing and growth response of the small heterotrophic dinoflagellate *Gymnodinium sp.* and the ciliate *Balanion comatum* – a comparative study. Mar Ecol Prog Ser 158: 75-86

Jeong HJ, Yoo DY, Seong KA, Kim JH, Park JY, Kim S, Lee SH, Ha JH, Yih WH (2005) Feeding by the mixotrophic red-tide dinoflagellate *Gonyaulax polygramma*: mechanisms, prey species, effects of prey concentration, and grazing impact. Aquat Microb Ecol 38: 249-257

Jobling M (1983) Towards an explanation of specific dynamic action. J Fish Biol 23: 549-555

Jobling M, Davies PS (1980) Effects of feeding on metabolic rate and the specific dynamic action in plaice *Pleuronectes platessa*. J Fish Biol 16: 629-638

Jonsson PR (1986) Particle size selection, feeding rates and growth dynamics of marine planktonic oligotrichous ciliates (Ciliophora Oligotrichina). Mar Ecol Prog Ser 33: 265-277

Kiørboe T, Møhlenberg F, Hamburger K (1985) Bioenergetics of the planktonic copepod *Acartia tonsa*: relation between feeding, egg production and respiration, and composition of specific dynamic action. Mar Ecol Prog Ser 26: 85-97

Kleppel GS (1993) On the diets of calanoid copepods. Mar Ecol Prog Ser 99: 191-198

Leising AW, Horner RA, Pierson JJ, Postel JR, Halsband-Lenk C (2005a) The balance between microzooplankton grazing and phytoplankton growth in a highly productive estuarine fjord. Prog Oceanogr 67: 366-383

Leising AW, Pierson JJ, Halsband-Lenk C, Horner RA, Postel JR (2005b) Copepod grazing during spring blooms: does *Calanus pacificus* avoid harmful diatoms? Prog Oceanogr 67: 384-405

Lessard EJ (1991) The trophic role of heterotrophic dinoflagellates in diverse marine environments. Mar Microb Foods Webs 5: 49-58

Lessard EJ, Swift E (1985) Species-specific grazing rates of heterotrophic dinoflagellates in oceanic waters, measured with a dual-label radioisotope technique. Mar Biol 87: 289-296

Levinsen H, Turner JT, Nielsen TG, Hansen BW (2000) On the trophic coupling between protists and copepods in arctic marine ecosystems. Mar Ecol Prog Ser 204: 65-77

Liu H, Dagg MJ, Strom SL (2005) Grazing by the calanoid copepod *Neocalanus cristatus* on the microbial foodweb in the coastal Gulf of Alaska. J Plankton Res 27: 647-662

Mcmanus GB, Fuhrman JA (1988) Control of marine bacterioplankton populations: measurement and significance of grazing. Hydrobiologia 159: 51-62

Moal J, MartinJezequel V, Harris RP, Samain JF, Poulet SA (1987) Interspecific and intraspecific variability of the chemical-composition of marine-phytoplankton. Oceanol Acta 10: 339-346

Montagnes DJS, Berges JA, Harrison PJ, Taylor JFR (1994) Estimating carbon, nitrogen, protein, and chlorophyll alpha from volume in marine phytoplankton. Limnol Oceanogr 39: 1044-1060

Nakamura Y, Yamazaki Y, Hiromi J (1992) Growth and grazing of a heterotrophic dinoflagellate, *Gyrodinium dominans*, feeding on a red tide flagellate, *Chattonella antiqua*. Mar Ecol Prog Ser 82: 275-279

Nakamura Y, Suzuki SY, Hiromi J (1995) Growth and grazing of a naked heterotrophic dinoflagellate *Gyrodinium dominans*. Aquat Microb Ecol 9: 157-164

Olson MB, Lessard EJ, Wong CHJ, Berhhardt MJ (2006) Copepod feeding selectivitity strongly influences the population ecology of the microplankton, including the toxigenic diatom, *Pseudo-nitzschia spp.*, in the coastal Pacific Northwest. Mar Ecol Prog Ser 326: 207-220

Owen RW (1989) Microscale and finescale variations of small plankton in coastal and pelagic environmnents. J Mar Res 47:197-240

Pomeroy LR, Johannes RE (1968) Occurrence and respiration of ultraplankton in the upper 500 meters of the ocean. Deep-Sea Res 15: 381-391

Sherr EB, Sherr BF (2000) Marine microbes - an overview. In Kirchmann DL (ed) Microbial Ecology of the Oceans. Wiley AJ & Sons, pp. 13-46

Sherr EB, Sherr BF (2007) Heterotrophic dinoflagellates: a significant component of microzooplankton biomass and major grazers of diatoms in the sea. Mar Ecol Prog Ser 352: 187-197

Stoecker DK, Capuzzo JM (1990) Predation on protozoa: its importance to zooplankton. J Plankton Res 12: 891-908

Stoecker DK, Michaels AE (1991) Respiration, photosynthesis and carbon metabolism in planktonic ciliates. Mar Biol 108: 441-447

Straile D (1997) Gross growth efficiencies of protozoan and metazoan zooplankton and their dependence on food concentration, predator-prey weight ratio, and taxonomic group. Limnol Oceanogr 42: 1375-1385

Strathmann RR (1967) Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. Limnol Oceanogr 12: 411-418

Strom SL (1991) Growth and grazing rates of the herbivorous dinoflagellate *Gymnodinium sp*. from the open subarctic Pacific Ocean. Mar Ecol Prog Ser 78: 103-113

Strom SL, Buskey EJ (1993) Feeding, growth, and behavior of the thecate heterotrophic dinoflagellate *Oblea rotunda*. Limnol Oceanogr 38: 965-977

Suzuki K, Nakamura Y, Hiromi J (1999) Feeding by the small calanoid copepod *Paracalanus sp*. on heterotrophic dinoflagellates and ciliates. Aquat Microb Ecol 17: 99-103

Thor P (2000) Relationship between Specific Dynamic Action and protein deposition in calanoid copepods. J Exp Mar Biol Ecol 24: 171-182

Thor P (2002) Specific dynamic action and carbon incorporation in *Calanus finmarchicus* copepodites and females. J Exp Mar Biol Ecol 272: 159-169

Thor P, Cervetto G, Besiktepe S, Ribera-Maycas E, Tang KW, Dam HG (2002) Influence of two different green algal diets on specific dynamic action and incorporation of carbon into biochemical fractions in the copepod *Acartia tonsa.* J Plankton Res 24: 293-300

Thor P, Wendt I (2010) Functional response of carbon absorption efficiency in the pelagic calanoid copepod *Acartia tonsa* Dana. Limnol Oceanogr (in press)

Verity P (1985) Grazing, Respiration, Excretion, and Growth Rates of Tintinnids. Limnol Oceanogr 30: 1268-1282

Verity PG (1991) Measurement and simulation of prey uptake by marine planktonic ciliates fed plastidic and aplastidic nanoplankton. Limnol Oceanogr 36: 729-750

Verity PG, Robertson CY, Tronzo CR, Andrews MG, Nelson JR, Sieracki ME (1992) Relationships between cell-volume and the carbon and nitrogen-content of marine photosynthetic nanoplankton. Limnol Oceanogr 37: 1434-1446

Verity PG, Stoecker DK, Sieracki ME, Burkill PH, Edwards ES, Tronzo CR (1993) Abundance, biomass and distribution of heterotrophic dinoflagellates during the North Atlantic spring bloom. Deep-Sea Res 40: 227-244

Vincent D, Hartmann HJ (2001) Contribution of ciliated microprotozoans and dinoflagellates to the diet of three copepod species in the Bay of Biscay. Hydrobiologia 443: 193-200

CHAPTER III

The pelagic food web during the late winter bloom in subtropical waters: Are there cascade effects?

Claire Schmoker, Javier Arístegui, Santiago Hernández-León

Abstract

Planktonic biomass was estimated in the Canary Islands waters in order to study the characteristic late winter bloom of subtropical waters. Weekly sampling was performed between January and August 2005 at 6 stations around Gran Canaria Island. The bloom observed in this study presented a succession of peaks of the different planktonic communities. The bloom began with the increase of primary producers in winter followed by consecutive increases of heterotrophic nanoflagellates, microplankton and mesozooplankton. During the period studied, mesozooplankton, picoplankton and bacteria showed similar trends, whereas nano- and microplankton depicted an inverse pattern. This was explained by the predation of mesozooplankton upon nano- and microplankton fractions and the fertilization promoted by mesozooplankton excretion, both factors increasing primary production and the efficiency of the food web to recycle the nutrient input during winter. Our results suggest the existence of a top-down trophic cascade control in the different plankton categories from mesozooplankton to bacteria. Thus, the late winter bloom in subtropical waters is much more complex than the simple increase in chlorophyll found during the minimum temperature recorded during the annual cycle.

Introduction

The marine zooplankton community is composed by a high variety of organisms with different feeding abilities, which exploit a wide diversity of food types available in the environment. Trophic interactions, direct or indirect, are important regulators of the structure of planktonic communities. Pelagic food webs are quite complex (Sanders and Wickham 1993; Kiøboe 1997) because of the interaction between organisms differing in lengths, feeding modes and predation abilities. Autotrophs, heterotrophs and mixotrophs are normally observed in a wide range of sizes and taxonomic groups.

The main predators of bacterioplankton and planktonic prokaryotes (picoplankton 0.2-2 µm) are small heterotrophic nanoflagellates, mainly in the range of 3-5 µm (Fenchel 1986; Sherr and Sherr 2002). Nanoplankton is at its turn consumed by microzooplankton (20-200 µm), mainly ciliates and dinoflagellates, which generally dominate this size fraction. Finally, mesozooplankton are efficient predators on planktonic ciliates (Calbet and Saiz 2005), being thus the connection between the microbial food web and the classical algae-predator-fish food chain in the marine environment (Pomeroy 1974). However, this rather complex food web is not well known in the warm oligotrophic subtropical waters, especially their temporal variability.

Subtropical waters are characterized by a quasi-permanent thermocline caused by a strong surface heating throughout the year. The thermocline restrains the pumping of nutrients from deeper waters to upper layers and limits phytoplankton growth. During February-March, the cooling of surface waters erodes the thermocline promoting an increase in nutrients (De León and Braun 1973; Braun 1980), and therefore primary production and chlorophyll concentration in the euphotic zone. This productive period is known as the 'late winter bloom' (Menzel and Ryther 1961). In April-May, the thermocline starts to reform, leading to the normal situation of a surface euphotic zone with reduced inorganic nutrients. Despite the ecological relevance of the process there are only few studies describing the changes in the planktonic community during the blooming season (Arístegui et al. 2001, Hernández-León et al. 2004, Arístegui and Montero 2005, Tsai et al. 2008). This fact contrasts with the amount of detailed studies on seasonal cycles in temperate regions (e.g. Sverdrup 1953; Purdie 1996; Broglio et al. 2004).

The waters surrounding the Canary Archipelago have been described as oligotrophic, with low variability in plankton biomass and productivity (De León and Braun 1973; Braun 1980). Mesozooplankton biomass evolves with a short lag respect

to chlorophyll (Arístegui et al. 2001; Hernández-León et al. 2004) and the control of diel vertical migrants on epipelagic mesozooplankton also promotes a succession of biomass peaks related to the lunar cycle (see Hernández-León et al. 2004). In these oligotrophic waters, most of the primary production is due to small autotrophic cells and most of the primary production seemed to be controlled by microzooplankton, as deduced from the proportionally low grazing exerted by mesozooplankton (Arístegui et al. 2001; Hernández-León et al. 2004). The latter feeds on a large proportion of non-pigmented microzooplanktonic organisms (Hernández-León et al. 2001, 2002, 2004), similar to the behavior of mesozooplankton in other warm water ecosystems (Dam 1995; Gaudy et al. 2003; Calbet and Saiz, 2005). However, the abundance and biomass of microzooplankton and its role in the development of the late winter bloom is almost unknown in these waters. Strikingly, Hernández-León (2009) observed that the increase in copepods during the bloom coincided with the increase in primary production and no lag was found between both increments. This author suggested that the consumption of microzooplankton by mesozooplankton released primary production, thus inducing a top-down effect on autotrophic and heterotrophic organisms. However, this hypothesis has not been yet tested. This trophic cascade effect could have important consequences for the flux of energy and matter in the pelagic realm as mesozooplankton would play an important role in structuring the epipelagic food web and, therefore, in the flux of carbon to the mesopelagic zone. The flow of matter through microzooplankton would promote recycling and respiration in the epipelagic zone and therefore low gravitational flux. In contrast, enhanced concentrations of mesozooplankton would control microzooplankton favoring the transport of carbon due to active flux by diel vertical migrants (Longhurst et al. 1989; Dam et al. 1995; see Hernández-León 2009) and sinking fecal pellets. Here we present results that reveal a striking interplay between bottom-up and top-down effects during

the development of the late winter bloom, showing the importance of large predators on the structure and function of plankton communities.

Materials and methods

Sampling was carried out weekly from January to August 2005 on board the RV Solana II. Six stations separated by 10 nautical miles were sampled around Gran Canaria Island (Fig. 1).

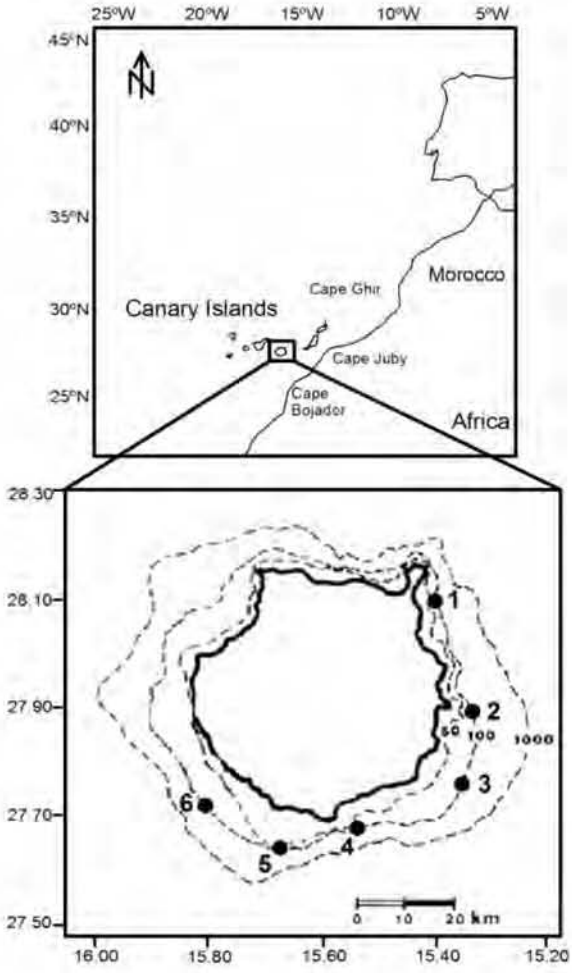


Fig. 1: Map of the study area, showing the location of the six sampling stations around Gran Canaria Island, Canary Islands (subtropical NE Atlantic)

The stations were located at the edge of the narrow island shelf along the isobath of 100 m depth. Vertical profiles of temperature, conductivity and fluorescence were obtained using a CTD SBE25 probe (Sea-Bird Electronics Inc., Bellevue, WA, USA). Samples for chlorophyll, bacterio- and microplankton were taken in the mixed layer at 15 m depth with a Niskin bottle.

Depth profiles of *in situ* fluorescence were converted to chlorophyll from a fluorescence versus chlorophyll relationship (Chl *a* = 0.292·fluorescence + 0.083; r^2 = 0.515, $p < 0.001$). Chlorophyll was measured fluorometrically on a Turner Designs bench fluorometer 10A, previously calibrated with pure chlorophyll *a* (Sigma-Aldrich Inc., St. Louis, MO, USA; Yentsch and Menzel 1963). Samples of 500 ml of sea water were filtered on board through 25 mm Whatman GF/F fiber glass filters and preserved in liquid nitrogen until their analysis. Pigments were extracted during 24 h in 10 ml of 90% acetone at 4°C in the dark. For the final determinations of chlorophyll *a,* the acetonic extracts were acidified allowing chlorophyll *a* and phaeopigments to be independently estimated.

Heterotrophic prokaryotes (HP), small photosynthetic eukaryotic cells (autotrophic picoeukaryotes, APE), *Prochlorococcus* (Pro) and *Synechococcus* (Syn) type cyanobacteria, were counted by flow cytometry using a FACScalibur instrument (Becton and Dickinson). Immediately after collection, samples were fixed with paraformaldehyde (2% final concentration), incubated for 30 min at 4°C and then stored frozen in liquid nitrogen until analysis. To count heterotrophic prokaryotes, 200 µl samples were stained with a DMS-diluted SYTO-13 (Molecular Probes Inc.) stock solution (10:1) at 2.5 µM final concentration. HP were identified by their signatures in a plot of side scatter (SSC) versus green fluorescence (FL1) (Gasol et al. 1999). The identification of small phytoplankton groups was based on interactive analyses of multiple bivariate scatter plots of side scatter, red and orange fluorescence. Samples

were run at low speed for HP and at medium or high speed for autotrophic picoplankton until 10,000 events were acquired or 2 minutes were passed. HP abundances were converted to biomass using a factor of 20 fgC $cell^{-1}$ (Cho and Azam 1990; Lee and Furhman 1987; Bode et al. 2001). Pro cell numbers were converted to biomass assuming a mean biovolume of 0.1 μm^3 $cell^{-1}$ (Sieracki et al. 1995), and a conversion factor of 220 fgC μm^{-3} (Christian and Karl 1994; Zubkov et al. 2000). Syn cell numbers were converted to biomass by using a conversion factor of 250 fgC $cell^{-1}$ (Kana and Glibert 1987; Li et al. 1992). APE abundances were transformed to biomass using a conversion factor of 2100 fgC $cell^{-1}$ (Campbell et al. 1997).

Samples for autotrophic (ANF) and heterotrophic (HNF) nanoflagellates (2-20 µm) were preserved following the procedure given by Haas (1982). Immediately after collection, the sample was fixed with glutaraldehyde (0.3% final concentration). The sample was placed into a filtration tower and fixed with diamidino-2-phenylindole for 5 minutes, and filtered onto a 0.2 µm black polycarbonate membrane filter, 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 20 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. ANF and HNF cell numbers were converted to biomass assuming a mean volume of 20 μm^3 $cell^{-1}$ for autotrophs, and of 10 μm^3 $cell^{-1}$ for heterotrophs and a conversion factor of 220 fgC μm^{-3} (Børsheim and Bratbak 1987).

Autotrophic biomass was calculated from the pico- and nanoplankton components (A) computed as the sum of the Syn, Pro, PE and ANF biomasses. The biomass of heterotrophs (H) was obtained by adding the HP and HNF biomasses.

Microplankton samples were preserved in acid Lugol's iodine (2% final concentration) and stored in the dark. Microplankton samples from stations 1, 3 and 5 were analysed, using a FlowCAM (Sieracki et al. 1998; Zarauz et al. 2009) to determine the biomass and size structure of this community. Fluorescence measurements were not included in the analysis; hence every particle (cells and detritus) was counted and imaged. For each sample, a maximum of either 2000 particles or 10 ml were analyzed. An x4 objective was used in the sample analysis. The instrument was calibrated using beads of a known size. Invalid recordings (i.e. bubbles, repeated images) were removed from the image database through visual recognition. The biovolume of each particle was calculated from its equivalent spherical diameter (ESD), and was converted to biomass according to the equation of Montagnes et al. (1994) for marine phytoplankton (Zarauz et al. 2009).

