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XXIX CICLO DEL DOTTORATO DI RICERCA IN AMBIENTE E VITA

ECOLOGY AND DIVERSITY OF MARINE MICROZOOPLANKTON

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Table of contents

Summary

Protists are a taxonomic group of organisms world wild distributed with high abundance and biodiversity; their countless forms, sizes, and trophic activities constitute a continuum of species ranging from bacterial-sized cells for the smallest known species of chlorophytes to meters in length for the largest colonies of radiolaria. The enormous size range of protists, their many nutritional modes, and their rapid metabolic rates result in their pivotal ecological roles as primary producers and consumers at and near the base of marine food webs. Protists, in particular the heterotophic ones, are, with few metazoans larval stage, the major components of microzooplankton, on which this study focuses.

Microzooplankton assemblage is described as a group of planktonic organisms in the size range of 10/20-200 μm; they are consumers of bacteria, cyanobacteria, other protists, viruses, and some metazoans. The quantitative importance of microzooplankton as consumers of primary production in the ocean has been recognized in the last decades; at the same time copepod predation on heterotrophic dinoflagellates and ciliates constitutes a trophic link more important than the phytoplankton–copepod link in many situations. Phagotrophic protists are the primary trophic link between minute cyanobacterial and bacterial production and higher organisms, a concept formalized more than 35 years ago in the microbial food web by Pomeroy (1974).

In this study I want to provide more information on microzooplankton assemblages, describing the community composition and the role of this important component in different environments. I point out some of the factors influencing the distribution of these organisms with the aims to increase the current knowledge on microzooplankton and to contribute to the understanding of the phenomena that regulate the efficiency of the trophic web of the marine ecosystem.

In the first chapter of the thesis entitled “Microzooplankton composition in the winter sea ice of the Weddell Sea” diversity, abundance and carbon biomass of sympagic microzooplankton were studied during late winter in the northern Weddell Sea. In order to asses the role of

microzooplanktonic component as food supply for the upper levels of the trophic web in this particular environment, the ice-cores were collected on an ice floe along three dive transects, and sea water was taken from under the ice through the central dive hole from which all transects were connected. The areal and vertical microzooplankton distributions in the ice and water were compared. They showed high abundance (max 1300 cells L^{-1}) and biomass (max 28 µg C L^{-1}) in the ice-cores, and were lower in the water, below sea ice (maxima, 19 cells L^{-1}; 0.15 µg C L^{-1}, respectively). The highest amounts were found in the lower 10 cm section of ice cores. The microzooplankton community within sea ice comprised mainly aloricate ciliates, foraminifers and micrometazoans. In winter, microzooplankton represent an important fraction of the sympagic community in the Antarctic sea ice. They can potentially control microalgal production and can contribute to *particulate organic carbon* concentrations when released into the water column due to ice melting in spring. Continued reduction of the sea ice might undermine these roles of microzooplankton, leading to reduction or completely loss in diversity, abundance and biomass of these sympagic protists.

The second Chapter of this work focuses on the microbial community along the Ligurian coast in correspondence of two marine canyons anylising the effect of this stuctures on the microzooplankton population . Community structures along the water column were studied using microscopic techniques, and their relationships with the environmental factors recorded along the canyons were investigated. The study considered pico-, nano- and micro-planktonic fractions, their abundances and composition. We also considered the mean cells' size of the studied group in order to identifying the trophic patterns that regulate and shape the entire microbial population.

Profiles of temperature and salinity showed the same trend for all stations and no significant difference were found among stations in correspondence of the canyons profiles and the ones detected on the adjacent slope.

Our results highlight that the main factor regulating the communities composition is the depth and that community recorded in the samples belonging to each transect were not separated on the base of geographic distribution.

A similar trend for abundances and biomasses was observed at all stations: higher values were generally measured in the surface layer and they decreased with increasing depth and a significant linear regression was highlighted in each of the three transects.

Results of the distribution of all microbial heterotrophs from the surface down to 2500 m pointing out that prey abundance was generally higher than the feeding threshold of predators; this evidence suggests that the interaction between different size classes is bottom-up regulated in the study area.

The third Chapter of the study concerns the horizontal variability of the microzooplankton assemblages; abundance, biomass, and taxonomical compositions were analysed and the community structures were discussed on the base of abiotic variables measured during the summer 2015 in the Western Mediterranean Sea. The purpose was to highlight a connection between population composition and the hydrology that characterizes the specific basins, contributing to the understanding of the phenomena that make the Mediterranean area a hotspot of variability and diversity. A significant effect of the interaction between Transect and Depth factors, were detected. Total MCZ community abundance and number of taxa decreased from the surface to 500 m depth. Furthermore, PERMANOVA results showed a significant effect of the variable depth in the community structures and abundances, this variable explain 28.6% of the total variance recorded in our sampling units. A significant effect is also highlighted for the variable Transect accounting for 11.6 % of the total variance; conversely the Site variable resulted not significant. The value of similarity recorded between the community of the transects analyzed in this study appeared closely related to the circulation that characterizes the Western Mediterranean basin highlighting that the water masses modifications in salinity along the Mediterranean circulation could play a significant role in shaping the protis assemblages.

Riassunto

I protisti rappresentano un gruppo di organismi distribuiti in tutti gli oceani del mondo e si distinguono in ogni ecosistema per la loro grande abbondanza e i loro elevati tassi di biodiversità; presentano un elevatissima eterogeneità sia in termini morfologici che in termini dimensionali: sono stati descritti organismi appartenenti a questo gruppo dalle dimensioni di una cellula procariotica, come alcune clorofite, fino a metri di lunghezza per le grandi colonie di alcuni radiolari. Le specie presenti in questo gruppo sono caratterizzati dalle più diverse strategie trofiche, esse vanno dalla autotrofia alla eterotrofia passando per strategie nutrizionali intermedie come la mixotrofia. La vasta gamma di dimensioni, le loro molteplici strategie trofiche e soprattutto il loro alto tasso metabolico, inducono a considerare i protisti una componente fondamentale nel funzionamento ecologico dell'ambiente marino; essendo in grado di svolgere sia il ruolo di produttori primari che quello dei primi consumatori, questi organismi svolgono infatti un ruolo fondamentale nel controllare i passaggi di energia nei primi livelli della rete trofica marina.

I protisti, in particolare la frazione eterotrofa, sono insieme ad alcune forme larvali di metazoi, la componente fondamentale del microzooplancton: il gruppo di organismi su cui si focalizza questo lavoro di tesi.

Il comparto microzooplantonico è descritto come l'insieme degli organismi che presentano eterotrofia di dimensioni comprese tra i 10-20 e i 200 μm; queste specie esercitano pressione predatoria su batteri e cianobatteri, su altri protisti, virus e in alcuni casi su i primi stadi larvali di alcuni metazoi.

Nell'ultimo decennio, l'importanza del microzooplancton come consumatore della produzione primaria in ambiente acquatico, è stato pienamente riconosciuto; è stato anche evidenziato che la predazione sulla frazione microzooplanctonica possa, in condizioni di oligotrofia e di scarsa

luminosità, costituire la principale risorsa per la componente mesozooplantonica superando come importanza la relazione trofica tra fitoplancton e mesozooplancton.

Il microzooplancton costituisce quindi l'anello di congiunzione primario tra la produzione di batteri e cianobatteri e i livelli più alti della rete trofica, un concetto formalizzato più di 35 anni fa nella formulazione della rete trofica microbica da Pomeroy (1974).

L'obbiettivo di questo studio è quello di fornire maggiori informazioni relativamente alla componente microzooplanctonica, descrivendo la struttura dei diversi popolamenti e analizzando quale sia il ruolo di questi organismi nei diversi ambienti in cui sono stati studiati. Si sono analizzati i fattori biotici ed abiotici che influenzano la distribuzione e la composizione del microzooplancton al fine di contribuire alla comprensione di quelli che sono i fenomeni che regolano l'efficienza della rete trofica in ambiente marino.

Nel primo capitolo di questo lavoro di tesi dal titolo: " Microzooplankton composition in the winter sea ice of the Weddell Sea" la componente simpagica del microzooplancton è stata studiata durante la stagione tardo invernale nella zona settentrionale del mare di Weddell, nel corso dello studio sono state raccolte informazioni relative alla diversità, abbondanza e biomassa di carbonio che caratterizzavano i popolamenti di questo ambiente. L'obiettivo era valutare il ruolo della componente microzooplanctonica come risorsa alimentare per i livelli superiori della rete trofica in questo ambiente peculiare. Il campionamento è avvenuto attraverso il prelievo di carote di ghiaccio, sono stati individuati 3 transetti che si dipartivano da un unico foro centrale dal quale invece sono stati effettuati i prelievi d'acqua sottostante. Le distribuzioni verticali degli organismi nella colonna d'acqua e all'interno della carota, sono state analizzate e messe a confronto. Le abbondanze maggiori si riscontravano all'interno della carota (max 1300 ind. L^{-1}) così come la maggior frazione di biomassa (max 28 μg C L^{-1}), mentre invece valori molto più bassi di abbondanza e biomassa sono stati registrati nella colonna d'acqua presente sotto il pack (max, 19 ind. L^{-1}; 0,15 μg C L^{-1}, rispettivamente). I valori più alti in generale sono stati registrati negli ultimi 10 cm della carota di ghiaccio, in prossimità dell'interfaccia acqua ghiaccio, i popolamenti analizzati all'interno della

carota risultavano principalmente composti da grandi ciliati aloricati, foraminiferi e micrometazoi. È stato evidenziato come in inverno il microzooplancton rappresenti una frazione importante della comunità simpagica nel ghiaccio marino, rappresentando una potenziale fonte di nutrimento per la componente mesozooplanctonica. Questa frazione è potenzialmente in grado di controllare la produzione microalgale e può contribuire alla concentrazione di carbonio organico particolato quando viene rilasciata nella colonna d'acqua a causa dello scioglimento del ghiaccio in primavera. La continua riduzione del ghiaccio marino, potrebbe portare ad una notevole riduzione dell'habitat disponibile per questa importante frazione, minando cosi quello che è il fragile equilibro che al momento attuale garantisce il mantenimento della biodiversità in questo ambiente.

Il secondo capitolo di questo lavoro si concentra sulla comunità microbica lungo la costa ligure, i campioni sono stati raccolti in corrispondenza di due canyon seguendo il loro profilo, dalla costa al piattaforma sottostante. Lungo i transetti, i campioni sono stati raccolti in tutta la colonna d'acqua studiando i popolamenti che caratterizzavano le masse d'acqua dalla superficie fino al fondo, sono stati inoltre registrati i fattori ambientali che caratterizzavano l'area di studio e questi sono stati messi in relazione con le composizioni della comunità microbica. Lo studio ha considerato le frazioni pico, nano e micro-planctoniche, le loro abbondanze e ed i rapporti tra queste diverse componenti. Abbiamo anche considerato dimensioni medie delle cellule provando ad identificare quali siano le dinamiche trofiche che regolano e strutturano l'intera popolazione microbica.

I profili di temperatura e di salinità delle stazioni studiate lungo il profilo dei canyon, non mostravano differenze significative con quelle registrate sulla scarpata adiacente, esse mostravano infatti valori simili lungo tutti i profili analizzati.

I nostri risultati evidenziano come il fattore principale che regola la composizione delle comunità in questo ambiente sia la profondità e come i campioni raccolti alle stesse profondità lungo il profilo dei canyon e al difuori di questi, non fossero significativamente diversi e non si dividessero in base alla loro distribuzione geografica.

Un trend analogo è stato registrato per abbondanza e biomassa in tutte le stazioni: i valori più alti sono stati generalmente misurati nello strato superficiale e diminuivano con l'aumentare della profondità, una significativa regressione lineare è stata evidenziata in ciascuno dei tre transetti.

L'analisi della distribuzione di tutte le componenti eterotrofe dalla superficie ai 2500m di fondo, ha evidenziato che la disponibilità delle prede superava la soglia minima di presenza necessaria per i predatori; questa evidenza ha suggerito che nell'area di studio le interazioni tra le diverse frazioni dimensionali fossero regolate da processi bottom-up.

Il terzo capitolo dello studio si concentra sulla distribuzione orizzontale della componente microzooplanctonica; abbondanza, biomassa e composizione tassonomica dei popolamenti sono stati analizzate e le strutture delle comunità sono state discusse sulla base delle variabili abiotiche misurate durante l'estate 2015 nel Mediterraneo occidentale. Il lavoro si pone come obbiettivo quello di indagare le connessioni che intercorrono tra le composizioni dei popolamenti microzooplantonici e le caratteristiche idrologiche che interessano i diversi bacini oggetto di studio, andando a contribuire alla comprensione dei fenomeni che rendono l'area mediterranea un hotspot di variabilità e diversità. Nel corso dello studio è stato rilevato un effetto significativo dell'interazione tra i fattori area geografica e profondità ($p < 0{,}001$). Le abbondanze ed il numero di taxa presenti all'interno dei diversi popolamenti, diminuivano lungo il gradiente di profondità dalla superficie fino ai 500 m; inoltre, i risultati della analisi PERMANOVA hanno evidenziato un effetto significativo della variabile profondità sulle strutture e le abbondanze dei popolamenti studiati. I risultati indicano come la variabile profondità spieghi da sola il 28,6% della varianza totale registrata nelle nostre unità di campionamento. Altrettanto significativo è risultato l'effetto della variabile area geografica, il diverso bacino di origine dei campioni infatti spiegava l' 11,6% della varianza totale; Al contrario la variabile sito nella stessa area geografica non è risultata significativa..

I valori di somiglianza che sono stati calcolati tra i popolamenti che caratterizzavano le diverse aree di studio, hanno restituito un quadro strettamente correlato alla circolazione che caratterizza il bacino occidentale del mar Mediterraneo.

Questo risultato potrebbe essere imputato al gradiente di salinità che interessa le masse d'acqua che si spostano all'interno bacino ed evidenzia, infine, come circolazione e fattori abiotici influenzino la struttura dei popolamenti a protisti, giocando un ruolo fondamentale nella trofia dell'intero ecosistema.

Introduction

The marine system is very complex and the current knowledge on its dynamics is often sporadic (Siokou-Frangou et al., 2010). Today the role that microbial organisms play in the marine ecosystems is widely recognized (Sherr & Sherr, 1988; Azam, 1998; Arrigo, 2005; Karl, 2007; Andersen et al., 2009). Microbes, which seem to be the most diverse group of organisms (Fuhrman, 2009), represent a major portion of the biomass at the sea and are responsible for the flow of matter and energy of the marine system (e.g. Azam et al., 1983; 1993; Fenchel, 2008). Since the dynamics and functioning of ecosystems are based on the channelling of solar energy in trophic networks and in biogeochemical cycles, and because microbes are located at the base of the latter in many pelagic systems, descriptions of the structure and processes of microbial communities can contribute to the understanding of the ecosystems operation (Fuhrman, 2009).

Marine plankton

In 1887 Hensen coined the term "plankton" to indicate all natural organic particles floating freely and involuntarily in open water. Marine plankton consists of a wide variety of organisms belonging to different taxonomic groups, and can be classified on the basis of structural, functional or dimensional criteria.

Traditionally marine plankton is subdivided according to trophic characteristic, in phytoplankton (autotrophic organisms) and zooplankton (heterotrophic organisms).

Phytoplankton can be defined as a corporation, or as the set of organisms, heterogeneous at a taxonomic and dimensional level, but similar at a functional level, that are part of a community

(Root, 1967). Phytoplankton includes organisms living in the photic zone of aquatic environments involved in the process of primary production. This fenomena is due to prokaryotes (essentially cyanobacteria) and nano- and micro-eukaryotic algae belonging to different taxa.

Zooplankton is made of heterotrophic organisms belonging to different taxonomic subdivisions. It contributes to the structuring of the planktonic communities, to the control of production and to phytoplankton dynamics in the processes of nutrients regeneration.

Mixotrophic organisms are the intermediate state between those mentioned above, namely the presence in the same body of heterotrophy and autotrophy (Stoecker, 1999). This strategy allows photosynthetic organisms to support their metabolism through fagotrophy and the utilization of dissolved organic carbon (DOC) that allows predators to photosynthesize. This nutritional mode brings ecological benefits to organisms, as it allows them to survive even in environments in which light, nutrients, or food particles are limited (e.g. Havskurn & Riemann, 1996).

To distinguish the various components of plankton, Sieburth et al. (1978) proposed a system based on size classes (Figure 1). The cell size raises several implications in the physiology and ecology of organisms (Verity & Smetacek, 1996; Jürgens & Massana, 2008).

Femtoplankton 0.02-0.2 µm Virus, Bacteria

Picoplankton 0.2-2 µm Bacteria, Archea, Cyanobacteria, Proclorophytes

Nanoplankton 2-20 µm Phytoflagellates, Coanoflagellates, Dinoflagellates, Ciliates

Microplankton 20-200 µm Diatoms, Dinoflagellates, Radiolarians, Ciliates, Metazoans

Mesoplankton 0.2-20 mm Crustaceans (Copepods, Eufasiaceans, Cladocerans)

Macroplancton 2-20 cm Jellyfish

Megaplancton 20-200 cm Jellyfish, Tunicates

Figure 1- size classes of plankton organisms

In this work I will focus on the heterotrophic and mixotrophic organisms that compose the microplanktonic fraction.

Microzooplankton

Microzooplankton is the group of heterotrophic and mixotrophic organisms of a size between 10-20 and 200 m (Margalef, 1963; Sieburth et al., 1978), which includes aloricate and loricate (tintinnids) ciliates, dinoflagellates, radiolarians, foraminiferans and, to a lesser extent, micrometazoans (nauplii, copepodits, meroplanktonic larvae).

The protozoans, ciliates and dinoflagellates in particular, represent the largest component of the entire microzooplaktonic fraction. In coastal waters ciliates and dinoflagellates reach abundances between 10^3 and 10^5 cells L^{-1}, while the metazoans are much less abundant, generally 10^0-10^2 individuals L^{-1} (Fonda et al., 2010).

The success of these organisms seems bound to a double food strategy adopted by many microzooplanktonic species (Jones, 1994; Jeong et al., 2010).

Mixotrophy can give a competitive advantage within the planktonic component, particularly when main nutrients are limited (Caron, 2000; Pitta & Giannakourou, 2000). Mixotrophy may be due to either the symbiosis with bacteria (the only known case among ciliates is the one of *Strombidium purpureum* (Fenchel & Bernard, 1993) or to the ingestion and retention of plastids coming from the ingested algae (kleptoplastids) (Stoecker, 1998; Esteban et al., 2010).

Ciliates are typically heterotrophic, but there are examples of mixotrophic species, often for the maintenance of plastids derived from ingested algae (Stoecker, 1998). The methods of the food intake can be phagocytosis or ingestion by modified cilia that serve as a filter, depending on the size of the prey and the presence or absence of a specialized region that acts as oral opening. The

planktonic ciliates prey on suspended particles in a selective manner, by different mechanisms (Fenchel, 1980). They feed on a wide spectrum of prey, all those that fall in the correct size range: algae, bacteria, dinoflagellates and other ciliates (Fenchel, 1980; Fenchel, 1987; Vaqué et al., 1994; Sherr & Sherr, 2002).

Dolan et al. (1999) found that the mixotrophic ciliates are more abundant in the Eastern Mediterranean Sea in respect to the Western. In 2001 Pitta et al. confirmed these observations; the mixotrophs represent 17-18% in abundance and biomass and are 3 to 18 times more abundant in the Eastern basin, despite the abundance of total ciliates in the Western basin.

Marine tintinnids (Ciliophora, Spirotrichea, Tintinnid) are represented by 925 species (Zhang et al., 2011). By analyzing the morphology of the lorica it is possible to recognize those organisms even in cases when the ciliates are damaged as a result of a particularly traumatic sampling effort and fixation procedures (Paranjape & Gold, 1982). It has been seen that there is a certain polymorphism in the morphology of the lorica within the same species that might be misleading to ensure proper identification (Gold & Morales, 1976; Laval-Peuto, 1983). Although ubiquitous in marine systems, they are generally a smaller proportion of marine plankton accounting for only 5-10% of the total number of ciliates in microzooplankton (eg. Dolan & Marassé, 1995). However, occasionally tintinnids can dominate the population and be the main consumers of pico- and nanoplankton (Karayanni et al., 2005). The ingestion rate of tintinnids is 3-4 times greater than that reported for aloricate ciliates, although they are numerically often less abundant (Strom & Morello, 1998; Pitta et al., 2001).

The free-living marine dinoflagellates are represented by 1555 species classified in 117 genera (Gómez, 2005). The most numerous genera are *Protoperidinium* (264 species), *Gymnodinium* (173 species), *Dinophysis* and *Phalacroma* (104 + 41 species), *Gyrodinium* (87 species), *Amphidinium* (76 species), *Histioneis* (65 species), *Neoceratium* (64 species = Ceratium) and *Gonyaulax* (60 species).

Dinoflagellates tend to dominate in regions with low concentrations of nutrients. Recent studies revealed that many dinoflagellates considered exclusively autotrophic, are actually mixotrophs (Jeong et al., 2010). Mixotrophy is widespread in many dinoflagellates orders: Prorocentrales, Dinophysiales, Gymnodiniales, Noctilucales, Peridinialese, Gonyaulacales, Blastodiniales, Phytodiniales, Dinamoebales (e.g. Stoecker et al., 1997; Smalley & Coats, 2002).

It has been shown that dinoflagellates can prey on different taxa, including autotrophic bacteria (Jeong et al., 2005B), and heterotrophic ones (Jeong et al., 2008) on picoeukaryotes (Jeong et al., 2008), on cryptophytes (Li et al., 2000) and haptophytes (Berge et al., 2008), diatoms (Jacobson & Anderson, 1986; Jeong et al., 2004; Menden-Deuer et al., 2005; Yoo et al., 2009) other dinoflagellates (Adolf et al., 2007; Tillmann, 2004), nano-heterotrophs (Jeong et al., 2007), ciliates (Hansen, 1991) and eggs or larvae of copepods (Jeong, 1994). Predation is allowed by the particular trophic adaptations, which allow the capture and digestion of prey larger than themselves: phagocytosis, nutrition using pedicle or through pallium (Hansen & Calado, 1999). In the first case the prey is swallowed up in certain areas of the groove, in the second the body pulls out a stalk to suck the cytoplasmic contents of the prey, while in the third case a pseudopod: plastic extension of the digestive vacuole, is rolled down and around the prey until it is totally enveloped.

In all three cases, the enzymatic digestion is carried out after by specialized vacuoles. A common distinction is between thecate dinoflagellates, or with a cellulosic organized wall plaque, and athecate ones. In fact the latter are also provided with a theca characterize by thinner and less organized plaques that are difficult to detect with an optical microscope (Avancini et al., 2006). The larval stages and the eggs of the Metazoans belong to the Microzooplankton. Metazoans are larger multicellular eukaryotes that, if planktonic, belong to the higher classes (meso-, macro- or megaplankton). It is, for example, the larvae of Crustaceans Copepods, Molluscs: veliger of Bivalve or pelagic Tunicates larvae. It is more rare to find the presence of rotifers or microscopic metazoans pseudo coelomates.

Microzooplankton plays an important role in marine environments as it rules the structure, production and dynamics of plankton communities (Froneman & Perissinotto, 1996; Lessard & Murrell, 1998; James & Hall, 1998; Strom et al., 2007). In fact, microzooplanktonic organisms, in particular ciliates, can have growth rates equal to or higher than those of phytoplankton: this gives them advantages over larger metazoans, allowing them to adapt quickly to changes in food availability (Calbet & Landry 2004; Strom et al., 2000). Furthermore, the wide variety of shapes and food plasticity (especially dinoflagellates) makes this functional group able to prey on a wide range of prey, also of much larger dimension of the predator, including the colonial diatoms.

Food webs and carbon cycle

Thienemann originally introduced lexicon used in the description of the energy cycles in 1926.

The photosynthetic organisms, autotrophs, produce complex organic compounds in which solar energy is stored in carbon-based molecules flowing in the different ecosystem compartments through the consumers, the heterotrophic organisms.

From this scheme a hierarchical structure for the energy flows was formulated by Lindeman (1942), which since the early trials appeared to be complex and more like a "network" that a "chain". In the 80s the concept of marine food web has been further developed, and the "classical" trophic network view, which included a linear flow from phytoplankton to predators belonging to higher trophic levels, has been integrated with that of the microbial loop.

The microbial loop, a term coined in the 1983 by Azam et al. (Fenchel, 2008), outlines the role of bacteria in marine food webs and in nutrient cycles. The dissolved organic carbon (DOC) is generated by microalgae exudation (Alldredge et al., 1993), during the processes of predation (sloppy feeding) (Eppley et al., 1981), by the activities of degradation of faecal pellets produced by zooplankton (Honjo & Roman, 1978), by spontaneous or caused by viral infection cell lysis (Bratbak et al., 1993, 1998; Fuhrman & Suttle; 1993; Fuhrman & Noble; 1995). The DOC flows to

higher trophic levels in the "classic" food network through the processes of predation, after being used by prokaryotes for their growth and metabolism (Ducklow & Carlson 1992; Azam et al., 1993; Azam & Worden, 2004; Azam & Malfatti, 2007). In this context the concept of mistivourus network (mistivourus food web), was used to define the interaction of the classical with the microbial food web.

Microzooplankton is considered the transition element between the microbial compartment and the traditional food network (Sherr et al., 1986; Calbet, 2008) and as such is a structurally and functionally important element of pelagic ecosystems. Numerous studies have identified microzooplanktonic organisms as the main grazers of phytoplankton in many marine ecosystems, from the ultra-oligotrophic one to those of the upwelling regions (e.g. Verity et al., 1993; Gallegos et al., 1996; Strom & Strom, 1996; Landry et al., 1998; Calbet, 2001; Quevedo & Anadón 2001; Liu et al., 2005; Calbet & Landry, 2004).

According to some estimates the organisms belonging to this size class consume an average 67% of the daily phytoplankton production (Landry & Calbet, 2004; see also, however Dolan & McKeon, 2005), while the remaining third is subject to larger size predators, viral lysis, natural mortality of the cells, to sedimentation, etc.

Microzooplankton is in turn preyed upon by mesozooplankton. In particular, the copepods, major representatives of mesozooplankton, mainly prey on ciliates (Sherr et al., 1986; Stoecker & Capuzzo, 1990; Roman et al., 2000; Calbet & Saiz, 2005), although mesozooplankton does not seem able to exert control over microplanktonic organisms (Broglio et al., 2004), because of the lower growth rates and low abundances.

Typically, the trophy of the system determines what is the dominant circuit, and the prevalence of one or of the other path allows defining if the biomass will be remineralized or transferred to higher trophic levels. Planktonic communities can range from a system based on the microbial loop to the one dominated by the traditional network in the medium-short periods.

The classic network belongs to the areas where important microalgal bloom and diatoms occur, such as coastal areas or upwelling, where there is availability of nutrients. In this condition the fixed carbon may be removed from the photic zone following the processes of sedimentation. These can occur due to predations by zooplankton, and then to the production of relatively heavy faecal pellets, or in the absence of predation, because of aggregation processes.

The microbial loop is considered characteristic of oligotrophic areas, where the nutrients contribution is limited, and is based mainly on regeneration processes (Andersen & Ducklow, 2001). In these areas the organic carbon is mainly photosynthesized by primary producers of smaller size, generally less than 5μm (Agawin et al., 2000a), which may be responsible for ≥50% of the total of the phytoplankton biomass. In these environments *Synechococcus* spp. may contribute to more than half of the fixed carbon (Iriarte & Purdie, 1994). The dominance of small component determines a poor transfer of energy to higher trophic levels (Turley et al., 2000).

In oligotrophic systems where the dominant fractions are pico- and nanoplankton both in planktonic terms of productivity and of biomass (Li et al., 1983; Platt et al., 1983), it is expected that heterotrophic flagellates and ciliates would be the main grazers because the higher size predators are not able to effectively prey on these components (Marshall, 1973; Pitta & Giannakourou, 2000). By predation of microzooplankton on pico- and nano-plankton the fraction of organic carbon represented by prokaryotes, DOC and POC is not lost, but it is part of the classical food chain and reaches the highest levels of the marine food web (Azam et al., 1983).

The amount of carbon that reaches higher trophic levels varies in reference to the nature of ecosystems (Barquero et al., 1998) and to the complexity of the trophic network. In this sense, the mistivorus trophic web is less efficient than the classic one in the transfer of matter from one trophic level to the next, but undoubtedly more realistic and advantageous in the regeneration of nutrients and the recycling of DOC (Legendre & Rassoulzadegan, 1995). It has been shown that the involvement of microzooplankton in the food web increases recycling rate of metal (Barbeau et al.,

2001), of bioactive elements (Calbet & Landry, 2004) and of inorganic nutrients (Sherr & Sherr, 2002) because the microzooplanktonic protists are characterized by a high growth rate and a very fast metabolism (Fenchel, 1987). This recycling takes on a greater significance in oligotrophic seas like the Mediterranean, where the availability of inorganic nutrients is essential for primary producers, who are the base of the food web, and thus fundamental to the proper functioning of the marine ecosystem.

New tools for studying protistan diversity.

Characterizing and understanding protistan diversity has been and continues to be an active area of research in marine science for the reasons noted above. Establishing the natural abundances and ecological activities of protists in aquatic ecosystems has involved visualization (historically, protists have been described and identified on the base of morphological features), culture, and laboratory experiments to establish basic physiology and behavior, and then extrapolation of that information to nature and verification via field-based observations and experiments. The information gathered from this work has provided fundamental understanding of many of the ecological roles performed by protists in natural communities and allowed for the development of models describing their activities.