Zooplankton was caught in oblique hauls using a Bongo net of 40 cm diameter, equipped with 200 µm mesh size nets and a flowmeter (General Oceanics Inc., Miami, FL, USA) to measure the volume of water filtered. The net was towed at 2-3 knots from a maximum of 90 m up to surface. Sample was fractionated with a 1 mm mesh and both size fractions were used to quantify mesozooplankton biomass as dry weight, following the method of Lovegrove (1966).

Statistical analyses were performed by multiple regression analysis and Pearson´s correlation coefficients (r) were calculated in order to examine the relationships between the planktonic groups. Non-parametric Krustal-Wallis tests were run to assess differences between stations 1 to 6 in mesozoo-, nano-, pico- and bacterioplankton biomasses and between stations 1, 3 and 5 in microplankton biomass.

Results

During the period of study, temperature dropped below 18°C at the end of February promoting mixing and the largest peak in chlorophyll *a* probably due to the input of macronutrients as previously observed (e.g. De León and Braun, 1973; Braun, 1980; Arístegui et al. 2001) (Fig. 2). Other two smaller peaks were found before temperature decreased to 18°C. At the beginning of April, temperature increased reforming the thermocline.

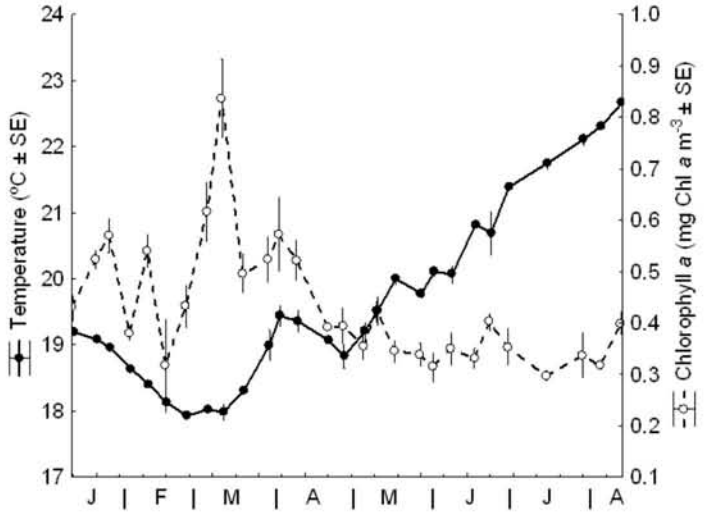


Fig. 2: Average values (± SE) of stations 1-6 for each sampling day of temperature (solid line) (°C) and of cholorophyll *a* (dotted line) (mg Chl a m^{-3}) in the mixed layer from January to August 2005

Non-significant statistical differences (K-W ANOVA, $p < 0.05$) were observed among the six oceanographic stations sampled for the different planktonic organisms except for mesozooplankton <1 mm size fraction, which were slightly higher to the south of the island.

Autotrophic picoplankton biomass was mainly composed by APE from January to May. Syn was also present from January to May peaking in March and April. However, Pro was present only from May to August coinciding with the highest surface temperatures. ANF showed the highest biomass at the beginning of January and remained low during the rest of the period studied. APE contributed for most of the total

autotrophic biomass from February to May (64% ± 16%), whereas ANF were dominant in January (47% ± 31%, Fig. 3D). HNF depicted an inverse pattern to APE (Fig. 3B), suggesting an alternate control of both groups by larger organisms. Microplankton (25-75 µm) showed a high variability with a succession of peaks from January to July (Fig. 3C). Its biomass showed rather high values due to the fact that the FlowCAM instrument counted all the particles (cells and detritus). Therefore, those values will be used only as a proxy for microplankton. The latter organisms and mesozooplankton also appeared to have an inverse pattern during the bloom (Fig. 3C). Increases in mesozooplankton were followed by lower values of microplankton, as observed in March and May. Strikingly, during March, April and May the patterns of mesozooplankton and autotrophic picoeukaryotic biomasses showed a similar trend (Fig. 3D). As expected, HP were present during the whole period of study and the pattern of mesozooplankton and bacterioplankton suggested a similar trend (Fig. 4). At each maximum in mesozooplankton biomass, corresponded a maximum in bacteria biomass.

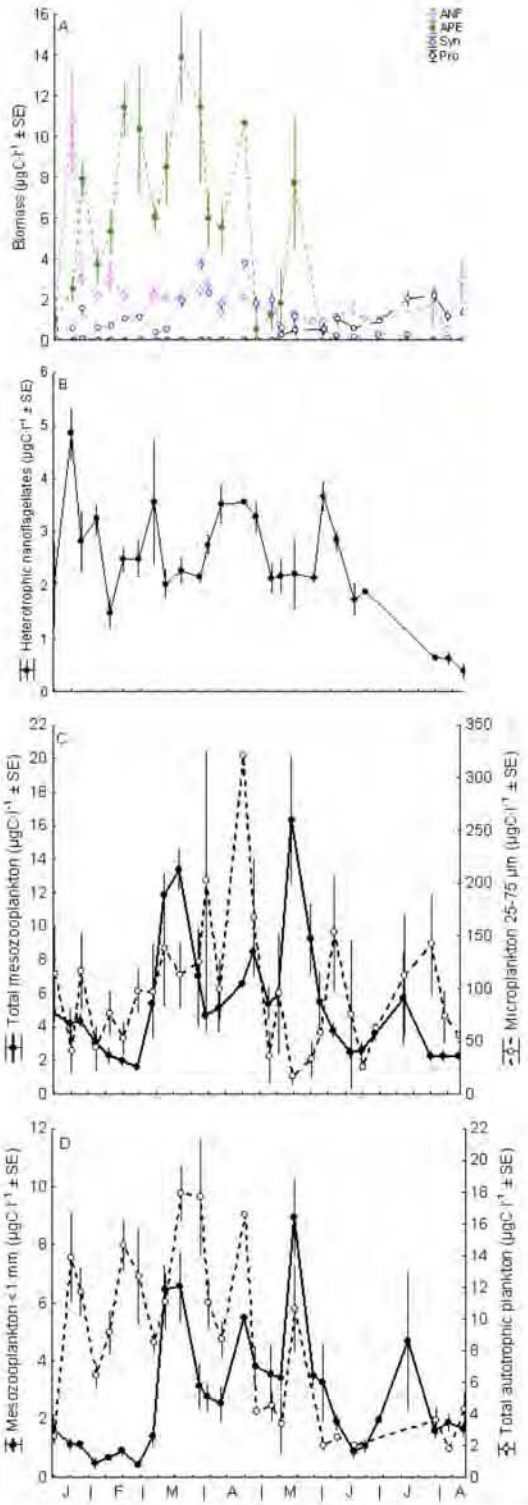


Fig. 3: Time-series from January to August 2005 of (A) *Prochlorococcus* (Pro) (black dotted line), *Synechococcus* (Syn) (blue dotted line), autotrophic picoeukaryote (APE) (green dotted line), autotrophic nanoflagellates (ANF) (pink dotted line) biomasses (µgC l^{-1}); (B) heterotrophic nanoflagellates biomass (µgC l^{-1}); (C) total mesozooplankton (solid line) and microplankton (25 - 75 µm) (dotted line) biomasses (µgC l^{-1}); (D) mesozooplankton <1 mm (solid line), and A (A is the sum of Pro, Syn APE and ANF biomasses) (dotted line) biomasses (µgC l^{-1})

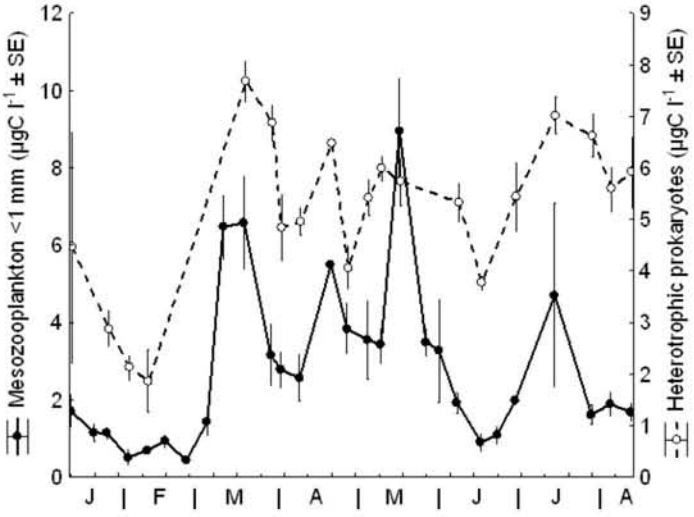


Fig. 4: Time-series from January to August 2005 of mesozooplankton <1mm (solid line) and heterotrophic prokaryotes (dotted line) biomasses (µgC l^{-1})

The most striking relationships were the ones observed between mesozooplankton <1 mm size fraction and heterotrophic prokaryote biomass, and between nanoflagellates and the different autotrophic picoplankton groups (Table 1). Autotrophic picoplankton also showed a significant correlation with chlorophyll *a*.

Tab.1: Pearson pairwise correlation coefficients (r) between chlorophyll *a* (Chl *a*), and biomasses of mesozooplankton (>1 and <1 mm) (MZP), heterotrophic (HNF) and autotrophic (ANF) nanoflagellates, *Prochlorococcus* (Pro), *Synechococcus* (Syn), autotrophic picoeukaryotes (APE), heterotrophic prokaryotes (HP) for data of 1-6 stations and biomasses of microplankton (Mic) for data at stations 1, 2 and 3. Bold numbers indicate significant correlations at *p < 0.05, **p < 0.01 and, ***p < 0.001

	Chl *a*	Temp.	MZP >1	MZP <1	Mic	ANF	HNF	APE	Syn	Pro
Temp.	**-0.493*****									
MZP >1	**0.034*****	**-0.412*****								
MZP <1	0.026	0.048	**0.423*****							
Mic	0.161	-0.138	-0.027	0.032						
ANF	0.352	**-0.338*****	0.107	-0.124	0.093					
HNF	0.066	**-0.256****	**0.262****	0.088	0.069	**0.314*****				
APE	**0.533*****	**-0.717*****	**0.398*****	0.055	0.017	**0.333*****	0.173			
Syn	**0.261****	**-0.447*****	**0.305*****	0.150	0.208	**0.227***	**0.329*****	**0.602*****		
Pro	**-0.554*****	**0.727*****	**-0.421*****	-0.076	-0.154	**-0.411*****	**-0.355*****	**-0.681*****	**-0.696*****	
HP	-0.083	0.170	-0.083	**0.392*****	0.165	-0.103	-0.133	-0.026	0.058	0.199

As expected, the total autotrophic biomass was the highest during winter months (Table 2), decreased in spring and was the lowest in summer with values 6 times higher in March than in June. On the other hand, total heterotrophic biomass was higher in summer than in early winter and was maximal in early spring (Table 2). The

ratio of autotrophic to heterotrophic biomass followed, thus, this seasonal variability being on average 8-fold lower during summer months than winter and early spring (Table 2).

Tab. 2: Mean values (± SE) of autotrophic (A) (A as the sum of Pro, Syn, APE and ANF biomasses) and heterotrophic (H) (H as the sum of HP and HNF biomasses) biomasses (µgC l^{-1}), and A:H ratios for the mixed layer from January to August 2005

	A	H	A:H
January	8.94 ± 1.82	5.86 ± 1.73	0.74 ± 0.22
February	11 ± 1.09	4.18 ± 0.70	2.86 ± 1.38
March	14 ± 1.48	9.48 ± 0.33	1.87 ± 0.20
April	9.20 ± 1.07	7.92 ± 0.36	1.05 ± 0.10
May	5.67 ± 1.28	7.87 ± 0.31	0.75 ± 0.19
June	2.29 ± 0.17	6.95 ± 0.64	0.35 ± 0.03
July	3.59 ± 0.95	6.70 ± 0.42	0.57 ± 0.19
August	3.18 ± 0.75	6.29 ± 0.39	0.59 ± 0.18

The relative biomass of each planktonic group showed that the bloom observed was composed by a succession of peaks (Fig. 5A). A pattern of autotrophic picoplankton (APic as sum of APE, Pro and Syn), HNF, microplankton (Mic) and mesozooplankton (MZP) successive peaks were observed during all the bloom. At least four cycles were observed. The first one (boldface APic, HNF, and MZP in Fig. 5A) started in February and was followed by a second cycle starting in March (italics). The third peak was observed in April (boldface) and the last one in May (italics). Interestingly, at the beginning of March and at the end of April and May, the increases in autotrophic picoplankton (APic) coincided with increases of mesozooplankton (MZP). Strikingly, peaks in MZP and APic showed a 30 days period matching the lunar cycle. Because these four cycles are rather difficult to follow, we standardized each cycle giving the values of 100 to each maximum value of APE, HNF, Mic and MZP, and by fitting the maximum value of APE to a fixed day (day 15). The common pattern of succession is observed by the increase of APE followed by HNF, Mic and MZP, the latter showing the maximum value coinciding with the peak of APE (Fig. 5B).

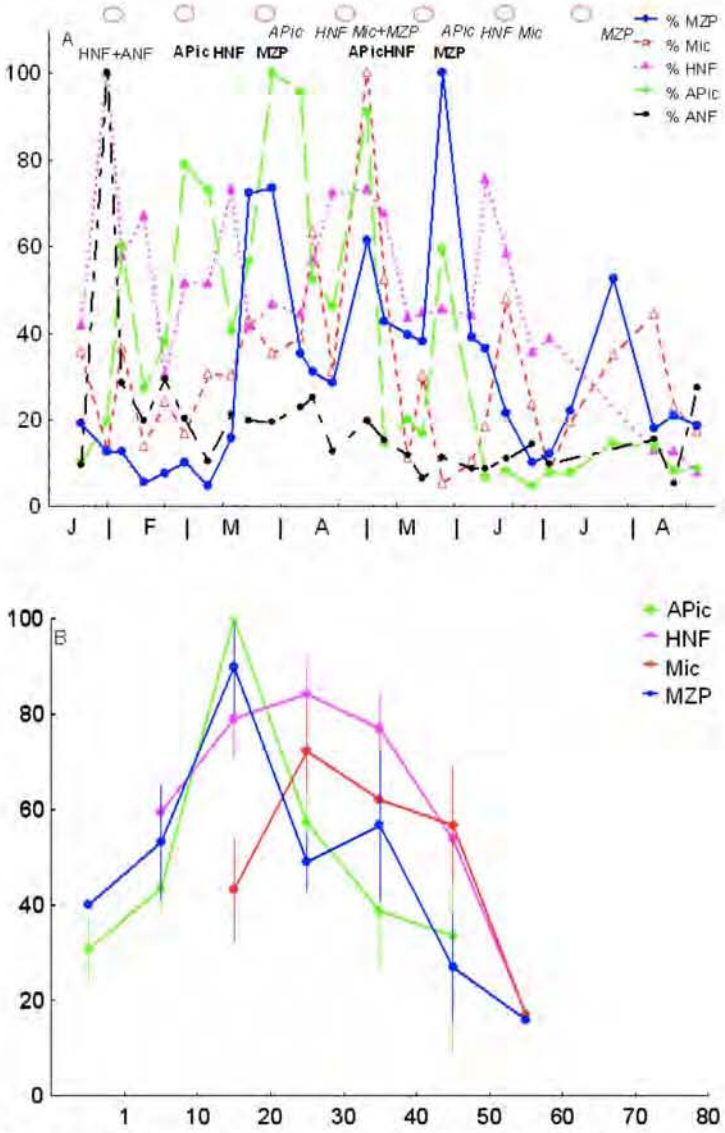


Fig. 5: Time-series of relative biomass (%) of (A) mesozooplankton (blue solid line), microplankton (red dotted line), heterotrophic flagellates (pink dotted line), autotrophic nanoflagellates (black dotted line) and autotrophic picoplankton (green dotted line) from January to August 2005; (B) standardized cycles of mesozooplankton (blue line), microplankton (red line), heterotrophic flagellates (pink dotted line), and autotrophic picoplankton biomasses. MZP stands for mesozooplankton, Mic for microplankton, NHF for heterotrophic nanoflagellates, ANF for autotrophic nanoflagellates and APE for photosynthetic picoplankton. Red circles correspond to the full moon period

Autotrophic picoplankton biomass dominated the plankton community biomass during the bloom (February, March, April) (Table 3). Bacterio- and nanoplankton biomasses were similar except in January where nanoplankton clearly dominated the community. MZP biomass was the lowest during the whole period studied.

Tab. 3: Biomass (µgC l^{-1}) mean values (± SE) of the different plankton communities by size ranges from January to August 2005. MZP stands for mesozooplankton, NF for nanoplankton, APic for autotrophic picoplankton, and HP for heterotrophic prokaryotes

	MZP >1 mm	MZP <1 mm	NF	APic	HP
January	3.15 ± 0.34	1.33 ± 0.18	8.64 ± 1.93	4.75 ± 0.92	3.87 ± 1.45
February	1.47 ± 0.21	0.70 ± 0.09	4.64 ± 0.28	8.57 ± 1.10	2.02 ± 0.25
March	4.99 ± 0.70	4.40 ± 0.62	4.74 ± 0.35	12.1 ± 1.52	7.28 ± 0.29
April	2.94 ± 0.53	3.18 ± 0.36	5.12 ± 0.26	7.20 ± 1.02	4.71 ± 0.28
May	3.95 ± 0.84	4.27 ± 0.56	3.16 ± 0.20	4.57 ± 1.22	5.71 ± 0.20
June	1.82 ± 0.17	1.73 ± 0.26	3.80 ± 0.23	1.16 ± 0.10	5.02 ± 0.38
July	0.78 ± 0.13	2.64 ± 0.90	2.31 ± 1.11	2.26 ± 0.22	6.82 ± 0.28
August	0.49 ± 0.06	1.78 ± 0.19	2.26 ± 0.66	1.34 ± 0.10	5.75 ± 0.41

Discussion

The main objective of this work was to describe the temporal variation of the planktonic community during the late winter bloom in the Canary Islands waters using a rather intensive eight-month study in order to know the short-term response of plankton to the seasonal nutrient enrichment. The late winter bloom observed in this study is characteristic of subtropical waters (Arístegui et al. 2001), and results from the erosion of the open ocean thermocline due to the cooling of surface waters, enhancing vertical mixing and the injection of nutrients into the euphotic layer. In this study, we observed that the bloom is much more complex than the simple increase in chlorophyll found during the minimum temperature recorded during the annual cycle. The two cyanobacterial genera *Synechococcus* and *Prochlorococcus* and eukaryotic picophytoplankton are well known to be widely distributed in the ocean and they are the principal groups of phytoplankton biomass (Stockner and Antia 1986; Partensky et al. 1999). The abundance of these organisms changes seasonally (Li 1994; Katano et al. 2005; Arístegui and Montero 2005) and their abundance is regulated by several environmental parameters as light (Armbrust et al. 1989; Vaquer et al. 1996; Jacquet et al. 2001), temperature (Iriarte and Purdie 1994; Agawin et al. 2000a, 2000b), nutrients (Katano et al. 2005) and grazing (Landry et al. 1995; Reckermann and Veldhuis 1997; Worden and Binder 2003; Hirose et al. 2008). The seasonal variability in the

autotrophic picoplankton community observed here is, in general, comparable to those in other subtropical regions of the ocean (Olson et al. 1990; Campbell et al. 1997; Durand et al. 2001; Liu et al. 2007; Arístegui and Montero 2005; Baltar et al. 2009). *Synechococcus* and autotrophic picoeukaryote biomasses increase in winter and early spring when temperatures are the coldest and when the water column is mixed. *Prochlorococcus* biomass, however, increases in summer when temperatures are higher and the water column is stratified. The winter bloom of *Synechococcus* and autotrophic picoeukaryotes occurs when the thermocline is eroded and the nutrients become available in the euphotic zone. It has been shown that *Synechococcus* is able to quickly respond to nitrate inputs (Glover et al. 1988), while *Prochlorococcus* residing in the high-light, low-nutrient surface mixed layers are adapted to use regenerated ammonium (Rippka et al. 2000; Moore et al. 2002). Temperature can also be a factor for the dominance of *Prochlorococcus* in summer and of *Synechococcus* in winter and early spring. The oceanic distribution of *Prochlorococcus* suggests that low temperatures have a lethal effect on them. The lowest surface temperature at which *Prochlorococcus* is recorded is about 10°C while *Synechococcus* is found at temperatures as low as 2°C (Partensky et al. 1999). In the Canary Current, even if these waters never reach such low temperatures, it is probable that *Synechococcus* is better adapted to winter water temperatures and, thus, dominate during this period of the year. In a recent study in Canarian waters, Baltar et al. (2009) also found a strong seasonal difference in *Prochlorococcus* abundance, being essentially absent at temperatures below 16.1°C. Heteronanoflagellates in almost all our study period depicted an inverse pattern to autotrophic picoeukaryotes (Fig. 3). As both communities can be grazed by microzooplankton (Rassoulzadegan et al. 1988, Jürgens et al. 1996, Guillou et al. 2001), when microzooplankton feeds on one of them, the other would be free of predation and its biomass can increase. This can explain the inverse relationship between autotrophic plankton and nanoflagellates.