Nevertheless, the goal of documenting the wealth of protistan taxa in a sample from a natural community has been greatly hindered by the magnitude of this task and by the difficulties and complexities of protistan taxonomies. These difficulties include multiple fixation and processing procedures as well as the diverse taxonomic characters that must be determined to identify different groups of protists. Species with small cell size and/or few morphological features (e.g., many amoebae, and photosynthetic and heterotrophic forms <10 μm in size) present difficulties for rapid and easy identification. Additionally, ecological research often requires the collection and processing of large numbers of samples, making traditional approaches impractical.

The incorporation of DNA sequence–based approaches for defining, identifying, and quantifying

protistan taxa is rapidly changing the landscape on this issue. These approaches have already begun to enable the development of molecular taxonomies that can be applied using improving genetic technologies to provide taxonomically broad and relatively rapid assessments of protistan diversity (Caron et al. 2009). Genetic information has been useful for identifying cryptic protistan taxa within morphologically defined species (Pfandl et al. 2009) and for providing additional characters to distinguish species with amorphous morphologies, such as lobose amoebae (Nassonova et al. 2010). These findings have prompted work to purposefully incorporate sequence information into protistan species descriptions.

Critics often argue that genetic differences among strains within a species could overestimate an assessment of the species richness of an assemblage by erroneously equating intraspecific genetic variability to species-level differences, or that the adoption of a DNA taxonomy will result in the loss of understanding of the form and function that epitomizes the species concept. Some have argued that the genetic variations that have been promulgated as evidence of cryptic species are merely accumulated neutral mutations within morphospecies (Fenchel 2005). The presence of pseudogenes and different mutational rates among different genes have also been cited as factors confounding the use of sequence information for defining taxonomy. These criticisms are valid and must be addressed, but the ability to dramatically increase the rate of analysis and decrease the cost required for sequence-based approaches provides a strong impetus for the establishment of molecular taxonomies.

Nevertheless molecular methods and approaches have significantly improved our tools for characterizing the diversity, abundances, and activities of protists in natural marine communities. The isolation and culture of protists from natural ecosystems provide specimens for laboratory studies of protistan physiology, biochemistry, trophic activities, etc., while microscopy provides identification and estimates of abundance; this information is gathered to understand the ecological and biogeochemical activities of individual taxa of protists, and is used to synthesize predictive

models of their activities in nature. Iterative testing and reformulation of these models, and our understanding of protistan activities, are accomplished through in situ observations and field-based experimental studies. The application of molecular approaches and techniques has significantly augmented our ability to identify protists, estimate their abundances in natural samples, and examine the metabolic activities of these species.

Recent molecular studies of protistan diversity have provided tantalizing glimpses of novel taxa, incredibly diverse assemblages, and potentially new ecological roles for protists in marine ecosystems. These studies have also provided fruitful avenues for new research directions. A more vexing issue relates to our limited ability at the present to assign species identifications to lists of OTUs generated by sequencing campaigns. Most environmental sequences have not been directly linked to protistan species descriptions that have been defined using traditional (morphological) approaches. Therefore, there is presently no easy way for ecologists to interpret the long lists of OTUs generated in studies of protistan diversity.

The availability of expanding databases of DNA sequence information for protistan taxa will play a pivotal role in the development of approaches that exploit this information to specifically identify and accurately enumerate species of interest in complex natural microbial communities.

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Chapter 1- Microzooplankton composition in the winter sea ice of the Weddell Sea

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Abstract

Sympagic microzooplankton were studied during late winter in the northern Weddell Sea, for diversity, abundance and carbon biomass. Ice-cores were collected on an ice floe along three dive transects, and sea water was taken from under the ice through the central dive hole from which all transects were connected. The areal and vertical microzooplankton distributions in the ice and water were compared. They showed high abundance (max 1300 ind. L^{-1}) and biomass (max 28 μg C L^{-1}) in the ice-cores, and were low in the water, below sea ice (maxima, 19 ind. L^{-1}; 0.15 μg C L^{-1}, respectively). The highest amounts were found in the lower 10-cm section of ice cores. The microzooplankton community within sea ice comprised mainly aloricate ciliates, foraminifers and micrometazoans. In winter, microzooplankton represent an important fraction of the sympagic community in the Antarctic sea ice. They can potentially control microalgal production and can contribute to particulate organic carbon concentrations when released into the water column due to ice melting in spring. Continued reduction of the sea ice might undermine these roles of microzooplankton, leading to reduction or completely loss in diversity, abundance and biomass of these sympagic protists.

Key words: Sympagic protists, aloricate ciliates, tintinnids, foraminifers, micrometazoans

1. Introduction

Antarctic sea is a key driver in Southern Ocean biogeochemical cycles and ecosystem function (Arrigo 2014) and covers during maximum extent in September-October an area of approximately 19 x 10^6 km^2 (ca. 40% of the Southern Ocean's surface). The sea ice contains an internal system of delicate brine channels and pockets, which serve as a habitat for a variety of organisms. Together with the under-ice organisms, these constitute the sympagic sea ice community (Schnack-Schiel 2003). The large standing stock of the sea ice biota has an ecologically important role in the Southern Ocean. Ice algae and the associated sea ice microbial community are considered as a highly enriched and spatially confined food source for Antarctic krill during winter (larvae) and early spring (adults), when food in the water column is scarce (Meyer 2012).

Although sympagic organisms include many autotrophic and heterotrophic organisms, studies of the ice communities have mainly concentrated on ice algae and bacteria (Roberts *et al.* 2007). Therefore, little attention has been paid to the faunal components, and especially to microzooplankton (Garrison & Buck 1989, Kramer *et al.* 2011).

Microzooplankton include organisms with dimensions between 20 µm and 200 µm in size, and in the Antarctic sea ice they comprise mainly ciliates, heterotrophic dinoflagellates, foraminifers and the first larval stage of micrometazoans. Detailed investigations into the composition of the microzooplankton sympagic communities in Antarctica have generally focused only on specific protozoan groups, such as aloricate ciliates (Song & Wilbert 2000), heterotrophic dinoflagellates (Archer *et al.* 1996) and foraminifers (Spindler *et al.* 1990, Schnack-Schiel *et al.* 2001). Moreover, studies during the winter season are particularly scarce (Garrison & Buck 1989, Garrison & Close 1993, Schnack-Schiel *et al.* 2001, Kramer *et al.* 2011).

Sympagic microzooplankton are important because they can accumulate in the sea ice to high concentrations. They can live in the ice at concentrations several orders of magnitude higher than in the water (Garrison 1991, Garrison & Close 1993), where they are grazing on bacteria and algae,

and are a food source for heterotrophic consumers (Caron & Gast 2010). The sea ice also represents a nursery ground for larvae of key species, such as Antarctic krill, *Euphausia superba* and many other metazoans.

The present study was aimed to expand our knowledge of Antarctic sympagic microzooplankton diversity, abundance and biomass within sea ice and the adjacent water layer in late Antarctic winter, in the northern Weddell Sea.

2. Materials and Methods

Sampling was carried out during expedition ANT29-7 (14 August to 13 October, 2013) with R.V. "Polarstern", in the frame of the project WISKY (Winter Sea Ice Study on Key species). Ice cores were taken using a Kovacs Mark II ice corer (0.09 m internal diameter), powered with an electric drill. Sampling was performed on dive transects (EB, POL, ROV) (Fig. 1) on an ice floe at 60°47.76'S and 26°19.73'W

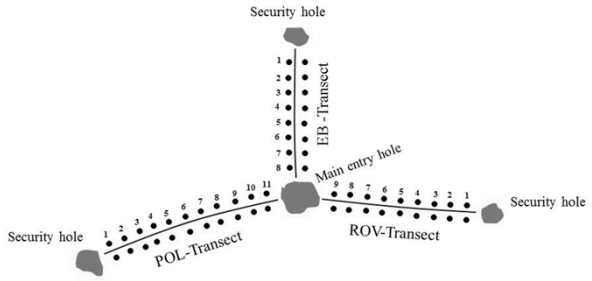

Fig. 1. Sampling transects on which the ice cores were performed from the security holes to the main entry hole of the divers. The dots represent the samplings on each transect. The distance between samples was two meters.

Three replicate ice cores were taken every second meter along the three transects (Fig. 1). One core was used for salinity and temperature measurements, whereas the two other cores were used for

analyses of microzooplankton composition, chlorophyll a (Chl a) as well as particulate organic carbon and nitrogen (POC and PON) concentration (Fig. 1). Core temperature was measured immediately after core extraction by drilling 5 mm holes into the ice core every 10 cm along the core in which a Pt 100 digital Thermometer was placed. The temperature along the core was not significantly different, so that the data were pooled. After than the core were cut into two parts as outlined below and melted separately onboard for analyzing the salinity with a YSI Inc. Model 30 conductivity meter. The salinity of both cores showed no differences and were pooled.

The two remaining ice cores for determining microzooplankton composition Chl a, POC and PON) concentration (Fig. 1) were also cut into two parts for comparisons and the replicate sections were pooled. One part was the last 10 cm of the core (the lower part), whereas the other, upper part was the rest of the ice core. Both parts were separately sealed in plastic tubes and were melted on board in a temperature constant room at -4 °C in the dark by adding 200 ml of 0.2 μm filtered sea water per cm ice core length to avoid osmotic stress (Garrison & Buck 1986). After 24 to 36 hours when the sea ice was melted, the volume was determined, and 3 to 5 L were concentrated using a 10-μm mesh. Once reduced to 250 mL, the specimens were immediately fixed with buffered formaldehyde (final concentration, 4%). The remaining volume of melted sea ice were used for Chl a measurements and elemental analysis of POC and PON. For Chl a analysis, 0.1-0.2L were filtered onto 25 mm diameter GF/F filters at pressures not exceeding 200 mbar. Filters were immediately transferred to centrifuge tubes with 6 ml 90% acetone and 1 cm^3 of glass beads. The tubes were sealed and stored at –20 °C for at least 30 min and up to 24 h. Chl a was extracted by placing the centrifuge tubes in a grinder for 20 seconds followed by centrifugation at 0 °C. The supernatant was poured in quartz tubes and measured for Chl content in a Turner 10-AU fluorometer. Calibration of the fluorometer was carried out at the beginning and at the end of the cruise. Chl a content estimated using the different calibration curves differed by <0.5%. Values presented here were calculated using average parameter values from the two calibrations and the equation given in Knap *et al.* (1996).

For POC and PON analysis 0.1-0.2L were filtered onto pre-combusted 25 mm Whatman GF/F filters. Filters were immediately transferred to pre-combusted glass petri dishes and dried overnight at 50 °C. Dried filters were stored at -20 °C until analysis. Before analysis, filters were treated with a few drops of 1N HCl and dried overnight at 50 °C to remove inorganic C. Filters were analysed using a EuraEA (Euro Vector) elemental analyser. An acetanilide standard series was measured at the beginning and end of each measurement cycle. POC concentrations in samples were estimated from the standart series after blank correction from measurements on blank filter taken during the cruise (processed as samples by filtering water from the ship's Milli-Q system). Total N and C content in blank filters was 3 ± 2 µg (SD) and 11 ± 6 µg (SD), respectively.

Water samples were collected through the entry hole of the Scuba divers (Fig.1) with a peristaltic pump. A tube was located under the ice in 1 m depth and the pump was switched on. Water samples of 5 L to 10 L were collected, concentrated and fixed, as described above.

Subsamples (50-100 mL) of melted ice and sea water were examined in a settling chamber using an inverted microscope (Leica DMI 3000B) equipped with phase-contrast and bright-field illumination (magnification, 200×), according to the Utermöhl method (1958). The entire surface of the chamber was examined. In total, 52 ice-core samples were analysed, as 26 lower sections and 26 upper sections, along with six seawater samples.

Among the microzooplankton community, four main groups were considered: ciliates (naked, tintinnids); heterotrophic dinoflagellates; micrometazoans and other protozoans, as Foraminifera, Radiolaria and Heliozoa. The identification to these groups were based on the descriptions of Alder (1999), Petz (2005) and Petz *et al.* (1995) for the ciliate, Balech (1976) and McMinn & Scott (2005) for the heterotrophic dinoflagellates, Larik & Westheide (2006) for micrometazoans, Kemle-von Mücke & Hemleben (1999) for the Foraminifera, Kling & Boltovskoy (1999) for the Radiolaria Phaeodaria, and Mikrjukov *et al.* (2000) for the Heliozoa.

Although identification of aloricate ciliates is uncertain without silver-staining procedures, in our samples the ciliates were very well preserved and their large dimensions allowed their identification

at the genus level, and sometimes even at the species level. Empty loricae were not differentiated from filled loricae, because the tintinnid protoplasts are attached to the loricae by fragile strands that can easily detach during collection and fixing of the samples.

The phototrophic ciliate *Mesodinium rubrum* is an obligate autotroph (Lindholm 1985), but it was considered in this study, as it was only a minor part of the ciliates biomass. Dinoflagellates were considered heterotrophic on the basis of previous studies (Lessard 1991). Only gyrodinoide species could not be identified to species level, and the contribution of autotrophy or mixotrophy could not be evaluated by this method, although their contribution to the total biomass was <1%.

For each taxon, the biomasses were estimated by measuring the linear dimensions of each organism using an eyepiece scale and relating the individual shapes to standard geometric figures. Cell volumes were converted to carbon values using the appropriate formulae and conversion factors, as: tintinnids, pg C $cell^{-1}$ = μm^3 ×0.053 + 444.5 (Verity & Langdon 1984); naked ciliates, pg C $cell^{-1}$ = μm^3 ×0.14 (Putt & Stoecker 1989); dinoflagellates, pg C $cell^{-1}$ = μm^3 ×0.11 (Edler 1979); micrometazoans, pg C $cell^{-1}$ = μm^3 ×0.08 (Beers & Stewart 1970); others protozoans, pg C $cell^{-1}$ = μm^3 ×0.089 (Gifford & Caron 2000).To elucidate the relationships between the main taxa in the ice with environmental parameters (temperature, salinity, Chl a, POC, PON) we performed a Canonical Correspondence Analysis (CCA) (Legendre & Legendre 1998) using PAST V3.14 software (Øyvind Hammer, Oslo, NO). Significance of axis was tested by Permutation analyses (Permutation number 999).

To test the variability of the main microzooplankton taxa present in the upper and lower ice-core sections, a cluster analysis was carried out (Warwick & Clarke 2001). Multivariate analyses were based on Bray–Curtis similarities or dissimilarities (Bray & Curtis 1957), as calculated from the square-root-transformed abundance and biomass data. The analyses were conducted using the PRIMER V.7 software (PRIMER-E Ldt, Plymouth, UK), and the significance level for all statistical tests was set at 5%.

3. Results

3.1 Lower ice-core sections

The microzooplankton abundance ranged from 128 ind. L^{-1} (EB1) to 1300 ind. L^{-1} (POL7). The aloricate ciliates were the most abundant group, and these ranged from 38 ind. L^{-1} (ROV2) to 1017 ind. L^{-1} (POL11). The micrometazoans represented mainly the nauplia of copepods, with a maximum of 282 ind. L^{-1} (POL10). Foraminifers were present in all the samples and reached a maximum abundance of 461 ind. L^{-1} (POL1). The heterotrophic dinoflagellates were almost absent, while tintinnids showed the maximum of 542 ind. L^{-1} (ROV1), although they generally showed lower values.

The microzooplankton biomasses ranged from 2.17 µg C L^{-1} (EB1) to 28.2 µg C L^{-1} (POL10). The higher biomasses were due to the large size of the aloricate ciliates, as the genera *Gymnozoum*, *Litonotus*, *Placus* and *Frontonia*, and to the large-sized foraminifer *Neogloboquadrina pachyderma.* The naupliar stages of copepods also contributed to the total amount of carbon in the ice. The average abundance and biomass of microzooplankton reached 568 ±325 ind. L^{-1} and 12.4 ±6.7 µg C L^{-1} (Fig. 2), respectively.

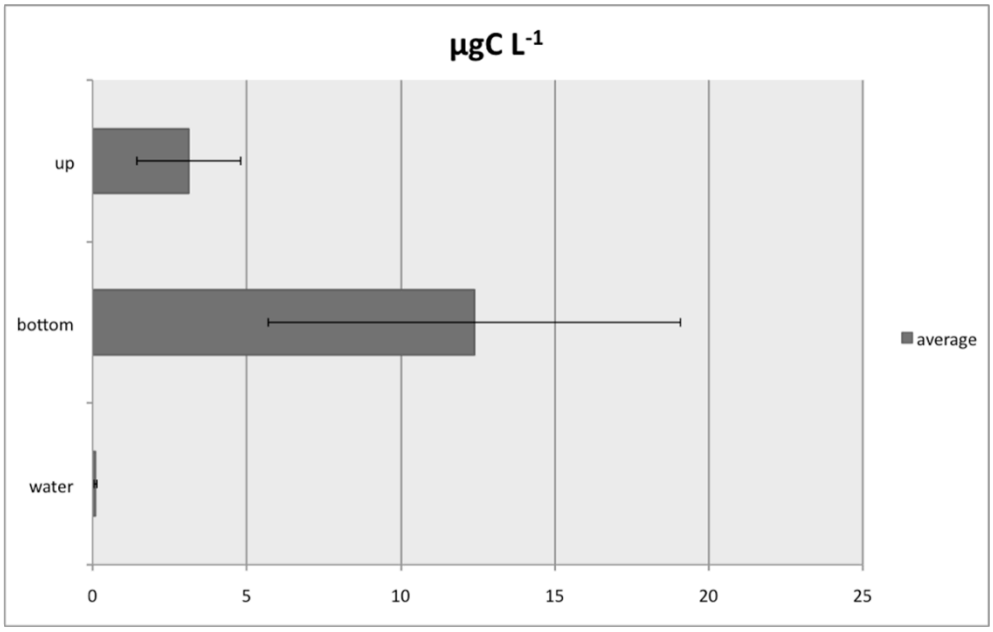

Fig. 2. Average biomass of microzooplankton (µg C L^{-1}) in the upper ice-core sections (up), lower ice-core sections (bottom) and in the sea water under the ice (water).

The analysis of the composition of the microzooplankton community in the lower ice-core samples identified 56 taxa (Supplementary Table 1). Among the ciliates, there was the aloricate genus *Gymnozoum* in all of the samples, with *Gymnozoum viviparum* (Fig. 3a), *G. sympagicum* (Fig. 3b), and at lower abundance, *Gymnozoum glaciale*. *Placus antarcticus* (Fig. 3c) and *Didinium gargantuan* (Fig. 3d) and the genera *Litonotus* (Fig. 3e) and *Euplotes* (Fig. 3f) were also found frequently. Many ciliates showed diatoms inside, generally as pennate diatoms (Fig. 4a, b). The tintinnids were assigned to only three genera (i.e., *Cymatocylis*, *Codonellopsis*, *Laackmanniella*) and six species (Supplementary Table 1). The most abundant tintinnids species were

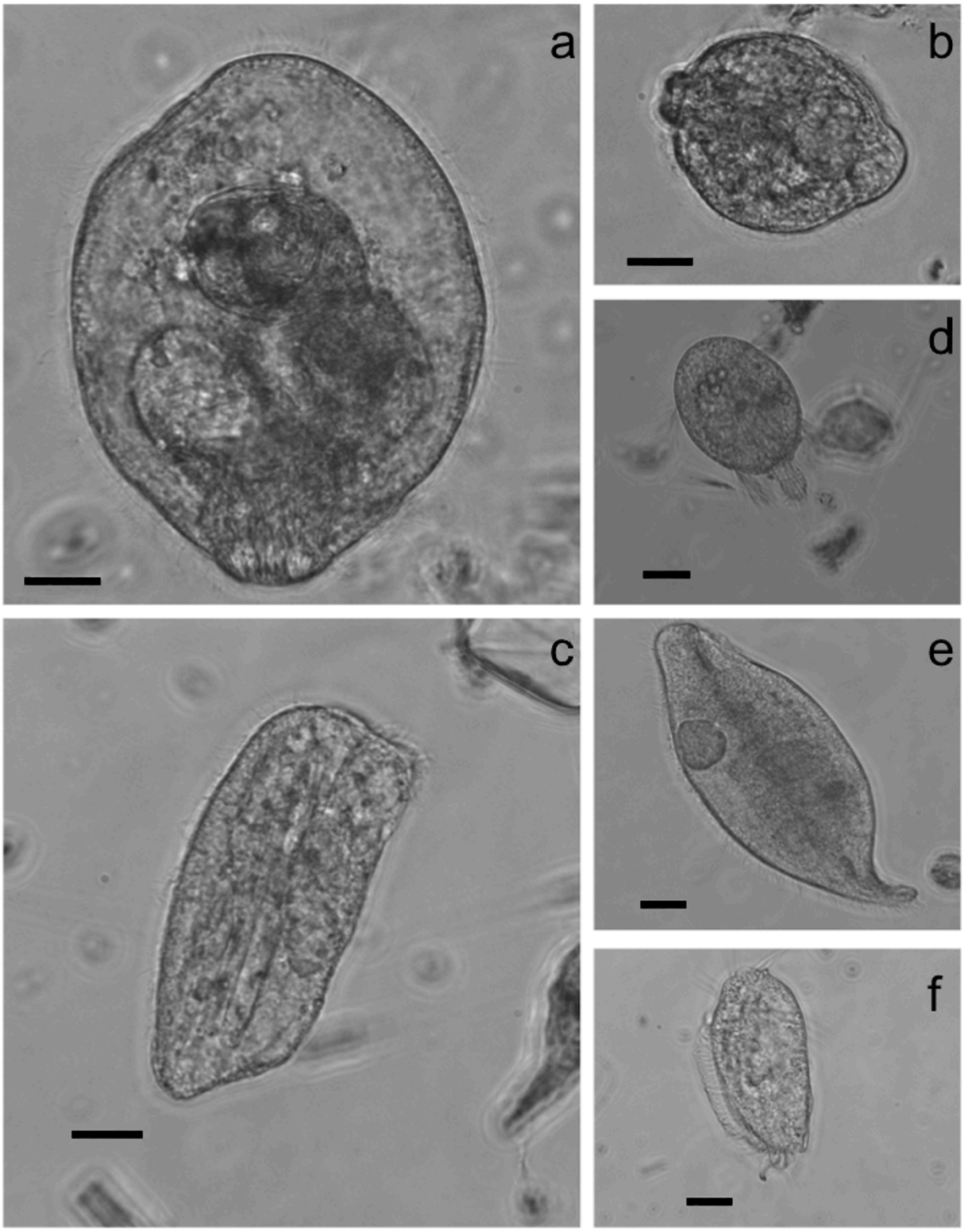

Fig 3. *Gymnozoum viviparum* (a), *G. sympagicum* (b), *Placus antarcticus* (c), *Didinium gargantua* (d), *Litonotus* sp. (e), *Euplotes* sp. (f). All scar bars indicate 20 μm.

Codonellopsis glacialis and *Laackmanniella naviculaefera*. Most of the tintinnids were empty, or as for the genus *Laackmanniella*, the presence of material attached to the lorica hindered the view of the inner cell. Foraminifers were present in almost all of the samples, mainly *N. pachyderma* (Fig. 5). The heterotrophic dinoflagellates were very rare, with only the genus *Gyrodinium* identified (Supplementary Table 1).

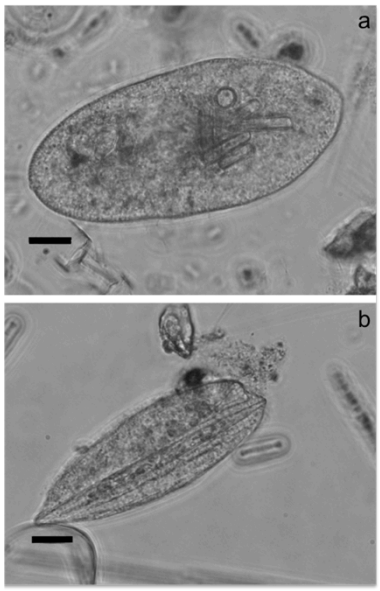

Fig. 4. *Fuscheria marina* (a), *Placus antarcticus* (b), with inside pennate diatoms. All scar bars indicate 20 μm.

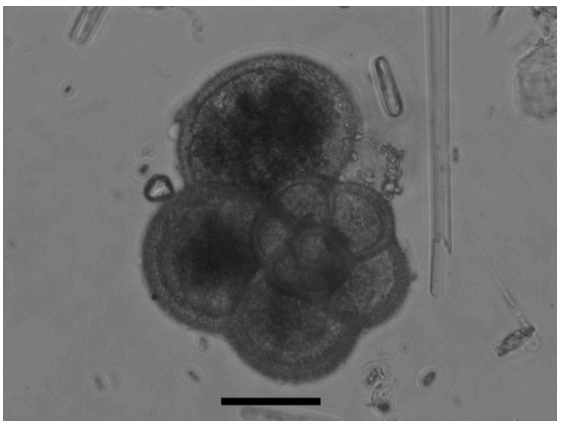

Fig. 5. *Neogloboquadrina pachyderma*. Scar bar indicates 40 μm.

3.2 Upper ice-core sections

The microzooplankton were less abundant in the upper ice-core sections compared to the last 10 cm of ice cores. In the upper ice-core sections, they ranged from 30 ind. L^{-1} (EB4) to 577 ind. L^{-1} (EB8). Only in two samples (EB8, ROV3) were the microzooplankton abundances higher than in the lower 10 cm of ice-core.

The aloricate ciliates were the most abundant group, and they ranged from 21 ind. L^{-1} (EB4) to 568 ind. L^{-1} (EB8). The micrometazoans were dominated by copepode nauplia with a maximum abundance of 43 ind. L^{-1} (POL2). The heterotrophic dinoflagellates and tintinnids showed very low values in the upper ice-core sections, with maximum abundance of 11 and 25 ind. L^{-1}, respectively. Foraminifers presented values below 70 ind. L^{-1}, only in EB5 and POL4 they reached 238 ind. L^{-1} and 135 ind. L^{-1}, respectively.

The microzooplankton biomasses in the upper ice-core sections ranged from 0.37 µg C L^{-1} (EMB4) to 7.14 µg C L^{-1} (POL9). The average abundance and biomass of microzooplankton here were lower than in the last 10 cm of the ice core sections. They only reached 199 ±109 ind. L^{-1} and 3.1 ±1.7 µg C L^{-1} (Fig. 2).

For these upper sections of the ice cores, the microzooplankton recorded covered 40 taxa (Supplementary Table 1). The aloricate ciliates were the most abundant group, with 12 genera, although this remained less than for the last 10 cm of the ice core sections. The genera *Spirostrombidium*, *Fuscheria*, *Dysteria*, *Frontonia* and *Uronema* were not found in the upper ice-core sections. However, the general microzooplankton species composition was very similar between the upper and the lower ice-core sections.

3.3 Sea water under the ice

In the sea water samples from under the ice, the microzooplankton abundance was low ranging from 8 ind. L^{-1} to 19 ind. L^{-1} according to the low chlorophyll values ranging from 0.08 to 0.9 μg L^{-1}.They were constituted mostly by micrometazoans and tintinnids. Among the tintinnids, there were five genera recorded, *Amphorellopsis*, *Cymatocylis*, *Codonellopsis*, *Laackmanniella* and *Salpingella* (Supplementary Table S1). The abundance of aloricate ciliates and thecate dinoflagellates were also low (below 2 L^{-1}). The former were represented by Strombididae and *Mesodinium* sp., and the latter by the genera *Protoperidinium* and *Cochlodinium* (Supplementary Table 1). According to the low microzooplankton abundance, the biomass was in all samples below 0.15 μg C L^{-1} (Fig. 2) and the largest proportion came from large-sized tintinnids, such as *L. naviculaefera* and the naupliar stages of copepods.

3.4 Statistical analyses

The CCA scatter plot (Fig. 6) showed that the first axis, explaining 70% of variance ($p < 0.05$), is characterized by a decreasing trend of temperature and an increasing trend of salinity, POC and PON concentration. The second axis explained only 22% of variance ($p < 0.05$) and can be associated with Chl a concentration. Tintinnids appeared to be positively correlated with salinity, POC and PON. The samples of the ROV transect occupied the right part of the scatter plot, with the exception of ROV 6, due to the higher abundance of aloricate ciliates. The samples of the other two transects were placed on the left part of the scatter plot and only some of them appeared positively correlated with Chl a concentration (EB8, POL 9, POL 8 and EB4).