Autrotrophic (A) plankton seems to be more abundant in proportion to heterotrophic (H) plankton during winter and spring than in summer, leading to a decrease with time in the A:H ratio, and suggesting that heterotrophs play a predominant role in summer. Arístegui and Montero (2005) and Baltar et al. (2009) found similar patterns in the Canary Island waters.

As observed in this study, planktonic blooms in subtropical waters are a complex process divided in various cycles of increasing and decreasing biomass of the different groups. The bloom observed in this work is composed by a succession of peaks beginning with the increase of autotrophic nanoflagellates which can benefit from the input of macronutrients in winter (Arístegui et al. 2001), followed by an increase of the smallest autrotrophic organisms (APE). After the autotrophs, the heteronanoflagellates increase followed by microplankton and mesozooplankton (Fig. 3). However, recognition of interactions between copepods, ciliates, nanoplankton and bacteria is gaining importance in the study of marine ecosystems (Kivi and Setälä 1995; Calbet and Landry 1999; Calbet and Saiz 2005). Vadstein et al. (2004) showed a clear effect of copepods controlling ciliates and releasing primary production as observed from the increase in chlorophyll. In another mesocosm study, Zöllner et al. (2009) showed cascading effects on the structure and function of bacterial assemblages, documenting a four-link predatory cascade affecting the entire functional groups of mainly heterotrophic organisms. Thus, zooplankton is potentially able to promote important changes in the community structure of the pelagic realm, affecting all the size spectrum of organisms. The latter organisms prey upon micro- and nanoheteroplankton releasing primary producers from grazing. The increase in mesozooplankton also gives rise to an increase in the amount of regenerated nutrients as they excrete ammonium (Hernández-León and Torres 1997), urea and different organic compounds (Miller and Gliber 1998). Therefore, they fertilize the mixed layer and control nano- and microplankton. Both factors seem to promote a parallel increase in primary production

as deduced from the increase in picoplanktonic cells. This parallel increase in mesozooplankton and primary production was observed in the Canary Islands waters by Hernández-León (2009). He found a striking match between ^{14}C primary production and copepod abundance. This coupling was explained as the effect of copepod predation upon microzooplankton, releasing grazing pressure on primary production. The absence of a lag between the increases in production and zooplankton (at least 2 weeks in these waters) supported the explanation of a top-down effect as small cells have a turnover time of about one day in this subtropical zone, allowing the parallel increase of copepods and small cells as microzooplankton is controlled by the formers. This parallel increase in mesozooplankton and autotrophic picoplankton, as observed in the present work, promotes a new increase of heteronanoflagellates, followed by micro- and mesozooplankton, which gives rise to another increase in primary production (see Fig. 5A). However, even if we observed these cycles during almost all the period of study, it was not clearly observable in April where NHF, although present in high numbers, did not follow this general pattern. In any case, standardization of the data (Fig. 5B) shows an increase in HNF and Mic after autotrophic picoeukaryotes, and a match between the latter and mesozooplankton, supporting the existence of the APE-HNF-Mic-MZP succession, which is re-started in every MZP increase. Bivariate or multivariable regressive techniques are used in the majority of studies examining the relationships between the environment and the different planktonic communities. However, as the process may not be linear, perfect correlations between all the planktonic groups are not expected. Using path analysis, Wells et al (2008) identified correlations between biotic and environmental variables in the California Current system in order to quantify how each of the variables relates to the other. Siuda and Dam (2008), using the same method of analysis to quantify links between planktonic groups, showed that zooplankton control of lower food web, even if pervasive, was

variable and subtle and that trophic links within the planktonic community are not linear.

In May, the heating of the surface layer stratifies the water column and the phytoplankton bloom is then finished. However, it seems that mesozooplankton still consumes microplankton, promoting a new increase of primary production and a new cycle of heteronanoflagellates and microplankton. Mesozooplankton appears, thus, to be determinant in these successions of peaks as it has a double role as predators and as fertilizers of the mixed layer.

Trophic cascade is defined as reciprocal predator-prey effects that alter the abundance, biomass and productivity of a population, a community or trophic level across more than one link in a food web (Pace et al. 1999). In natural systems, the effects of zooplankton on the microbial loop were extensively studied in freshwater systems (Burns and Schallenberg 1996, 1998; Wickham 1998; Zöllner et al. 2003). In marine systems, the previous studies were focused mainly on the copepod-ciliate link (Levinsen et al. 2000; Broglio et al. 2004; Calbet and Saiz 2005). Many dilution experiments (Landry and Hasset 1982) also allowed to study the microzooplankton impact on primary production but only a few field investigations showed whether predation effects on microzooplankton are transferred to heterotrophic nano- and picoplankton (Sipura et al. 2003; Zubkov and López-Urrutia 2003; Schnetzer and Caron 2005). In this sense, our results are in accordance with Sundt-Hansen et al. (2006) who showed that copepod predation on ciliates resulted in a stimulation of picocyanobacteria. The strong linkage between ciliates and heteronanoflagellates is also supported by numerous studies (see Kivi and Setälä 1995; Schnetzer and Caron 2005 among others) giving to microzooplankton the role of the main link between the microbial loop and classical food chain.

No taxonomic analyses were performed in the present study and microplankton could not be separated into autotroph and heterotroph organisms. Thus, we could not discern between microplankton acting as predator on nanoheterotrophs from mixo- and autotrophic microplankton. This could explain the absence of an inverse correlation between both communities. Moreover, unfortunately, we did not sample the 75-200 µm size fraction composed mainly by nauplius and small copepodites. Thus, we wonder whether the first autotrophic picoplankton peak in February was coincident or not with an increase in this size fraction, or if the start of the bloom occurred in January or December, before we started the sampling. In any case, the now common result of mesozooplankton promoting cascade effects in mesocosms can be envisaged in our field sampling. Due to this top-down effect, the bloom seemed to be re-started at least three times. This feature allowed the full use of the nutrient input by mixing, but also to recycle at least three times the energy and matter in the epipelagic zone.

Finally, the mesozooplankton outburst that occurred in these subtropical waters during the late winter bloom was observed to be driven by the interplay between bottom-up and top-down controls. Hernández-León (1998) and Hernández-León et al. (2001, 2002, 2004) found that mesozooplankton peaks matched the lunar cycle as observed in lakes by Gliwicz (1986). The explanation for this pattern was the presence or not of diel vertical migrants (DVMs) at night in the epipelagic zone during the dark or illuminated phases of the moon. Shortly, to avoid predation, DVMs do not reach the upper layers of the ocean during full moon, allowing the growth of epipelagic (non-migrant) zooplankton without predatory pressure. During new moon, DVMs occupy the shallower layers at night preying upon the epipelagic mesozooplankton. This would explain the decrease in mesozooplankton after the full moon and the start of a new cycle. Therefore, the top-down effect of these DVMs (large zooplankton and micronekton) promotes, to our view, an important cascade effect as the one described above. Thus, the links in end-to-end webs seem to affect the structure and function

(and therefore flux) in the ocean. This key process in the warm blue ocean deserves further research.

Acknowledgements

The authors would like to thank all the colleagues of the Biological Oceanography Group that contributed to the very hard field work. Also, A. Calbet and H. Dam are thanked for valuable comments on the manuscript. This study was funded by the projects "ConAfrica" (CTM2004-02319) and "Lucifer" (CTM2008-03538) from the Ministry of Education, and Science of Spain and from PhD fellowship to C. Schmoker from the Government of Canary Islands, Spain.

References

Agawin NSR, Duarte CM, Agustí S (2000a) Nutrient and temperature control of the contribution of picoplankton to phytoplankton biomass and production. Limnol Oceanogr 45: 591-600

Agawin NSR, Duarte CM, Agustí S (2000b) Response of Mediterranean *Synechococcus* growth and loss rates to experimental nutrient inputs. Mar Ecol Prog Ser 206: 97-106

Arístegui J, Hernández-León S, Montero MF, Gómez M (2001) The seasonal planktonic cycle in coastal waters of the Canary Islands Sci Mar 65: 51-58

Arístegui J, Montero MF (2005) Temporal and spatial changes in plankton respiration and biomass in the Canary Islands regions: The effect of mesoscale variability. J Mar Syst 54: 65-82

Armbrust EV, Bowen JD, Olson RJ, Chisholm SW (1989) Effect of light on the cell cycle of a Marine *Synechococcus* Strain. Appl Environ Microbiol 55: 425-432

Børsheim KY, Bratbak G (1987) Cell volume to cell carbon conversion factors for a bacterivorous *Monas sp.* enriched from seawater. Mar Ecol Prog Ser 36: 171-175

Bode A, Barquero S, Varela M, Braun JG, De Armas D (2001) Pelagic bacteria and phytoplankton in oceanic waters near the Canary Islands in summer. Mar Ecol Prog Ser 209: 1-17

Baltar F, Arístegui J, Montero MF, Espino M, Gasol JM, Herndl GJ (2009) Mesoscale variability modulates seasonal changes in the trophic structure of nano- and picoplankton communities across the NW Africa-Canary Islands transition zone. Prog Oceanogr 83: 180-188

Braun JG (1980) Estudios de producción en aguas de las Islas Canarias I. Hidrografía, nutrientes y producción primaria. Bol Inst Esp Oceanogr 5: 147-154

Broglio E, Saiz E, Calbet A, Trepat I, Alcaraz M (2004) Trophic impact and prey selection by crustacean zooplankton on the microbial communities of an oligotrophic coastal area (NW Mediterranean Sea). Aquat Microb Ecol 35: 65-78

Burns CW, Schallenberg M (1996) Relative impacts of copepods, cladocerans and nutrients on the microbial food web of a mesotrophic lake. J Plankton Res 18: 683-714

Burns CW, Schallenberg M (1998) Impacts of nutrients and zooplankton on the microbial food web of an ultra-oligotrophic lake. J Plankton Res 20: 1501-1525

Calbet A, Landry MR (1999) Mesozooplankton influences on the microbial food web: Direct and indirect trophic interactions in the oligotrophic open ocean. Limnol Oceanogr 44: 1370-1380

Calbet A, Saiz E (2005) The ciliate-copepod link in marine ecosystems. Aquat Microb Ecol 38: 157-167

Campbell L, Liu H, Nolla HA, Vaulot D (1997) Annual variability of phytoplankton and bacteria in the subtropical North Pacific Ocean at Station ALOHA during the 1991-1994 ENSO event. Deep-Sea Res I 44: 167-192

Cho BC, Azam F (1990) Biogeochemical significance of bacterial biomass in the ocean's euphotic zone. Mar Ecol Prog Ser 63: 253-259

Christian JR, Karl DM (1994) Microbial community structure at the U.S.-Joint Global Ocean Flux Study Station ALOHA: Inverse methods for estimating biochemical indicator ratios. J Geophys Res 99: 14269-14276

Dam HG, Zhang XS, Butler M, Roman MR (1995) Mesozooplankton grazing and metabolism at the equator in the central Pacific: Implications for carbon and nitrogen fluxes. Deep-Sea Res II 42: 735-756

De León AR, Braun JG (1973) Annual cycle of primary production and its relation to nutrients in the Canary Islands waters. Bol Inst Esp Oceanogr 167: 1-24

Durand MD, Olson RJ, Chisholm SW (2001) Phytoplankton population dynamics at the Bermuda Atlantic Time-series station in the Sargasso Sea. Deep-Sea Res II 48: 1983-2003

Fenchel T (1986) The ecology of heterotrophic microflagellates. Adv Microb Ecol 9: 57-95

Gasol JM, del Giorgio PA, Duarte CM (1997) Biomass distribution in marine planktonic communities. Limnol Oceanogr 42: 1353: 1363

Gasol JM, Zweifel UL, Peters F, Fuhrman JA, Hagstrom A (1999) Significance of size and nucleic acid content heterogeneity as measured by flow cytometry in natural planktonic bacteria. Appl Environ Microbiol 65: 4475-4483

Gaudy R, Champalbert G, Le Borgne R (2003) Feeding and metabolism of mesozooplankton in the equatorial Pacific high-nutrient low-chlorophyll zone along 180°. J Geoph Res 108: 8144-8156

Gliwicz P (1986) A lunar cycle in zooplankton. Ecology 67: 883-897

Glover HE, Prézelin BB, Campbell L, Wyman M (1988) Pico- and ultraplankton Sargasso Sea communities: variability and comparative distributions of *Synechococcus* spp. and algae. Mar Ecol Prog Ser 49: 127-139

Guillou L, Jacquet S, Chretiennot-Dinet MJ, Vaulot D (2001) Grazing impact of two small heterotrophic flagellates on *Prochlorococcus* and *Synechococcus*. Aquat Microb Ecol 26: 201-207

Haas LW (1982) Improved epifluorescence microscopy for observing planktonic micro-organisms. Ann Inst Oceanogr Paris 58: 261-266

Hernández-León S (2009) Top-down effects and carbon flux in the ocean: A hypothesis. J Mar Syst 78: 576-581

Hernández-León S, Torres S (1997) The relationship between ammonia excretion and GDH activity in marine zooplankton. J Plankton Res 19: 587-601

Hernández-León S, Almeida C, Gómez M, Torres S, Montero I, Portillo-Hahnefeld A (2001) Zooplankton biomass and indices of feeding and metabolism in island-generated eddies around Gran Canaria. J Mar Syst 30: 51-66

Hernández-León S, Almeida C, Becognée P, Yebra L, Arístegui J (2004) Zooplankton biomass and indices of grazing and metabolism during a late winter bloom in subtropical waters. Mar Biol 145: 1191-1200

Hirose M, Katano T, Nakano SI (2008) Growth and grazing mortality rates of *Prochlorococcus*, *Synechococcus* and eukaryotic picophytoplankton in a bay of the Uwa Sea, Japan. J Plankton Res 30: 241-250

Iriarte A, Purdie DA (1994) Size distribution of chlorophyll a biomass and primary production in a temperate estuary (Southampton water): the contribution of photosynthetic picoplankton. Mar Ecol Prog Ser 115: 283-297

Jacquet S, Partensky F, Marie D, Casotti R, Vaulot D (2001) Cell cycle regulation by light in *Prochlorococcus* strains. Appl Environ Microbiol 67: 782-790

Jürgens K, Wickham SA, Rothhaupt KO, Santer B (1996) Feeding rates of macro- and microplankton on heterotrophic nanoflagellates. Limnol Oceanogr 41: 1833-1839

Kana TM, Glibert PM (1987) Effects of irradiances up to 2000 µm E $m^{-2} \cdot s^{-1}$ on marine *Synechococcus* WH 7803-1. Growth, pigmentation, and cell composition. Deep-Sea Res I 26: 97-108

Katano T, Kaneda A, Takeoka H, Nakano S (2005) Seasonal changes in the abundance and composition of picophytoplankton in relation to the occurrence of 'Kyucho' and bottom intrusion in Uchiumi Bay, Japan. Mar Ecol Prog Ser 298: 59-67

Kivi K, Setälä O (1995) Simultaneous measurement of food particle selection and clearance rates of planktonic oligotrich ciliates (Ciliophora: Oligotrichina). Mar Ecol Prog Ser 119: 125-137

Landry MR, Constantinou J, Kirshtein J (1995) Microzooplankton grazing in the Central Equatorial Pacific during February and August, 1992. Deep-Sea Res II 42: 657-671

Landry MR, Hassett RP (1982) Estimating the grazing impact of marine microzooplankton. Mar Biol 67: 283-288

Lee S, Fuhrman JA (1987) Relationships between biovolume and biomass of naturally derived marine bacterioplankton. Appl Environ Microbiol 53: 1298-1303

Levinsen H, Turner JT, Nielsen TG, Hansen BW (2000) On the trophic coupling between protists and copepods in arctic marine ecosystems. Mar Ecol Prog Ser 204: 65-77

Li WKW (1994) Primary production of prochlorophytes, cyanobacteria, and eukaryotic ultraphytoplankton: Measurements from flow cytometric sorting. Limnol Oceanogr 39: 169-175

Li WKW, Dickie PM, Irwin BD, Wood AM (1992) Biomass of bacteria, cyanobacteria, prochlorophytes and photosynthetic eukaryotes in the Sargasso Sea. Deep-Sea Res A 39: 501-519

Liu HB, Chang J, Tseng CM, Wen LS, Liu KK (2007) Seasonal variability of picoplankton in the northern South China Sea at the SEATS station. Deep-Sea Res II 54: 1602-1616

Lovegrove T (1966) The determination of the dry weight of plankton and the effect of various factors on the values obtained. In Barnes H (ed) Some contemporary studies in Marine Sciences. George Allen and Unwin Ltd, pp. 429-467

Menzel DW, Ryther JH (1961) Zooplankton in the Sargasso Sea off Bermuda and its relation to organic production. J Du Conseil Pour L'Exploration De La Mer 26: 250-258

Miller CA, Glibert PM (1998) Nitrogen excretion by the calanoid copepod *Acartia tonsa*: results of mesocosm experiments. J Plankton Res 20: 1767-1780

Montagnes DJS, Berges JA, Harrison PJ, Taylor FJR (1994) Estimating carbon, nitrogen, protein, and chlorophyll alpha from volume in marine phytoplankton. Limnol Oceanogr 39: 1044-1060

Moore LR, Post AF, Rocap G, Chisholm SW (2002) Utilization of different nitrogen sources by the marine cyanobacteria *Prochlorococcus* and *Synechococcus*. Limnol Oceanogr 47: 989-996

Olson RJ, Chisholm SW, Zettler ER, Altabet MA, Dusenberry JA (1990) Spatial and temporal distributions of prochlorophyte picoplankton in the North Atlantic Ocean. Deep-Sea Res 37: 1033-1051

Pace ML, Cole JJ, Carpenter SR, Kitchell JF (1999) Trophic cascades revealed in diverse ecosystems. Trends Ecol Evol 14: 483-488

Partensky F, Hess WR, Vaulot D (1999) *Prochlorococcus*, a marine photosynthetic prokaryote of global significance. Microb Mol Biol Rev 63: 106-127

Purdie DA (1996) Marine phytoplankton blooms. In Summerhayes CP and Thorpe SA (eds) Oceanography. An illustrated Guide. Manson Publ, pp. 89-95

Rassoulzadegan F, Laval-Peuto M, Sheldon RW (1988) Partitioning of the food ration of marine ciliates between pico- and nanoplankton. Hydrobiol 159: 75-88.