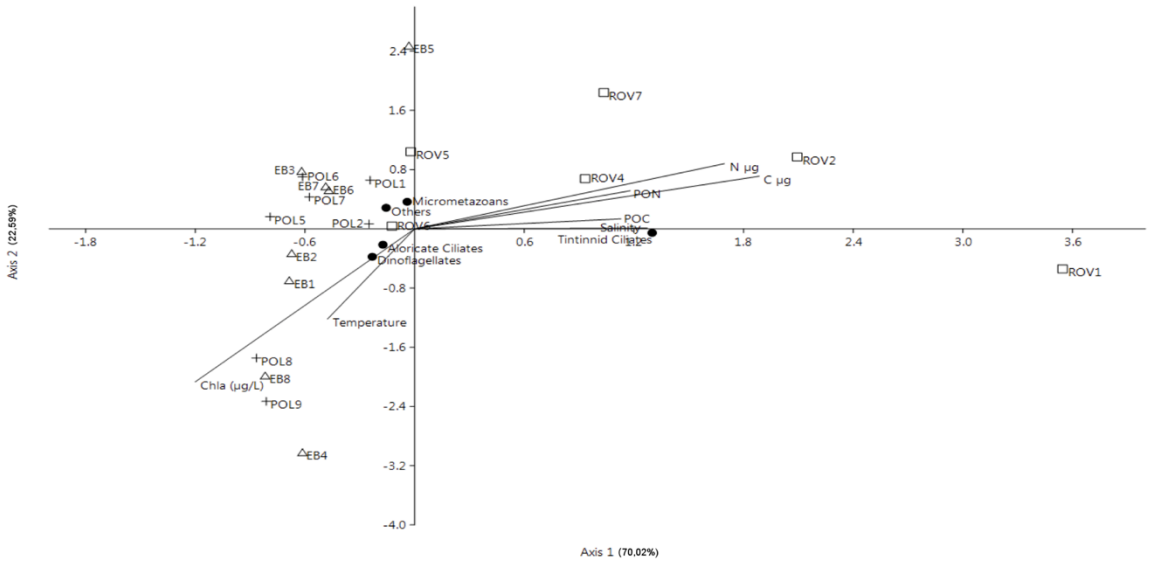

Fig. 6**.** CCA analyses on environmental data and microzooplankton groups (Aloricate ciliates, heterotrophic dinoflagellates, tintinnid ciliates, micrometazoans, others). Environmental variables are plotted as correlations with site scores, scaling 2 were used to emphasizes relationships between species.

The microzooplankton in all three of these transects (Fig. 1, EB, POL, ROV) were not restricted to the lower ice-core section. To define the community diversity, we applied cluster analyses to all of the identified species and identified four main groups (Fig. 7): Groups A and C were constituted by the lower ice-core samples, and Groups B and D by the upper ice-core samples. There was only one exception here, for a lower ice-core sample (EB4) that was included in Group B, as the larger group of the upper ice-core samples, because it showed less abundance than all of the lower ice-core samples and totally lacked copepod nauplia. The lower ice-core samples showed greater abundances of microzooplankton, and particularly for Group C, which was formed by the samples from the POL transect. Groups B and D were also divided on the basis of the presence/ absence of some of the aloricate ciliates (e.g., *Gymnozoum*, *Placus*).

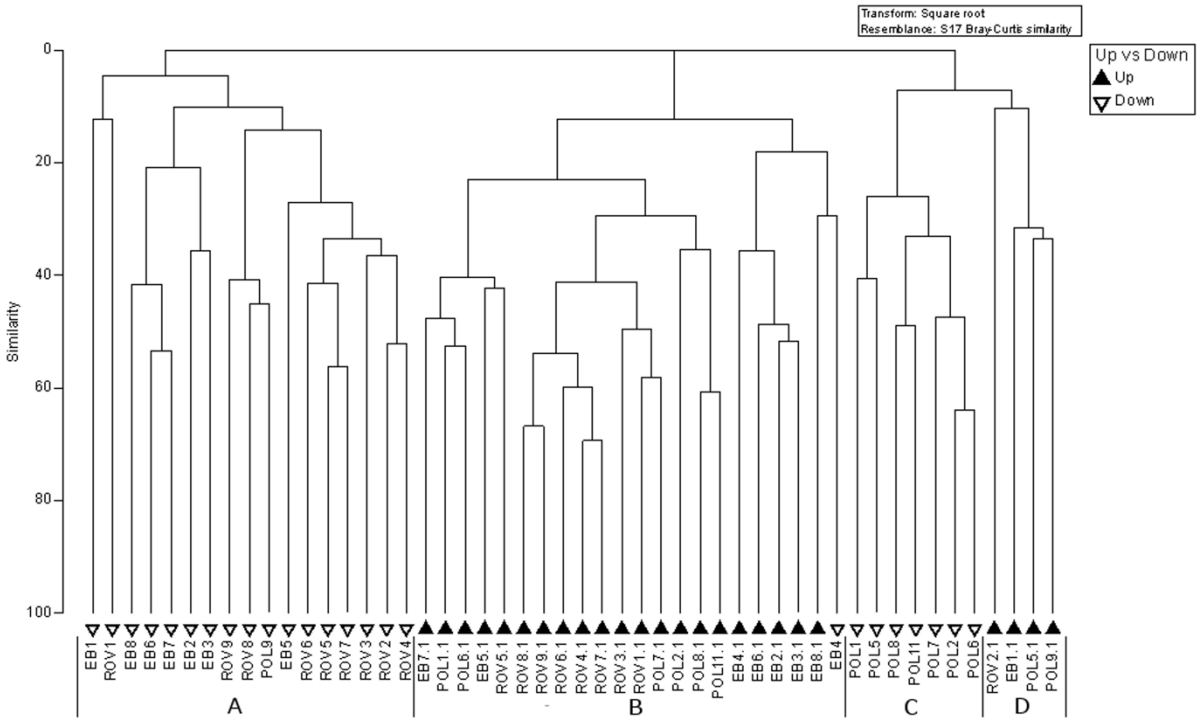

Fig. 7. Cluster analyses of all the upper ice-core (▲) and lower ice-core (▽) sections.

4. Discussion

The microzooplankton identified in the present study were in general agreement with previous studies in the same region (Supplementary Table S2), with the dominance of the aloricate ciliates and foraminifers in the sea ice communities (Garrison & Buck 1989, Garrison & Close 1993, Schnack-Schiel *et al.* 2001, Kramer *et al.* 2011). In the late winter in the perennially ice-covered western Weddell Sea, ciliates were shown to dominate the protozoan communities in terms of abundance and biomass (median abundance 20 $\times 10^3$ ind. m^{-2}; median biomass 2.38 mg C m^{-2}), followed by foraminifers (Kramer *et al*. 2011). Schnack-Schiel *et al.* (2001) reported that in the late winter ice of the Weddell Sea, foraminifers dominate the sea ice communities in terms of abundance (48%), while ciliates reached only 9% representation of the sea ice meiofauna. The foraminifer mean abundance was 47.85 $\times 10^3$ ind. m^{-2}, and mean biomass was 1.48 mg C m^{-2}, while the ciliate mean abundance was 10.36 $\times 10^3$ ind. m^{-2}, with a mean biomass of 0.11 mg C m^{-2} (Schnack-Schiel *et al.* 2001). Garrison & Close (1993) analysed pack-ice floes collected in the same

area during a winter cruise, and they reported that heterotrophic dinoflagellates entirely comprised of athecate forms dominated the protozoan biomass (39 μg C L^{-1}), while the ciliates average was 7.30 $\times 10^3$ ind. L^{-1}, with an average biomass of 9 μg C L^{-1}. In another study, Garrison & Buck (1989) reported that the maximum abundance for foraminifers, collected in drifting pack-ice near the Antarctic peninsula, was 200 ind. L^{-1}, which is lower than seen in the present study. To explain the paucity of foraminifers, the authors considered the particularly patchy distribution of these organisms.

In the samples analysed in the present study, nauplia were well represented (maxima, 282 ind. L^{-1}; 9 μg C L^{-1}), although this was lower than that reported by Garrison & Close (1993), who reported maximum nauplia abundance of 3.2 $\times 10^3$ ind. L^{-1}, and for biomass, 18 μg C L^{-1}, and by Garrison & Buck (1989), who reported for drifting pack-ice near the Antarctic peninsula 800 ind. L^{-1}. We did not classify the nauplia, but in previous studies in the same area, the majority of the nauplia in the sea ice belonged to pelagic calanoid copepods (Schnack-Schiel *et al.* 1998).

The majority of the ciliates identified in our samples are considered benthic species, typical in ice environments (Petz *et al.* 1995), and generally are not found in the planktonic community (Garzio & Steinberg 2013; Monti *et al.* 2016). The ice is a favorable environment for these ciliates, it provides shelter from predators and plenty of food to grow, documented by the large sizes of these organisms, which can transfer a remarkable amount of carbon to the upper trophic levels. In some cases (e.g., *Placus antarcticum*) they were filled with pennate diatoms, demonstrating that these ciliates were feeding on diatoms inside the ice channels. The initial incorporation of these ciliates into the ice remains unclear since such incorporation should require their presence in the water column during sea ice formation, as it is known for diatoms and dinoflagellates. The ciliate ice taxa are rarely encountered in the water because presumably the prey abundances are not sufficiently dense or the pelagic habitat not favourable to the development of significant robust ciliates populations. In the water, benthic ciliates can live in low numbers attached on particles but they are probably too rare to appear in routine surveys of water samples. On the contrary, inside the pack ice

these organisms can find a particularly favourable condition, which enhances their abundance at the expense of truly planktonic organisms. In addition, the fate of the ciliates after the ice melt is still not clear. Most probably, they will sink to the sea floor, either directly or mediated via faecal pellets. On the other hand, ciliates can be grazed before the complete melting of the ice, as krill and other organisms are frequently foraging under sea ice in spring (Garrison & Close 1993, Meyer 2012).

In the present study, only a few specimens of tintinnids and heterotrophic dinoflagellates were recorded. Tintinnids were occasionally recorded in ice-cores from the western Weddell Sea during winter by Kramer *et al.* (2011), and more often in the melt-water assemblages at the ice/ snow interface of pack-ice (Caron & Gast 2010). Most of the heterotrophic dinoflagellates in sea ice include the naked forms (e.g., *Gyrodinium*, *Gymnodinium*) (Garrison & Buck 1989) while thecate dinoflagellates can reach high abundances in spring-summer fast sea ice microhabitats (Stoecker *et al.* 1993). Both tintinnids and thecate dinoflagellates are unusual in compact ice, probably because their movements can be hampered by the presence of the lorica and theca. Tintinnids showed a positive correlation with salinity, PON and POC. Therefore, on the base of these results, we can hypothesize that tintinnids might be linked to organic matter (including bacteria) abundance.

The only recorded foraminifer species was *N. pachyderma*, which is a typical sea ice associated species, and can dominate sea ice communities, in terms of their abundance (Schnack-Schiel *et al.* 2001). *Neogloboquadrina pachyderma* is growing inside the brine channels and can survive salinities up to 82 (Spindler *et al.* 1990). The chamber formation rates are slower at higher salinities, and the final sizes of specimens decrease with increasing salinity. Reproduction of *N. pachiderma* was never observed at salinities >50, suggesting that *N. pachyderma* does not reproduce within sea ice.

The occurrence of organisms in sea ice can be highly variable for their areal and vertical distributions, due to differences in the sea ice conditions. The differences may arise from between deep-water pack-ice and land fast ice, from the interannual variability, or from the heterogeneity of

sympagic communities (Garrison & Buck 1989, Garrison & Close 1993, Schnack-Schiel *et al.* 2001). In general, sympagic organisms occupy the layer near the ice/ water interface because its temperature and salinity are more similar to those of the underlying water, although they can occupy the whole ice-core. Schnack-Schiel *et al.* (2001) suggested that the bulk of the meiofanua is concentrated in the lowest parts of the sea ice, especially in winter and autumn, because the ice is less porous in the upper sections. Garrison & Buck (1989) noted that although organisms were found throughout ice floes, the highest concentrations and diversity occurred in a slush-like layer near the snow-ice interface. Our study confirms that of Kramer *et al*. (2011), with sympagic protozoans present all through the ice-cores, and not restricted to a specific levels. It is still unknown which factors control the vertical distribution of sympagic organisms. Kramer *et al.* (2011) reported that the distribution of sympagic meiofauna in the western Weddell Sea was correlated with vertical pigment profiles, but not with any of the abiotic variables measured (i.e., temperature, salinity, brine volume). The ciliates diversity presumably reflects the physical and chemical complexity of the sea ice microhabitats. Here, despite the similarity in the taxonomic composition of the lower and upper ice-core samples, cluster analysis still distinguished samples belonging to the two different series, with only one exception. Thus, the main difference between the lower and upper ice-core series was of a quantitative character.

In our study the distribution of the majority of samples seemed not to be affected by environmental factors such as temperature, salinity, Chl a, POC or PON. Only samples of the ROV transect appeared positively correlated with salinity, POC and PON concentration but also with tintinnid abundance, and inversely correlated with temperature. Other few samples of POL and EB transects seemed to be positively correlate with Chl a. All the other samples showed no significant relationships with environmental variables and presented a typical patchy distribution reflecting the heterogeneity of the ice environment (Caron & Gast 2010).

In the present study, the microzooplankton community in the water below sea ice was profoundly different from those in the ice-cores. In the water below the ice the abundance never exceeded 20

ind. L^{-1} and was constituted mostly by micrometazoans and tintinnids. Our results differentiated from previous studies conducted during summer in the same area. The microzooplankton found during two cruises along the western Antarctic peninsula in summer 2010 and 2011 showed athecate dinoflagellates and aloricate ciliates as the dominant groups in terms of the abundance and biomass in the sea water (Garzio & Steinberg 2013). Also, tintinnids were encountered, and there was correlation between the biomass and the latitude, with tintinnids and larger dinoflagellates in particular showing higher biomass with increasing latitude (Garzio & Steinberg 2013). The aloricate ciliates in the water column during summer were mostly from genera *Strombidium*, *Strobilidium* and *Didinium*, while dinoflagellates mainly belong to the genus *Protoperidinium*. During a summer cruise in the Weddell Sea, Boltovskoy & Alder (1992) reported that dinoflagellates dominated the microzooplankton community both in terms of number and biomass, followed by tintinnids. The differences between our results in the sea water under the ice and the others researches can be impute to the different seasons of investigation.

The comparison between the sea water and ice community underlines as the microzooplankton in the sea ice is constituted by unique protozoan assemblages with organisms that do not appear to have pronounced effects as sources of the summer microzooplankton population. The ice microhabitat seems not to have to compete for food, as in the ice there is availability of abundant prey also in the winter, as diatoms, flagellates and bacteria. Due to the high carbon biomass and potentially high contributions as prey, microzooplankton appear to constitute an important food source for under-ice organisms, such as krill.

5. Conclusions

During the Antarctic winter, microzooplankton have high abundance and biomass in the sea ice. Although these organisms occupied the whole ice-core, their abundance and biomass were concentrated in the lower 10 cm of the ice-core sections, probably due to the frequencies of brine

channels, and their dimensions and levels of ramification in the different ice-core sections. The sea ice populations strongly differed from the ice-free water communities. The sea ice communities were very similar across all of the ice-core samples, so both in the lower and upper ice-cores, and these were mainly formed by benthic ciliates, while tintinnids and heterotrophic dinoflagellates were scarce. For this reason, the microzooplankton in sea ice do not appear to have pronounced effects as sources of the spring/ summer populations. The data from the present study demonstrate that microzooplankton represent an important fraction of the sympagic community in the Antarctic sea ice. The diversity, abundance and biomass of Antarctic sympagic microzooplankton have been underestimated to date. Microzooplankton contribute to the food supply for the upper levels of the trophic web, they have a potential role in the control of microalgal production and biomass, and they might contribute to the particulate organic carbon when they are released into the water in summer.

However, the continued reduction of the ice caused by the global warming could undermine these particular ice organisms, and thus lead to a reduction, or completely loss of the diversity, abundance and biomass of these sympagic protists.

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Supplementary

Table S1. Taxa observed during the sampling period, including the mean abundance of each taxa in ice (upper and lower ice-core sections) and sea water under the ice.
Abundances (ind. L^{-1}): - = 0, + = 0.1-10, ++ = 11-30, +++ = 31-50, ++++ = 51-80.

	UPPER	LOWER	WATER
PROTOZOA			
CLASS GLOBOTHALAMEA			
Order Rotaliida			
Neogloboquadrina pachyderma	++	++++	-
Foraminiferida unid.	++	++++	+
CLASS STICHOLONCHEA			
Order Sticholonchida			
Sticholonche zanclea	-	-	+
CLASS THECOFILOSEA			
Order Phaeogromida			
Protocystis sp.	-	+	-
CLASS POLYCYSTINA			
Polycystina unid.	-	-	+
CLASS DINOPHYCEAE			
Order Gymnodiniales			
Cochlodinium pupa	-	-	+
Cochlodinium sp.	-	-	+
Order Peridiniales			
Gyrodinium sp.	+	+	-
Gyrodinium cfr. *crassum*	-	+	-
Protoperidinium defectum	-	-	+
Protoperidinium sp.	-	-	+
Thecate Dinoflagellida unid.	-	-	+
CLASS HETEROTRICHEA			
Order Heterotrichida			
Condylostoma sp.	+	+	-
Heterotrichida unid.	-	+	-
CLASS SPIROTRICHEA			
Order Euplotidae			
Euplotes cfr. *acanthodus*	+	+	-
Euplotes cfr. *antarcticus*	+	+	-
Euplotes cfr. *rariseta*	+	++	-
Euplotes sp.	+	++	-

Spirotrichea unid.	+	-	-
Order Tintinnida			
Amphorellopsis quinquelata	-	-	+
Cymatocylis drygalskii	+	-	
Cymatocylis convallaria	+	+	+
Cymatocylis convallaria	+	+	+
Cymatocylis vanhöffeni	+	+	-
Codonellopsis gaussi	-	+	+
Codonellopsis glacialis	+	++	+
Codonellopsis sp.	-	+	+
Laackmanniella naviculaefera	+	++	+
Salpingella costata	-	-	+
Salpingella costata	-	-	+
Salpingella cfr. *decurtata*	-	-	+
Tintinnida unid.	-	+	+
Order Choreotrichida			
Choreotrichida unid.	-	+	+
Order Urostylida			
Urostylida unid.	+	+	-
Order Strombidiida			
Spirostrombidium sp.	-	+	-
Strombidiidae cfr. *rhyticollare*	-	+	-
Strombidiidae unid.	+	+	+
CLASS LITOSTOMATEA			
Order Cyclotrichida			
Mesodinium rubrum	+	+	-
Mesodinium sp.	+	+	+
Cyclotrichida unid.	-	+	-
Litostomatea unid.	+	+	-
Order Haptorida			
Didinium gargantua	+	+	-
Pseudotrachelocerca cfr. *trepida*	+	+	-
Lacrymaria cfr. *lagenula*	+	+	-
Lacrymaria cfr. *spiralis*	+	+	-
Lacrymaria sp.	+	+	-
Fuscheria marina	-	+	-
Chaenea teres	+	+	-
Haptorida unid.	-	+	-
Order Pleurostomatida			
Litonotus sp.	+	+	-
Loxophyllum rostratum	+	+	-
CLASS PHYLLOPHARYNGEA			
Order Chlamydodontida			
Gymnozoum glaciale	+	+	-
Gymnozoum sympagicum	+++	+++	-

Gymnozoum viviparum	+	++	-
Gymnozoum sp.	++	++++	-
Chlamydonella sp.	+	+	-
Chlamydodontida unid.	-	+	-
Phyllopharyngea unid.	+	++	-
Order Dysteriida			
Dysteria cfr. *monostyla*	-	+	-
CLASS NASSOPHOREA			
Order Synhymeniida			
Nassophorea unid.	-	+	-
CLASS PROSTOMATEA			
Order Prorodontida			
Placus antarcticus	++	++	-
Prostomatea unid.	-	+	-
CLASS OLIGOHYMENOPHOREA			
Order Peniculida			
Frontonia cfr. *frigida*	-	+	-
Oligohymenophorea unid.	+	+	+
Order Philasterida			
Uronema sp.	-	+	-
Uronematidae unid.	+	+	-
Ciliophora unid.	+	++	+
METAZOA			
ARTHROPODA			
CRUSTACEA			
COPEPODA			
Copepods (nauplius)	+	++++	+
Larvae unid.	+	+	-
Eggs unid.	-	-	+

Table S2. Comparison of abundance (ind. L^{-1}) and carbon biomass (µg C L^{-1}) of aloricate ciliates, foraminifers, heterotrophic dinoflagellates and nauplius larvae.

Microzooplankton groups	Sampling period	Abundance (mean)	Abundance (range)	Carbon Biomass (mean)	Carbon Biomass (range)	References
	August-October 2006					
Aloricate ciliates		20 x 10^3 ind m^{-2}	1.4-84.9 x10^3 ind m^{-2}	2.38 mg C m^{-2}	0.13-6.27 mg C m^{-2}	Kramer *et al.* 2011
Foraminifers		0.7 x 10^3 ind m^{-2}	0.2-3.1 x10^3 ind m^{-2}	1.14 mg C m^{-2}	0.02-2.62 mg C m^{-2}	
	September-October 1986					
Aloricate ciliates		10.36 x 10^3 ind m^{-2}	0-67 x 10^3 ind m^{-2}	0.11 mg C m^{-2}	0-0.7 mg C m^{-2}	Schnack-Schiel *et al.* 2001
Foraminifers		47.85 x 10^3 ind m^{-2}	0-440 x10^3 ind m^{-2}	1.48 mg C m^{-2}	0-14 mg C m^{-2}	
	June-July 1988					
Aloricate ciliates		7.30 x 10^3 ind L^{-1}	5.60x10^2 -2.70x10^4 ind L^{-1}	9 µg C L^{-1}	<1-37 µg C L^{-1}	Garrison & Close 1993
Foraminifers		1.26 x 10^3 ind L^{-1}		0.6 µg C L^{-1}		
Hetrotrophic dinoflagellates		5.40 x 10^4 ind L^{-1}	0.03-2.10x10^5 ind L^{-1}	39 µg C L^{-1}	0-150 µg C L^{-1}	
Nauplius larvae		3.74 x 10^2 ind L^{-1}	2.40x10^1-9.20x10^2 ind L^{-1}	7.7 µg C L^{-1}	2.9-18 µg C L^{-1}	
	June-July 1987					
Foraminifers		200 ind L^{-1}				Garrison & Buck 1989
Nauplius larvae		800 ind L^{-1}				
	August-October 2013					
Aloricate ciliates		320 ind L^{-1}	38-1017 ind L^{-1}	4.77 µg C L^{-1}	0.2-18.4 µg C L^{-1}	This study
Foraminifers		114 ind L^{-1}	0-462 ind L^{-1}	5.06 µg C L^{-1}	0-22.6 µg C L^{-1}	
Hetrotrophic dinoflagellates		7 ind L^{-1}	0-35 ind L^{-1}	0.01 µg C L^{-1}	0-0.06 µg C L^{-1}	
Nauplius larvae		81 ind L^{-1}	0- 282 ind L^{-1}	1.98 µg C L^{-1}	0-9.1 µg C L^{-1}	
Tintinnids		46 ind L^{-1}	0-541 ind L^{-1}	0.58 µg C L^{-1}	0-8.4 µg C L^{-1}	

Chapter 2- Vertical Distribution of Microbial Community along Mediterranean Sub-Marine Canyons

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Abstract

The aim of the present study was to investigate the microbial community along the Ligurian coast in correspondence of two marine canyons. Community structures along the water column were studied using microscopic techniques, and their relationships with the environmental factors recorded along the canyon were investigated.

The study considered pico-, nano- and micro-planktonic fractions, their abundances and composition. We also considered the mean cells' size in the studied groups in order to identifying the trophic patterns that regulate and shape the entire microbial population.

Profiles of temperature and salinity showed the same trend for all stations and no significant difference were found among stations in correspondence of the canyons profiles and the ones detected on the adjacent slope.

Our results highlight that the main pattern regulating the communities composition is the depth and that community recorded in the samples belonging to each transect were not separated on the base of geographic distribution.

A similar trend for abundances and biomasses was observed at all stations: higher values were generally measured in the surface layer and they decreased with increasing depth and a significant linear regression was highlighted in each of the three transects.

Results of the distribution of all microbial heterotrophs from the surface down to 2500m pointing out that prey abundance was generally higher than the feeding threshold of predator; this evidence suggests that the interaction between different size classes is bottom-up regulated in the study area.

The increase of mean cell biovolume of both heterotrophic nanoflagellates and microzooplankton should be the solely evidence of the expected "canyon effect", and can be ascribe to the decreasing of temperature and to the major input at the bottom of the canyon of organic matter (POC) that can be used by heterotrophic nanoflagellates also directly as food source.

1. Introduction

Marine micro-organisms are recognized to contribute to >95% of the particulate organic carbon in the oceans, and to be the major drivers of global biogeochemical cycles (Pomeroy, 2007). Microorganisms are represented by a number of functional types, from Bacteria and Archaea to phytoplankton and heterotrophic and mixotrophic flagellates, up to large protists.

Heterotrophic protists play a key role in the general pelagic ecosystem functioning by linking the microbial food web (bacteria, archea and small eukaryotic phytoplankton) to the classic food web (large phytoplankton, mesozooplankton and fish) (Azam et al., 1983).

Heterotrophic protists are an important component of nanoplankton (2–20 μm) and microplankton (20– 200 μm) assemblages in the marine pelagic ecosystem and include radiolarian, foraminifers, ciliates, heterotrophic and mixotrophic dinoflagellates, and heterotrophic and mixotrophic nanoflagellates (Stoecker & Capuzzo, 1990; Sherr, 1994). With their small size, rapid metabolism, and high growth rates, ciliates and heterotrophic flagellates may contribute significantly to the trophic flux and nutrient cycling (Fenchel, 1968; Rassoulzadegan & Sheldon, 1986). Heterotrophic protists ingest a broad size spectrum of prey, from bacteria to microphytoplankton, and are themselves important prey for mesozooplankton.

Distribution patterns of the marine plankton are strongly influenced by the physical processes occurring in a particular area (Boucher et al., 1984; 1987; Margalef & Estrada, 1987).

Submarine canyons are deep incisions in the continental margins that stretch from continental shelves to deep ocean environment (Shepard & Dill, 1966).

Many studies have described marine canyon structures as one of the main factors influencing the oceanographic characteristics of an area, as they are the major pathways for the transport and translocation of organic carbon.

They influence the biogeochemical cycling of carbon and other elements down to the abyssal depths (Canals et al., 2006). Several Authors (e.g. McHugh et al., 1992; Vetter, 1995) studying

some canyons in the North Pacific Ocean, have observed large accumulations of sediments and detritus near the floor that form a persistent deposit of organic and inorganic debris.

This feature is in large part produced by the intense vertical currents near the canyon floor along its axis (Alvarez & Tintord, 1996), which helps to create a special habitat characterized by a great density and diversity of benthic and pelagic fauna exceeding that of other habitats along the continental shelf and slope (Vetter & Dayton, 1998; De Leo et al., 2010; McClain & Barry, 2010).

The hydrodynamism and sedimentology of these environments are well studied (Gili & Coma, 1998; Palanques et al., 2005; Allen & Durrieu de Madron, 2009; Solé et al., 2016) as well as the nutrient flows and changes in chemical and physical conditions along the canyon depth profile.

Many studies focused on the biodiversity and biomass of the benthic domain analyzing the macrofaunal aspects; these studies highlighted that the sinking flux of organic debris is one of the most important factors determining the habitat heterogeneity (Harris & Whiteway, 2011; Puig et al., 2014).

Conversely, up to now not much is known about the planktonic domain, in particular about the heterotrophic microbial fraction that should play a pivotal role in the recycle of sinking organic matter and the transport to the upper level of the trophic food web.

While there is some literature on heterotrophic bacteria living on particles (e.g. marine snow) throughout the water column (Azam & Long, 2001; Arístegui et al., 2009) details on the vertical distribution of protist morphospecies are limited (Silver et al., 1978; Caron et al., 1986; Davoll & Silver, 1986; Fontanez et al., 2015).

The aim of this work was to identify the major drivers, which control the distribution of protist communities in these peculiar environments.

We studied the community structures along the water column using microscopic techniques, and investigated their relationships with the environmental factors recorded along the canyon.

We also considered the availability of prey by measuring the abundances of picoplankton and nanoplankton with the aim of identifying the trophic patterns that regulate and shape the protists population.

2. Material and methods

Samples were collected from 30th of April 2013 to 20th of May 2013 during the oceanographic cruise BioLig (Biodiversity, ecosystem functioning and pelagic-benthic coupling in Ligurian submarine canyons) in the Ligurian Sea along the canyons Polcevera and Bisagno. These two canyons converge into a single one, witch is commonly referred to as the "Canyon of Genoa", and represents one of the largest submarine canyons of the entire Mediterranean.

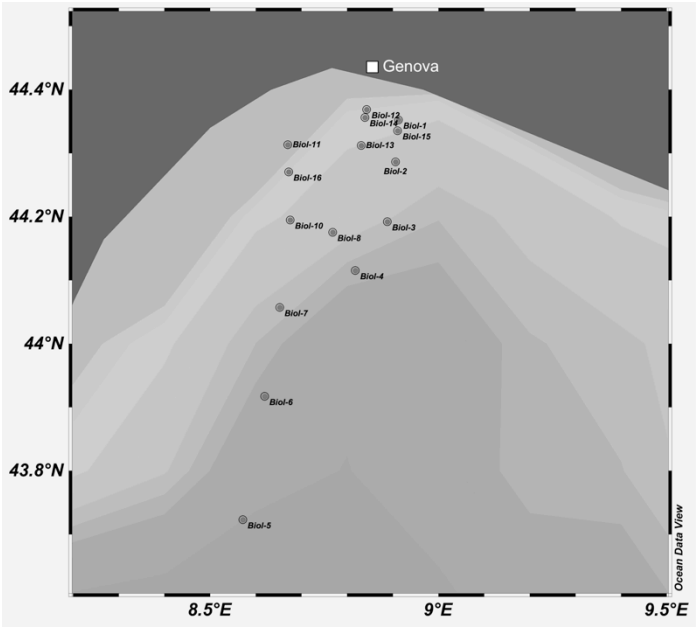

Fig. 1 Map of sampling stations

Sample design provides for the identification of 3 transects, two in correspondence of the canyon profile and one on the adjacent slope; a total of 15 stations have been identified (Table S1), and samples were collected along the entire water column, in general on 4 layers: 5m, DCM (Deep

Chlorophyll Maximum), intermediate (in the different water masses observed along the downcast) and near the bottom.

In order to investigate the whole water column in the deepest stations, samples were collected every 500m.

Water samples were collected by an Oceanics rosette equipped with 24 12-liters Niskin Bottles and CTD SBE911 plus (pressure, temperature and conductivity plus dissolved oxygen SBE43, Chelsea Aqua 3 fluorometer and Chelsea/Seatech transmissometer) that provided data about temperature, salinity, oxygen and fluorescence in correspondence of the sampling depth.

In order to enumerate and characterize the pico- and nano-planktonic fractions, from each depth, 250 mL of seawater were prefiltered on 200 μm mesh and fixed with 2% buffered formalin. For the analysis of protists community, 5L of surface and DCM water, and 10 L for the deeper depths were collected. The water was prefiltered through a 200 μm mesh. Organisms were concentrated to a final volume of 250 mL through gently filtration on a 10 μm mesh and fixed with 2% buffered formalin. All samples were stored at 4°C in the dark until subsequent analysis.

2.1 Fluorescence microscopy analysis

The enumeration of pico- and nano-plankton, was carried out following the protocol developed by Porter & Feig (1980). This technique is based on the microbial cells' staining with the fluorescent dye DAPI (4'6-diamino-2-pheylindole). After staining for 15 minutes in the dark with the fluorocrome DAPI at 1 μg/mL final concentrations, for each sample three subsamples were analyzed: 3 aliquots were filtered (volumes ranged from 2 mL to 50 mL according to the depth) onto 0.22 μm pore-size black polycarbonate filters (diameter, 25 mm; Nucleopore) for picoplanktonic fraction and 0.8 μm pore-size for nanoplanktonic fraction; filtrations were carried out exerting a depression comprised between 0.2 and 0.3 atm., immediately after the filters were

placed directly onto a microscope slide between two drops of immersion oil, and stored at -20°C until counting

Enumeration was performed using an Olympus BX60 epifluorescence microscope equipped with a 100 W high-pressure mercury burner (HPO 100W/2) with a 100X immersion objective and ocular 10 X (Olympus WH10x-H/22), getting a final magnification of 1000 X.

In order to detect natural pigment, light sets (BP, 480–550 nm; BA, 590 nm) for green and (BP, 420–480 nm; BA, 515 nm) for blue were used, while for the DAPI excitation an UV filter (BP 330–385 nm; BA 420 nm) were used. For each filter, 30 to 70 grid filed were randomly selected, and at least 200 cells were counted in every sample and divided in autotrophic and heterotrophic organisms.

According to Christaki et al. (2001) nanoplanktonic organisms were divided in three dimensional classes: 2–3 μm, 3–5 μm, and 5–10 μm and their biovolumes were calculated approximating organisms to sphere with the diameter of 2.5 μm, 4 μm and 7.5 μm, respectively.

In order to assess the heterotrophic bacteria (HB) biomass, a conversion factor of 20 fg C $cell^{-1}$ was used as reported by Ducklow & Carlson (1992) while 200 fg C $cell^{-1}$ was used for the autotrophic picoplankton (Caron et al., 1991); for the nanoplanktonic fraction, both heterotrophic nanoflagellates (HNF) and phototrophic nanoflagellates (PNF), the factor of 183 fg C μm^{-3} (Caron et al., 1995) was multiplied by the calculated biovolumes.

2.2 Inverted microscopy analysis

Analyses of microzooplankton (MCZ) were carried out using an Olympus IX51 inverted microscope equipped with 10X, 20X, and 40X objectives, obtaining a final magnification of 400X.

For species identification and enumeration subsamples of 100 mL (corresponding to 2.5-5 L of sea water) were settled according to the Uthermöhl method (1958). Settling time was calculated on the

base of the length of the settling tube (3h for 1 cm). The entire surface of the settling chamber was examined and during the analysis organisms were measured with an eyepiece scale.

The main groups of the MCZ considered in the study were: aloricate ciliates and tintinnids, radiolarians, foraminifers, heliozoans and micrometazoans, as well as heterotrophic dinoflagellates (Sherr & Sherr, 2007).

Organisms have been identified up to the species level when possible.

Taxonomical assignations were based on Tomas & Haste (1997), Faust & Gulledge (2002), Kofoid & Campbell (1939) Rampi & Zatera (1982), the nomenclature was revised according to WoRMS Editorial Board (2016).

In order to assess biomass, every organism, according to its shape and dimension, was approximated to a geometrical object (Edler, 1979), and biovolume was calculated. Biovolumes were then transformed in carbon content according to Putt & Stoecker (1989) for aloricate ciliates, Verity & Langdon (1984) for tintinnids, Olenina et al. (2006) for dinoflagellates, and Michaels et al. (1995), and Beers & Stewart (1970) for the other protists and micro metazoans.

2.3 Statistical analysis

In order to study similarity between the samples a Non-metric multidimensional scaling (NMDS) based on a Bray- Curtis distance matrix was carried out using PAST V3.14 software (Øyvind Hammer, Oslo, NO). The algorithm implemented is based on the approach developed by Taguchi & Oono (2005). This software permits to include one or more initial columns containing additional "environmental" variables for the analysis. These variables are not included in the ordination. The correlation coefficients between each environmental variable and the NMDS scores are presented as vectors from the origin. The length of the vectors are arbitrarily scaled to make a readable plot, so only their directions and relative lengths should be considered.

A two-way analysis of variance (2-way ANOVA) was calculated to test the effects of transect and temperature on the abiotic factor; we applied also simple linear regression to assess the relationship between abundances and depth. All these analyses were performmed in R statistical programming language (v. 3.3.2, http://www.R-project.org).

3. Results

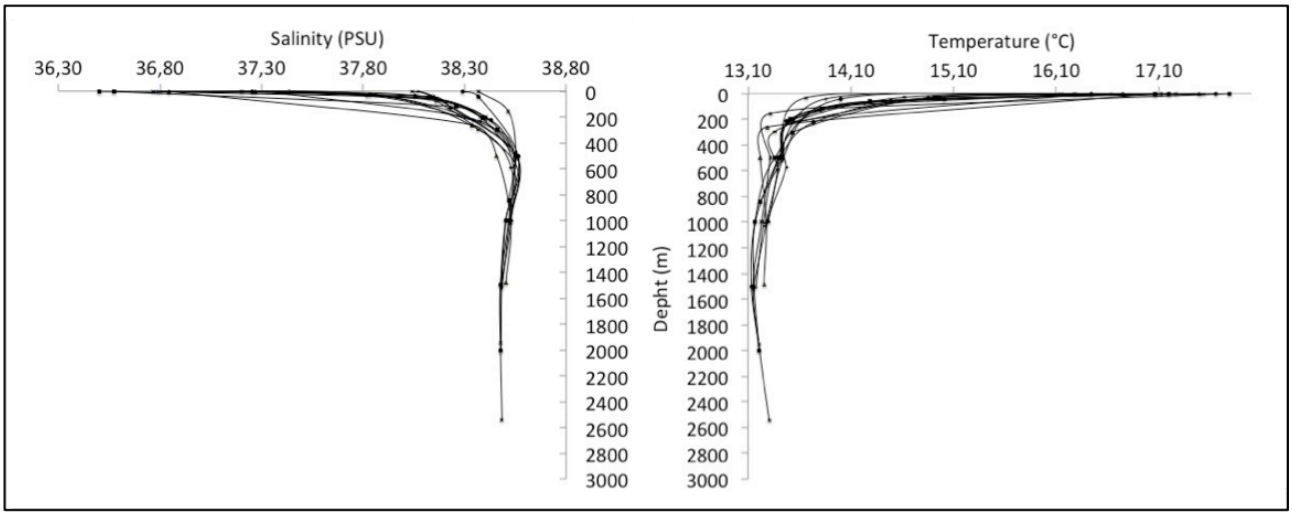

Fig. 2 Vertical profiles of temperature (°C) and salinity (PSU) for each station

3.1 Abiotic factors

We observed low values of chl-a concentration and dissolved oxygen in the surface layer (0-5m). Salinity ranged from 36.5 to 38.3 highlighting an increasing gradient from coastal to offshore sampling sites; temperature ranged from 16.14°C to 17.78 °C and ΔT for the 0 - 75 m layer was 4.11 °C, highlightening a stratified water column in the study area (Table S1).

In the deeper layers, temperature decreased to 13.2±0.05°C, while salinity increased to 38.42±0.02 below 1000m depth.

Profiles of temperature and salinity showed the same trend for all stations (Fig.2) and no significant differences were found among stations in the different transects; a 2-way ANOVA, showed a

significant effect of depth on both temperature (F (1-64)=31.25, p > 0.01) and salinity (F (1-64)=21.04, p > 0.01), whereas the variation among transects was not significant (p> 0.05)

3.2 Autotrophic components

The abundances of photo-picoplankton in the surface layer ranged from 0.31 * 10^7 cells L^{-1} at the station Biol-8 to 5.33 * 10^7 cells L^{-1} at the station Biol-6 with a mean value of 1.30 * 10^7 (±1.19 * 10^6) cells L^{-1}. The lowest abundance of PNF fraction was registered at station Biol-15 (1.42 * 10^5 cells L^{-1}) while the maximum of 8.44 * 10^5 cells L^{-1} was found at station Biol-5 with a mean value of 4.30 * 10^5 (±2.00 * 10^5) cells L^{-1}; microphytoplankton abundances varied from 0.95 * 10^4 to 30.2 * 10^4 cells L^{-1} at station Biol-5 and station Biol-2 respectively, showing a mean value of 5.34 * 10^4 (± 7.04 * 10^4) cells L^{-1} over the entire study area.

Abundances and biomass distribution did not show a clear in-shore / off-shore pattern, while they sharply decreased below the photic zone and the decrease was significant (p < 0,001 t-test). However it is possible to notice as PNF and even more photo- picoplankton augmented where microphytoplankton was scarce or absent.

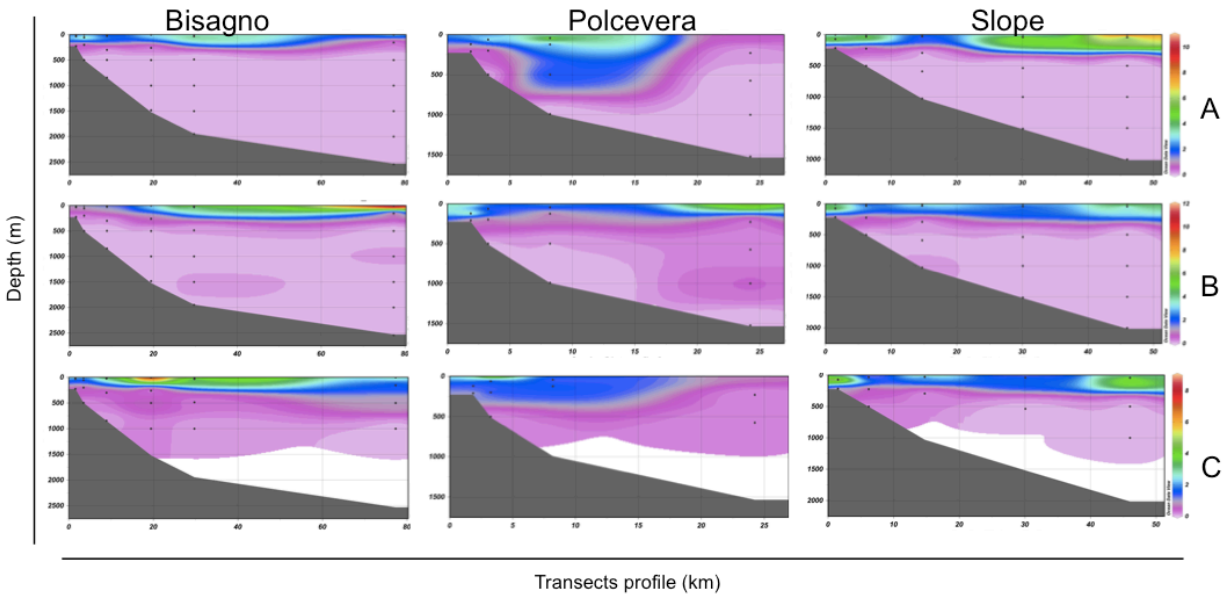

Fig. 3 Biomass of the autotrophic component of pico- (A), nano- (B), and micro-planktonic (C) fractions along the three transects

In the figure 3 biomass values were resumed for the three transects, mean values of 2.59±2,37 µgC L^{-1}, 4.57±2,98 µgC L^{-1} and 3.29±2,82 µgC L^{-1} were calculated for photo- picoplankton, PNF and microphytoplankton, respectively. Maximum values were registered for photo-picoplankton at station Biol-6 (10.65 µgC L^{-1}) while PNF showed a maximum at station Biol-5 (12.38 µgC L^{-1}) and microphytoplankton at station Biol_14 (8.08 µgC L^{-1}).

High values for both biomass and abundances were recorded at station Biol-13 at the depth of 500m were the total autotrophic biomass was 2.6 µgC L^{-1}, mainly due to photo-picoplankton biomass (Fig. 3).

3.3 Heterotrophic fractions

Heterotrophic organisms belonging to the three dimensional classes were collected at all stations throughout the entire water column highlighting values ranging from 1.56 * 10^7 to 92.2 * 10^7 cells L^{-1} for HB, from 0.16 * 10^4 to 76.5 * 10^4 cells L^{-1} for HNF and from 1 to 73.5 cells L^{-1} for MCZ.

Higher abundances were generally measured in the surface layer and they decreased with increasing depth (Fig. 4); this trend was observed at all stations of the three transects where a significant linear regression was registered ($p < 0{,}001$) (Tab. 1).

	Abundance											
	Bacteria				HNF				Microzooplankton			
Transect	*slope*	*se*	*sig.*	r^2	*slope*	*se*	*sig.*	r^2	*slope*	*se*	*sig.*	r^2
Bisagno	-0,5474	0,04875	***	0,82	-0,623	0,0649	***	0,77	-0,5245	0,04255	***	0,84
Polcevera	-0,4441	0,0618	***	0,7748	-0,5649	0,1084	***	0,6443	-0,417	0,0672	***	0,7198
Slope	-0,588	0,0574	***	0,8329	-0,4947	0,0737	***	0,6818	-0,506	0,0463	***	0,85

Tab. 1 Log-Log regression results for the decrease of abundance of bacteria (HB), heterotropic nanoflagellates (HNF) and microzooplankton (MCZ) over depth (5-2500 m)

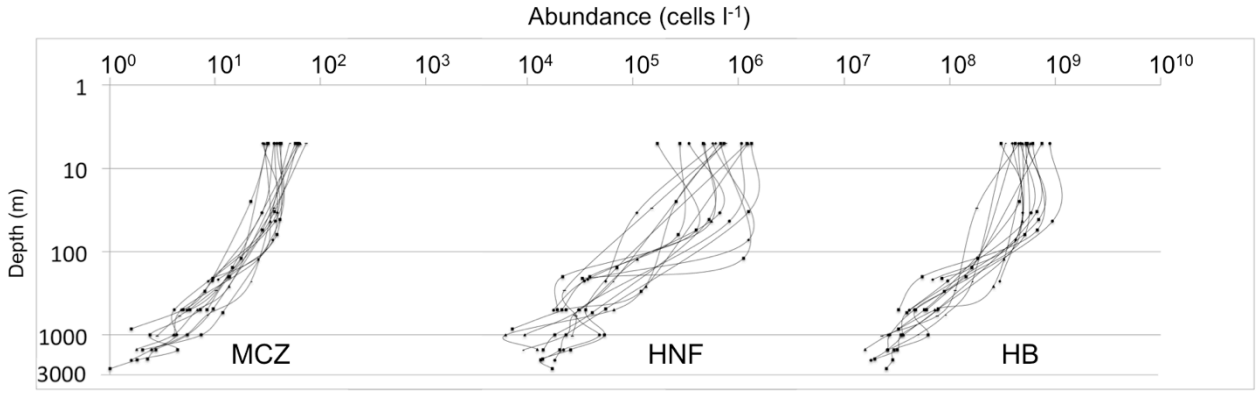

Fig. 4 Vertical distributions of abundance (cells l^{-1}) of bacteria (HB), heterotropic nanoflagellates (HNF) and microzooplankton (MCZ) over depth (5-2500 m) for each station

Data of abundance were grouped into three different depth ranges: 1-150m (photic layer), 150-1000m (intermediate layer) and 1000-2500m (deep layer).

Each of the three fractions decreased by one order of magnitude from the photic to the deep layers, HB abundances decreased from the mean value of $48.6 * 10^7 \pm 17.9 * 10^7$ cells L^{-1} registered in the photic layer to $2.95 * 10^7 \pm 1.14 * 10^7$ cells L^{-1} in the deeper layer; HNF as well decreased from $212 * 10^3 \pm 188 * 10^3$ to $11.2 * 10^3 \pm 6.73 * 10^3$ cells L^{-1} from the surface to the bottom; similarly, MCZ abundances ranged from 38.29±14.37 to 3.18±1.69 cells L^{-1}.

The ratio of HB to HNF and HNF to MCZ (Fig.5) remained almost constant over depth, ranging from 10^3 to 10^4.

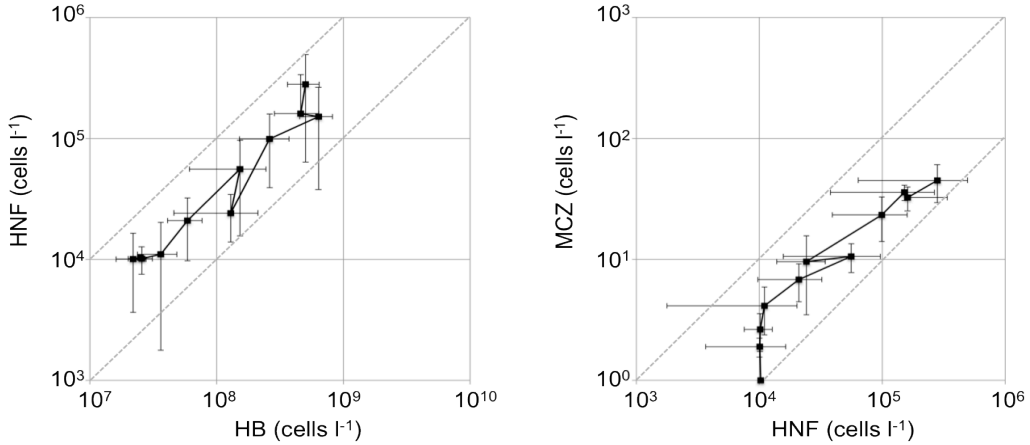

Fig. 5 Relationships in mean abundance (±SD) of bacteria (HB) vs. heterotrophic nanoflagellates (HNF) and heterotropic nanoflagellates (HNF) vs. microzooplankton (MCZ) at each depth (5-2500 m). Dotted lines denote ratios of 10^3:1 and 10^4:1. Mean values are connected with line in order of depth.

Distribution of biomass is consistent with the abundances trend, showing maximum values in the photic-layer and decreasing with the increasing depth ($p<0,001$) (Tab.2).

Maximum values of biomass (Fig.6) were due to the HB fraction ranging from 0.58±0.22 to 9.71±3.58 µgC L^{-1} while HNF fraction determined values between 0.04±0.03 and 1.55±2.03 µgC L^{-1}. Microzooplanktonic fraction represented the minimum percentage of biomass ranging from 0.05±0.03 to 0.66±0.39 µgC L^{-1}.

	Biomass											
	Bacteria				HNF				Microzooplankton			
Transect	*slope*	*SE*	*sig.*	r^2	*slope*	*SE*	*sig.*	r^2	*slope*	*SE*	*sig.*	r^2
Bisagno	-0,5852	0,05147	***	0,822	-0,7862	0,08432	***	0,76	-0,5104	0,06579	***	0,6822
Polcevera	-0,46553	0,0762	***	0,731	-0,6856	0,1073	***	0,7312	-0,3642	0,0628	***	0,6915
Slope	-0,5852	0,0514	***	0,82	-0,7862	0,0843	***	0,7564	-0,51004	0,0658	***	0,68

Tab. 2 Log-Log regression results for the decrease of biomass of bacteria (HB), heterotropic nanoflagellates (HNF) and microzooplankton (MCZ) over depth (5-2500 m)

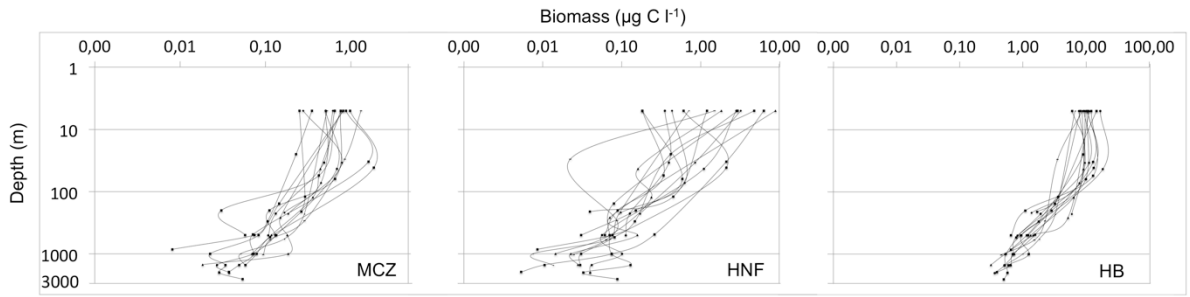

Fig. 6 Vertical distributions of biomass (µg C l^{-1}) of bacteria (HB), heterotropic nanoflagellates (HNF) and microzooplankton (MCZ) over depth (5-2500 m) for each station

Biomass values were strictly related to the abundance ones. In Fig. 7 mean values of cell biovolumes ± SD are resumed for each depth. No significant trend was found over depth for both MCZ ($11.1 * 10^4 \pm 2.11 * 10^4$ μm^3 to $17.5 * 10^4 \pm 9.00 * 10^4$ μm^3) and HNF (from 12.8 ± 6.51 μm^3 to 39.5 ± 16 μm^3) volumes.

Surprisingly, a significant increase of biovolumes was registered at 2500 m for both components; at this depth biovolumes of MCZ and HNF were $3.02 * 10^5$ and 46.5 μm^3, respectively.

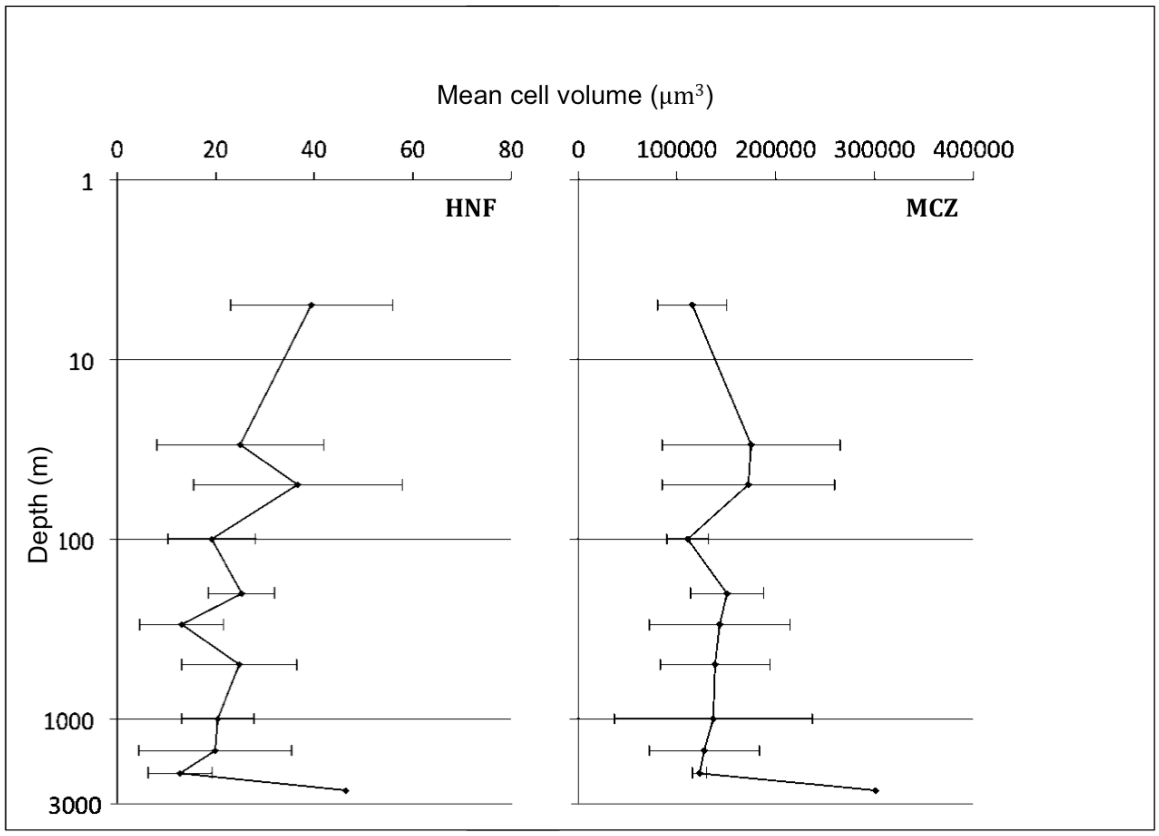

Fig. 7 The mean cell volumes (± SD) of HNF and MCZ along the whole water column

3.4 Microplankton composition

Microphytoplankton highest abundance was found in the photic layer (0-150m). In this part of the water column diatoms prevailed (average contribution of 88%) followed by dinoflagellates (9%), dichtiochophyceae and cryptophyeceae (1%).

In the intermediate layer (150-1000m) the abundance was significantly lower compared to the photic layer ($p<0.01$). As in the upper layer diatoms dominated (77%) followed by the increasing contribution of dinoflagellates (19%). cryptophyeceae (2%), euglenophyceae and dichtiochophyceae (1%) presented always low values.

About species composition, there was a clear decrease of abundance of all the main species. Indeed, the differences between surface and intermediate layers were especially due to dissimilarities in the abundance and frequency of a pool of taxa common to all stations (diatoms as *Thalassionema nitzschioides* and *Cylindrotheca closterium*) and the presence of specific taxa that are associated, albeit to different degrees, with deep stations (pennate diatoms and *Nitzschia* spp.). On average, the three transects were characterized by decreasing abundance.

Microzooplanktonic populations were well structured in all the water layers analyzed (Table S2 and S3). The highest abundances were registered at the surface (Fig. 8), where the population was dominated by aloricate ciliates (30%), and micrometazoans (28%) reaching the abundances of 36 cells L^{-1} and 25 cells L^{-1}, respectively.

Heterotrophic dinoflagellates represented 24% of the total abundances while tintinnids accounted for 14%. Radiolarians, foraminifera and other protists (2%), represented a small fraction of surface communities.

Analysing abundances observed in the deepest layers, it should be noted that between 150m and 1000m tintinnids represented the majority of the organisms (28% of the total abundance), showing abundances just higher than those of micrometazoans, aloricate ciliates and dinoflagellates. Data highlighted constant value of abundances for foraminifers, radiolarians and other protists along the water column. At increasing depths this last fraction resulted more and more important in the composition of MCZ populations in terms of percentage and biomass.

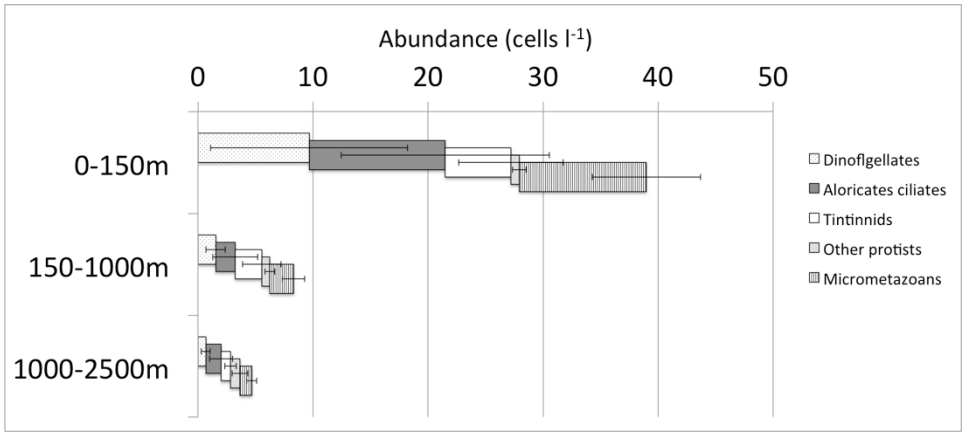

Fig. 8 Mean abundance of the main MCZ groups in the entire study area binned for depth

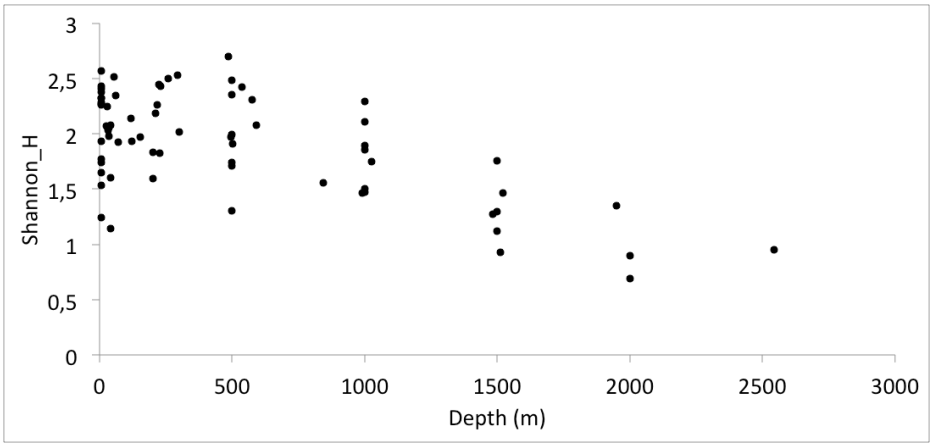

Fig. 9 Value of Shannon index over the deph

In Fig. 9 values of Shannon index calculated on tintinnids are reported. It is evident a reduction at increasing depth.