Reckermann M, Veldhuis MJW (1997) Trophic interactions between picophytoplankton and micro- and nanozooplankton in the western Arabian Sea during the NE monsoon 1993. Aquat Microb Ecol 12: 263-273

Rippka R, Coursin T, Hess W, Lichtle C, Scanlan DJ, Palinska KA, Iteman I, Partensky F, Houmard J, Herdman M (2000) *Prochlorococcus marinus* Chisholm et al. 1992 subsp. pastoris subsp. nov strain PCC 9511, the first axenic chlorophyll a_2/b_2-containing cyanobacterium (Oxyphotobacteria). Internat. J Syst Evol Microb 50: 1833-1847

Sanders RW, Wickham SA (1993) Planktonic protozoa and metazoa: Predation, food quality and population control. Aquat Microb Ecol 7: 197-223

Schnetzer A, Caron DA (2005) Copepod grazing impact on the trophic structure of the microbial assemblage of the San Pedro Channel, California. J Plankton Res 27: 959-971

Sherr EB, Sherr BF (2002) Significance of predation by protists in aquatic microbial food webs. Antonie Van Leeuwenhoek 81: 293-308

Sieracki CK, Sieracki ME, Yentsch CS (1998) An imaging-in-flow system for automated analysis of marine microplankton. Mar Ecol Prog Ser 168: 285-296

Sieracki ME, Haugen EM, Cucci TL (1995) Oversestimation of heterotrophic bacteria in the Sargasso Sea: direct evidence by flow and imaging cytometry. Deep-Sea Res 42: 1399-1409

Sipura J, Lores E, Snyder RA (2003) Effect of copepods on estuarine microbial plankton in short-term microcosms. Aquat Microb Ecol 33: 181-190

Siuda AN, Dam HG (2008) Cascading trophic effects, omnivory and ecological stoichiometry. Proc IMBER IMBIZO, Miami

Stockner JG, Antia A (1986) Algal picoplankton from marine and freshwater ecosystems: a multidisciplinary perspective. Can J Fish Aquat Sci 43: 2472-2503

Sundt-Hansen L, Olsen Y, Stibor H, Heldal M, Vadstein O (2006) Trophic cascades mediated by copepods, not nutrient supply rate, determine the development of picocyanobacteria. Aquat Microb Ecol 45: 207-218

Sverdrup HU (1953) On conditions for the vernal blooming of phytoplankton. J Du Conseil Pour L'Exploration De La Mer 18: 287-295

Tsai AY, Chiang KP, Chang J, Gong GC (2008) Seasonal variations in trophic dynamics of nanoflagellates and picoplankton in coastal waters of the western subtropical Pacific Ocean. Aquat Microb Ecol 51: 263-274

Vadstein O, Stibor H, Lippert B, Løseth K, Roederer W, Sundt-Hansen L, Olsen Y (2004) Moderate increase in the biomass of omnivorous copepods mayease grazing control of planktonic algae. Mar Ecol Prog Ser 270: 199-207

Vaquer A, Troussellier M, Courties C, Bibent B (1996) Standing stock and dynamics of picophytoplankton in the Thau Lagoon (northwest Mediterranean coast). Limnol Oceanogr 41: 1821-1828

Wells BK, Field JC, Thayer JA, Grimes CB, Bograd SJ, Sydeman WJ, Schwing FB, Hewitt R (2008) Untangling the relationships among climate, prey and top predators in an ocean ecosystem. Mar Ecol Prog Ser 364: 15-19

Wickham SA (1998) The direct and indirect impact of Daphnia and Cyclops on a freshwater microbial food web. J Plankton Res 20: 739-755

Worden AZ, Binder BJ (2003) Application of dilution experiments for measuring growth and mortality rates among *Prochlorococcus* and *Synechococcus* populations in oligotrophic environments. Aquat Microb Ecol 30: 159-174

Yentsch CS, Menzel DW (1963) A method for the determination of phytoplankton chlorophyll and phaeophytin fluorescence. Deep-Sea Res I 10: 221-231

Zarauz L, Irigoien X, Fernandes JA (2009) Changes in plankton size structure and composition, during the generation of a phytoplankton bloom, in the central Cantabrian sea. J Plankton Res 31: 193-207

Zöllner E, Santer B, Boersma M, Hoppe HG, Jürgens K (2003) Cascading predation effects of Daphnia and copepods on microbial food web components. Fresh Biol 48: 2174-2193

Zöllner E, Hoppe HG, Sommer U, Jürgens K (2009) Effect of zooplankton-mediated trophic cascades on marine microbial food web components (bacteria, nanoflagellates, ciliates). Limnol Oceanogr 54: 262-275

Zubkov MV, López-Urrutia A (2003) Effect of appendicularians and copepods on bacterioplankton composition and growth in the English Channel. Aquat Microb Ecol 32: 39-46

Zubkov MV, Sleigh MA, Burkill PH, Leakey RJG (2000) Picoplankton community structure on the Atlantic Meridional Transect: a comparison between seasons. Prog Oceangr 45: 369-386

CHAPTER IV

Patterns of plankton communities in subtropical waters during the late winter bloom: An end-to-end approach

Claire Schmoker, Alicia Ojeda, Santiago Hernández-León

Abstract

The microbial planktonic community of the subtropical waters around Gran Canaria Island, Canary Islands, was studied before, during and after the typical late winter bloom. The study consisted of a weekly sampling from October 2005 to June 2006 at five stations. During all the period of study, microplankton abundance was dominated by small athecate dinoflagellates (15-20 µm) whereas its biomass was dominated by aloricate ciliates (20-30 µm). The bloom began with the increase of autotrophic picoplanktonic cells and small diatoms. Consecutively, nano-, micro-, and mesozooplankton biomass also increased. During the development of the winter bloom, picoplankton, heterotrophic nanoflagellates, microzooplankton, mainly aloricate ciliates, and mesozooplankton showed inverse trends suggesting that the bloom is a succession of rather complicated bottom-up and top-down controls.

Introduction

The Canary Islands are located in the subtropical waters of the Northeast Atlantic Ocean. This region is rather oligotrophic except during a short period of the year, the so-called late winter bloom (Menzel and Ryhter 1961). During most of the year, the strong heating of surface waters causes a quasi-permanent thermocline, which restrains the nutrient pumping to upper layers and limits phytoplankton growth. During winter months, in February-March, the surface waters cool off, the thermocline is eroded and the nutrients become available in the euphotic layer (De León and Braun 1973, Braun 1989) promoting an increase in primary production and chlorophyll. In April-May, the thermocline starts to reform, leading to the usual situation of a surface euphotic zone with reduced inorganic nutrients. Although the late winter bloom is now well known and described (Arístegui et al. 2001, Hernández-León et al. 2004, Arístegui and Montero 2005), only few studies were done on the temporal variability of

planktonic communities, especially on the lower end of the food web. Even if these waters have been described with low variability in plankton biomass and productivity (De León and Braun 1973, Braun 1980), recent studies have shown a clear and strong seasonality of the picoplankton community (Baltar et al. 2009).

Knowledge of these communities in warm oligotrophic waters is increasing (Tsai et al. 2008, Baltar et al. 2009). However, there are still some important gaps about the functioning of the microbial loop and its connection to upper trophic levels in these rather extensive areas of the ocean. One of them is their temporal and seasonal variability. Because much of the research in oceanic waters is related to episodic sampling on board large of oceanographic vessels, our knowledge about the evolution of these communities along seasons is rather short. In this sense, oceanic islands are excellent platforms to afford this problem because the easy accessibility to the open ocean.

In these warm oligotrophic waters, small cells (<2 μm) account for more than 60% of the primary production (Arístegui et al. 2001), as well as in other oligotrophic areas (Zubkov et al. 2000a). Here, unicellular picoplanktonic prokaryotes and eukaryotes (Sieburth et al. 1978) play a fundamental role as their small size, reduced diffusion boundary layer and large surface area per unit volume are an advantage to uptake nutrients (Azam et al. 1983, Raven 1998, Sherr and Sherr 2007). There is also a lack of information about the temporal variability of heterotrophic nanoflagellates, dinoflagellates and ciliates in this oceanic area of the Canary Current. Even less known is how mesozooplankton affects the development of these communities due to their feeding on nano- or microzooplankton.

The main objective of this work was to describe the temporal variability of plankton communities before, during and after the late winter bloom in the subtropical area off the Canary Islands. Autotrophs and heterotrophs in a wide range of sizes and

taxonomic groups were studied. Bacterioplankton, small autotrophic plankton (picoplankton 0.2-2 µm), heterotrophic nanoflagellates (nanoplankton mainly in the range of 3-5 µm), microzooplankton (mainly ciliates and dinoflagellates 20-200 µm) and mesozooplankton were sampled in order to know the effect of the winter mixing, the main annual perturbation, on the food web in an end-to-end approach in order to know their fluctuations and, therefore, envisage the biogeochemical consequences of their variability.

Material and methods

Weekly sampling has been carried out from October 2005 to June 2006 on the RV Solana II. Five stations separated by 10 nautical miles and located at the edge of the island slope (about 100 m depth) were sampled around Gran Canaria Island (Fig. 1).

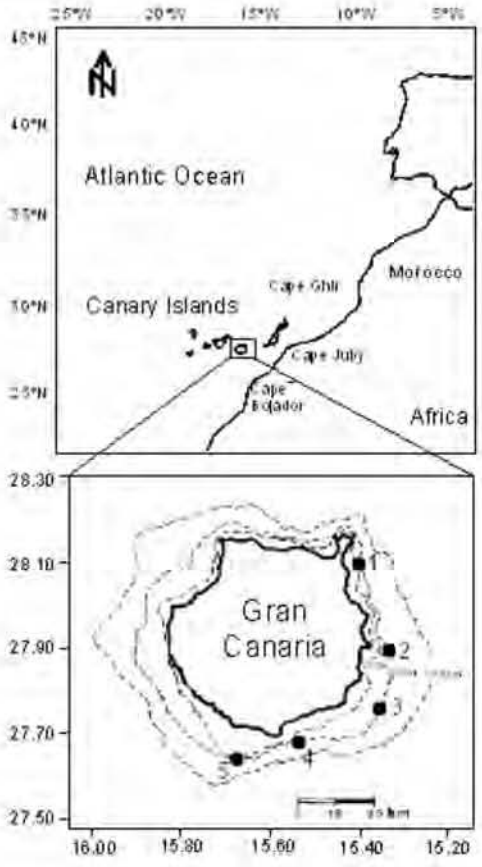


Fig. 1: Map of the study area showing the location of the five sampling stations at the edge of the island shelf around Gran Canaria, Canary Islands

Vertical profiles of temperature, conductivity and fluorescence were obtained using a CTD SBE25 probe (Sea-Bird Electronics Inc., Bellevue, WA, USA). Samples for chlorophyll, bacterio- and microplankton were taken in the mixed layer at 15 m depth with a 5 l Niskin bottle.

Chlorophyll was measured fluorometrically on a Turner Designs bench fluorometer 10AU, previously calibrated with pure chlorophyll *a* (Sigma-Aldrich Inc., St. Louis, MO, USA; Yentsch and Menzel 1963). Seawater samples (500 ml) were filtered on board through 25 mm Whatman GF/F filters and preserved in liquid nitrogen until analysis. Pigments were extracted during 24 h in 10 ml of 90% acetone at 4ºC in the dark. Depth profiles of *in situ* fluorescence were converted to chlorophyll from a fluorescence versus chlorophyll relation (Linear regression: Chl *a* = 0.2515*fluorescence + 0.0362; r^2 = 0.61; p <0.001).

Heterotrophic prokaryotes (HP), small photosynthetic eukaryotic cells (autotrophic picoeukaryotes, APE), *Prochlorococcus* (Pro) and *Synechococcus* (Syn) type cyanobacteria, were counted by flow cytometry using a FACScalibur instrument (Becton and Dickinson). Immediately after collection, samples were fixed with paraformaldehyde (2% final concentration), incubated for 30 min at 4ºC and then stored frozen in liquid nitrogen until analysis. To count heterotrophic prokaryotes, 200 µl were stained with a DMS-diluted SYTO-13 (Molecular Probes Inc.) stock (10:1) at 2.5 µM final concentration. HP were identified by their signatures in a plot of side scatter (SSC) versus green fluorescence (FL1) (Gasol et al. 1999). The identification of small phytoplankton groups was based on interactive analyses of multiple bivariate scatter plots of side scatter, red and orange fluorescence. Samples were run at low speed for HP and at medium or high speed for autotrophic picoplankton until 10,000 events were acquired or 2 minutes were passed.

Samples for autotrophic (ANF) and heterotrophic nanoflagellates (HNF) were preserved following the procedure given by Haas (1982). Immediately after collection, the samples were fixed with glutaraldehyde (0.3% final concentration). The samples were placed into a filtration tower and stained with diamidino-2-phenylindole for 5 minutes, and filtered onto a 0.2 µm black polycarbonate membrane filter, placed over a Whatman GF/C backing filter. The filter was mounted on a microscope slide with low-fluorescence paraffin oil. Samples from station 1 were analyzed. At least 300 cells, or 20 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.

Microplankton samples were preserved in Lugol's iodine solution (final concentration 2%) and stored in the dark. In the laboratory, subsamples from station 1 were analyzed. Subsamples (100 ml) were allowed to settle for 48 h in a composite chamber. The entire chamber bottom was examined using the Utermöhl technique. Ciliates and tintinnids were assigned to genus when possible following Lynn and Small (2002). Dinoflagellates were identified following Steidinger and Tangen (1997) and Ojeda (2005) and diatoms following Ricard (1987), and, Hasle and Syvertsen (1997). The length and diameter of each individual (up to a maximum of 20 cells for each taxon) were measured at 400x magnification using a calibrated ocular micrometer. Mean cell volume was calculated by equating the shape of each taxa to a standard geometric configuration (Hillebrandt et al. 1999).

HP abundances were converted to biomass using a factor of 20 fgC·cell^{-1} (Lee and Fuhrman 1987, Cho and Azam 1990, Bode et al. 2001). Pro cell numbers were converted to biomass assuming a mean biovolume of 0.1 µm^3·cell^{-1} (Sieracki et al. 1995), and a conversion factor of 220 fgC·µm^{-3} (Christian and Karl 1994, Zubkov et al. 2000b). Syn cell numbers were converted to biomass by using a conversion factor of

250 fgC·cell^{-1} (Kana and Glibert 1987, Li et al. 1992). APE abundances were transformed to biomass using a conversion factor of 2100 fgC·cell^{-1} (Campbell et al. 1997).

ANF and HNF cell-numbers were converted to biomass assuming a mean volume of 20 μm^3·cell^{-1} for autotrophs, and of 10 μm^3·cell^{-1} for heterotrophs and a conversion factor of 220 fgC·μm^{-3} (Børsheim and Bratbak 1987).

Ciliate biovolumes estimated were converted to carbon equivalents by the factor experimentally derived for Lugol's-fixed marine oligotrichs from Putt and Stoecker (1989), except for tintinnid carbon, which was estimated using experimental factor determined by Verity and Langdon (1984). Dinoflagellate abundances were converted to biomass using a Mender-Deuer and Lessard (2000) carbon content conversion factor. Finally, diatom abundances were converted to biomass according to the equation of Strathmann (1967). The use of Lugol´s fixative precluded identification of mixotrophic from autotrophic and heterotrophic microorganisms. Taxa were placed into size categories (<20 μm, 20-40 μm and >40 μm).

Zooplankton was caught in oblique hauls using a Bongo net of 40 cm diameter, equipped with 200 μm mesh size nets (General Oceanics Inc., Miami, FL, USA). The net was towed at two or three knots from a maximum of 90 m up to surface. Samples were fractionated with a 1 mm mesh and both size fractions were used to quantify mesozooplankton biomass as dry weight, following the method of Lovegrove (1986) and was converted to carbon biomass assuming a carbon:dry weight ratio of 0.4 (Båmstedt 1986).

Statistical analyses performed consisted in multiple regression analysis and Pearson´s correlation coefficients (r) tests calculated in order to examine the relationships between the planktonic groups. Non-parametric Krustal-Wallis tests were

also run to assess differences between stations 1 to 5 in mesozoo-, pico- and bacterioplankton biomass.

Results

Temperature and chlorophyll *a* followed the typical seasonal cycle previously observed around the Canary Islands. During October and November, surface temperatures were high, while at the beginning of winter, surface waters cooled and chlorophyll *a* concentration started to increase (Fig. 2).

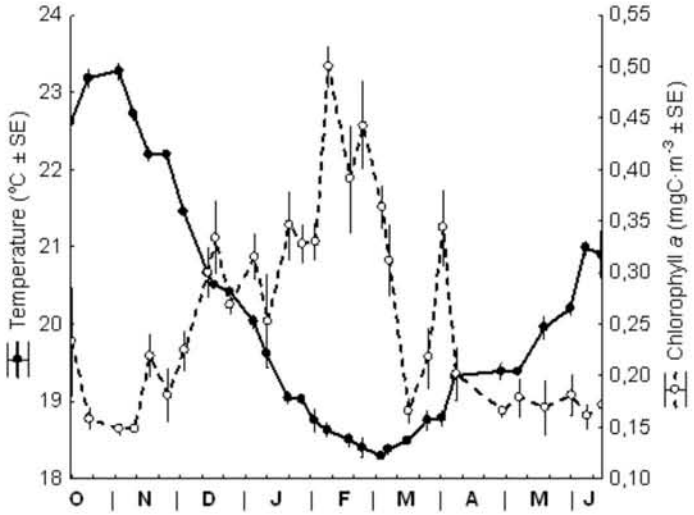


Fig. 2: Temporal distribution of average (stations 1 to 5) temperature and chlorophyll *a* in the mixed layer

During late winter, chlorophyll *a* concentration reached the maximum coinciding with temperature below 19°C in the mixed layer. In spring, when temperature increased, the thermocline was reestablished, leading to rather low chlorophyll *a* values.

Heterotrophic prokaryotes and autotrophic picoplankton communities did not show significant differences in biomass between stations. Mesozooplankton, however, was not homogeneously distributed along the island shelf edge, showing slightly but significant higher values south of the island (K-W ANOVA, $p < 0.05$).

Heterotrophic prokaryotes (HP) were present during the whole period of study with a

rather constant biomass but decreasing at the beginning of January and April (Fig. 3A). Autotrophic picoplankton (APic) showed a succession of the different groups. *Prochlorococcus* (Pro) showed quite low values in winter while autotrophic eukaryotic picoplankton (APE) and *Synechococcus* (Syn) showed high biomass from December to April. Pro and APE made up most of the autotrophic biomass during the whole period of study and clearly depicted the late winter bloom observed as chlorophyll *a*. Maximum biomass of autotrophic nanoflagellates (ANF) was observed in April and May but only contributed to a small fraction of the total autotrophic biomass (Fig. 3B). The variability of HNF and APE biomass suggested an inverse pattern along the period studied (see below, Fig. 3C).