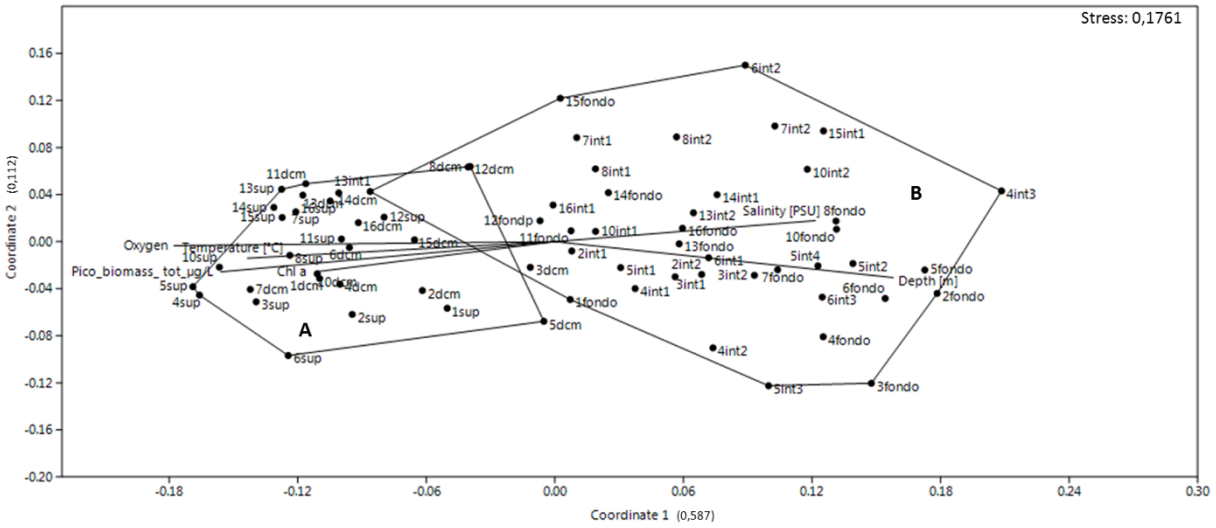

Fig. 10 Plot of non metric MDS analysis

The statistical analysis applied on the matrix build up with the abundances of the main microzooplanktonic groups (dinoflagellates, aloricate ciliates, tintinnids, micrometazoans and other protists) highlighted a clear division between samples collected in the photic layers (surface and DCM) and those collected at intermediate and bottom depths (Fig 10). High values of temperature, oxygen, and abundances of nanoplankton and picoplankton characterize photic layers samples. The samples belonging to the aphotic layers are characterized by higher values of salinity and obviously by depth. Conversely samples of the three transects at the same depth were grouped together as they did not diversified each other neither in term of abundances or qualitative composition.

4. Discussion and conclusion

Our results show that the samples belonging to each transect were not separated on the base of geographic distribution, but only on the base of the depth at which they were collected. This translates in the lack of differences among canyons (Polcevera and Bisagno) and the near slope in term of plankton composition and abiotic factors (temperature and salinity).

In general, the abundances of pico- and nanoplankton found during our study fall within the range reported by Tanaka & Rassoulzadegan (2002) for a western Mediterranean site (DIFAMED), while MCZ abundance, although still within their range, generally occupied the lowest rank.

The abundances of the main taxonomic groups of microphytoplankton evidenced their quantitative scarcity. Results obtained in this study are in line whit the values recorded in other oligotrotrophic areas of Mediterranean sea during the spring period (Psarra et al., 2000; Ignatiades et al., 2002; Lasternas et al., 2011).

The autotrophic biomass in the three transects did not show any evident in- shore – off –shore gradient and was almost equally divided into the three dimensional classes (pico – nano- and microphytoplankton). This is an index of oligotrophic conditions, which established in the western Mediterranean at the end of spring. MCZ is considered the major loss term for microphytoplankton (Calbet & Landry, 2014) although it is well known that they can use other energetic sources, especially pico- and nanoplankton (Zoccarato et al., 2016). In our study, anyway, the scarcity of microphytoplankton at the surface can justify the paucity of the MCZ surface populations recorded in our samples.

The most evident pattern was the decreasing trend with increasing depth, observed in all three transects for all the biotic components considered. All three fractions decreased by one order of magnitude irrespectively of their abundance. Regression coefficients for abundances calculated from the surface to the bottom, ranged from -0,440 to -0,588 for HB, from -0,494 to -0, 623 for HNF and from -0,417 to -0,524 for MCZ and are of the same order found by Aristegui at al. (2009) for the prokaryotic abundance and HNF.

These results represent a common feature not only for the Mediterranean Sea (Tanaka & Rassoulzadegan, 2002) where a strong trophic link between HB, HNF and MCZ was found with an increasing prey: predator ratio, but generally on the ocean world (Yamaguchi et al., 2002; Sohrin et al., 2010).

Indeed the over the depth decreasing of abundances and biomass should reflect the different balance between growth and loss processes. In our case only small differences was observed between the regressions slopes for each dimensional components, therefore we can infer that balance between growth and loss is in the same order of magnitude for the three classes over the depth. The constant ratio of both HB vs HNF and HNF vs MCZ over the depth saggest that their abundances and biomass were controlled more by resources (bottom-up control) than by predation (top-down control). The distribution of all microbial heterotrophs from the surface down to 2500m suggests that prey abundance was generally higher than the feeding threshold of predator. HNF prey on small phytoplankton as well on HB in the photic layer, but they can feed only on HB (and archea) in the aphotic layer, MCZ prey on microphytoplankton as well as on HNF in the euphotic layer while in the aphotic layer MCZ ingest solely HNF and HB (Zoccarato et al., 2016). In the euphotic layer all microbial community included small microphytoplankton and ANF should be included in what is called the microbial food web. In the aphotic layer the microbial loop (sinking POC➔DOC➔HB➔HNF) became dominant.

The decrease of HNF was a little sharper than that of the other two dimensional classes to indicate a major unbalance between growth and loss of this fraction.

Values of biovolumes calculated for HNF were in the range measured by Tanaka & Rassoulzadegan (2002) while concerning MCZ values are normally higher than that measured in the same study solely for ciliates, anyway no significant correlation was found between biovolumes and depth.

The great value recorded in our study near the bottom may be explained by several factors: the cell size of HNF and MCZ is dependent upon the individual species (Esteban & Finlay, 2007), temperature (Weisse et al., 2002; Atkinson et al., 2003), size-selective grazing by predators (Fenchel, 1982) and mainly upon food availability and prey concentration (Weisse et al., 2002).

Despite at the bottom the picoplanktonic abundance decreased, an increase of the HNF biovolumes was measured, this might suggest that HNF utilized a source of food other than prokaryotes cells in

this layer, such as colloids and organic matter like marine snow. These elements could be actively export from surface in the canyon in the POC fraction (McHugh et al., 1992; Vetter, 1995; Canals et al., 2006) and can be utilized mainly by large HNF as food source (Sherr, 1988; Tranvik, 1994). Finally, the availability of larger prey and the low concentration of food source, could have promoted the presence of large MCZ predators like micrometazoans that showed a best efficiency in movement and predation strategy.

Summing up the major results of this study were the evident decreasing trend for all the considered components along the water column, which is a very common feature in the ocean world, but it is even more evident in the Mediterranean Sea. This pattern is so strong that probably hides all other possible differences among transects and indeed the non metric-MDS calculated on the biological matrix identify only a separation between surface and DCM samples and aphotic samples. The expected "canyon effect" was limited to the increase of biovolume of both HNF and MCZ (the only measured), which we ascribe to the major input at the bottom of the canyon of organic matter (POC) that can be used by HNF also directly as food source (Sohrin et al., 2010). The other striking evidence is the constant ratio between prey and predators (HB vs HNF and HNF vs MCZ) along the water column that indicates the availability of food sources also at the bottom. In the bathypelagic realm the efficiency of HNF predation was experimentally estimated and resulted very high if compared to other surface trophic conditions (Zoccarato et al., 2016) therefore it is not surprising to count a relative high number of heterotrophic components also in this extreme environment.

Acknowledgment

We want to thank Luca Zoccarato who collected all samples and the crew of the research vessel MINERVA-1. We want to thenk Fabrizio Aubry for microphytoplankton data. We are grateful to all students that collected data taking part in the epifluorescence-microscopy analysis and to the colleague Enrico Tordoni for his helpful support with statistical analysis.

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Supplementary

Table S1- station coordinates and abiotic factors

TRASECT	STATION	SAMPLE ID.	Lon (°E)	Lat (°N)	Depth [m]	Temperature [°C]	Salinity [PSU]	Chl a [µgC L-1]	Oxygen
BISAGNO	Biol-1	1sup	8,9126	44,3521	5,00	16,75	36,79	0,18	2,89
BISAGNO	Biol-1	1dcm	8,9126	44,3521	34,00	14,90	37,82	0,25	2,80
BISAGNO	Biol-1	1fondo	8,9126	44,3521	226,00	13,53	38,34	0,04	2,46
BISAGNO	Biol-2	2sup	8,9063	44,2861	5,00	17,06	36,50	0,13	2,91
BISAGNO	Biol-2	2dcm	8,9063	44,2861	25,00	14,93	37,83	0,22	2,80
BISAGNO	Biol-2	2int1	8,9063	44,2861	300,00	13,53	38,46	0,01	2,31
BISAGNO	Biol-2	2int2	8,9063	44,2861	496,00	13,43	38,55	0,01	2,11
BISAGNO	Biol-2	2fondo	8,9063	44,2861	843,00	13,21	38,52	0,01	1,99
BISAGNO	Biol-3	3sup	8,8881	44,1921	5,00	17,06	36,84	0,41	2,98
BISAGNO	Biol-3	3dcm	8,8881	44,1921	260,00	13,28	38,34	0,02	2,47
BISAGNO	Biol-3	3int1	8,8881	44,1921	500,00	13,22	38,46	0,01	2,25
BISAGNO	Biol-3	3int2	8,8881	44,1921	1000,00	13,28	38,53	0,01	1,96
BISAGNO	Biol-3	3fondo	8,8881	44,1921	1482,00	13,25	38,50	0,01	1,88
BISAGNO	Biol-4	4sup	8,8181	44,1151	5,00	16,15	38,04	0,14	2,87
BISAGNO	Biol-4	4dcm	8,8181	44,1151	33,00	13,66	38,13	0,72	2,74
BISAGNO	Biol-4	4int1	8,8181	44,1151	487,00	13,40	38,55	0,02	2,14
BISAGNO	Biol-4	4int2	8,8181	44,1151	1000,00	13,17	38,50	0,01	1,97
BISAGNO	Biol-4	4int3	8,8181	44,1151	1500,00	13,16	38,48	0,01	1,91
BISAGNO	Biol-4	4fondo	8,8181	44,1151	1950,00	13,20	38,48	0,01	1,85
BISAGNO	Biol-5	5sup	8,5716	43,7228	5,00	16,28	38,37	0,09	2,95
BISAGNO	Biol-5	5dcm	8,5716	43,7228	155,00	13,32	38,52	0,03	2,26
BISAGNO	Biol-5	5int1	8,5716	43,7228	500,00	13,31	38,55	0,01	2,06
BISAGNO	Biol-5	5int2	8,5716	43,7228	1000,00	13,23	38,52	0,01	1,98
BISAGNO	Biol-5	5int3	8,5716	43,7228	1500,00	13,15	38,48	0,13	1,89
BISAGNO	Biol-5	5int4	8,5716	43,7228	2000,00	13,21	38,48	0,01	1,84
BISAGNO	Biol-5	5fondo	8,5716	43,7228	2545,00	13,31	38,48	0,01	1,77
SLOPE	Biol-6	6sup	8,6196	43,9171	5,00	16,45	38,29	0,21	2,92
SLOPE	Biol-6	6dcm	8,6196	43,9171	43,00	14,00	38,37	1,07	2,84
SLOPE	Biol-6	6int1	8,6196	43,9171	500,00	13,36	38,56	0,01	2,05
SLOPE	Biol-6	6int2	8,6196	43,9171	1000,00	13,17	38,50	0,01	1,98
SLOPE	Biol-6	6int3	8,6196	43,9171	1500,00	13,13	38,48	0,01	1,93
SLOPE	Biol-6	6fondo	8,6196	43,9171	2002,00	13,21	38,48	0,01	1,85
SLOPE	Biol-7	7sup	8,6525	44,0571	5,00	16,64	38,07	0,05	2,88
SLOPE	Biol-7	7dcm	8,6525	44,0571	41,00	13,86	38,13	0,70	2,77
SLOPE	Biol-7	7int1	8,6525	44,0571	537,00	13,39	38,54	0,03	2,16
SLOPE	Biol-7	7int2	8,6525	44,0571	1000,00	13,25	38,52	0,01	1,96
SLOPE	Biol-7	7fondo	8,6525	44,0571	1512,00	13,17	38,48	0,01	1,90
POLCEVERA	Biol-8	8sup	8,7685	44,1753	5,00	16,41	37,33	0,13	3,01
POLCEVERA	Biol-8	8dcm	8,7685	44,1753	230,00	13,51	38,38	0,02	2,45
POLCEVERA	Biol-8	8int1	8,7685	44,1753	575,00	13,47	38,54	0,01	2,09
POLCEVERA	Biol-8	8int2	8,7685	44,1753	1000,00	13,28	38,53	0,01	1,95
POLCEVERA	Biol-8	8fondo	8,7685	44,1753	1522,00	13,15	38,48	0,01	1,91
SLOPE	Biol-10	10sup	8,6753	44,1946	5,00	17,53	36,80	0,15	2,94
SLOPE	Biol-10	10dcm	8,6753	44,1946	30,00	14,62	37,86	0,34	2,76
SLOPE	Biol-10	10int1	8,6753	44,1946	294,00	13,36	38,37	0,02	2,42
SLOPE	Biol-10	10int2	8,6753	44,1946	590,00	13,39	38,53	0,01	2,13
SLOPE	Biol-10	10fondo	8,6753	44,1946	1026,00	13,27	38,52	0,01	1,94
SLOPE	Biol-11	11sup	8,6703	44,3131	5,00	17,13	37,44	0,16	2,90
SLOPE	Biol-11	11dcm	8,6703	44,3131	72,00	14,24	38,16	0,26	2,76
SLOPE	Biol-11	11fondo	8,6703	44,3131	216,00	13,47	38,41	0,02	2,38
POLCEVERA	Biol-12	12sup	8,8431	44,3686	5,00	17,78	36,57	0,37	2,93
POLCEVERA	Biol-12	12dcm	8,8431	44,3686	120,00	13,81	38,26	0,05	2,59
POLCEVERA	Biol-12	12fondp	8,8431	44,3686	210,00	13,48	38,39	0,02	2,42
POLCEVERA	Biol-13	13sup	8,8311	44,3121	5,00	17,49	37,27	0,19	2,89
POLCEVERA	Biol-13	13dcm	8,8311	44,3121	43,00	15,00	38,15	0,28	2,86
POLCEVERA	Biol-13	13int1	8,8311	44,3121	123,00	13,79	38,23	0,13	2,64
POLCEVERA	Biol-13	13int2	8,8311	44,3121	500,00	13,44	38,56	0,01	2,08
POLCEVERA	Biol-13	13fondo	8,8311	44,3121	991,00	13,30	38,53	0,01	1,95
POLCEVERA	Biol-14	14sup	8,8391	44,3561	5,00	17,20	36,76	0,23	2,88
POLCEVERA	Biol-14	14dcm	8,8391	44,3561	62,00	14,28	38,17	0,12	2,74
POLCEVERA	Biol-14	14int1	8,8391	44,3561	200,00	13,51	38,38	0,02	2,43
POLCEVERA	Biol-14	14fondo	8,8391	44,3561	499,00	13,43	38,55	0,01	2,08
BISAGNO	Biol-15	15sup	8,9108	44,3351	5,00	17,27	37,20	0,17	2,89
BISAGNO	Biol-15	15dcm	8,9108	44,3351	55,00	14,49	38,13	0,20	2,83
BISAGNO	Biol-15	15int1	8,9108	44,3351	200,00	13,54	38,40	0,02	2,42
BISAGNO	Biol-15	15fondo	8,9108	44,3351	498,00	13,42	38,56	0,01	2,06
SLOPE	Biol-16	16sup	8,6721	44,2703	5,00	17,66	37,26	0,19	2,86
SLOPE	Biol-16	16dcm	8,6721	44,2703	34,00	14,85	38,06	0,35	2,81
SLOPE	Biol-16	16int1	8,6721	44,2703	225,00	13,74	38,43	0,01	2,40
SLOPE	Biol-16	16fondo	8,6721	44,2703	501,00	13,39	38,55	0,01	2,08

Table S2- Abundances expressed in cells L-1 for the main groups

TRASECT	STATION	SAMPLE ID.	HB	PhotoPico	HNF	PNF	Dinoflgellates	Aloricate Ciliates	Tintinnids	Other Protist	Micrometazoans
BISAGNO	Biol-1	1sup	3,83E+08	7,08E+06	4,15E+05	1,96E+05	7,60	3,60	1,20	0,00	6,00
BISAGNO	Biol-1	1dcm	4,81E+08	1,10E+07	8,69E+04	2,23E+04	11,82	2,73	3,18	2,27	19,09
BISAGNO	Biol-1	1fondo	2,96E+08	1,74E+06	3,65E+04	1,89E+04	2,50	0,91	2,50	0,00	2,73
BISAGNO	Biol-2	2sup	3,01E+08	6,05E+06	3,65E+05	3,65E+05	17,20	6,80	2,00	0,00	6,00
BISAGNO	Biol-2	2dcm	4,47E+08	1,08E+07	1,32E+05	1,27E+05	7,27	3,18	2,73	0,00	8,64
BISAGNO	Biol-2	2int1	8,85E+07	0,00E+00	9,99E+04	1,96E+04	1,80	0,40	2,20	0,00	3,60
BISAGNO	Biol-2	2int2	5,75E+07	0,00E+00	1,99E+04	3,31E+03	1,33	0,67	1,00	0,00	2,00
BISAGNO	Biol-2	2fondo	3,25E+07	0,00E+00	5,71E+03	1,49E+03	0,80	0,20	0,20	0,00	0,40
BISAGNO	Biol-3	3sup	5,64E+08	1,47E+07	7,65E+05	4,77E+05	41,40	5,60	5,60	0,00	11,60
BISAGNO	Biol-3	3dcm	2,58E+08	9,99E+05	4,72E+04	8,69E+04	3,20	3,60	3,20	0,00	3,60
BISAGNO	Biol-3	3int1	7,21E+07	0,00E+00	5,30E+04	1,32E+04	1,82	0,45	2,27	0,23	2,05
BISAGNO	Biol-3	3int2	2,60E+07	0,00E+00	6,21E+03	0,00E+00	1,20	0,20	1,00	0,00	1,60
BISAGNO	Biol-3	3fondo	1,56E+07	2,82E+04	9,43E+03	2,98E+03	0,00	0,80	0,60	0,00	0,40
BISAGNO	Biol-4	4sup	5,30E+08	1,22E+07	6,43E+05	6,95E+05	10,45	35,91	0,00	0,00	15,00
BISAGNO	Biol-4	4dcm	6,45E+08	2,15E+07	4,67E+05	7,93E+05	9,20	9,60	2,80	0,40	14,80
BISAGNO	Biol-4	4int1	7,72E+07	0,00E+00	2,73E+04	2,81E+04	1,60	3,00	3,20	0,00	1,80
BISAGNO	Biol-4	4int2	3,45E+07	0,00E+00	2,15E+04	1,66E+03	1,36	1,36	0,00	2,05	0,68
BISAGNO	Biol-4	4int3	3,18E+07	0,00E+00	7,03E+03	1,32E+04	0,45	0,00	0,45	1,36	0,45
BISAGNO	Biol-4	4fondo	1,94E+07	0,00E+00	3,55E+03	1,04E+04	0,00	0,45	0,00	0,00	1,82
BISAGNO	Biol-5	5sup	7,32E+08	1,37E+07	3,68E+05	8,44E+05	7,73	23,18	15,00	0,00	15,45
BISAGNO	Biol-5	5dcm	1,60E+08	6,76E+05	5,29E+04	1,76E+04	3,18	0,91	6,36	0,45	3,64
BISAGNO	Biol-5	5int1	4,72E+07	0,00E+00	2,73E+04	8,28E+03	2,50	1,00	0,25	1,50	2,00
BISAGNO	Biol-5	5int2	6,20E+07	0,00E+00	1,57E+04	3,81E+04	0,20	0,00	0,80	0,00	1,40
BISAGNO	Biol-5	5int3	2,94E+07	0,00E+00	1,41E+04	1,16E+04	0,20	3,00	0,20	0,40	0,60
BISAGNO	Biol-5	5int4	2,86E+07	0,00E+00	1,02E+04	3,10E+03	0,00	0,00	0,45	0,00	1,36
BISAGNO	Biol-5	5fondo	2,49E+07	0,00E+00	1,03E+04	6,86E+03	0,00	0,23	0,00	0,23	1,00
SLOPE	Biol-6	6sup	8,35E+08	5,33E+07	3,53E+05	7,23E+05	3,64	22,73	3,64	0,00	8,64
SLOPE	Biol-6	6dcm	9,22E+08	1,92E+07	3,47E+05	4,82E+05	8,18	6,82	7,73	0,45	14,09
SLOPE	Biol-6	6int1	4,61E+07	0,00E+00	1,66E+04	1,41E+04	0,45	0,23	0,23	0,45	2,73
SLOPE	Biol-6	6int2	2,70E+07	0,00E+00	2,90E+04	1,90E+04	0,91	1,82	0,91	0,23	0,45
SLOPE	Biol-6	6int3	2,54E+07	0,00E+00	1,24E+04	9,52E+03	0,68	0,45	0,00	0,00	1,36
SLOPE	Biol-6	6fondo	1,78E+07	0,00E+00	1,63E+04	1,77E+03	0,40	0,00	0,00	0,00	1,20
SLOPE	Biol-7	7sup	5,31E+08	3,76E+06	1,37E+05	3,30E+05	3,64	22,27	2,27	0,45	12,73
SLOPE	Biol-7	7dcm	6,62E+08	3,41E+07	1,04E+05	4,22E+05	5,00	9,55	2,27	0,00	24,55
SLOPE	Biol-7	7int1	3,91E+07	0,00E+00	2,07E+04	2,07E+04	2,95	2,05	4,77	1,14	0,91
SLOPE	Biol-7	7int2	3,54E+07	0,00E+00	4,97E+03	1,32E+04	0,40	3,00	1,80	1,20	1,00
SLOPE	Biol-7	7fondo	2,57E+07	0,00E+00	8,87E+03	5,21E+03	0,68	0,00	0,00	0,00	1,36
POLCEVERA	Biol-8	8sup	3,33E+08	3,06E+06	1,76E+05	4,97E+05	13,20	22,00	4,00	0,00	11,60
POLCEVERA	Biol-8	8dcm	1,62E+08	7,35E+05	1,49E+04	4,97E+04	2,27	9,09	6,36	0,91	3,18
POLCEVERA	Biol-8	8int1	9,00E+07	0,00E+00	1,66E+04	1,08E+04	0,91	2,27	3,18	0,45	1,59
POLCEVERA	Biol-8	8int2	4,93E+07	0,00E+00	1,66E+03	2,15E+04	0,00	2,00	1,50	1,00	0,75
POLCEVERA	Biol-8	8fondo	2,56E+07	0,00E+00	8,99E+03	0,00E+00	0,00	0,80	0,40	0,20	1,00
SLOPE	Biol-10	10sup	4,36E+08	1,70E+07	3,06E+05	4,58E+05	23,00	32,00	5,50	0,00	13,00
SLOPE	Biol-10	10dcm	1,77E+08	2,82E+06	1,49E+04	1,37E+05	6,40	10,80	9,20	1,20	8,80
SLOPE	Biol-10	10int1	1,12E+08	0,00E+00	2,07E+04	1,66E+03	0,68	1,82	3,64	1,36	2,73
SLOPE	Biol-10	10int2	7,60E+07	0,00E+00	1,32E+04	1,57E+04	0,40	1,40	1,60	0,40	0,80
SLOPE	Biol-10	10fondo	2,27E+07	0,00E+00	1,05E+04	1,61E+04	1,00	0,80	0,00	0,20	0,80
SLOPE	Biol-11	11sup	4,40E+08	1,00E+07	1,12E+05	4,62E+05	12,73	8,64	5,45	0,00	10,00
SLOPE	Biol-11	11dcm	3,91E+08	2,89E+07	1,65E+05	1,09E+06	0,91	15,91	7,73	0,00	10,91
SLOPE	Biol-11	11fondo	6,88E+07	1,50E+05	2,11E+04	1,62E+04	1,14	2,27	4,77	0,00	2,50
POLCEVERA	Biol-12	12sup	4,05E+08	1,26E+07	2,59E+05	4,23E+05	9,55	12,73	2,73	0,00	6,36
POLCEVERA	Biol-12	12dcm	1,80E+08	3,97E+06	1,35E+05	9,87E+05	4,09	5,00	6,36	0,00	2,27
POLCEVERA	Biol-12	12fondp	8,40E+07	2,68E+05	1,56E+04	1,74E+04	2,50	1,25	1,75	0,50	3,50
POLCEVERA	Biol-13	13sup	4,85E+08	8,14E+06	1,59E+05	3,21E+05	0,91	4,55	5,45	0,45	18,18
POLCEVERA	Biol-13	13dcm	4,54E+08	3,37E+07	5,29E+04	5,05E+05	8,00	13,50	2,00	0,00	10,00
POLCEVERA	Biol-13	13int1	3,17E+08	9,52E+06	4,39E+04	6,62E+04	0,91	5,45	11,36	0,00	7,95
POLCEVERA	Biol-13	13int2	6,64E+07	9,52E+06	1,49E+04	1,66E+04	0,91	0,00	0,68	1,36	1,82
POLCEVERA	Biol-13	13fondo	3,65E+07	0,00E+00	3,79E+03	5,68E+03	1,36	0,23	0,00	0,45	2,05
POLCEVERA	Biol-14	14sup	5,15E+08	1,10E+07	2,98E+04	2,49E+05	13,20	13,60	2,40	0,00	13,20
POLCEVERA	Biol-14	14dcm	4,94E+08	2,11E+07	1,35E+05	1,35E+05	2,73	2,73	22,27	0,45	10,45
POLCEVERA	Biol-14	14int1	1,42E+08	0,00E+00	3,89E+04	0,00E+00	0,00	0,75	0,50	0,75	1,75
POLCEVERA	Biol-14	14fondo	6,01E+07	0,00E+00	1,42E+04	6,98E+03	1,14	0,91	3,64	0,68	2,05
BISAGNO	Biol-15	15sup	6,00E+08	1,36E+07	2,98E+04	1,42E+05	23,00	20,50	5,50	0,00	9,00
BISAGNO	Biol-15	15dcm	6,45E+08	2,82E+07	1,18E+05	2,82E+05	5,91	5,00	8,64	0,91	7,73
BISAGNO	Biol-15	15int1	5,47E+07	0,00E+00	1,49E+04	6,62E+03	0,00	0,23	2,73	0,00	0,45
BISAGNO	Biol-15	15fondo	3,24E+07	2,12E+04	1,38E+04	5,32E+03	0,25	4,25	0,50	0,25	0,50
SLOPE	Biol-16	16sup	4,63E+08	8,20E+06	7,05E+04	2,70E+05	16,80	8,40	2,40	0,00	8,80
SLOPE	Biol-16	16dcm	5,56E+08	3,29E+07	9,99E+04	5,70E+05	2,80	9,60	3,60	0,40	11,60
SLOPE	Biol-16	16int1	9,56E+07	0,00E+00	2,73E+04	7,45E+03	1,20	1,80	3,00	0,20	3,20
SLOPE	Biol-16	16fondo	4,12E+07	3,17E+04	1,42E+04	3,43E+03	2,20	0,20	1,00	0,40	1,60

Table S3- Biomass expressed in µgC L^{-1} for the main groups

TRASECT	STATION	SAMPLE ID.	HB	PhotoPico	HNF	PNF	Dinoflgellates	Aloricate Ciliates	Tintinnids	Other Protist	Micrometazoans
BISAGNO	Biol-1	1sup	7,652	1,416	3,203	1,946	0,043	0,023	0,003	0,000	0,207
BISAGNO	Biol-1	1dcm	9,629	2,205	0,848	0,033	0,075	0,012	0,051	0,043	0,603
BISAGNO	Biol-1	1fondo	5,917	0,348	0,170	0,096	0,020	0,003	0,013	0,000	0,148
BISAGNO	Biol-2	2sup	6,024	1,210	4,760	2,622	0,135	0,049	0,008	0,000	0,159
BISAGNO	Biol-2	2dcm	8,932	2,157	0,416	0,707	0,053	0,019	0,004	0,000	0,151
BISAGNO	Biol-2	2int1	1,770	0,000	0,147	0,068	0,012	0,001	0,007	0,000	0,086
BISAGNO	Biol-2	2int2	1,149	0,000	0,076	0,007	0,006	0,006	0,006	0,000	0,057
BISAGNO	Biol-2	2fondo	0,650	0,000	0,009	0,002	0,002	0,002	0,001	0,000	0,003
BISAGNO	Biol-3	3sup	11,281	2,944	8,801	2,895	0,438	0,042	0,030	0,000	0,298
BISAGNO	Biol-3	3dcm	5,164	0,200	0,071	0,779	0,021	0,017	0,017	0,000	0,093
BISAGNO	Biol-3	3int1	1,442	0,000	0,157	0,044	0,132	0,004	0,006	0,002	0,035
BISAGNO	Biol-3	3int2	0,519	0,000	0,023	0,000	0,112	0,001	0,003	0,000	0,069
BISAGNO	Biol-3	3fondo	0,313	0,006	0,028	0,011	0,000	0,003	0,007	0,000	0,008
BISAGNO	Biol-4	4sup	10,606	2,433	6,279	8,179	0,046	0,214	0,000	0,000	0,612
BISAGNO	Biol-4	4dcm	12,909	4,295	2,102	1,826	0,468	0,099	0,008	0,004	1,021
BISAGNO	Biol-4	4int1	1,544	0,000	0,260	0,096	0,007	0,010	0,023	0,000	0,031
BISAGNO	Biol-4	4int2	0,690	0,000	0,074	0,000	0,007	0,011	0,000	0,041	0,014
BISAGNO	Biol-4	4int3	0,636	0,000	0,011	0,166	0,003	0,000	0,003	0,031	0,013
BISAGNO	Biol-4	4fondo	0,389	0,000	0,005	0,096	0,000	0,003	0,000	0,000	0,034
BISAGNO	Biol-5	5sup	14,630	2,744	2,931	12,387	0,033	0,167	0,074	0,000	0,541
BISAGNO	Biol-5	5dcm	3,210	0,135	0,079	0,026	0,017	0,008	0,024	0,004	0,092
BISAGNO	Biol-5	5int1	0,944	0,000	0,070	0,038	0,016	0,008	0,002	0,014	0,043
BISAGNO	Biol-5	5int2	1,240	0,000	0,075	0,126	0,002	0,000	0,001	0,000	0,020
BISAGNO	Biol-5	5int3	0,588	0,000	0,129	0,050	0,000	0,005	0,001	0,004	0,024
BISAGNO	Biol-5	5int4	0,571	0,000	0,033	0,005	0,000	0,000	0,003	0,000	0,026
BISAGNO	Biol-5	5fondo	0,499	0,000	0,088	0,026	0,000	0,001	0,000	0,001	0,054
SLOPE	Biol-6	6sup	16,700	10,651	2,825	6,945	0,015	0,215	0,018	0,000	0,256
SLOPE	Biol-6	6dcm	18,445	3,837	1,091	1,566	0,072	0,039	0,028	0,004	0,541
SLOPE	Biol-6	6int1	0,921	0,000	0,056	0,019	0,002	0,000	0,001	0,001	0,129
SLOPE	Biol-6	6int2	0,540	0,000	0,101	0,092	0,005	0,021	0,007	0,002	0,035
SLOPE	Biol-6	6int3	0,507	0,000	0,042	0,037	0,003	0,004	0,000	0,000	0,021
SLOPE	Biol-6	6fondo	0,356	0,000	0,040	0,003	0,003	0,000	0,000	0,000	0,034
SLOPE	Biol-7	7sup	10,629	0,752	0,607	3,719	0,029	0,240	0,009	0,011	0,687
SLOPE	Biol-7	7dcm	13,235	6,810	2,103	1,518	0,029	0,077	0,011	0,000	1,739
SLOPE	Biol-7	7int1	0,782	0,000	0,081	0,081	0,024	0,010	0,023	0,009	0,046
SLOPE	Biol-7	7int2	0,707	0,000	0,030	0,044	0,002	0,033	0,003	0,021	0,021
SLOPE	Biol-7	7fondo	0,514	0,000	0,030	0,023	0,002	0,000	0,000	0,000	0,056
POLCEVERA	Biol-8	8sup	6,652	0,611	0,701	5,836	0,089	0,077	0,022	0,000	0,336
POLCEVERA	Biol-8	8dcm	3,233	0,147	0,091	0,224	0,008	0,028	0,037	0,016	0,195
POLCEVERA	Biol-8	8int1	1,800	0,000	0,071	0,214	0,002	0,019	0,012	0,007	0,125
POLCEVERA	Biol-8	8int2	0,986	0,000	0,007	0,455	0,000	0,021	0,004	0,009	0,016
POLCEVERA	Biol-8	8fondo	0,513	0,000	0,013	0,000	0,000	0,004	0,000	0,005	0,048
SLOPE	Biol-10	10sup	8,722	3,408	1,492	5,828	0,164	0,237	0,053	0,000	0,861
SLOPE	Biol-10	10dcm	3,535	0,564	0,022	1,130	0,040	0,092	0,056	0,011	0,647
SLOPE	Biol-10	10int1	2,247	0,000	0,087	0,000	0,005	0,009	0,016	0,034	0,222
SLOPE	Biol-10	10int2	1,520	0,000	0,063	0,063	0,001	0,016	0,009	0,007	0,080
SLOPE	Biol-10	10fondo	0,454	0,000	0,025	0,148	0,008	0,004	0,000	0,002	0,080
SLOPE	Biol-11	11sup	8,792	2,004	0,432	4,539	0,082	0,029	0,042	0,000	0,461
SLOPE	Biol-11	11dcm	7,815	5,773	0,628	6,318	0,005	0,078	0,036	0,000	0,327
SLOPE	Biol-11	11fondo	1,377	0,030	0,097	0,074	0,011	0,014	0,025	0,000	0,116
POLCEVERA	Biol-12	12sup	8,094	2,527	1,204	5,170	0,072	0,029	0,011	0,000	0,138
POLCEVERA	Biol-12	12dcm	3,605	0,793	0,447	6,124	0,069	0,010	0,018	0,000	0,193
POLCEVERA	Biol-12	12fondp	1,680	0,054	0,039	0,067	0,016	0,004	0,011	0,005	0,226
POLCEVERA	Biol-13	13sup	9,699	1,628	1,817	3,665	0,006	0,014	0,027	0,004	0,711
POLCEVERA	Biol-13	13dcm	9,071	6,743	0,161	1,655	0,027	0,035	0,014	0,000	0,364
POLCEVERA	Biol-13	13int1	6,336	1,904	0,238	0,375	0,005	0,015	0,064	0,000	0,275
POLCEVERA	Biol-13	13int2	1,327	1,904	0,071	0,094	0,002	0,000	0,005	0,012	0,099
POLCEVERA	Biol-13	13fondo	0,729	0,000	0,014	0,026	0,021	0,000	0,000	0,008	0,040
POLCEVERA	Biol-14	14sup	10,304	2,209	0,183	1,774	0,083	0,038	0,012	0,000	0,623
POLCEVERA	Biol-14	14dcm	9,885	4,222	0,584	0,366	0,028	0,015	0,113	0,004	0,490
POLCEVERA	Biol-14	14int1	2,847	0,000	0,150	0,000	0,000	0,007	0,001	0,007	0,096
POLCEVERA	Biol-14	14fondo	1,202	0,000	0,031	0,026	0,008	0,003	0,013	0,014	0,092
BISAGNO	Biol-15	15sup	12,002	2,713	0,183	2,120	0,147	0,157	0,025	0,000	0,318
BISAGNO	Biol-15	15dcm	12,909	5,650	0,339	0,804	0,046	0,030	0,068	0,016	0,263
BISAGNO	Biol-15	15int1	1,093	0,000	0,089	0,201	0,000	0,000	0,020	0,000	0,010
BISAGNO	Biol-15	15fondo	0,649	0,004	0,061	0,017	0,000	0,015	0,003	0,002	0,037
SLOPE	Biol-16	16sup	9,257	1,639	0,351	1,004	0,112	0,047	0,012	0,000	0,335
SLOPE	Biol-16	16dcm	11,118	6,575	0,395	2,847	0,023	0,032	0,019	0,010	0,400
SLOPE	Biol-16	16int1	1,912	0,000	0,127	0,034	0,009	0,006	0,013	0,002	0,102
SLOPE	Biol-16	16fondo	0,825	0,006	0,112	0,021	0,011	0,000	0,004	0,003	0,090

Chapter 3- Microzooplankton Composition and Horizontal Distribution in the Western Mediterranean Sea

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Abstract

In this study microzooplanktonic abundance, biomass, and taxonomical compositions were analysed and the community structures were discussed on the base of abiotic variables measured during the summer 2015 in the Western Mediterranean Sea.

The purpose was to highlight a connection between population composition and the hydrology that characterizes the specific basins, contributing to the understanding of the phenomena that make the Mediterranean area a hotspot of variability and diversity.

A significant effect of the interaction between Transect and Depth factors, were detected ($p < 0.001$). Total MCZ community abundance and number of taxa decreased from the surface to the 500m depth. Furthermore, PERMANOVA results showed a significant effect of the variable depth ($p < 0.001$) in the community structures and abundances, this variable explain 28.6% of the total variance recorded in our sampling units. A significant effect is also highlighted for the variable Transect ($p < 0.001$) accounting for 11.6 % of the total variance; conversely the Site variable resulted not significant ($p > 0,05$). The value of similarity recorded between the community of the transects analyzed in this study appeared closely related to the circulation that characterizes the Western Mediterranean basin highlighting that the water masses modifications in salinity along the Mediterranean circulation could play a significant role in shaping the protis assemblages.

1. Introduction

The western Mediterranean Sea has been considered oligotrophic since the study of Jespersen (1923).

Many studies consider the basin of the Mediterranean Sea as an oligotrophic reservoir (Sournia, 1973), with the exception of the Northern Adriatic (Fonda Umani, 1996), in terms of nutrients concentration (Krom et al., 1991), primary production (Turley et al., 2000) and autotrophic biomass (Dolan et al., 1999). Primary production of the Mediterranean Sea is generally low and chlorophyll concentration offshore rarely exceeds 2-3 mg m^{-3}.

Marine environment in this area, both in coastal and oceanic domains, is characterized by a microbes dominated trophic network (Fogg, 1995; Agawin et al., 2000).

In oligotrophic systems, where the dominant fractions in terms of productivity and biomass are pico and nano-plankton (Li et al., 1983; Platt et al., 1983), it is expected that heterotrophic protists and small micrometazoans, which compose the microzooplankton (MCZ) fraction, would be the main grazers since the larger size predators are not able to effectively prey on these components (Marshall, 1973; Pitta and Giannakourou, 2000).

Nowadays, there is a general consensus that MCZ occupies a key position in marine food webs as major consumers of primary production (Calbet and Landry, 2004), as intermediaries between primary producers and copepods (Gifford, 1991; Calbet and Saiz, 2005; Calbet, 2009) and as key component of the microbial loop (Azam et al., 1983; Sherr and Sherr, 2002). MCZ is considered the transition element between the microbial compartment and the traditional food web (Sherr et al., 1986; Calbet, 2008) and they can be deemed a structurally and functionally important element of pelagic ecosystems.

MCZ include heterotrophic or mixotrophic planktonic organisms whose size is between 10 μm (Margalef, 1963) or 20 μm (Sieburth et al., 1978; Fenchel, 1987) and 200 μm (Travers, 1972;

Sieburth et al., 1978; Revelante and Gilmartin, 1987). It is a functional group: its members share the same ecological role and not always a common evolutionary origin (Dolan, 2012). The taxa most represented in this fraction belong to the protists, different and polyphyletic group of unicellular eukaryotes: ciliates, both aloricate and loricate (tintinnids), some heterotrophic and mixotrophic genera of dinoflagellates and, to a lesser extent, radiolarians and foraminifers. Another important component is that of micrometazoans, or larval forms of metazoans. Numerous studies have identified MCZ as the main grazers of phytoplankton in many marine ecosystems, from the ultra-oligotrophic one to those of the upwelling regions (Verity et al., 1993; Gallegos et al., 1996; Strom and Strom, 1996; Landry et al., 1998; Calbet, 2001; Quevedo & Anadón 2001; Liu et al., 2002; Calbet and Landry, 2004) and MCZ abundance is often correlated with chlorophyll-*a* and the composition of phytoplankton community, indicating a clear influence of food supply on their distribution (Heinbokel and Coats, 1986; Burkill et al., 1995; Archer et al., 1996; Becquevort, 1997; Klaas, 2001). Hydrodynamism and abiotic factors are strictly related to plankton distribution, directly involved in the shaping of the autotrophic component (Siokou-Frangou et al., 2010), hydrodynamic characteristics should indeed play a pivotal role in the composition of the MCZ community.

The aim of this study was to characterize MCZ communities established in the whole water column of the main macro-areas of the Western Mediterranean Sea.

MCZ population abundance, biomass, and taxonomical compositions were analysed and the population structures were discussed on the base of abiotic variables measured during the summer 2015 in the Western Mediterranean Sea.

The purpose was to highlight a connection between population composition patterns and the hydrology that characterizes the specific basins, contributing to the understanding of the phenomena that make the Mediterranean area a hotspot of variability and diversity (Lejeusne, et al., 2010).

2. Materials and methods

2.1 Water circulation in the study area

The Mediterranean Sea is a marginal enclosed sea, which covers 2.542 x 106 Km^2, with an average depth of ca. 1.500 m. It is a unique system because of its permanent homeothermy below 200 - 300 m, with warm deep waters (13 – 14 ºC). At the surface, temperature varies between winter minima ≈ 13 °C and summer maxima ≈ 26°C. It is characterized by strong climatic and trophic gradients as illustrated by the ranges of primary production from a mean in the western sector of 502.7 ± 342.2 mg C m^{-2} d^{-1} to 151 ± 91.6 mg C m^{-2} d^{-1} in the eastern sector (Turley et al. 2000). Nutrient concentrations are very low, particularly in the open waters, and Phosphorus is the most limiting factor (Thingstad and Rassoulzadegan, 1995).

Hydrological factors in the basin result to be highly influenced from wind regime and river inputs (Bougis et al., 1957; Lacombe and Tchernia, 1960) and nutrient concentration and productivity show high variation as a result of local and seasonal variations of water characteristics and hydrodynamics.

These variations in nutrient and hydrodynamic characteristic strongly affected the phytoplankton distribution (Moran et al 2001; Isern- Fontanet et al., 2004; Kahru et al., 2007)

The Western Mediterranean includes the Alboran Sea, the Algero-Ligurian region, the Tyrrhenian Sea and the northern part of the Sicilian Strait.

Several studies quite well describe the water circulation and the related patterns in nutrients and productivity in the Algerian basin since 1980 (Wald, 1980; García Lafuente et al., 1995; Lopezjurado et al., 1995; Salat, 1996).

In this area, the Atlantic water flows in the Alboran Sea through the Gibraltar Strait (Millot, 1999) determining cyclonic and anticyclonic gyres in the entire basin among Balearic Islands, Sardinia and Algeria; this large variability in the hydrodynamic characteristics determines sporadic

upwelling along the coastline, resulting in temporary high nutrient levels and primary production in coastal areas (Estrada, 1981, Vargas-Yanez at al., 2002).

The influence of the Atlantic water is delineated from the Balearic front that is relative superficial (200 m) and represents the northern boundary of the Algerian basin.

At the entrance of the Strait of Sardinia, the Eastward flow of Atlantic water is modulated by eddy-shaped unstable sporadic events (Salat, 1995).

In proximity of the Sardinian Channel the Atlantic current splits into two parts: one goes along Sardinian coasts heading North towards Tyrrhenian Sea, the other goes to the East flowing through the Sicilian Strait.

From the Tyrrhenian sea, Atlantic water flows mainly through Corsican Channel following the east Corsican coast and it mix northward with the West flow, forming the Ligurian current in the Gulf of Genova (Millot, 1999) that has been long investigated (Albérola et al., 1995; Astraldi et al., 1995; Conan and Millot, 1995; Font et al., 1995; Sparnocchia et al., 1995). The Ligurian current flows from the Gulf of Genova along the continental slope of Provence to the Channel of Ibiza (the so-called Liguro–Provenco–Catalan Current or Northern Current by Millot, 1992; 1999). In winter, its temperature is lowered both by the wind cooling the surface layer and by mixing with the upwelling water.

In the continental slope of the Gulf of Lions, the hydrodynamics are very variable, so the circulation tending southward is influenced by the upwelling phenomena, the continental winds and the Northern current (Hua and Thomasset, 1983; Millot 1990).

The Liguro-Provencal current is also affected by flows originating from depth mesoscale currents (Rouault, 1971; Millot, 1990). At intermediate depth the basin is characterised from the LIW (Levantine Intermediate Water) that flows from the Estern Mediterranean basin via the Strait of Sicily at depth between the ≈ 200 to 700 m, this water mass moves around Sicily into the Tyrrhenian Sea and westward over the south of Sardinia where a part of it move North into the Ligurian Sea and the rest continue westward toward the Alborán Sea and Gibraltar (Millot, 1990).

This variability is strictly related to the planktonic community structure and distribution.

2.2 Sampling design and analyses

In order to assess the MCZ community composition in the different macro areas of the Mediterranean Sea, 5 transects were investigated: PA (Palma de Mallorca - Algeri), SC (Sicily - Tunis), SB (Sardinia – Balearic Island), MB (Minorca- Barcellona) and VC (Ventimiglia – Calvì). A total of 25 stations, at 4 increasing depths, were sampled: 5 m (surface layer), deep chlorophyll maximum (DCM), 200 m and 500 m (Fig. 1).

In the late Mediterranean summer (4-31 of August 2015) samplings operations were carried out on board of the research vessel MINERVA1, in the framework of Ocean Certain Project during the VENUS 3 oceanographic cruise.

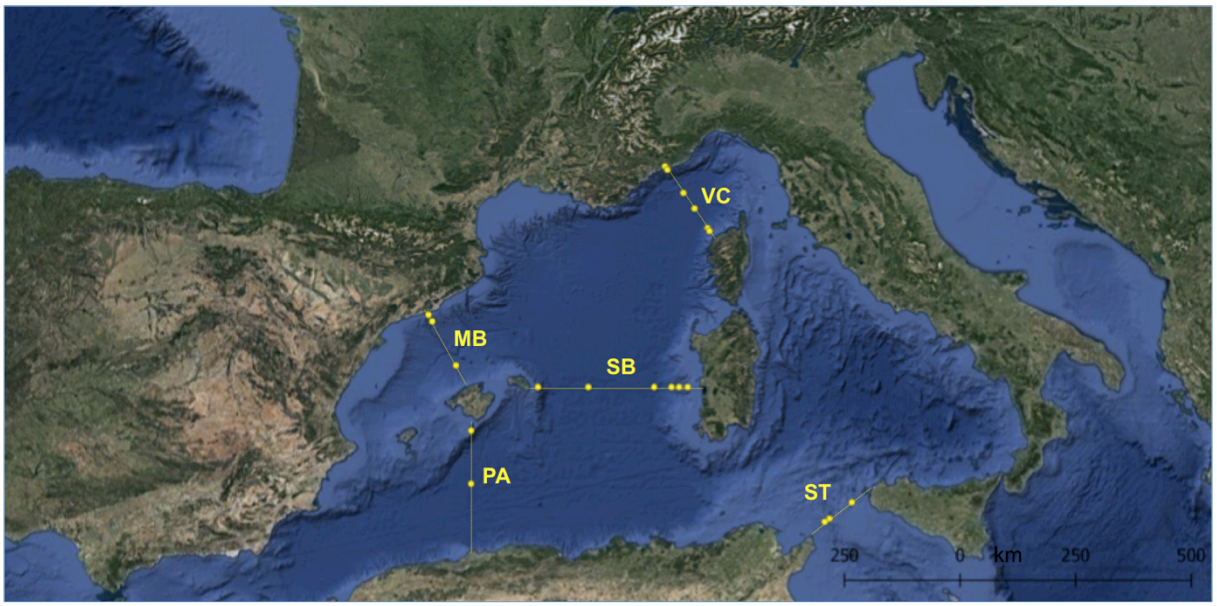


Fig. 11 study area

For each station, abiotic variables were measured: CTD SBE911 plus (pressure, temperature and conductivity plus dissolved oxygen SBE43, Chelsea Aqua 3 fluorometer and Chelsea/Seatech transmissometer) provided data for temperature, salinity, oxygen and fluorescence in correspondence of the sampling depth. Water samples were collected by an oceanic rosette

equipped with 24 Niskin bottles V=12 L.

In order to obtain representative samples different water volumes were collected in relation of the increasing depth: 5 L were collected for the samples in the photic layer (5-100 m) while 10 L were required for the deeper layer (200-500 m). With the aim of reducing samples volumes and fixative utilization, samples were prefiltered through a 200 μm mesh, and concentrated to a final volume of 250 mL.

Through gently filtration, organisms in the samples were collected on a 10μm mesh, resuspended in the final volumes, and fixed with 2 % buffered formalin. All samples were stored at 4 °C in the dark until subsequent analysis.

For identification and enumeration of the main taxa composing MCZ, subsamples of 100 mL, corresponding respectively to 2.5-5 L of original seawater, were settled in a settling tube and collected in a settling chamber.

According to the Uthermöhl method (1958) settling time was calculated on the base of the length of the settling tube (11cm) and the settling speed of the organisms (3 cm h^{-1}). The entire surface of the settling chamber was examined and every organism was counted and measured.

Measurement and taxonomical assignation were carried out using an inverted microscope Olympus IX51 equipped with 10X, 20X and 40X objectives obtaining a final magnification of 400X and with an eyepiece scale for the determination of cells dimension.

In this study, the main groups of the MCZ were considered: heterotrophic dinoflagellates (Sherr and Sherr, 2007) aloricate ciliates and tintinnids as well as radiolarians, foraminifers, heliozoans and micrometazoans were enumerated end assigned at the lower taxonomical level as possible.

Because of the difficult identification of the taxonomical characters, a common distinction between Holotrichids, with cilia evenly distributed around the entire cell, and Oligotrichids, with cilia arranged only in certain areas of the cell body (Petz, 1999) was used.

Taxonomical assignations were carried out following the identification-key proposed by Tomas and Haste (1997) Faust and Gulledge (2002) Kofoid and Campbell (1939) and other specific for the

Mediterranean area (Rampi and Zatera, 1982), the nomenclature was revised according to WoRMS Editorial Board (2017).

Biomass for each taxa were determined: every organism was measured by a eye piece scale and approximated to geometrical object (Edler, 1979) according to its shape and dimension, and biovolumes were calculated. Biovolumes were then transformed in carbon content according to Putt and Stoecker (1989) for aloricate ciliates, Verity and Langdon (1984) for tintinnids, Olenina et al. (2006) for dinoflagellates, and Michaels et al. (1995), and Beers and Stewart (1970) for the other protists and micro metazoans.

2.3 Statistical analyses

In order to test the influence of the considered variables (sampling site, transect, and depth) on the community structures highlighted in the study, we performed a Permutational Multivariate Analysis of Variance (hereafter called PERMANOVA) (Anderson and Walsh, 2013) on square-rooted Bray-Curtis dissimilarity matrix for abundance of each taxon.

The PERMANOVA design was built considering transect as random factor, site as random factor nested in transect and depth as fixed factor.

With the aim to measure the distances among transects for each depth layer, a pair-wise test were applied. The analysis were carried out using PRIMER-6 + PERMANOVA software package (Anderson et al., 2008).

The influence of abiotic factors on the community structures were assessed through distance based Redundancy Analysis (dbRDA) using vegan package (Okanen at al., 2017) in R environment (R Core Team, 2016)

3. Results

3.1 Water masses and abiotic factor

A strong stratification was detected in the entire basin: results for abiotic factors are resumed in Table S1. Generally, in the surface layer (20 m) the water columns showed the direct influence of the atmospheric conditions. Surface waters were characterized by high values of temperature, maximum values were recorded in the southern basin along the transect PA were the mean temperature in the first 10m of the water column was 26.98 ± 0.76 °C while lower values were recorded in the northern basin: 24.37 ± 1.06°C along the transect VC. In this transect salinity showed the highest value (38.33 ± 0.07) while the lowest values were observed in the transect PA (37.28 ± 0.16).

The deep chlorophyll maximum (DCM) varied along the different transects, maximum of fluorescence were measured at 52 ± 6 m depth in the transect MB while in the other transects it was recorded at greater depth (maximum in transect PA of 77 ± 4 m).

Water temperature at DCM varied from 13.98 °C at station S2 along the transect SB to 16.26 °C at station B06 along the transect MB, and the salinity showed higher values in the transects VC and ST.

At 200 m depth, at the interface between Modified Atlantics Water (MAW) and Levantine Intermediate Water (LIW), salinity and temperature resulted similar in the entire basin with the exception of transect ST that showed high values for both temperature and salinity (14.98 ± 0.06 °C and 38.85 ± 0.04, respectively).

The same conditions were detected at 500 m depth where the basin showed a mean value of 13.49 ± 0.14 °C for temperature and 38.60 ± 0.03 for salinity, while a temperature of 14.17 ± 0.05 °C and a salinity of 38.85 ± 0.01 were recorded in correspondence of the Sicilian Channel.

3.2 Microzooplankton composition

Data showed a significant difference in community composition according to the sampled water layer and the geographical region; Table 1 reported the PERMANOVA output for each factor considered in the analysis.

Factors	*df*	*SS*	*MS*	*Pseudo-F*	*perms*	*P(MC)*	*sig.*	*% of variance*
Transect	4	26934	6733.4	4.3345	4961	0,0002	***	11.66
Depth	4	55235	13809	6.2112	4984	0,0002	***	28.59
Site(Transect)	15	23478	1565.2	1.1381	4892	0,0546	-	1.54
TransectxDepth	16	37954	2372.1	1.7249	4893	0,0002	***	10.46

Tab. 1 Results of permanova analysis

A significant effect of the interaction between Transect and Depth factors, were detected ($p < 0.001$). Furthermore, results showed a significant effect of the variable depth ($p < 0.001$) in the community structures and abundances, this variable explain 28.6% of the total variance recorded in our sampling units. A significant effect is also highlighted for the variable Transect ($p < 0.001$) accounting for 11.6 % of the total variance; conversely the Site variable resulted not significant ($p > 0,05$).

These results allowed us to analyse the data aggregated by transect.

Analysing MCZ composition by summing the organisms counted in each transect, dinoflagellates resulted the most abundant and diverse group (Table S2). Tintinnids and aloricate ciliates were respectively the second and the third most abundant groups. Within aloricate ciliates holotrichids resulted more abundant than oligotrichids in the entire study area with the only exception of transect MB. Micrometazoans and other protists represented only a small fraction of the total abundances in all transects (Table S3).

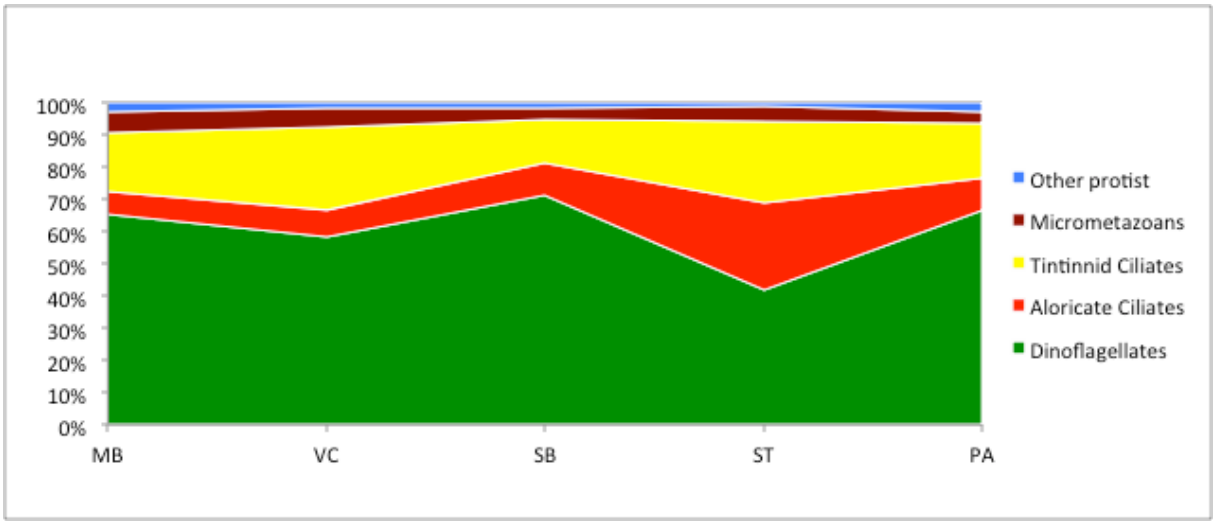


Fig. 12 Total abundances 0-500m for each transect (%)

Concerning dinoflagellates, in general small thecate dinoflagellates (10-40 μm) resulted the most abundant group representing from 22 % of the total organisms in the transect ST to 40 % c.a. in the transects SB and PA, followed by athecate dinoflagellates of the order Gymnodiniales that account from 0.9 % to 4.7 % respectively in the transects ST and MB. The transect MB was characterized by high abundance of the species belonging to the genus *Prorocentrum* (*Procentrum triestinum,* 3.7 % and *Prorocentrum micans*. 1.5 %) while high abundances of the genera *Gonyaulax* (4 % of the population) were detected along the transect VC; the species *Prorocentrum micans*, *Dinophyisis rotundata* and *Protoperidinum steinii*, exceed the 1 % of the relative abundance in this transect. Along the transect SB dinoflagellates strongly dominate the population and all the most abundant taxa were dinoflagellates, the genus *Prorocentrum* accounted for more than 6 % of the population and the species *Goyiaulax poligramma* and *Dinophysis rotundata* showed relative abundance over 2 %; at the same time the transect ST showed lower value of dinoflagellates, any way small thecate dinoflagellates resulted the most abundant group in this area, and *Prorocentrum*, *Dinophysis* and *Protoperidinum* genera exceed the 3 % of relative abundances, the lowest value for athecate dinoflagellatase of the order Gimnodiniales were detected along this transect (0.9 %). Along the transect PA the most abundant genera were *Prorocentrum, Protoperidinium, Oxytoxum* (over 3 % of relative abundance). High abundance of *Neoceratium* genus was also recorded (2 %).