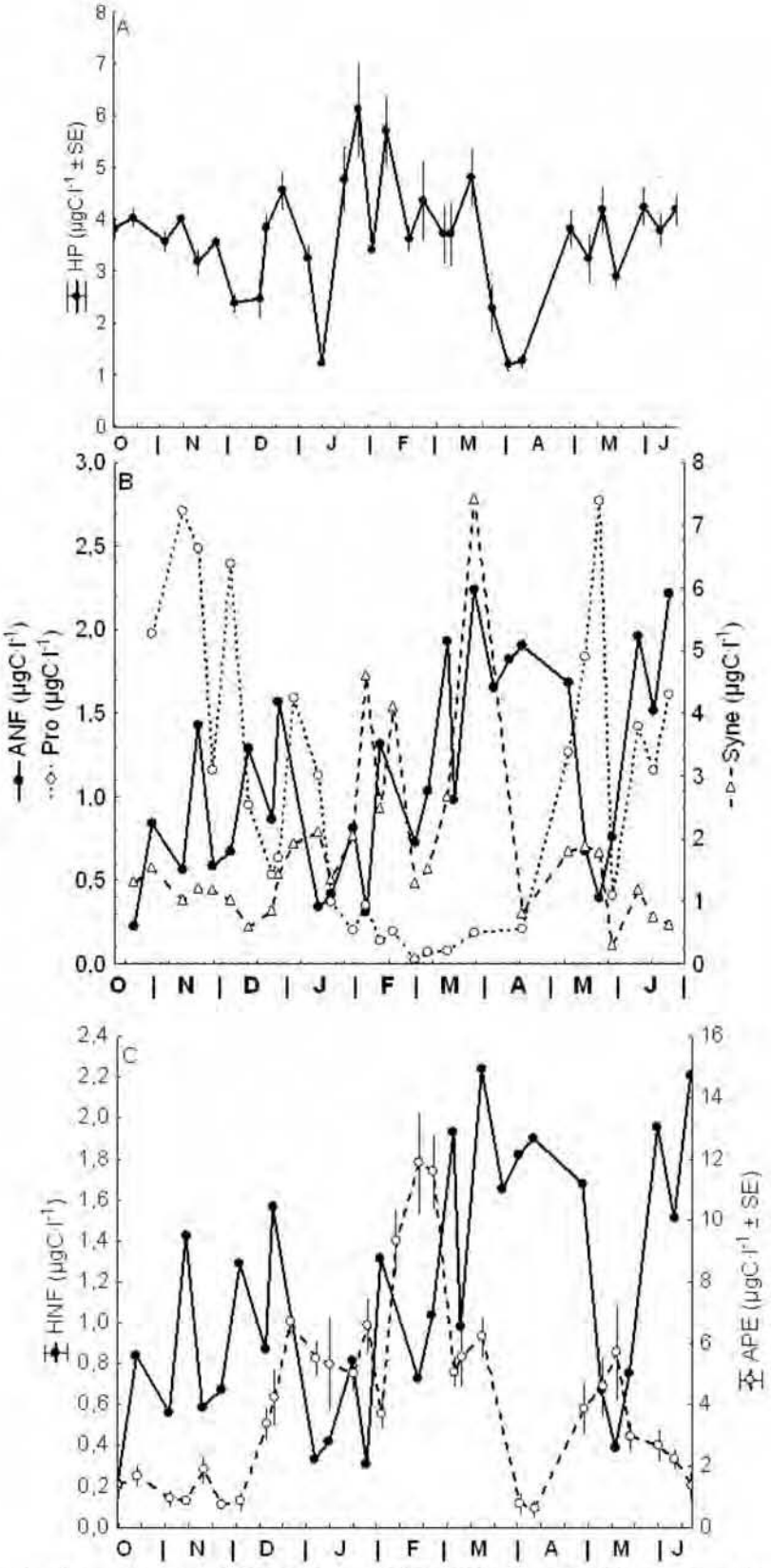


Fig. 3: Time-series from October 2005 to June 2006 of (A) averaged heterotrophic prokaryote biomass at stations 1 to 5; (B) *Prochlorococcus* (Pro), *Synechococcus* (Syn), autotrophic picoeukaryote (APE), autotrophic nanoflagellate (ANF) biomass at station 1; (C) heterotrophic nanoflagellates (HNF) at station 1 biomass and average autotrophic picoeukaryote (APE) biomass

Diatom biomass peaked only at the beginning of mixing (January) and when the thermocline was reestablished in May (Fig. 4A). The diatom blooms were mostly composed by the genera Rhizolenia, Chaetoceros and Navicula. Among dinoflagellates, small specimens (<20 µm) dominated their abundance (Table 1).

Tab.1: Dinoflagellate and aloricate ciliate abundances (N) (cells·ml^{-1}) and biomass (B) (µgC·m^{-3}) by size fractions for station 1

	Dinoflagellates						Aloricate Ciliates					
	<20 µm		20-40 µm		>40 µm		<20 µm		20-40 µm		>40 µm	
	N	B	N	B	N	B	N	B	N	B	N	B
November	10.40	0.103	1.23	0.165	0.22	0.062	0.25	0.041	0.24	0.229	0.13	0.552
December	11.93	0.118	1.36	0.182	0.09	0.025	0.52	0.092	0.47	0.715	0.17	1.156
January	7.98	0.079	1.10	0.148	0.19	0.055	0.55	0.092	0.51	0.745	0.19	1.170
February	9.91	0.098	1.37	0.184	0.07	0.020	1.93	0.348	2.07	2.649	0.27	1.229
March	12.34	0.122	1.10	0.147	0.07	0.020	0.81	0.100	0.30	0.390	0.11	0.516
April	9.19	0.09	0.72	0.096	0.14	0.040	0.67	0.133	0.25	0.365	0.11	0.303
May	12.95	0.128	1.69	0.227	0.14	0.040	0.92	0.192	0.77	1.29	0.16	0.872

The most important genera were Gymnodinium, Gyrodinium, Amphidinium, Cochlodinium and Torodinium. Organisms >40 µm were mainly thecate dinoflagellates from the genera Gonyaulax, Ceratium, Podolampas, Oxytum, Dinophysis, Ornithocercus. In terms of biomass, ciliates, however, were the most abundant group of microzooplankton (Fig. 4B). Large (>40 µm) aloricate ciliates dominated during the period of study, except in February and May, which were the medium (20-40 µm) size ciliates (Table 1). Aloricate ciliates clearly dominated the total microplankton biomass while tintinnids did not represent a significant proportion (Fig. 4B).

was grazed by microzooplankton (Fig. 4B). The same pattern was observed when we used the grazing rates estimated from assimilation efficiency and the primary production grazed varying from 61 to 92 ± 6% (Fig. 4C).

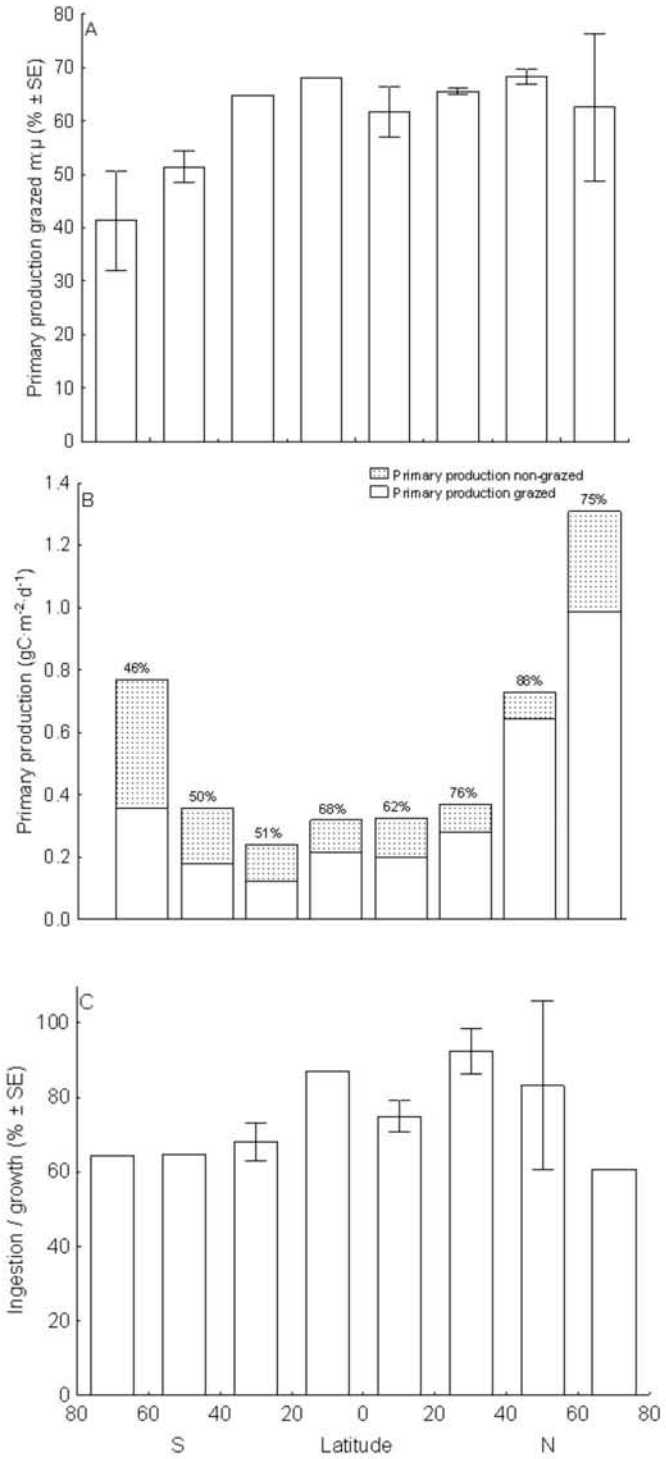


Fig. 4: Average values and error plots of (A) primary production grazed by microzooplankton as m:μ ratio from dilution experiment results, (B) primary production grazed and non-grazed by microzooplankton by latitude ranges and, (C) primary production grazed by microzooplankton assuming that grazing is the sum of assimilation and defecation by latitude ranges

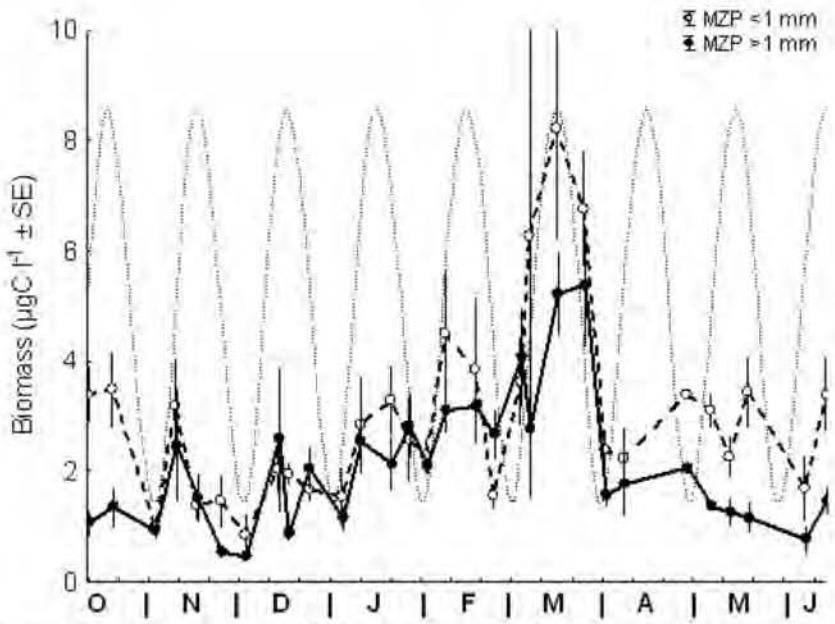


Fig. 5: Time-series from October 2005 to June 2006 of mesozooplankton <1 mm and mesozooplankton >1 mm

Chlorophyll *a* and temperature were correlated significantly with the biomass of all the autotrophic picoplankton groups and with the large size fraction of mesozooplankton (Table 2). Moreover, splitting the data in bloom (February, March, April) and non-bloom (rest of months) periods, we obtained negative correlations between heterotrophic nanoflagellates and autotrophic picoplankton. These correlations were significant between HNF and total autotrophic organisms (APic plus ANF) during the non-bloom period (Pearson's rank; $r = -0.665$; $p < 0.005$). Finally, a striking result is the high correlation found between large mesozooplankton and chlorophyll and autotrophic organism (Table 2).

Tab. 2: Pearson pairwise correlation coefficients (r) between monthly average values of chlorophyll *a* (Chl *a*), temperature (Temp.) and biomass of heterotrophic prokaryotes (HP), *Prochlorococcus* (Pro), *Synechococcus* (Syn), autotrophic picoeukaryotes (APE), heterotrophic (HNF) and autotrophic (ANF) nanoflagellates, microplankton (Mic) and mesozooplankton (MZP). APic is the sum of APE, Pro and Syn. A is the sum of APic and ANF. Bold numbers represent significant correlations at *p < 0.05, **p < 0.01 and ***p < 0.001

	Chl a	Temp.	HP	Pro	Syn	APE	Apic	ANF	A	HNF	Mic	MZP <1
Temp.	-0.667											
HP	0.250	-0.100										
Pro	**0.733***	**0.983*****	-0.067									
Syn	**0.833****	**-0.733***	0.083	**-0.750***								
APE	**0.717***	**-0.917*****	0.333	**-0.900*****	**0.833****							
Apic	**0.883****	**-0.700***	0.367	**-0.717***	**0.917*****	**0.850****						
ANF	-0.167	0.233	-0.383	0.283	-0.267	-0.333	-0.250					
A	0.767	**-0.683***	0.300	-0.633	**0.767***	**0.700***	**0.783***	0.067				
HNF	-0.383	-0.200	0.450	-0.217	-0.233	0.000	0.417	-0.233	0.433			
Mic	0.393	-0.428	**0.857***	-0.357	0.428	**0.786***	0.750	-0.286	0.536	-0.464		
MZP <1	0.267	-0.350	0.017	-0.283	0.400	0.333	0.267	0.200	**0.667***	0.017	0.357	
MZP >1	**0.800****	**0.917*****	-0.117	**-0.933*****	**0.850****	**0.817****	**0.767***	-0.117	**0.733***	0.033	0.214	0.333

Discussion

The main objective of this work was to reveal and describe the temporal succession of the different plankton groups in these oceanic waters from bacteria to large zooplankton in a rather intensive (weekly) sampling. The structure of the plankton community changed before, during and after the bloom. Before the bloom, the only group showing a relatively higher biomass was *Prochlorococcus*. The oceanic distribution of this cyanobacterium suggests that temperature can have a lethal effect on it (Partensky et al. 2009). In a recent study, Baltar et al. (2009) found that *Prochlorococcus* was almost absent at temperatures below 16.1ºC. In the present work, its biomass was minimal from January to May, when surface temperatures were the lowest.

The bloom started in December coinciding with the sharp decrease in temperature and the outburst of APE. As temperature dropped below 20°C, a short bloom of diatoms also developed. However, they represented a rather small fraction of autotrophic biomass. Diatom population dynamics usually show a short-lived biomass peak in these waters (Ojeda 1998), which indicates the start of the late winter bloom. In February, small autotrophic cells (APE <2 µm) replaced diatoms. Those cells and *Synechococcus* made the bloom in these waters due to the availability of nutrients as a consequence of mixing, as observed elsewhere (Jacquet et al. 2001, Agawin et al. 2000a, 2000b). Their fluctuations are also a consequence of predation by heterotrophic organisms such as nanoflagellates or microzooplankton (Reckermann and Veldhuis 1997, Guillou et al. 2001). In this work, we observed that autotrophic picoplankton biomass depicted an inverse pattern to heterotrophic nanoflagellate biomass. The negative correlation was stronger out of the bloom period, when the autotrophic picoplankton is less abundant. Microzooplankton preys upon nanoflagellates or picoplankton, in function of their availability (Rassoulzadegan et al. 1988, Jürgens et al. 1996, Guillou et al. 2001). In this sense, to explain the inverse relationship between

autotrophic plankton and nanoflagellates when microzooplankton feeds on nanoflagellates, picoplankton is free of predation and its biomass can increase. Oppositely, when microzooplankton feeds on picoplankton, nanoflagellates are not preyed and their biomass can increase.

Microplankton community was clearly dominated by aloricate ciliates. During the bloom, ciliate biomass was higher in December, January and February coinciding with the sharp decrease in heterotrophic nanoflagellates. In February, the main peak of ciliate biomass corresponded to the highest biomass of APE but lower biomass of HNF (Figs. 3, 4). This is an expected result as there is a consensus about the importance of microzooplankton, especially ciliates, in controlling HNF (Rassoulzadegan et al. 1988). In March, the abrupt increase of mesozooplankton biomass probably controlled ciliates, allowing a new increase of heterotrophic nanoflagellates. After the bloom, in May, we observed a small spring bloom in the presence of higher biomass of Pro, ANF, APE and diatoms, coinciding with the start of stratification and the use of the still available nutrients (new and regenerated) by autotrophic organisms. This small bloom was also observed as an increase of aloricate ciliates.

Mesozooplankton biomass variability was rather high (Fig. 5). This result is in agreement with several works performed around the Canary Islands (Hernández-León et al. 2004, Moyano et al. 2009). In previous works (Hernández-León 1998, Hernández-León et al. 2001, 2002, 2004), epipelagic mesozooplankton biomass was observed to vary with the lunar illumination cycle as the effect of the presence/absence of diel vertical migrants (DVMs, mainly large crustaceans and mesopelagic fishes) in the upper layers of the ocean during night. During full moon DVMs do not reach the upper layers of the ocean (<100 m depth) to avoid predation. Their absence allows epipelagic mesozooplankton to increase in abundance and biomass. After full moon, DVMs reach the upper layers feeding on the zooplankton crop. Thus, the sharp increase of small and large mesozooplankton in March (Fig. 5) is suggested to promote

the decrease in microzooplankton (Fig. 4) and the increase in HNF, controlling therefore picoplankton. This pattern suggests a top-down control of higher trophic levels at the end of the late winter bloom. Therefore, the presence or not of large organisms seems to have important consequences in the development of the bloom as, firstly, gives evidence of a top-down control of the bloom at the end-to-end perspective. Secondly, the presence or not of mesozooplankton promotes a change in the structure of the plankton community, conforming the landscape in the mixed layer. Thirdly, the rather high correlation between large mesozooplankton and chlorophyll *a* and autotrophic organisms suggests that the top-down control on microzooplankton releasing nanoflagellates from predation, promotes the increase in autotrophs. The latter should also benefits from the ammonia excretion by those organisms. Finally, the predation of DVMs on mesozooplankton seems to shunt the energy and matter to the mesopelagic zone (see also Hernández-León et al. 2004).

To summarize, we have characterized the planktonic community before, during and after a winter bloom in these subtropical waters. Mixing in winter promoted the conditions for the outburst of autotrophic picoplankton and small diatoms, fuelling the increase in microplankton and mesozooplankton, the latter promoting a top-down control at the end of the bloom. The biogeochemical consequences of these changes in the structure of the pelagic realm deserve further research.

Acknowledgements

The authors would like to thank all the colleagues of the Biological Oceanography Group that contributed to the hard field work. Also, A. Calbet is thanked for valuable comments on the manuscript. This study was funded by projects “ConAfrica” (CTM2004-02319) and “Lucifer” (CTM2008-03538) from the Ministry of Science and

Innovation of Spain and from a PhD fellowship to C. Schmoker from the Government of Canary Islands, Spain.