Tintinnids, the second most abundant group considered in this study, resulted highly diverse in terms of species composition: a total of 100 taxa included in 26 different genera were detected in the entire study area. Along the transects MB and VC the most abundant species among tinitnnids resulted *Salpingella acuminata*, *Dadayella ganymedes*, *Eutinitinnus tubulosus*, this three species represented respectively the 4- 2.5- 2 % of the total abundances in the transect MB, while in the transect VC their relative abundance increased to the 4.6- 4.4 and 5.6 % of total organisms. In the transect MB the relative abundance of tintinnids decreased to its minimum, any way the most abundant species resulted: *Salpingella decurtata* (2%)*, Craterella torulata* (1.5 %)*, Dictyocysta mitra* (1.3 %)*,* while *Eutinitinnus tubulosus* was the most abundant species in the transect VC accounted only for 0.4 % of the population. Higher relative abundances of the genus *Salpingella* were recorded in the transect ST, along this transect the species *Salpingella acuminata*, *Salpingella decurtata*, *Salpingella curta* account together for 9.2 %, *Acanthostomella conicoides* (3.6 %) and *Craterella torulata* (2.1 %), were also abundant. In the transect PA like in the transect ST the most abundant species resulted *Salpingella acuminata* (3.4 %) followed by *Rhabdonella amor* and *Acantostomella conicoides*: 1.4 % and 1.3 % of the total abundances, respectively.

	5 m		DCM		200 m		500 m	
	H	s.d.	H	s.d.	H	s.d.	H	s.d.
MB	1.68	0.09	2.40	0.28	2.47	0.25	1.95	0.11
VC	1.91	0.33	1.95	0.37	1.99	0.52	1.49	0.52
SB	1.64	0.43	2.27	0.20	2.25	0.28	1.57	0.51
ST	1.53	0.54	2.10	0.11	1.71	0.21	1.17	0.76
PA	1.50	0.30	2.19	0.27	2.19	0.27	1.78	0.28

Tab. 2 Shannon index value, calculated on tintinnids composition for each transect.

Generally tintinnids showed higher value of diversity (Shannon index in tab. 2) at intermediate layers: higher value of Shannon index were detected at DCM and 200m in all transects while lower values were detected at the surface that was characterized by dominant species, and in the 500m layer where the lowest number of organisms and taxa were detected.

Surface layer

Total MCZ abundance in the surface layer (5m depth) ranged from 105 to 584 cells L^{-1} (Fig. 3), maximum and minimum values were recorded along the transect SB that showed a decreasing trend from the Balearic Islands to the Sardinian coast.

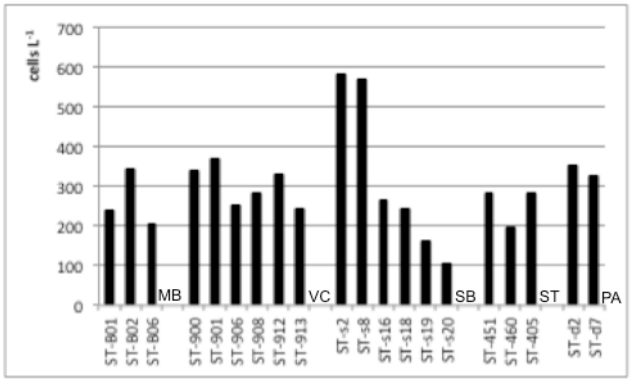
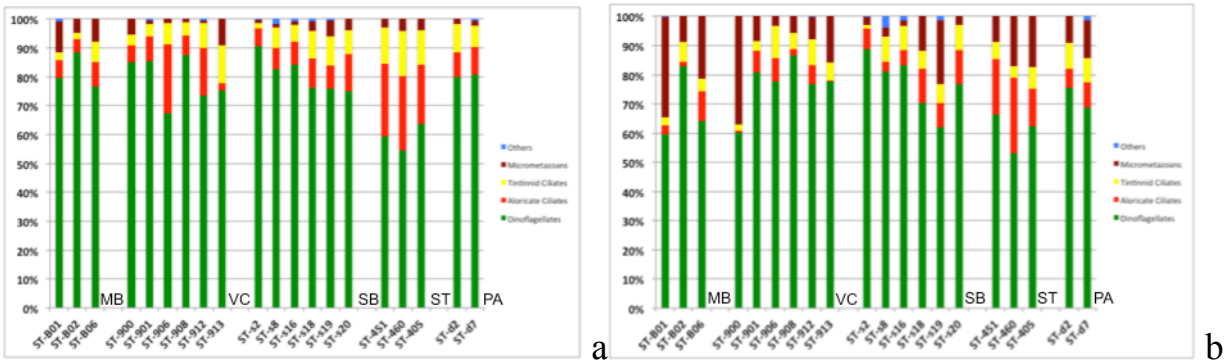


Fig. 13 Total abundances at the surface layer

Despite this, the five transects showed similar mean abundances: lower value of 254 ± 50 cells L^{-1} was recorded along the ST transect, while the maximum was registered along the transect PA (338 ± 17 cells L^{-1}).

Dinoflagellates resulted the most important group both in abundances and biomass, in the superficial water layer (Fig. 4a-b) accounting for values near 80% of relative abundance in the transects MB, VC, SB and PA while in correspondence of the transect ST the relative abundances of this component decreased to 60± 5 %.



a b

Fig. 14 Surfaces samples composition in abundances (a) and biomass (b).

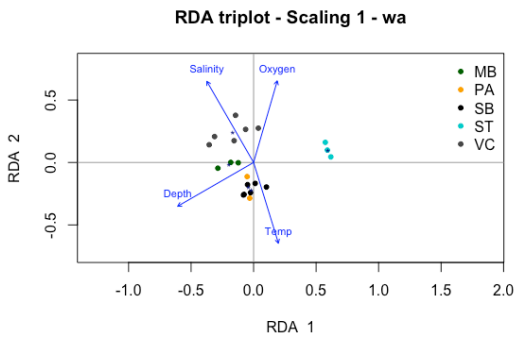
Aloricate ciliates were the second most abundant group in each transect (6-23% of total abundance) followed by tintinnids that represent 6-13 % of total abundance, the two groups showed the highest values along the transect ST. Micrometazoans showed the highest value along the transect MB accounting in this transect for 8% of total abundances and despite their relative low abundances they showed high value of biomass, while other protists never exceeded 1% in relative abundances and their carbon content resulted negligible.

Groups	*t*	*perms*	*P(MC)*	*sig.*
MB, VC	1.2683	84	0.1636	*NS*
MB, SB	1.542	84	0.0486	*
MB, ST	2.1291	10	0.0206	*
MB, PA	1.352	10	0.1968	*NS*
VC, SB	1.6671	462	0.0148	*
VC, ST	1.8385	84	0.0152	*
VC, PA	1.2663	28	0.1766	*NS*
SB, ST	1.9881	84	0.0086	**
SB, PA	1.1127	28	0.3004	*NS*
ST, PA	1.889	10	0.059	*NS*

a

	MB	VC	SB	ST	PA
MB	57.950				
VC	50.298	51.137			
SB	52.745	48.967	60.288		
ST	43.012	43.803	49.334	65.082	
PA	53.564	49.762	59.571	48.717	63.203

b



c

Fig. 15 Pair- wise test results: significant difference among transects (a) average similarity between/within transect (b) and dbRDA plot (c) for the surface layer

Pair-wise test results (Fig. 5a) highlighted significant difference ($p < 0.05$) between the transects MB and SB, MB and ST, VC and SB, VC and ST, and finally between SB and ST ($p < 0.001$) that consequently showed low similarity values (Fig. 5b).

Higher values in similarity were recorded for the interactions between the transects VC-MB, SB-MB, PA-MB and the highest value were recorded between PA and SB.

The dbRDA plot (Fig. 5c) grouped together the samples collected along the transects PA and SB while samples of the transect ST were isolated from the others.

DCM

Concerning total abundance of MCZ at the DCM layer, the transect VC showed the highest variability; in this transect low value of abundances were detected near the cost while high abundances were registered in the central part of the transect (Fig. 6). Total values ranged from 65 to 471 cells L^{-1}. Finally, along the transect SB the decreasing trend from the Balearic Islands to the Sardinian coast, registered for the surface layer, was confirmed and the transect MB always showed high value of abundances (mean value of 419 ± 41 cells L^{-1} were detected along this transect).

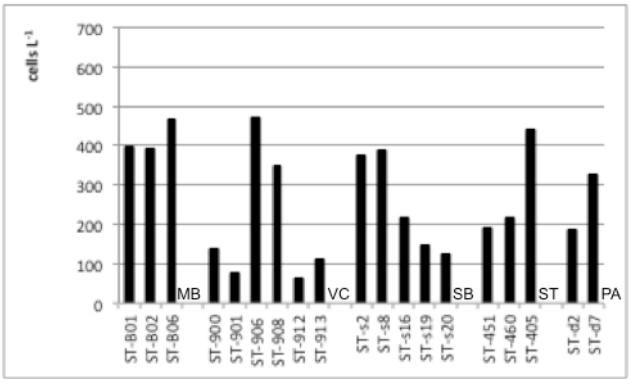
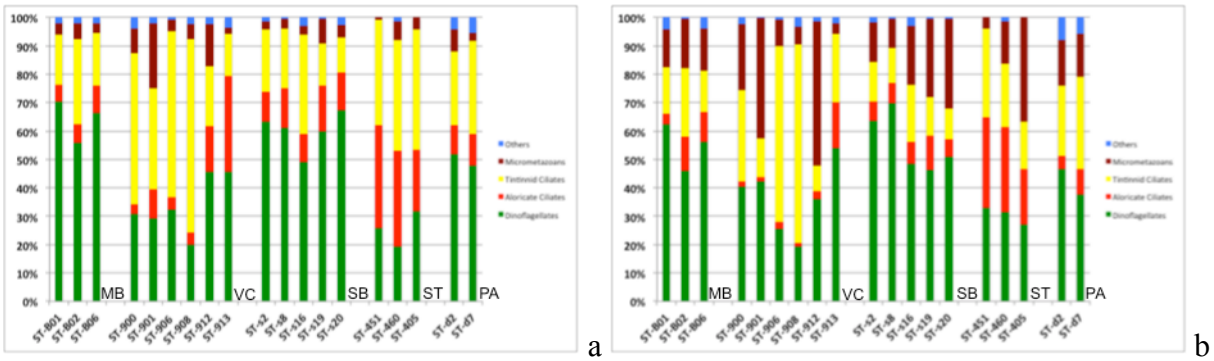


Fig. 16 Total DCM abundances

Relative abundances for the 5 main groups of MCZ (Fig. 7) highlighted that dinoflagellates represented the majority of the population both in terms of abundances and biomass in the transects MB, SB and PA (64, 60, 50 % of the total abundances, respectively). Tintinnids represented the 41% of detected organisms in the transect VC, they showed a relative abundances of 39% in the transect ST and generally the highest value of abundances after dinoflagellates.



a b

Fig. 17 DCM samples composition in abundances (a) and biomass (b).

Aloricate ciliates, like tintinnids, increased their relative abundances in this water layer in comparison to the surface values. High abundances of micrometazoans were detected along the transect VC where both for their dimension and abundances this fraction showed higher values in carbon content. Other protists always showed low values of abundances and biomass; nevertheless this fraction showed the highest relative abundance in correspondence of the transect PA.

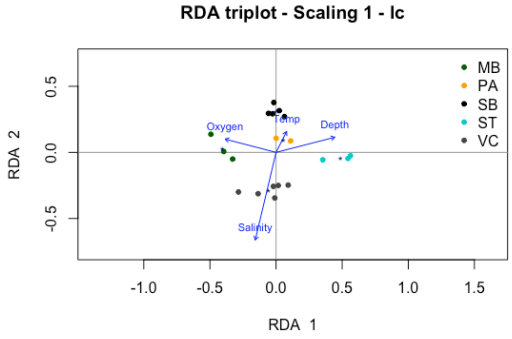
Like in the surface layer Pair-wise test results (Fig. 8a) highlighted significant difference ($p < 0.05$) between the transects MB and SB, MB and ST, VC and SB, VC and ST, and finally between SB and ST ($p < 0.001$); while the highest values in similarity (Fig.8b) were observed between the transects PA and ST and between the transects PA and SB (48.35 and 49.03, respectively)

Groups	*t*	*perms*	*P(MC)*	*sig.*
MB, VC	1.5039	84	0.0616	*NS*
MB, SB	1.9548	56	0.0146	*
MB, ST	2.289	10	0.0184	*
MB, PA	1.6419	4	0.1552	*NS*
VC, SB	1.5794	462	0.0352	*
VC, ST	1.6186	84	0.0478	*
VC, PA	0.96422	7	0.4794	*NS*
SB, ST	1.9977	56	0.0094	**
SB, PA	1.2243	6	0.2354	*NS*
ST, PA	1.5151	4	0.1842	*NS*

a

	MB	VC	SB	ST	PA
MB	55.669				
VC	39.722	39.881			
SB	41.055	39.526	55.371		
ST	36.019	36.837	43.374	62.067	
PA	35.262	39.609	49.034	48.355	63.203

b



c

Fig. 18 Pair- wise test results: significant difference among transects (a) average similarity between/within transect (b) and dbRDA plot (c) for the DCM layer

dbRDA plot (Fig, 8c) showed how the samples collected along the same transect groups together on the base of the community structures and how the abiotic factor influenced the distribution: samples of the transect ST presented similar MCZ community composition and were characterized by high value for the variable depth, while the samples of the transect VC showed higher variability in terms of composition, anyway they were characterize by high value of salinity.

200m layer

MCZ population decreased with the increasing depth ($p < 0.001$). In this layer total abundances ranged from a minimum of 16 cells L^{-1}, registered at station D7 along the transect MC, to the maximum value, recorded at station B02 along the transect PA of 57 cells L^{-1} (Fig. 9). The PA transect showed the highest mean abundances of 55 cells L^{-1} while along the other transects total abundance rarely exceeded 30 cells L^{-1}. Mean lowest value were recorded along the transect VC (20 ± 4 cells L^{-1}).

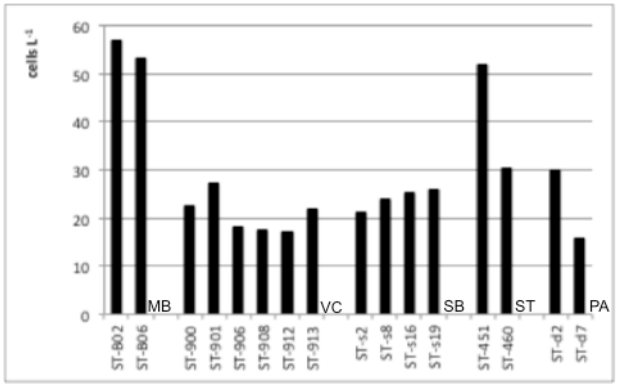


Fig. 19 Total abundances at 200m

Despite the low number of organisms the abundances resulted equally distributed among the main groups of MCZ (Fig. 10a). Dinoflagellates relative abundances ranged from 13% of the total organism along the transect ST to 28% in the transect MB.

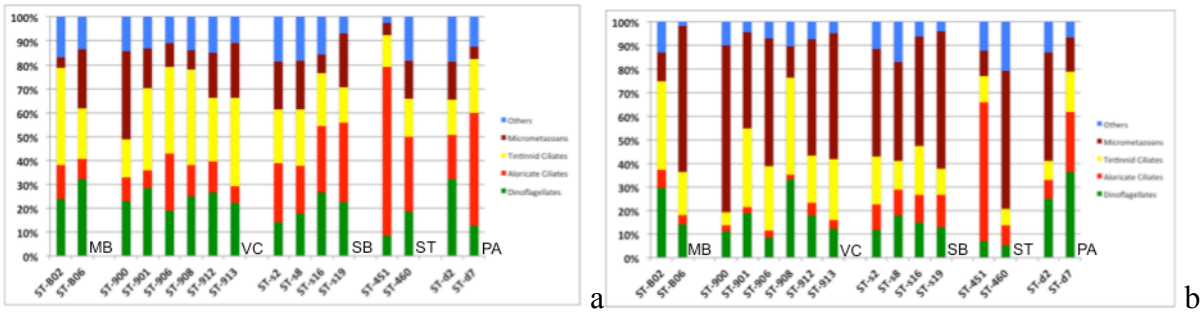


Fig. 20 200m samples composition in abundances (a) and biomass (b).

Tintinnids resulted very abundant in transects MB and VC (31% and 32% of the total abundances, respectively) while they showed lower values in the transect ST (14%). Aloricate ciliate represented a small fraction of MCZ community, both in terms of abundances and biomass, along transects MB and VC, while in correspondence of the transect ST those organisms accounted for 51% of total abundances. Micrometazoans represented from 10% (transects PA and ST) to 18% c.a. (transects VC and SB) of the abundance; despite the modest number of organism, micrometazoans resulted a pivotal component in biomass composition (Fig. 10b).

At this depth other protists increased, both in terms of relative abundances and carbon content, in comparison to the upper layers.

Groups	*t*	*perms*	*P(MC)*	*sig.*
MB, VC	1.3116	28	0.1478	*NS*
MB, SB	1.5758	15	0.0856	NS
MB, ST	1.2797	3	0.2782	NS
MB, PA	1.1582	3	0.3532	*NS*
VC, SB	1.597	210	0.0336	*
VC, ST	1.6901	28	0.0324	*
VC, PA	1.3939	28	0.1068	*NS*
SB, ST	1.7226	15	0.0544	NS
SB, PA	1.6715	15	0.0664	*NS*
ST, PA	1.1151	3	0.364	*NS*

a

	MB	VC	SB	ST	PA
MB	33.161				
VC	33.886	43.656			
SB	35.007	30.849	55.991		
ST	32.879	30.505	39.885	51.047	
PA	29.643	33.021	34.667	40.098	36.317

b

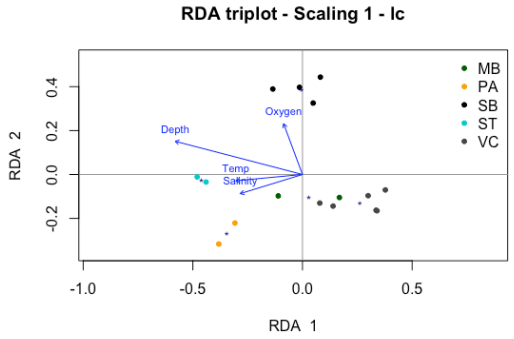


Fig. 21 Pair- wise test results: significant difference among transects (a) average similarity between/within transect (b) and dbRDA plot (c) for the 200m layer

Significant differences were highlighted only between the transects VC and SB and between VC and ST ($p < 0.05$) but the not-significant results could be due to the low permutation number (Fig. 11a). The highest values in community similarity were recorded among transects of the southern basin (ST-PA, ST-SB, PA-SB). Anyway, similarity matrix (Fig. 11b) showed similar low value among all transects suggesting a more heterogeneous situation in the study area.

The dbRDA (Fig. 11c) sharply divided the transects PA, ST and SB while transects MB and VC resulted more dispersed in the ordination.

500m layer

Total abundance achieved its minimum value in this water layer (Fig. 12), maximum value exceeded 20 cells L^{-1} only in two cases.

The highest values were recorded at station 460 along the transect ST (36 cells L^{-1}), while lower value were recorded in the transects MB and PA (10 ± 2 and 9 ± 0.5 cells L^{-1}).

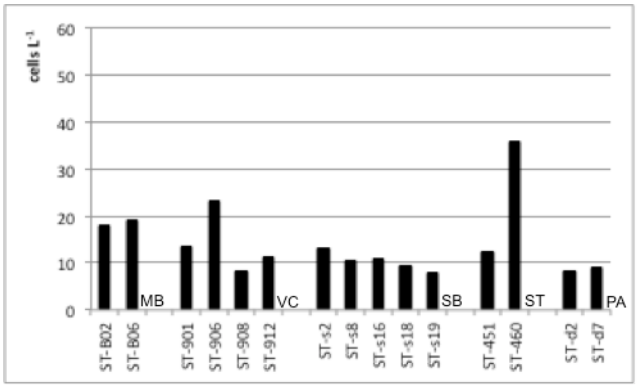
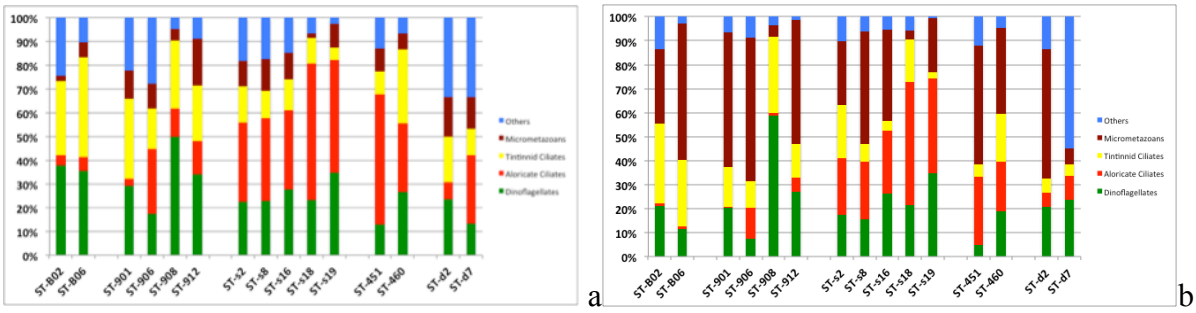


Fig. 22 Total abundances 500m

Some groups of heterotrophic Dinoflagellates resulted still present at this depth; this group showed higher value in abundances along the transects performed in the north basin, accounting for 33% c.a. in the transects MB and VC (Fig. 13a). The mean values of relative abundance of dinoflagellates decreased in the transects ST and PA where this component did not exceed 20% of the total organisms, tintinnids followed the same trend showing higher abundances in the northern transects and decreasing in the southern ones.



a b

Fig. 23 500m samples composition in abundances (a) and biomass (b).

Aloricate ciliates resulted the most important fraction along the transects SB and ST (41% c.a. of the total abundances) while represented only 5 ± 1 % of the organisms in the transect MB.

Micrometazoans and other protists showed the highest values along the transect PA. These two components increased their relative abundances over the increasing depth and represented the most important fraction of biomass (Fig. 13b) because of their huge dimensions.

Groups	*t*	*perms*	*P(MC)*	*sig.*
MB, VC	1.1248	15	0.3238	*NS*
MB, SB	1.8195	21	0.0334	*
MB, ST	1.3943	3	0.2354	NS
MB, PA	1.7864	3	0.1282	*NS*
VC, SB	1.8086	126	0.0168	*
VC, ST	1.3556	15	0.1806	NS
VC, PA	1.5471	15	0.1048	*NS*
SB, ST	1.5979	21	0.0624	NS
SB, PA	1.8814	21	0.0222	*
ST, PA	1.3277	3	0.258	*NS*

a

	MB	VC	SB	ST	PA
MB	39.005				
VC	31.560	33.098			
SB	26.776	27.139	48.148		
ST	17.631	20.909	26.792	25.770	
PA	17.956	22.463	28.418	24.939	47.445

b

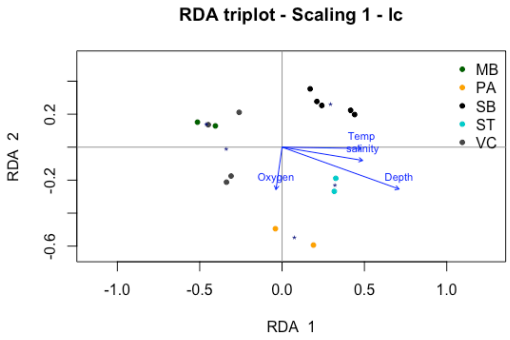


Fig. 24 Pair- wise test results: significant difference among transects (a) average similarity between/within transect (b) and dbRDA plot (c) for the 500m layer

A significant difference were highlighted for the populations recorded in the transects MB and SB, VC and SB, SB and PA (Fig. 14a). Values of similarity resumed in Fig. 14b resulted very low if compared to those recorded in the upper layers (surface and DCM). The highest value in similarity were recorded between the transects VC and MB that also were distributed in the same area of the bdRDA plot (Fig. 14c). However, low values of similarity and great distances in the dbRDA ordination confirmed a high heterogeneity among populations in the study area.

4. Discussions

The number of described protist species is rapidly growing in last decades, in Hofrichter (2002) c.a 4.400 species were described in the Mediterranean area, this estimate is based on the morphospecies description and may requires interpretation, this method may include a number of cryptic or pseudocryptic variants (Pedros-Aliò, 2006). In the last years molecular methods uncovered new sequences trying to associate them with the organisms they represent (Massana et al., 2006); as well as morphospecies description. These data should require cautious interpretations, for example, polymerase chain reaction (PCR) and pyrosequencing errors can cause marked diversity overestimations interpreting errors as rare haplotypes (Santoferrara et al., 2014). Recent applications of new methodologies like metagenomics will in the near future provide more accurate estimation on the OUT's diversity in marine environment.

The biodiversity distribution patterns are often the result of the interaction of multiple factors and are therefore difficult to identify separately, methods for identifying the independent entity and influences of these factors are important in both basic and applied ecology (Navarro et al., 2015). In this study we investigated the influence of environmental variables and spatial distribution to explain spatial patterns of compositions and biodiversity of the MCZ populations in the Western Mediterranean Basin, and we registered a significant effect of both depth and geographical area.

In the coastal zone of north-western Mediterranean, Schauer et al. (2000) determined that the seasonality was more important than the exact location in shaping bacterial community structure. Acinas et al. (1997) and Ghiglione et al. (2005) showed that microbial communities tend to be similar in the horizontal scale and much more variable on the vertical scale.

Our results on MCZ community agree with this last hypothesis, highlighting that the variable depth explain the highest value of variance among populations.

A large amount of investigation on marine biodiversity uses this variable to describe the distribution pattern of abundances and compositions (Macpherson, 2003; Kendall and Haedrich, 2006; Rex and Etter, 2010). Most popular theory showed that one of the most important response of biodiversity metrics for the majority of the marine groups to bathymetry was a parabolic or hump-shaped response, so the maximum value of biodiversity patterns occurred at an intermediate depth (Colwell and Lees, 2000; Kendall and Haedrich, 2006). In our results we found a similar trend for tintinnids biodiversity that increased from the surface layer to the DCM and 200m layers and decreased at the maximum depth.

Total MCZ community abundance and number of taxa decreased from the surface to the 500m depth. These results represent a common feature not only for the Mediterranean Sea (Tanaka and Rassoulzadegan, 2002; Diociaiuti et al., in press) but generally in the world ocean (Pernice et al., 2015)

The decrease in abundance should be considered as the direct consequence of the bottom-up control that characterizes the trophic interaction of the microbial realm at increasing depth (Zoccarato et al., 2016a). Several studies describe a decreasing trend in the abundance of the MCZ prey over depth (Yamaguchi et al., 2002; Tanaka and Rassoulzadegan, 2002; Arisegui et al 2009; Sohrin et al., 2010), and describe that abundances of pico- and nano- plankton rapidly decrease below the photic zone by one or two orders of magnitude.

The significance of different geographical areas in the composition of the analyzed community was also relevant in our results.

There are evidence that highlight as the composition of prokaryotic assemblages of the North Atlantic (Agogué et al., 2011) the arctic (Gland at al., 2010) and the southern Oceans (Celussi et al., 2010; Wilkins et al., 2013) change among different water masses and different physiochemical characters in the meso- and bathypelagic communities but at the same time very few studies are available on the protistan component. Some works demonstrated a strong correlation of eukaryotes

assemblage composition with the basin of origin (De Vargas et al., 2015) or even with water masses (Pernice et al., 2015; Zoccarato et al., 2016b).

Our results highlighted that the similarity within transects was always higher than the one between transects. This evidence, according with the results of De Vargas (2015) that highlighted this pattern on the macroscale, support the hypothesis that basins of origin play a pivotal role in shaping MCZ assemblage.

The value of similarity recorded between the community of the transects analyzed in this study appeared closely related to the circulation that characterizes the Western Mediterranean basin. At the surface as at the DCM, the assemblage recorded along the transects MB and SB showed the highest value in similarity, while the transect VC showed the highest value in similarity whit the transect MB; finally assemblage recorded in the transect ST showed the highest value of similarity with the ones recorded in the southern side of the basin (MB and PA). This similarity pattern followed the scheme of the Mediterranean circulation proposed in several works (Champalbert, 1996; Millot, 2005); stations of the western Alboran Sea (transect MB) and the stations in the north side of the Algerian basin (transect SB) are directly influenced by the Atlantic water masses that flow through Gibraltar, characterized by low values of salinity. Water masses, mixing with resident surface water, became saltier, and following the circulation of the western basin, move northward in the Ligurian basin (transect VC). From the Ligurian basin the water masses move to the Catalan coast (transect MB) following the Liguro-Provençal current. Millot (1999) well describe the salinity gradient that characterizes this water masses along the Mediterranean circulation, and our results detected the same increasing gradient, from Gibraltar to the Ligurian Sea.

In the deeper layers, where the MCZ is particularly scarce, the small volume and number of samples might contribute to hidden any significant similarity between the population of the transects. However, the similarity matrices and the plot of dbRDA showed a more heterogeneous situation showing lower similarity values than the ones recorded in the upper layers both within and between transects.

The salinity gradient combined to the circulation of the water masses might explain the shift recorded in the communities of the different areas of the Western Mediterranean basin.

The result of the dbRDA analysis showed that the vector salinity plays a significant role in the distribution of the samples on the plan.

Previous studies detected the influence of the salinity on different protistan taxa, influencing their physiology and diversity. In 1996 Ahel described the effect of this variable on phytoplankton, Bijma (1990) showed how salinity combined with temperature determines different grow rate in the planktonic foraminifers, and Dolan (2001) showed a significant role of salinity on tintinnids biodiversity.

This work highlights that MCZ composition is closely related to the physiochemical parameters that characterize their environment; the water masse's modifications along the Mediterranean circulation could have a significant effect in shaping the protist assemblages, and playing a significant role in the efficiency of the trophic web.

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Supplementary

Table S1- Coordinates, abiotic variable, and water masses assignation for each collected sample

Station	lat	lon	Depth [m]	Temperature [°C]	Salinity [PSU]	Oxygen [ml/l]	Water mass
staz-B01	41.128	1.980666	4.96	26.72	38.15	4.66	Surface water
staz-B01	41.128	1.980666	59.53	14.15	38.13	5.29	MMAW
staz-B01	41.128	1.980666	99.20	13.65	38.27	4.74	MMAW
staz-B02	41.007166	2.068833	5.95	25.97	38.06	4.71	Surface water
staz-B02	41.007166	2.068833	49.61	15.75	38.08	5.92	MAW
staz-B02	41.007166	2.068833	64.49	14.19	38.13	5.41	MAW
staz-B02	41.007166	2.068833	198.35	13.61	38.52	4.20	LIW
staz-B02	41.007166	2.068833	495.52	13.42	38.58	4.04	LIW
staz-B06	40.201	2.638833	4.96	26.73	38.09	4.65	Surface water
staz-B06	40.201	2.638833	49.61	16.26	38.17	6.18	MAW
staz-B06	40.201	2.638833	99.21	13.47	38.32	4.82	mixed
staz-B06	40.201	2.638833	198.37	13.44	38.50	4.35	LIW
staz-B06	40.201	2.638833	495.56	13.32	38.56	4.03	LIW
STAZ-900	43.753333	7.658333	4.96	25.63	38.34	4.84	Surface water
STAZ-900	43.753333	7.658333	48.60	15.59	37.97	5.94	MAW
STAZ-900	43.753333	7.658333	84.30	14.21	38.09	5.38	MAW
STAZ-900	43.753333	7.658333	198.30	13.65	38.42	4.49	LIW
STAZ-901	43.6915	7.7165	4.96	24.74	38.29	4.85	Surface water
STAZ-901	43.6915	7.7165	49.59	14.80	38.01	5.71	MAW
STAZ-901	43.6915	7.7165	84.30	14.31	38.18	5.03	MAW
STAZ-901	43.6915	7.7165	198.30	13.78	38.50	4.29	LIW
STAZ-901	43.6915	7.7165	495.40	13.70	38.65	3.88	LIW
STAZ-906	43.289666	8.099833	4.96	23.22	38.21	5.04	Surface water
STAZ-906	43.289666	8.099833	39.68	13.85	38.33	5.22	MAW
STAZ-906	43.289666	8.099833	99.18	13.44	38.44	4.46	mixed
STAZ-906	43.289666	8.099833	198.31	13.68	38.61	3.96	LIW
STAZ-906	43.289666	8.099833	495.42	13.49	38.61	3.91	LIW
STAZ-908	43.0165	8.366833	4.96	22.92	38.42	5.03	Surface water
STAZ-908	43.0165	8.366833	49.60	14.29	38.32	5.50	MAW
STAZ-908	43.0165	8.366833	99.18	13.32	38.40	4.63	mixed
STAZ-908	43.0165	8.366833	198.32	13.58	38.59	3.99	LIW
STAZ-908	43.0165	8.366833	495.43	13.48	38.61	3.95	LIW
STAZ-912	42.673833	8.6815	4.96	24.87	38.38	4.87	Surface water
STAZ-912	42.673833	8.6815	49.60	15.14	38.00	5.87	MAW

STAZ-912	42.673833	8.6815	75.39	14.06	38.09	5.21	MAW
STAZ-912	42.673833	8.6815	198.32	13.71	38.47	4.22	LIW
STAZ-912	42.673833	8.6815	495.45	13.79	38.68	3.88	LIW
STAZ-913	42.621166	8.724833	4.96	24.86	38.37	4.84	Surface water
STAZ-913	42.621166	8.724833	49.60	15.41	38.02	5.89	MAW
STAZ-913	42.621166	8.724833	79.35	14.06	38.09	5.24	MAW
STAZ-913	42.621166	8.724833	198.32	13.70	38.44	4.26	LIW
STAZ-s2	39.803333	4.607166	4.96	27.43	37.61	4.64	Surface water
STAZ-s2	39.803333	4.607166	49.61	16.31	37.79	6.08	MAW
STAZ-s2	39.803333	4.607166	89.29	13.98	38.04	5.00	MAW
STAZ-s2	39.803333	4.607166	198.38	13.35	38.40	4.55	LIW
STAZ-s2	39.803333	4.607166	495.58	13.31	38.56	4.03	LIW
STAZ-s8	39.803	5.813333	4.96	26.99	37.36	4.68	Surface water
STAZ-s8	39.803	5.813333	67.47	14.57	37.66	5.39	MAW
STAZ-s8	39.803	5.813333	99.21	14.00	38.01	4.95	mixed
STAZ-s8	39.803	5.813333	198.38	13.43	38.37	4.38	LIW
STAZ-s8	39.803	5.813333	495.58	13.45	38.59	3.85	LIW
STAZ-s16	39.802833	7.3955	4.96	25.86	38.05	4.75	Surface water
STAZ-s16	39.802833	7.3955	49.61	17.32	37.85	5.94	MAW
STAZ-s16	39.802833	7.3955	79.37	14.73	38.02	5.41	MAW
STAZ-s16	39.802833	7.3955	198.38	13.47	38.36	4.47	LIW
STAZ-s16	39.802833	7.3955	495.58	13.53	38.61	3.81	LIW
STAZ-s18	39.803833	7.816833	4.96	24.88	38.02	4.88	Surface water
STAZ-s18	39.803833	7.816833	74.41	14.13	38.09	4.90	MAW
STAZ-s18	39.803833	7.816833	495.58	13.95	38.73	3.91	LIW
STAZ-s19	39.8025	7.998666	7.94	26.08	38.21	4.70	Surface water
STAZ-s19	39.8025	7.998666	64.49	14.49	38.01	5.36	MAW
STAZ-s19	39.8025	7.998666	99.21	13.93	38.14	4.82	MAW
STAZ-s19	39.8025	7.998666	198.38	14.04	38.61	4.02	LIW
STAZ-s19	39.8025	7.998666	495.58	13.92	38.72	3.89	LIW
STAZ-s20	39.8025	8.203833	4.96	26.13	38.19	4.68	Surface water
STAZ-s20	39.8025	8.203833	59.53	14.86	37.95	5.60	MAW
STAZ-s20	39.8025	8.203833	96.24	13.88	38.24	4.40	MAW
STAZ-451	37.338333	11.600666	3.97	26.97	37.95	4.71	Surface water
STAZ-451	37.338333	11.600666	49.62	16.19	37.79	5.76	MAW
STAZ-451	37.338333	11.600666	74.43	15.17	38.05	5.29	MAW
STAZ-451	37.338333	11.600666	198.42	14.94	38.88	4.48	LIW
STAZ-451	37.338333	11.600666	495.69	14.21	38.86	4.12	LIW
STAZ-460	37.279666	11.486166	4.47	27.31	37.64	4.68	Surface water
STA- 460	37.279666	11.486166	49.62	15.98	37.82	5.70	MAW
STAZ-460	37.279666	11.486166	74.43	15.11	38.13	5.23	MAW
STAZ-460	37.279666	11.486166	198.42	15.03	38.82	4.54	LIW
STAZ-460	37.279666	11.486166	495.69	14.13	38.84	4.10	LIW
STAZ-405	37.647833	12.144166	3.97	24.18	38.03	5.16	Surface water
STAZ-405	37.647833	12.144166	46.64	15.05	38.19	5.28	MAW

STAZ-405	37.647833	12.144166	91.29	14.78	38.48	4.70	mixed
STAZ-d2	38.997	3.004333	4.96	27.52	37.40	4.62	Surface water
STAZ-d2	38.997	3.004333	49.62	17.15	37.61	6.10	MAW
STAZ-d2	38.997	3.004333	74.42	15.00	37.96	5.75	MAW
STAZ-d2	38.997	3.004333	198.39	13.39	38.39	4.28	LIW
STAZ-d2	38.997	3.004333	495.62	13.24	38.54	4.08	LIW
STAZ-d7	38.000333	3.003166	4.96	26.44	37.17	4.70	Surface water
STAZ-d7	38.000333	3.003166	49.62	16.28	37.45	5.93	MAW
STAZ-d7	38.000333	3.003166	79.39	14.39	37.90	5.21	MAW
STAZ-d7	38.000333	3.003166	198.41	13.42	38.41	4.03	LIW
STAZ-d7	38.000333	3.003166	495.66	13.51	38.61	3.86	LIW

Table S2- List of taxa detected in each transect

DINOFLAGELLATI	MB	VC	SB	ST	PA
Amphisolenia sp.	+	+	+		+
Centrodinium sp. 1	+				
Ceratium concilians	+				
Ceratium fusus	+	+	+	+	+
Ceratium gibberum		+	+		
Ceratium horridum	+				
Ceratium Kofoidii	+	+			
Ceratium lineatum	+				
Ceratium platycorne	+				+
Ceratium symmetricum	+	+	+		
Ceratium trichoceros	+	+	+	+	
Ceratium tripos	+	+	+	+	+
Ceratium sp. 1		+		+	+
Ceratocorys horrida	+	+	+	+	+
Cladopyxis sp. 1	+	+	+		+
Corythodinium tessellatum	+	+	+	+	+
Dinophysis acuta	+	+			+
Dinophysis caudata	+	+	+		+
Dinophysis fortii		+	+		+
Dinophysis hastata			+		
Dinophysis odiosa		+			
Dinophysis rotundata	+	+	+	+	+
Dinophysis saccolus	+	+		+	+

Dinophysis tripos		+		+	+
Diplopsalis sp. 1	+	+	+	+	+
Gonyaulax polygramma	+	+	+	+	+
Gonyaulax spiniferum	+		+		
Gonyaulax sp.	+	+	+	+	+
Gymnodinium impudicum		+	+		
Gymnodinium sp.		+	+		
Histioneis sp.		+	+	+	+
Kofoidinium velleloides	+	+	+		+
Lingulodinium polyedrum	+				
Noctiluca scintillans	+		+		
Ornithocercus magnificus		+	+	+	+
Ostreopsis ovata	+	+			
Oxytoxum caudatum	+	+	+	+	+
Oxytoxum conscrictum	+	+			
Oxytoxum milneri		+	+		
Oxytoxum sceptrum	+	+	+	+	+
Oxytoxum scolopax	+	+	+	+	+
Oxytoxum variabile	+	+	+	+	+
Oxytoxum sp 1	+	+			
Phalacroma rapa	+	+	+	+	+
Phalacroma rotundatum			+	+	+
Podolampas bipes			+		
Podolampas palmipes	+	+	+	+	+
Podolampas spinifera	+	+	+	+	+
Pronoctiluca acuta	+	+			
Pronoctiluca sp. 1	+	+	+	+	+
Pronoctiluca sp. 2		+			
Prorocentrum compressum	+	+	+	+	+
Prorocentrum gracile	+	+			
Prorocentrum micans	+	+	+	+	+
Prorocentrum minimum	+	+	+	+	+
Prorocentrum triestinum	+	+	+		
Prorocentrum sp.	+	+	+	+	+
Protoperidinium crassipes	+	+	+	+	+
Protoperidinium depressum		+			
Protoperidinium diabolum		+	+		
Protoperidinium divergens	+	+			
Protoperidinium oceanicum	+			+	+
Protoperidinium steinii	+	+	+	+	+
Protoperidinium sp. 1	+	+	+	+	+
Pyrocystis lunula	+	+	+		+
Spiraulax kofoidii	+	+	+		+
Triadinium polyedricum	+	+			+
Tripos candelabrus	+	+	+	+	+
Tripos declinatus	+	+	+		+
Tripos furca	+	+	+	+	+
Tripos limulus	+			+	+
Tripos pentagonus	+	+	+	+	+

Tripos pulchellus	+	+		+	+
Triposolenia sp.	+		+	+	+
Athecate dinoflagellates und.	+	+	+	+	+
Thecate dinoflagellates und.	+	+	+	+	+
ALORICATE CILIATES					
Gymnozoum sp. 1	+				
Gymnozoum sp. 2	+	+		+	+
Gymnozoum sp. 3		+	+	+	
Lacrymaria lagenula	+				
Lacrymaria sp. 1	+				
Mesodinium rubrum	+	+	+		+
Tiarina fusus			+		+
Holotricha sp.	+	+	+	+	+
Oligotrichea sp.	+	+	+	+	+
TINTINNIDI					
Acanthostomella conicoides	+	+	+	+	+
Acanthostomella lata	+	+	+	+	
Acanthostomella norvegica	+	+	+		+
Amphorides amphora	+	+	+		+
Amphorides laakmanni	+	+			+
Amphorides quadrilineata		+	+	+	+
Amphorides quadrilineata minor	+	+			
Amphorides tetragona	+		+	+	
Canthariella pyramidata	+		+	+	+
Climacocylis scalaria	+	+		+	+
Codoonella amphorella	+	+			
Codonella aspera	+	+	+		+
Codonella brevicollis	+				
Codonella sp. 1		+			
Codonellopsis sp. 1	+	+			+
Coxliella fasciata			+		
Coxliella helix	+	+			
Coxliella lacinosa	+	+			
Ascampbelliella armilla			+	+	
Ascampbelliella tortulata	+	+	+	+	+
Dadayiella ganymedes	+	+	+	+	+
Dadayiella pachytoecus			+	+	+
Dictyocysta duplex		+			
Dictyocysta elegans	+	+	+	+	
Dictyocysta mitra	+	+	+	+	+
Epiplocylis acuminata	+	+	+		+
Eutintinnus apertus	+	+	+	+	+
Eutintinnus fraknoii	+	+	+	+	+
Eutintinnus lususundae	+	+	+		+
Eutinitinnus macilentus	+				+
Eutintinnus stramentus		+	+	+	+
Eutinitinnus tubulosus	+	+	+	+	+
Eutintinnus sp.1	+	+	+		
Favella azorica			+		+

Favella sp. 1	+	+		+	+
Favella sp. 2					+
Ormosella bresslaui		+	+		
Ormosella trachelium	+	+		+	+
Parundella aculeata	+	+	+		+
Parundella caudata		+			
Parundella difficilis		+			
Parundella lohmanni	+	+	+	+	
Parundella longa		+			
Parundella messinensis	+	+	+		
Cyttarocylis ampulla			+		
Proplectella claparedei	+	+	+	+	+
Rhabdonella amor	+	+	+	+	+
Rhabdonella spiralis	+	+	+		
Salpingella acuminata	+	+	+	+	+
Salpingella curta	+	+	+	+	+
Salpingella decurtata	+	+	+	+	+
Salpingella rotundata	+	+	+		+
Salpingella sp subconica	+				
Salpingella unguiculata		+			
Steenstrupiella gracilis		+	+		
Steenstrupiella steenstrupii	+	+	+	+	+
Stenosemella nivalis		+		+	
Stenosemella ventricosa		+		+	
Tintinnpsis angulata	+				
Tintinnopsis cincta	+				
Tinitinnopsis cylindrica		+	+	+	
Tintinnopsis levigata	+	+	+		
Tintinnopsis lindeni	+	+			
Tintinnopsis nana	+		+		
Tintinnopsis radix	+	+			+
Tintinnopsis tregouboffi	+				
Undella angustior	+				+
Undella clevei	+			+	
Undella hyalina	+	+	+	+	+
Undella ostenfeldi	+	+	+	+	
Undella subcaudata subsp. acuta	+	+	+		+
Undellopsis marsupialis	+	+	+	+	+
Undellopsis sp. 1		+			
Xystonella lohmanni	+	+	+		
Xystonella longicauda	+	+	+	+	+
Xystonella treforti		+	+	+	+
Xystonellopsis cymatica		+	+		
Xystonellopsis scyphium		+	+		
Tintinnids sp. 1	+	+			+
OTHER PROTISTS					
Hermissinum adriaticum		+	+		+
RADIOLARIA					
Challengeron channeri	+		+		+

Radiolaria sp. 1	+	+			
Radiolaria sp. 2	+	+	+		+
Radiolaria sp. 3	+	+	+	+	+
Radiolaria sp. 4	+	+	+	+	+
Radiolaria sp. 5	+	+		+	
Radiolaria sp. 6		+			
Radiolaria sp. 7		+			
Radiolaria sp. 8			+	+	+
FORAMINIFERA					
Foraminifera sp.1	+	+	+	+	
Foraminifera sp.2	+	+	+	+	+
Foraminifera sp.3		+			
Foraminifera sp. 4	+		+		+
HELIOZOA					
Sticholonche zanclea	+	+	+	+	+
METAZOA					
metazoans eggs	+	+	+	+	+
metazoa larvae			+	+	+
Copepods nauplio	+	+	+	+	+

Table S3- values of abundance and biomass for the main taxonomical groups at each station

	Abundances [cells L-1]					Biomass [µg C L-1]				
	Dinoflagellates	Aloricate Ciliates	Tintinnid Ciliates	Micrometazoans	Others	Dinoflagellates	Aloricate Ciliates	Tintinnid Ciliates	Micrometazoans	Others
STAZ B01 sup	191,200	14,400	6,400	26,400	1,600	1,189	0,065	0,053	0,687	0,005
STAZ B01 DCM	280,800	23,200	70,400	16,000	8,000	1,609	0,096	0,425	0,345	0,111
STAZ B01 100 m	38,400	8,000	10,400	14,400	1,600	0,192	0,045	0,053	0,536	0,002
STAZ B02 sup	303,200	16,000	7,200	16,000	0,000	0,781	0,014	0,064	0,083	0,000
STAZ B02 DCM	218,400	26,400	116,800	22,400	8,000	1,589	0,419	0,835	0,593	0,019
STAZ B02 100 m	28,800	7,200	66,400	8,800	4,000	0,194	0,048	0,588	0,218	0,018
STAZ B02 200 m	13,600	8,000	23,200	2,400	9,600	0,113	0,029	0,143	0,047	0,049
STAZ B02 500 m	6,800	0,800	5,600	0,400	4,400	0,030	0,002	0,047	0,045	0,019
STAZ B06 sup	157,600	17,600	14,400	16,000	0,000	0,764	0,121	0,050	0,256	0,000
STAZ B06 DCM	310,400	44,800	87,200	16,000	9,600	1,413	0,270	0,363	0,372	0,100
STAZ B06 100 m	34,400	3,200	11,200	5,600	5,600	0,296	0,012	0,036	0,099	0,010
STAZ B06 200 m	17,200	4,400	11,200	13,200	7,200	0,079	0,024	0,102	0,349	0,010
STAZ B06 500 m	6,800	1,200	8,000	1,200	2,000	0,020	0,002	0,048	0,100	0,005
STAZ 900 SUP	291,200	19,200	12,800	18,400	0,000	1,702	0,016	0,061	1,048	0,000
STAZ 900 50m	128,800	4,800	52,800	19,200	0,000	0,664	0,011	0,302	0,372	0,000
STAZ 900 DCM	42,400	4,800	73,600	12,000	5,600	0,553	0,024	0,439	0,318	0,031
STAZ 900 200m	5,200	2,200	3,600	8,400	3,200	0,040	0,010	0,020	0,255	0,035
STAZ 901 SUP	317,600	31,200	16,000	4,800	1,600	2,502	0,228	0,103	0,266	0,000
STAZ 901 50m	83,200	2,400	16,000	11,200	0,800	0,403	0,016	0,089	0,291	0,004
STAZ 901 DCM	22,400	8,000	27,200	17,600	1,600	0,376	0,013	0,121	0,375	0,003
STAZ 901 200m	7,800	2,000	9,400	4,600	3,600	0,036	0,005	0,064	0,078	0,008
STAZ 901 500m	4,000	0,400	4,600	1,600	3,000	0,029	0,000	0,024	0,081	0,009
STAZ 906 SUP	170,400	60,800	19,200	3,200	0,000	1,693	0,178	0,242	0,069	0,000
STAZ 906 DCM	152,800	20,000	275,200	20,000	3,200	0,863	0,081	2,084	0,320	0,025
STAZ 906 100m	5,600	0,800	19,200	4,000	0,800	0,034	0,002	0,122	0,017	0,004
STAZ 906 200m	3,400	4,400	6,600	1,800	2,000	0,014	0,005	0,046	0,091	0,012
STAZ 906 500m	4,000	6,400	4,000	2,400	6,400	0,012	0,021	0,018	0,097	0,014
STAZ 908 SUP	248,000	19,200	12,800	3,200	0,000	2,841	0,073	0,183	0,186	0,000
STAZ 908 DCM	69,600	14,400	237,600	18,400	8,000	0,444	0,030	1,612	0,149	0,072
STAZ 908 100m	12,000	4,000	34,400	6,400	4,000	0,049	0,004	0,220	0,214	0,008
STAZ 908 200m	4,400	2,200	7,000	1,400	2,400	0,036	0,002	0,045	0,014	0,011
STAZ 908 500m	4,200	1,000	2,400	0,400	0,400	0,025	0,000	0,013	0,002	0,002
STAZ 912 SUP	243,200	54,400	28,000	3,200	1,600	1,868	0,162	0,216	0,186	0,002
STAZ 912 50m	88,800	7,200	27,200	8,800	4,800	0,587	0,025	0,139	0,194	0,027
STAZ 912 DCM	29,600	10,400	13,600	9,600	1,600	0,249	0,019	0,062	0,354	0,009
STAZ 912 200m	4,600	2,200	4,600	3,200	2,600	0,031	0,009	0,034	0,085	0,012
STAZ 912 500m	3,800	1,600	2,600	2,200	1,000	0,028	0,006	0,014	0,054	0,001
STAZ 913 SUP	184,000	6,400	32,000	22,400	0,000	3,270	0,007	0,265	0,665	0,000
STAZ 913 50m	113,600	5,600	51,200	8,800	0,800	0,755	0,032	0,377	0,368	0,005
STAZ 913 DCM	51,200	37,600	16,800	2,400	4,000	0,411	0,124	0,183	0,029	0,016
STAZ 913 200m	4,800	1,600	8,000	5,000	2,400	0,024	0,007	0,051	0,104	0,009
STAZ s2 SUP	529,600	36,800	10,400	6,400	1,600	2,778	0,222	0,043	0,082	0,008
STAZ s2 50m	391,200	25,600	33,600	12,000	3,200	1,975	0,097	0,105	0,325	0,031
STAZ s2 DCM	239,200	40,000	82,400	11,200	4,800	2,116	0,229	0,465	0,468	0,056
STAZ s2 200m	3,000	5,200	4,800	4,200	4,000	0,020	0,019	0,035	0,079	0,019
STAZ s2 500m	3,000	4,400	2,000	1,400	2,400	0,013	0,017	0,016	0,019	0,007
STAZ s8SUP	473,600	41,600	40,800	6,400	9,600	2,977	0,116	0,319	0,118	0,138
STAZ s8 DCM	237,600	54,400	81,600	13,600	1,600	1,524	0,155	0,270	0,223	0,008
STAZ s8 100m	51,200	21,600	16,800	4,000	9,600	0,346	0,069	0,068	0,063	0,085
STAZ s8 200m	4,200	4,800	5,600	4,800	4,400	0,040	0,024	0,026	0,093	0,037
STAZ s8 500m	2,400	3,600	1,200	1,400	1,800	0,012	0,019	0,006	0,037	0,005
STAZ s16 SUP	224,800	21,600	15,200	3,200	2,400	1,490	0,094	0,148	0,033	0,027
STAZ s16 50m	191,200	24,000	66,400	12,800	4,000	1,405	0,140	0,376	0,403	0,027
STAZ s16 DCM	105,600	21,600	76,000	6,400	6,400	0,957	0,153	0,400	0,409	0,058
STAZ s16 200m	6,800	7,000	5,600	2,000	4,000	0,034	0,027	0,047	0,106	0,014
STAZ s16 500m	3,000	3,600	1,400	1,200	1,600	0,013	0,013	0,002	0,019	0,003
STAZ s18 SUP	188,000	24,800	23,200	8,800	1,600	0,797	0,134	0,070	0,134	0,000
STAZ s18 175m	16,800	12,000	12,000	8,000	0,000	0,073	0,026	0,067	0,250	0,000
STAZ s18 500m	2,200	5,400	1,000	0,200	0,600	0,006	0,014	0,005	0,001	0,002
STAZ s19 SUP	121,600	12,800	16,000	8,800	0,800	0,561	0,076	0,059	0,200	0,011
STAZ s19 DCM	89,600	24,000	22,400	12,800	0,800	0,407	0,107	0,118	0,244	0,004
STAZ s19 100m	82,400	9,600	35,200	8,800	2,400	0,536	0,034	0,146	0,249	0,010
STAZ s19 200m	5,800	8,600	3,800	5,800	1,800	0,028	0,029	0,024	0,126	0,008
STAZ s19 500m	2,800	3,800	0,400	0,800	0,200	0,013	0,015	0,001	0,008	0,000
STAZ s20 SUP	79,200	13,600	8,800	4,000	0,000	0,478	0,073	0,052	0,019	0,000
STAZ s20 DCM	85,600	16,800	16,000	5,600	3,200	0,339	0,041	0,073	0,210	0,003
STAZ s20 FONDO	49,600	8,000	13,600	0,800	3,200	0,216	0,027	0,088	0,029	0,084
STAZ 451 SUP	168,800	71,200	36,000	8,000	0,000	1,804	0,515	0,154	0,242	0,000
STAZ 451 50m	52,000	74,400	36,800	5,600	0,000	0,611	0,400	0,096	0,145	0,000
STAZ 451 DCM	48,800	68,800	70,400	1,600	0,000	0,358	0,349	0,338	0,044	0,000
STAZ 451 200m	4,400	36,800	6,800	2,800	1,200	0,020	0,167	0,031	0,031	0,034
STAZ 451 500m	1,600	6,800	1,200	1,200	1,600	0,007	0,038	0,007	0,066	0,016
STAZ 460 SUP	106,400	50,400	30,400	8,000	0,000	1,058	0,516	0,081	0,338	0,000
STAZ 460 50m	228,800	75,200	96,000	24,000	1,600	1,262	0,489	0,555	1,052	0,057
STAZ 460 DCM	42,400	73,600	84,800	14,400	3,200	0,443	0,428	0,313	0,213	0,019
STAZ 460 200m	5,600	9,600	4,800	4,800	5,600	0,021	0,033	0,028	0,230	0,082
STAZ 460 500m	9,600	10,400	11,200	2,400	2,400	0,051	0,056	0,053	0,097	0,013
STAZ 405 SUP	179,200	57,600	33,600	11,200	0,000	1,440	0,303	0,166	0,405	0,000
STAZ 405 DCM	140,800	96,000	187,200	19,200	0,000	0,862	0,616	0,536	1,163	0,000
STAZ 405 92m	34,400	32,800	22,400	3,200	11,200	0,249	0,229	0,153	0,119	0,063
STAZ d2 SUP	280,800	30,400	34,400	5,600	0,000	1,231	0,105	0,146	0,148	0,000
STAZ d2 50m	235,200	35,200	51,200	9,600	6,400	1,507	0,190	0,146	0,289	0,083
STAZ d2 DCM	96,000	19,200	48,000	14,400	8,000	0,604	0,061	0,321	0,213	0,102
STAZ d2 200m	9,600	5,600	4,400	4,800	5,600	0,069	0,022	0,022	0,127	0,035
STAZ d2 500m	2,000	0,600	1,600	1,400	2,800	0,019	0,005	0,006	0,049	0,012
STAZ d7 SUP	264,000	30,400	24,000	6,400	1,600	1,052	0,129	0,129	0,196	0,023
STAZ d7 50m	220,800	24,000	46,400	14,400	1,600	1,122	0,099	0,327	0,228	0,009
STAZ d7 80m	156,800	36,800	107,200	9,600	17,600	0,714	0,170	0,614	0,289	0,108
STAZ d7 200m	2,000	7,600	3,600	0,800	2,000	0,032	0,022	0,015	0,013	0,006
STAZ d7 500m	1,200	2,600	1,000	1,200	3,000	0,021	0,009	0,004	0,006	0,048