References

Arístegui J, Hernández-León S, Montero MF, Gómez M (2001) The seasonal planktonic cycle in coastal waters of the Canary Islands. Sci Mar 65: 51-58

Arístegui J, Montero MF (2005) Temporal and spatial changes in plankton respiration and biomass in the Canary Islands regions: The effect of mesoscale variability. J Mar Syst 54: 65-82

Azam F, Fenchel T, Field JG, Gray JS, Meyerreil LA, Thingstad F (1983) The ecological role of water-column microbes in the sea. Mar Ecol Prog Ser 10: 257-263

Båmstedt U (1986) Chemical composition and energy content. In Corner EDS, O'Hara SCM (Eds) The biological Chemistry of Marine Copepods. Clarendon Press, Oxford, pp. 1-58

Baltar F, Arístegui J, Montero MF, Espino M, Gasol JM Herndl GJ (2009) Mesoscale variability modulates seasonal changes in the trophic structure of nano- and picoplankton communities across the NW Africa-Canary Islands transition zone. Prog Ocean Special issue 83: 180-188

Bode A, Barquero S, Varela M, Braun JG, De Armas D (2001) Pelagic bacteria and phytoplankton in oceanic waters near the Canary Islands in summer. Mar Ecol Prog Ser 209: 1-17

Børsheim KY, Bratbak G (1987) Cell volume to cell carbon conversion factors for a bacterivorous Monas sp. enriched from seawater. Mar Ecol Prog Ser 36: 171-175

Braun JG (1980) Estudios de producción en aguas de las Islas Canarias I. Hidrografía, nutrientes y producción primaria. Bol Inst Esp Oceanogr 5: 147-154

Campbell L, Liu H, Nolla HA, Vaulot D (1997) Annual variability of phytoplankton and bacteria in the subtropical North Pacific Ocean at Station ALOHA during the 1991-1994 ENSO event. Deep-Sea Res I 44: 167-192

Cho BC, Azam F (1990) Biogeochemical significance of bacterial biomass in the ocean's euphotic zone. Mar Ecol Prog Ser 63: 253-259

Christian JR, Karl DM (1994) Microbial community structure at the U.S.-Joint Global Ocean Flux Study Station ALOHA: Inverse methods for estimating biochemical indicator ratios. J Geophys Res 99: 14269-14276

De León AR, Braun JG (1973) Annual cycle of primary production and its relation to nutrients in the Canary Islands waters. Bol Inst Esp Oceanogr 167: 1-24

Gasol JM, Zweifel UL, Peters F, Fuhrman JA, Hagström A (1999) Significance of size and nucleic acid content heterogeneity as measured by flow cytometry in natural planktonic bacteria. Appl Environ Microbiol 65: 4475-4483

Gliwicz P (1986) A lunar cycle in zooplankton. Ecology 67: 883-897

Guillou L, Jacquet S, Chrétiennot-Dinet MJ, Vaulot D (2001) Grazing impacto f two small heterotrophic flagellates on *Prochlorococcus* and *Synechococcus*. Aquat Microb Ecol 26: 201-207

Haas LW (1982) Improved epifluorescence microscopy for observing planktonic micro-organisms. Ann Inst Oceanogr Paris 58: 261-266

Hasle G, Syvertsen EE (1997) Marine diatoms in Identifying marine diatoms and dinoflagellates pp. 5-385

Hernández-León S (1991) Accumulation of mesozooplankton in a wake area as a causative mechanism of the “island-mass effect”. Mar Biol 109: 141-147

Hernández-León S, Torres S (1997) The relationship between ammonia excretion and GDH activity in marine zooplankton. J Plankton Res 19: 587-601

Hernández-León S, Almeida C, Gómez M, Torres S, Montero I, Portillo-Hahnefeld A (2001) Zooplankton biomass and indices of feeding and metabolism in island-generated eddies around Gran Canaria. J Mar Syst 30: 51-66

Hernández-León S, Almeida C, Becognée P,Yebra L, Arístegui J (2004) Zooplankton biomass and indices of grazing and metabolism during a late winter bloom in subtropical waters. Mar Biol 145: 1191-1200

Hillebrand H, Durselen CD, Kirschtel D, Pollingher U, Zohary T (1999) Biovolume calculation for pelagic and benthic microalgae. J Phycol 35: 403-424

Jürgens K, Wickham SA, Rothhaupt KO, Santer B (1996) Feeding rates of macro- and microplankton on heterotrophic nanoflagellates. Limnol Oceanogr 41: 1833-1839

Kana TM, Glibert PM (1987) Effects of irradiances up to 2000 µm E m•2•s•1 on marine Synechococcus WH 7803-1. Growth, pigmentation, and cell composition. Deep-Sea Res I 26: 97-108

Lee S, Fuhrman JA (1987) Relationships between biovolume and biomass of naturally derived marine bacterioplankton. Appl Environ Microbiol 53: 1298-1303

Li WKW, Dickie PM, Irwin BD, Wood AM (1992) Biomass of bacteria, cyanobacteria, prochlorophytes and photosynthetic eukaryotes in the Sargasso Sea. Deep-Sea Res A 39: 501-519

Lovegrove T (1966) The determination of the dry weight of plankton and the effect of various factors on the values obtained. In: Barnes H (ed) Some contemporary studies in Marine Sciences. George Allen and Unwin Ltd pp. 429-467

Lynn DH, Small EB (2002) Ciliophora. In Lee: Leedale J, Bradbury P (eds) The illustrated guide to the Protozoa. Lawrence, Kansas, pp. 371-656

Menden-Deuer S, Lessard EJ (2000) Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. Limnol Oceanogr 45: 569-579

Menzel DW, Ryther JH (1961) Zooplankton in the Sargasso Sea off Bermuda and its relation to organic production. J Du Conseil Pour L'Exploration De La Mer 26: 250-258

Moyano M, Rodríguez JM, Hernández-León S (2009) Larval fish abundance and distribution during the late winter bloom off Gran Canaria Island, Canary Islands. Fish Oceanogr 18: 51-61

Ojeda A (2005) Dinoflagelados de Canarias estudio taxonómico y ecológico. Monografía LXV del Instituto de Estudios Canarios 1-301

Putt M, Stoecker DK (1989) An experimentally determined carbon: volume ratio for marine "oligotrichous" ciliates from estuarine and coastal waters. Limnol Oceanogr 34: 1097-1103

Rassoulzadegan F, Laval-Peuto M, Sheldon RW (1988) Partitioning of the food ration of marine ciliates between pico- and nanoplankton. Hydrobiol 159: 75-88

Raven JA (1998) The twelf Tansley Lecture. Small is beautiful: the picoplankton. Funct Ecol 12: 503-513

Reckermann M, Veldhuis MJW (1997) Trophic interactions between picophytoplankton and micro- and nanozooplankton in the western Arabian Sea during the NE monzón 1993. Aquat Microb Ecol 12: 263-273

Ricard M (1987) Atlas du phytoplancton marin. 2: Diatomophycées. 1-297

Sherr EB, Sherr BF (2007) Heterotrophic dinoflagellates: a significant component of microzooplankton biomass and major grazers of diatoms in the sea. Mar Ecol Prog Ser 352: 187-197

Sieburth J McN, Smetacek V, Lenz J (1978) Pelagic ecosistema structure: heterotrophic compartments of the plankton and their relationship to plankton size fractions. Limnol Oceanogr 23:1256-1263

Sieracki ME, Haugen EM, Cucci TL (1995) Oversestimation of heterotrophic bacteria in the Sargasso Sea: direct evidence by flow and imaging cytometry. Deep-sea Res 42: 1399-1409

Steidinger KA, Tangen K 1(997) Dinoflagellates in Identifying marine diatoms and dinoflagellates pp. 387-584

Strathmann RR (1967) Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. Limnol Oceanogr 12 (3): 411-418

Tsai AY, Chiang KP, Chang J, Gong GC (2008) Seasonal variations in trophic dynamics of nanoflagellates and picoplankton in coastal waters of the western subtropical Pacific Ocean. Aquat Microb Ecol 51: 263-274

Verity PG, Langdon C (1984) Relationships between lorica volume, carbon, nitrogen, and ATP content of tintinnids in Narragansett Bay. J Plankon Res 6: 859-868

Yentsch CS, Menzel DW (1963) A method for the determination of phytoplankton chlorophyll and phaeophytin fluorescence. Deep-Sea Res I 10: 221-231

Zubkov MV, Sleigh MA, Burkill PH (2000a) Assaying picoplankton distribution by flow cytometry of underway samples collected along a meridional transect across the Atlantic Ocean. Aquat Microb Ecol 21: 13-20

Zubkov MV, Sleigh MA, Burkill PH, Leakey RJG (2000b) Picoplankton community structure on the Atlantic Meridional Transect: a comparison between seasons. Prog Ocean 45: 369-386

CHAPTER V

End-to-end comparison of cold and warm years in the subtropical waters of the Canary Current

Claire Schmoker, Santiago Hernández-León

Abstract

The Canary Current has experienced a progressive warming and a decrease in productivity over the last decades. This overall trend promotes a real concern about the consequences of global warming in this region. In order to study the effect of stratification in these waters, we performed a weekly sampling during two and a half years, covering three times the most productive season in subtropical waters, the so-called Late Winter Bloom. Significant differences were observed in most of the environmental and biological variables between years. During 2005, the mixing of the water column started in January, while in 2007 the mixing period was delayed by one month showing slightly higher temperatures and lower chlorophyll concentrations. Autotrophic picoplankton and heterotrophic prokaryotes also decreased between 2005 and 2007. By opposite, nano- and microplankton increased through the same period. Low mesozooplankton biomass was also recorded during the warmer year in 2007. The small mesozooplankton size-fraction (<1000 µm) showed a sharp decrease in 2007, while the large size-fraction (>1000 µm) gradually decreased since 2005. The results suggest that small inter-annual differences in temperature (~0.5ºC) give rise to important changes in the structure of the pelagic ecosystem in subtropical waters through bottom-up and top-down effects.

Introduction

Over the past 50 years, the rate of temperature increase has accelerated and has led to a rapid warming of the atmosphere and oceans (Richardson 2008). The average surface warming level is now close to 1ºC for the terrestrial environment and over 0.5ºC for the ocean (Levitus et al. 2005). Moreover, air and sea temperatures over most of the NE Atlantic increased since the late 1980s by at least 0.4ºC per decade

(Dias et al. 1992, Brander et al. 2003). The Canary Current has also experienced a progressive warming and a decrease in productivity over the last decades (Arístegui et al. 2009, Demarcq 2009). Since 1986, a general warming was observed through the region with highest variability in the Mauritanian-Senegalese sub-region.

The composition, abundance, physiology and therefore trophic efficiency of plankton communities are strongly linked to water temperature. It is probably the single most important physical variable structuring marine ecosystems and its critical influence makes marine systems acutely vulnerable to global warming (Richardson 2008). Autotrophic picoplankton is highly affected by environmental changes like temperature, nutrients and light (Zubkov et al. 2000b, Arístegui and Montero 2005, Baltar et al. 2009). *Prochlorococcus* has been described as typical of oligotrophic waters of the oceans (Campbell and Vaulot 1993) at temperatures above 17°C (Olson et al. 1990a). On the other hand, *Synechococcus* (Veldhuis et al 1993, Partensky et al. 1999) and autotrophic picoeukaryotes (APE) (Olson et al. 1990b) are characteristic of winter and early spring, when the water column is mixed. The ratio of autotrophic to heteretrophic biomass (A:H ratio) also changes through the seasons in subtropical waters (Arístegui and Montero 2005, Baltar et al 2009). These authors observed that the ratio decreased from winter to summer, suggesting that heterotrophs play a more important role in summer. Moreover, they also showed that the relative proportion of autotrophic biomass to phytoplankton carbon derived from chlorophyll *a* was higher in winter and spring than in summer, suggesting that large eukaryotes contributed more in winter and spring than in summer. In zooplankton there is also evidence of changes due to temperature in the distribution of individual species and assemblages, earlier timing of important life cycle events or phenology, and changes in abundance and community structure (Richardson 2008).

At the regional level, in oligotrophic systems such as the subtropical gyres the cell abundance and primary production is dominated by picoplankton (Zubkov et al. 2000a)

while in coastal and temperate areas, biomass and production are generally dominated by nano- and microplankton (Malone et al. 1991). In more productive areas like coastal upwelling zones, large-size phytoplankton cells dominate the pelagic food web. In zooplankton, members of the warm-water copepod assemblages have moved more than 1100 km polewards over the past 50 years (Richardson et al. 2006). Changes in species composition are also reported inside a region due to stratification (see Valdés et al. 2007). Therefore, it seems important to study the effect that variations in temperature have on the structure and function of plankton communities in order to predict changes due to a warming scenario. Stratification promoted by the increasing temperature will give rise to fluctuations in community structure of unknown consequences. Those changes could be envisaged *a priori* comparing cool and warm years at the regional scale.

In this sense, the waters surrounding the Canary Islands are oligotrophic with a seasonal thermocline separating low-nutrient, low-chlorophyll surface, from deep nutrient-rich waters (de León and Braun 1973). During the late winter, after the erosion of the thermocline due to atmospheric cooling, a weak bloom is observed (De León and Braun 1973, Arístegui et al. 2001, Hernández-León et al. 2004). During the first phases of the bloom, increase of primary production results from the development of APE and larger phytoplankton cells (>2 µm, mainly autotrophic nanoflagellates, ANF, and diatoms) while small cells predominate at the end of the bloom (Arístegui et al. 2001). Changes in this pattern due to inter-annual variability is still unknown in these subtropical waters, despite the increasing interest to tackle the effects of global warming in those large areas of the ocean. Moreover, the changes in other components of the microbial food web such as microzooplankton are poorly known.

Thus, the main objective of this work was to study the inter-annual variability of the warm water ecosystem around the Canary Islands with special emphasis to the

observed changes in temperature. As a decrease in productivity was observed in the Canary Current probably due to the registered increment in temperature, we wonder if those changes would affect the planktonic composition and structure as well as the relationships between the different groups in subtropical waters. To answer this question, an intensive weekly sampling was carried out during two years and a half, covering three winters, the more productive period of the year in these oceanic subtropical waters. An end-to-end approach from bacteria to mesozooplankton was adopted in order to fill the gap existing in studies compiling data from these communities in the Canary Current.

Material and methods

Weekly sampling was carried out from January 2005 to June 2007 on board of the RV Solana II. Due to technical problems, no sampling was performed during September 2005. One station (28º04'N 15º21'E) was chosen for sampling and was located ~ 3 nm offshore in the 100 m isobath, at the edge of the island shelf (Fig. 1).

Fig. 1: Map of the study area, showing the location of the sampling station in front of Gran Canaria Island, Canary Islands (Northeast Subtropical Atlantic)

Vertical profiles of temperature, conductivity and fluorescence were obtained using a CTD SBE25 probe (Sea-Bird Electronics Inc., Bellevue, WA, USA). Samples for chlorophyll, bacterio- and microplankton were taken from the mixed layer at 15 m depth with a 5 l Niskin bottle.

Depth profiles of *in situ* fluorescence were converted to chlorophyll from fluorescence versus chlorophyll relationship. Chlorophyll was measured fluorometrically on a Turner Designs bench fluorometer 10A, previously calibrated with pure chlorophyll *a* (Sigma-Aldrich Inc., St. Louis, MO, USA; Yentsch and Menzel 1963). Samples of seawater (500ml) were filtered on board through 25 mm Whatman GF/F filters and preserved in liquid nitrogen until their analysis. Pigments were extracted during 24 h in 10 ml of 90% acetone at 4ºC in the dark.

Heterotrophic prokaryotes (HP), small photosynthetic eukaryotic cells (autotrophic picoeukaryotes, APE), *Prochlorococcus* (Pro) and *Synechococcus* (Syn) type cyanobacteria, were counted by flow cytometry using a FACScalibur instrument (Becton and Dickinson, USA) Immediately after collection, samples were fixed with paraformaldehyde (2% final concentration), incubated for 30 min at 4ºC in dark and then stored frozen in liquid nitrogen until analysis. To count heterotrophic prokaryotes, 200 µl samples were stained with a DMS-diluted SYTO-13 (Molecular Probes Inc.) stock (10:1) at 2.5 µM final concentration. HP were identified by their signatures in a plot of side scatter (SSC) versus green fluorescence (FL1) (Gasol et al. 1999). The identification of small phytoplankton groups was based on interactive analyses of multiple bivariate scatter plots of side scatter, red and orange fluorescence. Samples were run at low speed for HP and at medium or high speed for autotrophic picoplankton until 10,000 events were acquired or 2 minutes were passed.

Samples for autotrophic (ANF) and heterotrophic (HNF) nanoflagellates were preserved following the procedure given by Haas (1982). Immediately after collection,

samples were fixed with glutaraldehyde (0.3% final concentration). The samples were then placed into a filtration tower and fixed with diamidino-2-phenylindole for 10 minutes, and filtered onto a 0.2 µm black polycarbonate membrane filter, 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 20 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.

Microplankton samples were preserved in Lugol's iodine solution (final concentration 2%) and stored in the dark. For technical reasons, only samples from October 2005 to June 2007 were counted by light microscopy. Subsamples of 100 ml were allowed to settle for 48 h in a composite chamber. The entire chamber bottom was examined using the Utermöhl technique. Ciliates and tintinnids were assigned to genus when possible following Lynn and Small (2002). Dinoflagellates were identified following Steidinger and Tangen (1997) and Ojeda (2005) and diatoms following Ricard (1987) and Hasle and Syvertsen (1997). The length and diameter of each individual (up to a maximum of 20 cells for each taxon) were measured at 400x magnification using a calibrated ocular micrometer. Mean cell volume was calculated by equating the shape of each taxa to a standard geometric configuration (Hillebrandt et al. 1999).

HP abundances were converted to biomass using a factor of 20 fgC·cell^{-1} (Lee and Fuhrman 1987, Cho and Azam 1990, Bode et al. 2001). Pro cell numbers were converted to biomass assuming a mean biovolume of 0.1 µm^3·cell^{-1} (Sieracki et al. 1995), and a conversion factor of 220 fgC·µm^{-3} (Christian and Karl 1994, Zubkov et al. 2000b). Syn cell numbers were converted to biomass using a conversion factor of 250 fgC·cell^{-1} (Kana and Glibert 1987, Li et al. 1992). APE abundances were transformed to biomass using a conversion factor of 2100 fgC·cell^{-1} (Campbell et al. 1997).

ANF and HNF cell-numbers were converted to biomass assuming a mean volume of 20 $\mu m^3 \cdot cell^{-1}$ for autotrophs, and of 10 $\mu m^3 \cdot cell^{-1}$ for heterotrophs and a conversion factor of 220 $fgC \cdot \mu m^{-3}$ (Børsheim and Bratbak 1987).

Autotrophic biomass was calculated from the pico- and nanoplankton components (A) computed as the sum of the Syn, Pro, PE and ANF biomasses. The biomass of heterotrophs (H) was obtained by adding the HP and HNF biomasses.

Ciliate biovolumes estimated were converted to carbon equivalents by the factor experimentally derived for Lugol's-fixed marine oligotrichs from Putt and Stoecker (1989), except for tintinnid carbon, which was estimated from the experimentally derived factor from Verity and Langdon (1984). Dinoflagellate abundances were converted to biomass using a Mender-Deuer and Lessard (2000) carbon content conversion factor. Finally, diatom abundances were converted to biomass according to the equation of Strathmann (1967). The use of Lugol´s fixative precluded identification of mixotrophic from autotrophic and heterotrophic microorganisms. Taxa were distributed into size categories (<20 µm, 20-40 µm and >40 µm).

Zooplankton was caught in oblique hauls using a Bongo net of 40 cm diameter, equipped with 200 µm mesh size nets and a flowmeter (General Oceanics Inc., Miami, FL, USA). The net was towed at two or three knots from a maximum of 90 m depth up to surface. Samples were fractionated with a 1 mm mesh and both size fractions were used to quantify mesozooplankton biomass as dry weight, following the method of Lovegrove (1986) and was converted to carbon biomass assuming a carbon:dry weight ratio of 0.4 (Båmstedt 1986).

Statistical analyses performed consisted in multiple regression analysis and Pearson´s correlation coefficients (r) tests were calculated in order to examine the relationships between the planktonic groups. Additionally, we performed a structural equation model (path analysis), which hypothesized causal relationships among

planktonic variables and tested the causal model with a linear equation model (Sokal and Rohlf, 1995). We built a path diagram that showed the interconnection between variables using the SPSS 15.0 and the AMOS 17.0.2 sofware packages. Krustal-Wallis ANOVA (K-W ANOVA) were run to assess inter-annual differences in environmental variables, chlorophyll *a* and temperature, and in planktonic variables, heterotrophic bacteria, autotrophic picoplankton (APic as the sum of APE, Syn and Pro), nanoflagellates and large and small mesozooplankton, across the late winter bloom period (December-May). As most of the variables did not meet the underlying conditions of normality (Kolmogorov-Smirnov) and homogeneity of variances (Levene´s test), non-parametric tests were used.

Results

The typical weak seasonal temperature pattern for the Canary Current was observed. The water column was stratified from June to December of every year. In the winter of 2005, water temperature was approximately 0.5-1ºC lower than in 2006 and 2007 (Fig. 2A). At that period of the year, nutrients become available for the surface layers (Arístegui et al. 2001) and this was reflected in the increase of the chlorophyll *a* in the mixed layer in February (Fig. 2B).

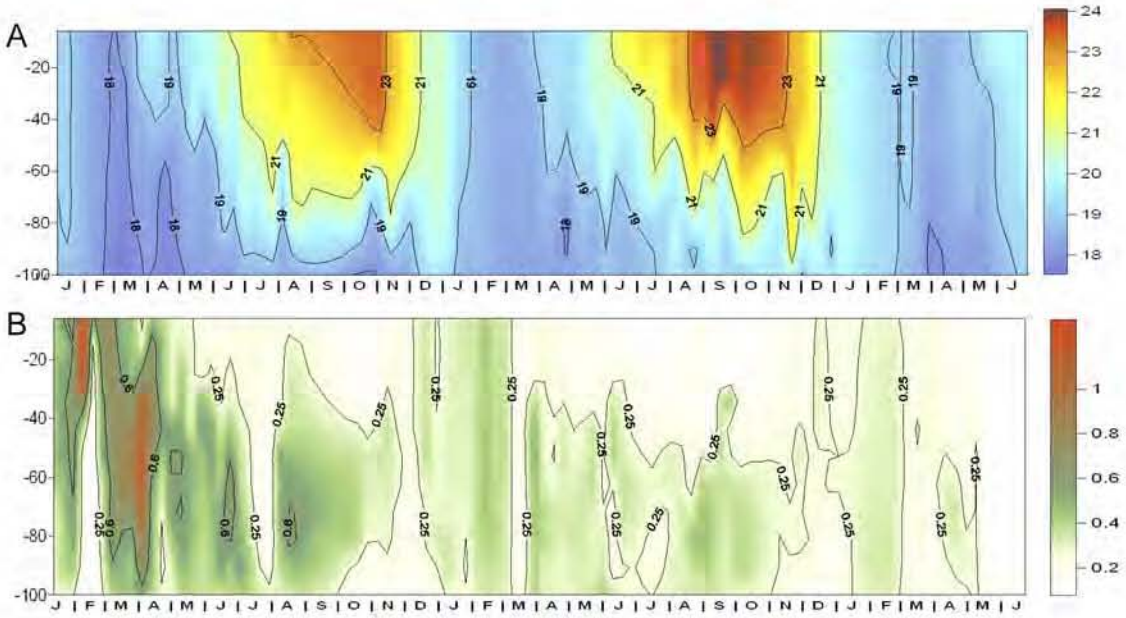


Fig. 2: Section up to 100 m depth of (A) temperature and (B) chlorophyll *a* from January 2005 to June 2007

This increase of primary producers was mainly driven by the dominance of APE at the beginning of winter, dominating the total autotrophic biomass during the different bloom periods (Fig. 3A). As mixing progressed (Fig. 2A), a peak in diatom biomass was also observed (Fig. 3B). Autotrophic prokaryote, *Prochlorococcus* and *Synechococcus*, biomasses clearly changed according to the period of the year. *Prochlorococcus* was present during summer while *Synechococcus* was present during winter and early spring (Fig. 3C).

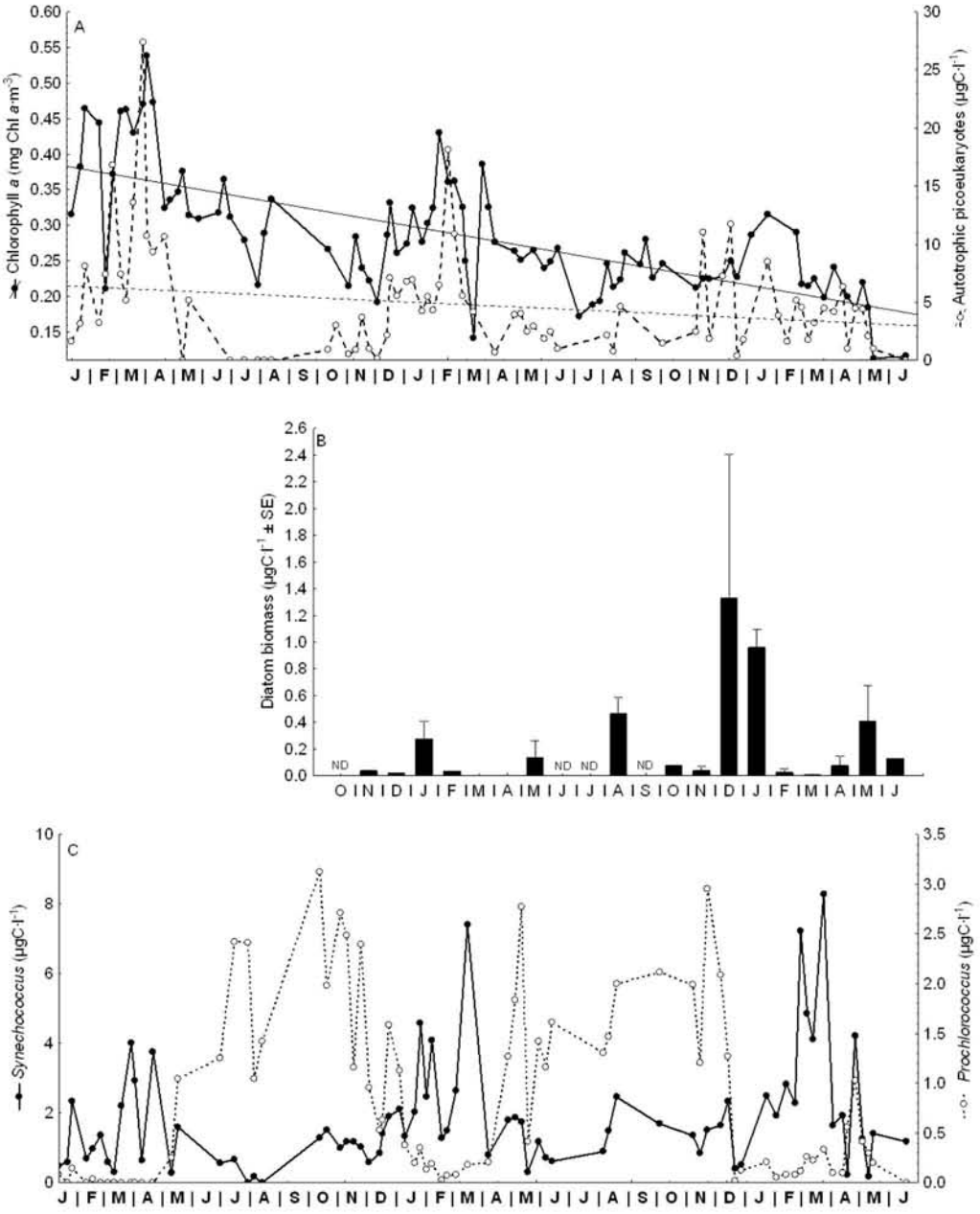


Fig. 3: Time series of (A) autotrophic picoeukaryote (APE) biomass and chlorophyll *a* concentration from January 2005 to June 2007, (B) monthly average of diatom biomass from November 2005 to June 2007, and (C) autotrophic bacteria biomass from January to June 2007. ND stands for No Data

Heterotrophic prokaryotes did not show a clear seasonal pattern but their biomass seemed to decrease through the almost 3-years of study (Fig. 4A). Autotrophic nanoflagellate biomass showed a peak in January 2005 and in February-March of 2007. However, no peak was observed during 2006 (Fig. 4B).

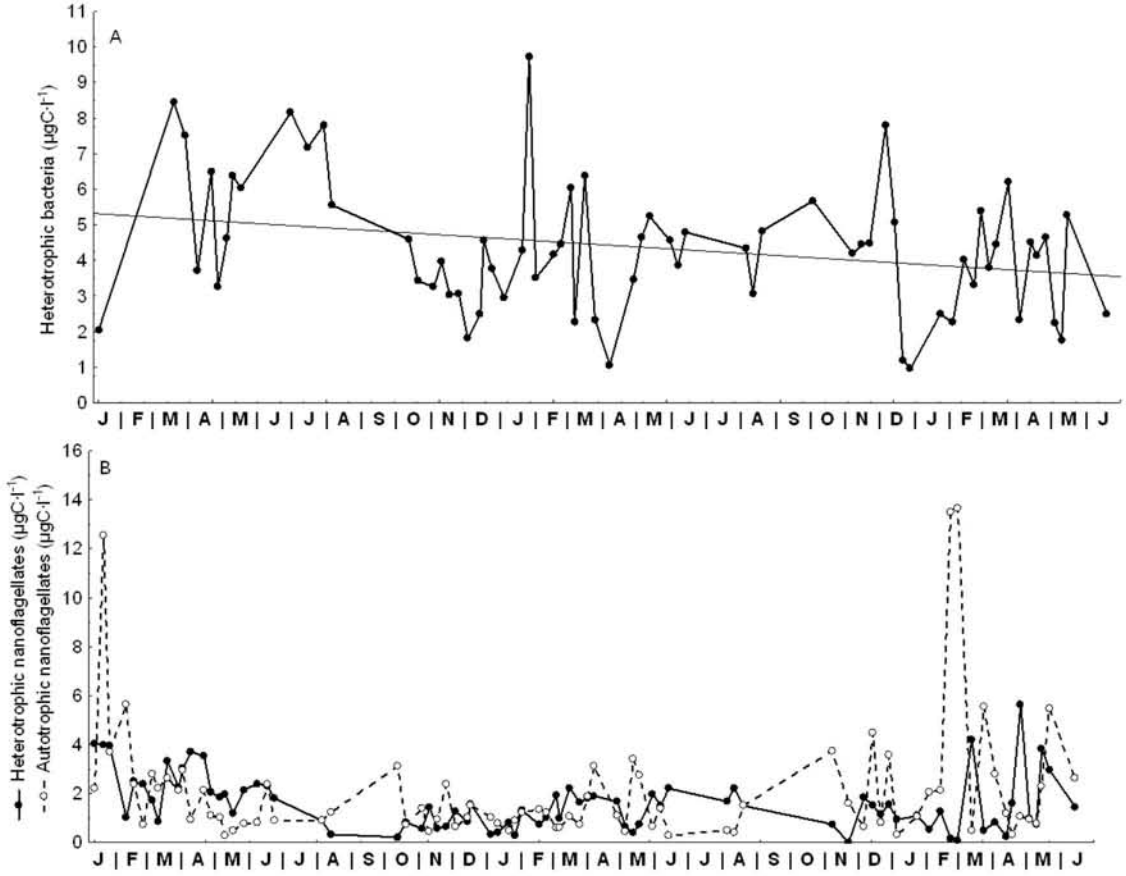


Fig. 4: Temporal evolution of (A) heterotrophic bacteria, and (B) autotrophic and heterotrophic nanoflagellate biomass from January 2005 to June 2007

Most of the microplankton biomass was composed by aloricate ciliates (20-30 µm) during the period studied. However, athecate dinoflagellates dominated in cell numbers. Most of the dinoflagellate cells were in the size range of 15-20 µm. We also observed a slight increase in total microplankton biomass from 2006 to 2007 (Fig. 5).

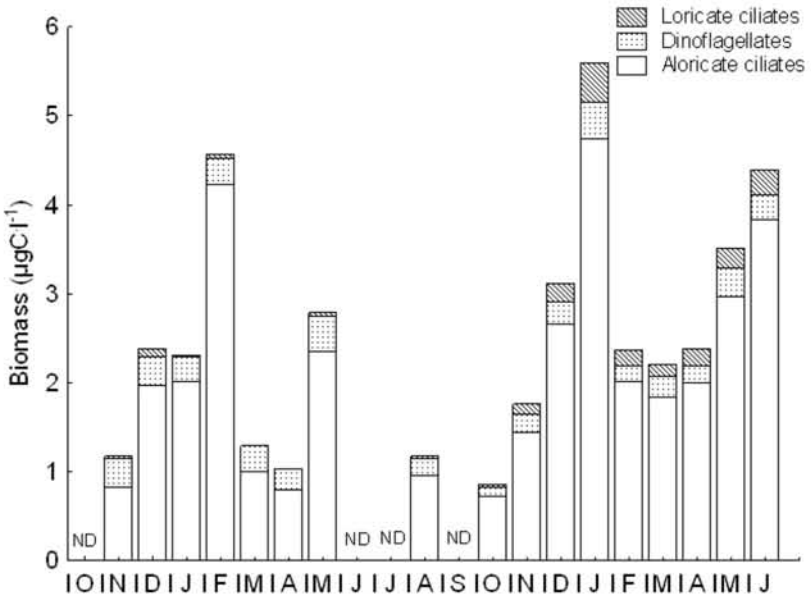


Fig. 5: Temporal evolution of monthly average values of aloricate and loricate ciliate and dinoflagellate biomass from October 2005 to June 2007. ND stands for No Data

Mesozooplanktonic biomass showed annual maxima during the bloom, which were normally coupled to chlorophyll (Fig. 6).

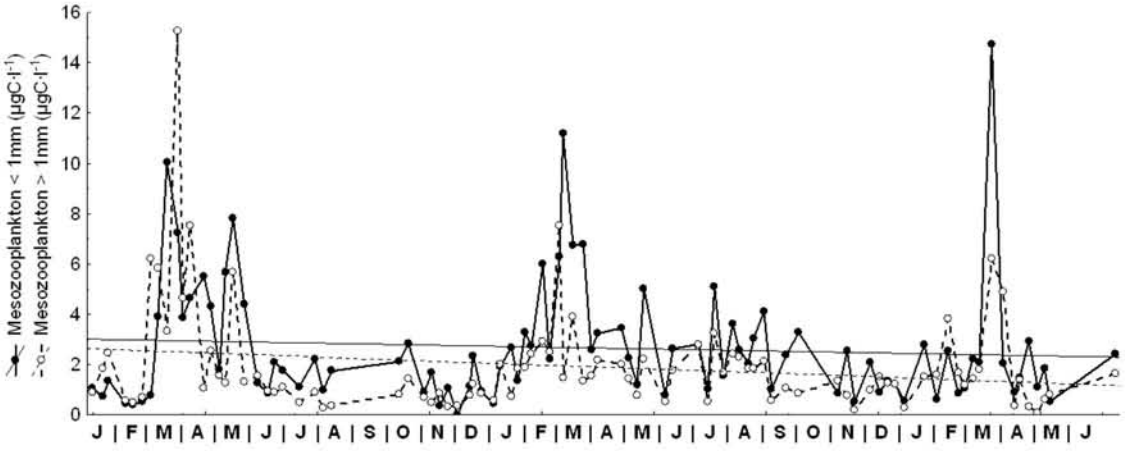


Fig. 6: Temporal evolution of mesozooplankton <1 mm and >1 mm from January 2005 to June 2007

Significant inter-annual variability was observed in the environmental variables. Minimum temperature exhibited higher values in 2007 (KW-ANOVA, $p < 0.001$) while chlorophyll *a* showed an inverse pattern to temperature (Pearson´s Rank $r = -0.207$; $p > 0.05$, Fig. 7A). Higher concentrations were reported in 2005, while 2007 registered

very low values (KW-ANOVA, p <0.001). Heterotrophic bacteria and autotrophic picoplankton biomass followed the same decreasing trend from 2005 to 2007 (Fig. 7B). Nanoflagellate biomass decreased only between 2005 and 2006 and then in 2007 reached their initial biomass of 2005. This variability was significant for heterotrophic nanoflagellates and APE (KW-ANOVA, p <0.001, Fig. 7C). The small fraction of mesozooplankton also showed a significant inter-annual variability (KW-ANOVA, p <0.001) with a slight increase in 2006. However, the large mesozooplankton fraction gradually decreased since 2005 (Fig. 7D).

Table 1: Pearson pairwise correlation coefficients (r) between chlorophyll a (Chl *a*), temperature (Temp.) and biomass of heterotrophic prokaryotes (HP), *Prochlorococcus* (Pro), *Synechococcus* (Syn), autotrophic picoeukaryotes (APE), heterotrophic (HNF) and autotrophic (ANF) nanoflagellates, microplankton (Mic), and mesozooplankton (MZP) <1 mm and >1 mm. Numbers in bold correspond to significant correlations at *p < 0.05, **p < 0.01 and ***p < 0.001

	Chl *a*	**Temp.**	**MZP<1**	**MZP>1**	**Mic**	**ANF**	**HNF**	**APE**	**Syn**	**Pro**
Temp.	-0.207									
MZP<1	0.200	**-0.328***								
MZP>1	0.178	**-0.374****	**0.639*****							
Mic	0.153	-0.211	**-0.330***	-0.118						
ANF	-0.117	0.000	-0.183	0.092	0.207					
HNF	-0.164	-0.085	**0.401****	0.236	-0.312	**-0.296***				
APE	**0.477*****	**-0.375****	0.158	0.225	0.095	0.063	-0.250			
Syn	0.093	**-0.404****	0.220	**0.308***	-0.023	0.167	0.104	**0.500*****		
Pro	-0.168	**0.797*****	-0.153	**-0.437****	-0.48**	-0.195	0.060	-0.221	-0.204	
HP	-0.042	0.030	0.185	0.156	-0.170	0.026	0.054	**0.342***	**0.493*****	0.253

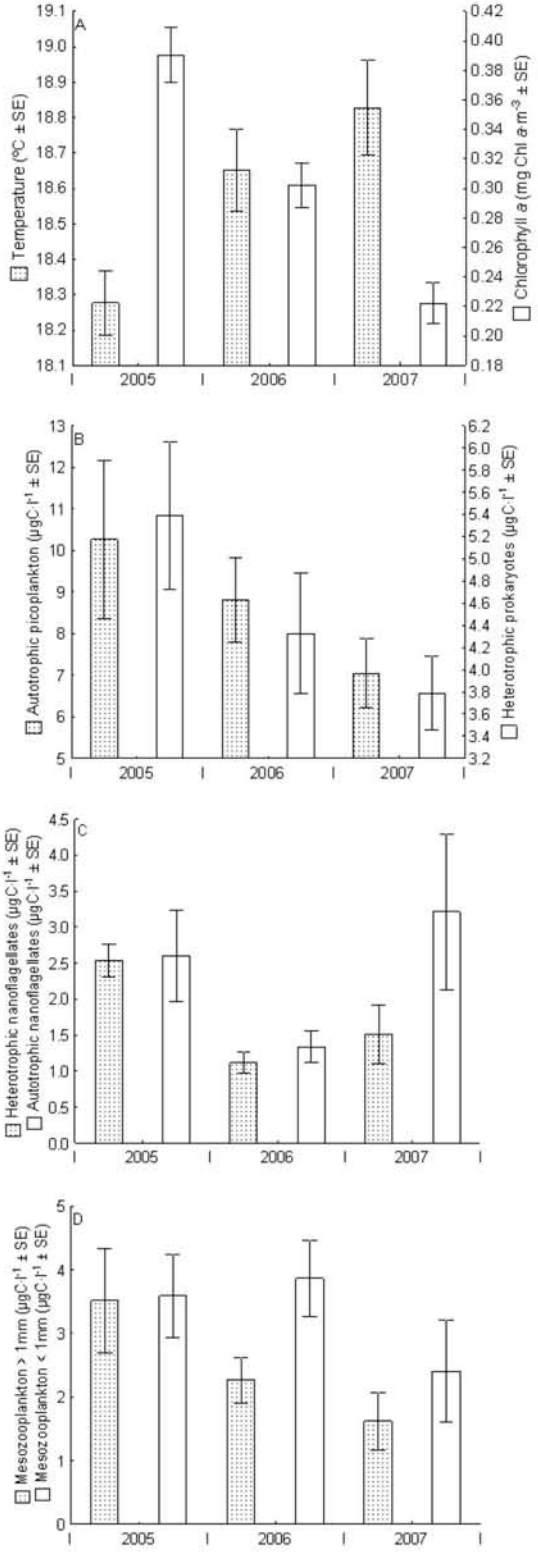


Fig. 7: Annual differences in the average values during the late winter bloom period (December-May) of (A) minimum temperature and chlorophyll *a*, (B) heterotrophic bacteria and autotrophic picoeukaryote (APic as the sum of APE, Syn and Pro) biomass, (C) autotrophic and heterotrophic nanoflagellate biomass and, (D) small and large mesozooplankton biomass

Finally, chlorophyll *a* and temperature were significantly correlated to autotrophic picoeukaryotes (Pearson's Rank, r = 0.477, p <0.001 and r = -0.375, p <0.01, respectively; Table 1). We also found a significant negative linear correlation (Pearson's Rank, r = -0.330, p <0.05) between small-size mesozooplankton fraction and microplankton as well as between autotrophic and heterotrophic nanoflagellates (Pearson's Rank, r = -0.296, p <0.05). Positive significant correlations were calculated for small-size mesozooplankton versus heterotrophic nanoflagellates (Pearson's Rank, r = 0.401, p <0.01), and between autotrophic picoeukaryotes and heterotrophic prokaryotes (Pearson's Rank, r = 0.342, p <0.05).

On the basis of these linear correlations, we built a path diagram where the best cause and effect relationships were assayed to make the model fitting (χ^2 test, p <0.1, Fig. 8).

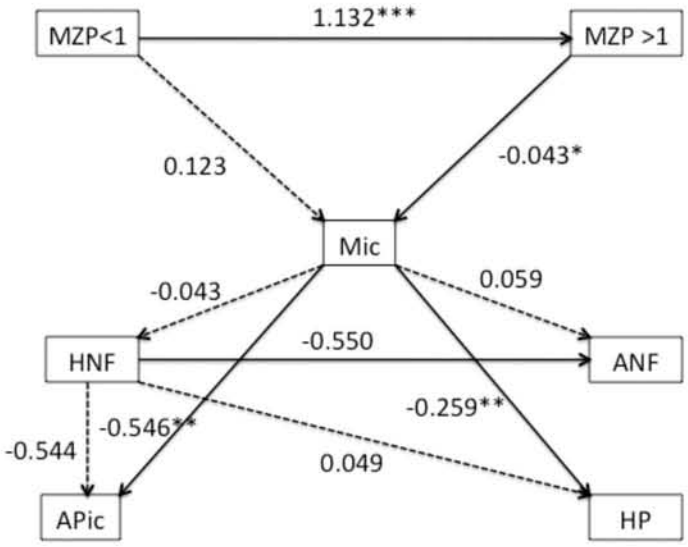


Fig. 8: Path diagram involving the different planktonic group biomasses (MZP stands for mesozooplankton, Mic for microplankton, APic for autotrophic picoplankton, HNF for heterotrophic nanoflagellates, ANF for autotrophic nanoflagellates and HP for heterotrophic prokaryotes). Numbers on the arrows indicate the path coefficients between variables. Solid lines represent significant relations and dotted lines represent non-significant relations *p < 0.1, **p < 0.05 and ***p < 0.001

The negative correlations between the small-size mesozooplankton fraction and microplankton, between microplankton and autotrophic picoplankton and between microplankton and heterotrophic prokaryotes were confirmed by the high level of significance.

Discussion

The effect of an increase of temperature on the planktonic community was studied during a long-term sampling at a fixed station off Gran Canaria Island. The main finding of this work was the evidence of significant changes in the whole plankton community as the effect of and increase in the minimal temperature of the mixed layer during the bloom period. A decrease in chlorophyll *a* and autotrophic picoeukaryotes was found through the period studied. On average, autotrophic picoplankton accounts for most of the photosynthetic biomass in the oceans. In the Canary Islands waters, picoplanktonic cells (<2 µm) account for more than 60% of primary production (Arístegui et al. 2001). This is reflected in the correspondence between chlorophyll a concentration and autotrophic picoeukaryote biomass peaks, the similar decreasing trend and the significant correlation between both biomass estimations through the years. Previous works showed that autotrophic picoplankton community was highly affected by environmental variables like temperature, light and nutrients (Zubkov et al. 2000b, Arístegui and Montero 2005, Baltar et al. 2009). This bottom-up effect on these organisms was reflected on the seasonal shift from a group to another. Undeniably, we could characterize and confirm the repetitive pattern of autotrophic picoeukaryotes present in winter months, *Synechococcus* in early spring and *Prochlorococcus* in summer. These patterns correspond to the ecological preferences of these autotrophic components of the food web. Indeed, *Prochlorococcus* is observed in oligotrophic waters (Campbell and Vaulot 1993) at temperatures above 17°C (Olson et al. 1990a).

In a recent study in Canary Islands waters, Baltar et al. (2009) confirm the absence of *Prochlorococcus* at temperatures below 16.1°C and showed a strong seasonal difference in cell abundance. Its dependence on temperature is clearly observed in the high correlation observed in Table 1. In fact, our results showed almost no *Prochlorococcus* from February to April of 2005, while it was present during the warmer winters of 2006 and 2007 (Fig. 3C).

In winter and early spring, when the water column is mixed, *Synechococcus* (Veldhuis et al 1993, Partensky et al. 1999) and autotrophic picoeukaryotes (Olson et al. 1990b) replace *Prochlorococcus*. Autotrophic picoplankton sensitivity to temperature and mixing could explain their decreasing pattern from 2005 to 2007. The bloom seemed, thus, to vary in function of the temperature. The mixing period started early in colder years such as 2005, while during the warmest year (2007), the bloom started later and may caused lower plankton densities during this period.

Although autotrophic picoplankton and bacterioplankton biomass showed a slight decrease through the period studied and especially during the bloom (Fig. 7), this was not observed in heterotrophic nanoflagellates or microplankton biomass. In fact, the biomass of microzooplankton increased between 2006 and 2007. A significant negative correlation was observed between those organisms and small mesozooplankton (Table 1), suggesting a top-down effect. Moreover, a significant positive relationship was also observed between the latter and heterotrophic nanoflagellates (Table 1), also supporting this top-down effect.

Trophic links and relationships between planktonic groups could also be highlighted by the path analysis taking into account the effect of one variable on another. The structuring model equation is useful to conceptualize food webs and discern the relative importance of pathways within the food web, allowing the enhancement of the significant correlations found between variables when using simple regressions. In that

sense, the correlation between two variables is the sum of the products of the chains of path coefficients along all the paths by which they are connected. Some path coefficients can be larger than 1 because no limitation was set on the magnitude of standard partial regression coefficients. Besides the effect of temperature and therefore, stratification on the development of the bloom, the results of the path analysis show the subtle control of mesozooplankton on lower food web levels and the central role of microplankton. In the Canary Islands waters, microzooplankton is dominated by small dinoflagellates (15-20 μm) in cell numbers and by aloricate ciliates (20-30 μm) in biomass. The small size of these organisms could explain their apparent feeding preference on autotrophic picoplankton, heterotrotrophic prokaryotes and, at a lower level, on nanoflagellates. On the other hand, the decrease in mesozooplankton biomass between 2005 and 2007 could explain the weak increase in microplankton biomass. It is therefore suggested that an indirect consequence of that release of predation on microplankton would be the decrease in autotrophic picoplankton, heterotrophic prokaryote and HNF biomass through the same period.

In summary, significant differences were observed in most of the environmental and biological variables between years. During 2005, the mixing of the water column started in January, while in 2007 the mixing period was delayed by one month showing slightly higher temperatures and lower chlorophyll concentrations. Autotrophic picoplankton and heterotrophic prokaryotes also decreased between 2005 and 2007. By opposite, nano- and microplankton seemed to increase through the same period. Low mesozooplankton biomass was also recorded during the warmer year in 2007. The results suggest that small inter-annual differences in temperature (~0.5ºC) give rise to important changes in the structure of the pelagic ecosystem in subtropical waters through bottom-up and top-down effects. Therefore, stratification was observed to change the structure of the food web explaining, at least in part, the decrease in productivity experienced over the last decades in the Canary Current. The warming

trend observed in this region and its end-to-end biological consequences deserve further research.

Acknowledgments

The authors would like to thank all the colleagues of the Biological Oceanography Group that contributed to the field work. This study was funded by the projects "ConAfrica" (CTM2004-02319) and "Lucifer" (CTM2008-03538) from the Ministry of Science and Innovation of Spain. C. Schmoker PhD fellowship was granted by the Government of Canary Islands, Spain.

References

Arístegui J, Hernández-León S, Montero MF, Gómez M (2001) The seasonal planktonic cycle in coastal waters of the Canary Islands. Sci Mar 65: 51-58

Arístegui J, Montero MF (2005) Temporal and spatial changes in plankton respiration and biomass in the Canary Islands regions: The effect of mesoscale variability. J Mar Syst 54: 65-82

Arístegui J, Barton ED, Álvarez-Salgado XA, Santos AMP, Figueiras FG, Kifani S, Hernández-León S, Mason E, Machú E, Demarcq H (2009) Sub-regional ecosystem variability in the Canary Current upwelling. Prog Oceanogr Special issue 83: 33-48

Baltar F, Arístegui J, Montero MF, Espino M, Gasol JM Herndl GJ (2009) Mesoscale variability modulates seasonal changes in the trophic structure of nano- and picoplankton communities across the NW Africa-Canary Islands transition zone. Prog Oceanogr Special issue 83: 180-188

Båmstedt U (1986) Chemical composition and energy content. In: Corner EDS, O'Hara SCM (Eds) The biological Chemistry of Marine Copepods. Clarendon Press, Oxford, pp. 1-58

Bode A, Barquero S, Varela M, Braun JG, De Armas D (2001) Pelagic bacteria and phytoplankton in oceanic waters near the Canary Islands in summer. Mar Ecol Prog Ser 209: 1-17

Børsheim KY, Bratbak G (1987) Cell volume to cell carbon conversion factors for a bacterivorous Monas sp. enriched from seawater. Mar Ecol Prog Ser 36: 171-175

Brander K, Blom G, Borges MF, Erzini K, Henderson G, MacKenzie BR, Mendes H, Santos AMP, Toresen R (2003) Changes in fish distribution in the eastern North Atlantic ; are seing a cohérent response to changing temperature ? ICES Mar Sci Symposia 219 : 261-270

Campbell L, Vaulot D (1993) Photosynthetic picoplankton community in the subtropical North Pacific Ocean near Hawaii (Station ALOHA). Deep-Sea Res 40: 2043-2060

Campbell L, Liu H, Nolla HA, Vaulot D (1997) Annual variability of phytoplankton and bacteria in the subtropical North Pacific Ocean at Station ALOHA during the 1991-1994 ENSO event. Deep-Sea Res 44: 167-192

Cho BC, Azam F (1990) Biogeochemical significance of bacterial biomass in the ocean's euphotic zone. Mar Ecol Prog Ser 63: 253-259

Christian JR, Karl DM (1994) Microbial community structure at the U.S.-Joint Global Ocean Flux Study Station ALOHA: Inverse methods for estimating biochemical indicator ratios. J Geophys Res 99: 14269-14276

de León AR, Braun JG (1973) Annual cycle of primary production and its relation to nutrients in the Canary Islands waters. Bol Inst Esp Oceanogr 167: 1-24

Demarcq H (2009) Trends in primary production, sea surface temperature and wind in upwelling systems (1998-2007). Prog Oceanogr Special issue 83: 376-385

Dias CA, Amorim A, das Dores Vacas M (1992) Sea-surface temperature: seasonal variation between the Iberian coast and the Madeira Islands, 1981-1987. ICES Mar Sci Symposia 195: 177-186

Gasol JM, Zweifel UL, Peters F, Fuhrman JA, Hagström A (1999) Significance of size and nucleic acid content heterogeneity as measured by flow cytometry in natural planktonic bacteria. Appl Environ Microbiol 65: 4475-4483

Hasle G, Syvertsen EE (1997) Marine diatoms in Identifying marine diatoms and dinoflagellates pp. 5-385

Haas LW (1982) Improved epifluorescence microscopy for observing planktonic micro-organisms. Ann Inst Oceanogr Paris 58: 261-266

Hernández-León S, Almeida C, Becognée P, Yebra L, Arístegui J (2004) Zooplankton biomass and indices of grazing and metabolism during a late winter bloom in subtropical waters. Mar Biol 145: 1191-1200

Hillebrand H, Durselen CD, Kirschtel D, Pollingher U, Zohary T (1999) Biovolume calculation for pelagic and benthic microalgae. J Phycol 35: 403-424

Kana TM, Glibert PM (1987) Effects of irradiances up to 2000 μm E m•2•s•1 on marine Synechococcus WH 7803-1. Growth, pigmentation, and cell composition. Deep-Sea Res I 26: 97-108

Lee S, Fuhrman JA (1987) Relationships between biovolume and biomass of naturally derived marine bacterioplankton. Appl Environ Microbiol 53: 1298-1303

Levitus S, Antonov JI, Boyer TP, Stephens C (2005) Warming of the world ocean. Science 287: 2225-2229

Li WKW, Dickie PM, Irwin BD, Wood AM (1992) Biomass of bacteria, cyanobacteria, prochlorophytes and photosynthetic eukaryotes in the Sargasso Sea. Deep-Sea Res 39: 501-519

Lovegrove T (1966) The determination of the dry weight of plankton and the effect of various factors on the values obtained, p. 429-467. In: Barnes H (ed) Some contemporary studies in Marine Sciences. George Allen and Unwin Ltd

Lynn DH, Small EB (2002) Ciliophora. In Lee: Leedale J, Bradbury P (eds) The illustrated guide to the Protozoa. Lawrence, Kansas, pp. 371-656

Malone TC, Ducklow HW, Peele ER, Pike SE (1991) Picoplankton carbon flux in Chesapeake Bay. Mar Ecol Prog Ser 78: 11-22

Menden-Deuer S, Lessard EJ (2000) Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. Limnol Oceanogr 45: 569-579

Ojeda A (2005) Dinoflagelados de Canarias estudio taxonómico y ecológico. Monografía LXV del Instituto de Estudios Canarios pp. 1-301

Olson RJ, Zettler ER, Altabet MA, Dusenberry JA, Chisholm SW (1990a) Spatial and temporal distributions of prochlorophyte picoplankton in the North Atlantic Ocean. Deep-Sea Res 37: 1033-1051

Olson RJ, Zettler ER, Altabet MA, Dusenberry JA, Chisholm SW (1990a) Spatial and temporal distributions of prochlorophyte picoplankton in the North Atlantic Ocean. Deep-Sea Res 37: 1033-1051

Partensky F, Hess WR, Vaulot D (1999) *Prochlorococcus*, a marine photosynthetic prokaryote of global significance. Microb Mol Biol Rev 106-127

Putt M, Stoecker DK (1989) An experimentally determined carbon: volume ratio for marine "oligotrichous" ciliates from estuarine and coastal waters. Limnol Oceanogr 34(6): 1097-1103

Ricard M (1987) Atlas du phytoplancton marin. 2: Diatomophycées. 1-297

Richardson AJ (2008) In hot water: zooplankton and climate change. ICES J Mar Sci 65: 279-295

Richardson AJ, Walne AW, John AWG, Jonas TD, Lindley JA, Sims DW, Witt M (2006) Using continuous plankton recorder data. Prog Oceanogr 68: 27-74

Sieracki ME, Haugen EM, Cucci TL (1995) Oversestimation of heterotrophic bacteria in the Sargasso Sea: direct evidence by flow and imaging cytometry. Deep-Sea Res I 42: 1399-1409

Steidinger KA, Tangen K (1997) Dinoflagellates in Identifying marine diatoms and dinoflagellates pp. 387-584

Sokal RR, Rohlf FJ (1995) Biometry. The principles and practice of statistics in biological research. Freeman WH and company (eds), New York, 887p

Strathmann RR (1967) Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. Limnol Oceanogr 12: 411-418

Valdés L, López-Urrutia A, Cabal J, Alvarez-Ossorio M, Bode A, Miranda A, Cabanas M, Huskin I, Anadón R, Alvarez-Marqués F, Llope M, Rodríguez N (2007) A decade of sampling in the Bay of Biscay: What are the zooplankton time series telling us? Prog Oceanogr 74: 98-114

Veldhuis MJW, Kreey GW, Gieskes WWC (1993) Growth and fluorescence characteristics of ultraplankton on a north-south transect in the eastern North Atlantic. Deep-Sea Res 40: 609-626

Verity PG, Langdon C (1984) Relationships between lorica volume, carbon, nitrogen, and ATP content of tintinnids in Narragansett Bay. J Plankton Res 6: 859-868

Yentsch CS, Menzel DW (1963) A method for the determination of phytoplankton chlorophyll and phaeophytin fluorescence. Deep-Sea Res 10: 221-231

Zubkov MV, Sleigh MA, Burkill PH (2000a) Assaying picoplankton distribution by flow cytometry of underway samples collected along a meridional transect across the Atlantic Ocean. Aquat Microb Ecol 21: 13-20

Zubkov MV, Sleigh MA, Burkill PH, Leakey RJG (2000b) Picoplankton community structure on the Atlantic Meridional Transect: a comparison between seasons. Progr Oceanogr 45: 369-386