On the role of local and global physical forcing to space–time dynamics of microbes: a case study from the Southern Seas

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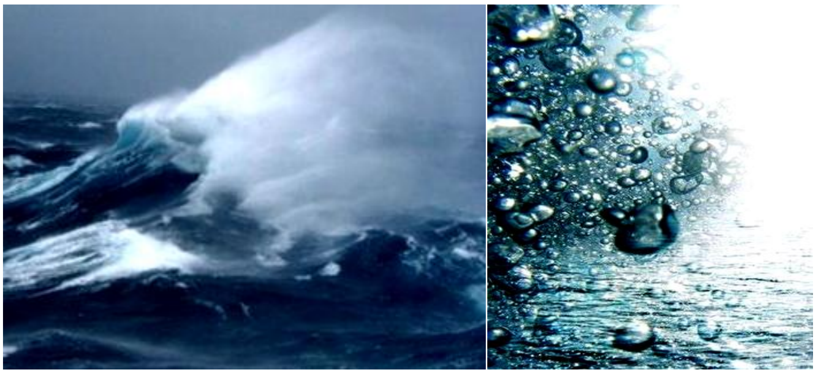
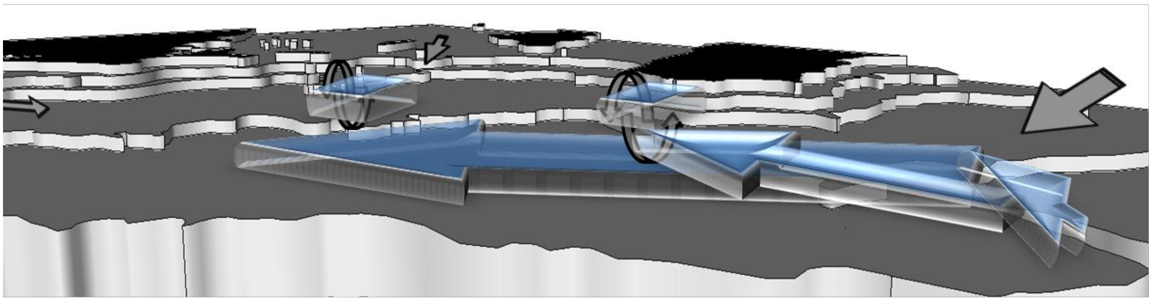


Thesis submitted for the degree of Doctor of Philosophy

Supervisor: Pr. Laurent Seuront
Co–supervisors: Assoc. Pr. Jim Mitchell, Dr. Justin Seymour



School of Biological Sciences
Flinders University of South Australia

To my parents (Titane, Marc), grand–parents (Anne,
Charly, Monique, Fernand), god–mother (Sophie), and
god–father (Christophe) who passed on to me their
enthusiasm for the ocean, travel, and nature

To my sister (Charlotte), brothers
(Alexandre, Edouard), and dearest friends
(Raphaèle, Julie, Florence, Benoit)

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Summary

Global climate changes urge scientists to understand the effect of plankton communities on carbon cycle in aquatic systems. Although the microbial food web was for long overlooked, its potential importance to future oceanic and coastal systems has been stressed. Being less than 3 µm diameter in size, picophytoplankton, heterotrophic bacteria and viruses are all major components of the microbial food web. These can reach abundances of respectively 10^5, 10^6, and 10^8 cells mL^{-1} and have a key role in marine carbon and energy transfer. However, the existence of diverse microbial populations may differently respond to changes in physical forcing, hence affecting the fate of organic matter and the efficiency of carbon and energy transfer of aquatic systems.

The present research aimed to improve our understanding on the temporal dynamics in microbial community structure, with specific emphasis on physical forcing. Flow cytometry was used throughout this work to identify and enumerate distinct microbial populations. First, the responses of discrete heterotrophic bacterial populations to local short–term environmental fluctuations were investigated within the Polar Frontal Zone of the Southern Ocean. Secondly, the local temporal dynamics of distinct picophytoplankton populations in relation to local physical events (i.e. upwelling, downwelling) influenced by local (wind) and global (El Niño/La Niña) climatic forcing were examined for the first time within the South Australian continental shelf waters. Finally, the relative importance of local to global hydroclimatic forcing on the long–term dynamics in picophytoplankton, heterotrophic bacteria, and viruses abundances and their relationships were investigated at the national reference station of the Southern Australian shelves.

Overall, results showed that the temporal variability in both picophytoplankton and heterotrophic bacterial community structure depended upon the sampled depth's properties, and, indicated that physical events of distinct nature differentially influenced various nodes of the microbial food web. The most abundant population or group often presented relatively little variability over time, but the least abundant population varied the most, suggesting that for different levels of organisation, microbes might present a constant *vs* sporadic behaviour over distinct time scales. In the South Australian continental shelf waters, the potential existence of distinct ecotypes of *Prochlorococcus*, *Synechococcus* and picoeukaryotes were reported with an unexpected southern extension of a High–Light and Low–Light adapted ecotypes of *Prochlorococcus*, likely due to advective transports. Upwelling and downwelling conditions associated to changes in the nature and intensities of stratification and mixing processes were found responsible for the local dominance of distinct picophytoplankton populations. The relationship between these dominant populations and upwelling conditions further showed the relative importance of local (wind field) and global (El Niño/La Niña) hydroclimatic forcing to picophytoplankton community structure. The distinct long–term temporal dynamics of picophytoplankton, heterotrophic bacteria, and viruses abundances reflected the temporal and vertical variability in salinity and temperature gradients associated to distinct upwelling and downwelling conditions. These

revealed for the first time a vertical decoupling of viruses and bacteria during upwelling of an El Niño event. The present research has major implications to the functioning of distinct ecosystems with global changes.

Acknowledgements

There is so much to learn from our environment, but we can only do one thing at a time. I was glad to accept the challenge presented by this field of oceanography, a fascinating world in itself, and one with so many still unanswered questions. For this, I am hugely indebted to both Jim and Laurent whom I met one day for lunch in France and for their faith in me since that first meeting. Or should I say, I am glad that I didn't miss my train that day and my flight to Australia a few months later, and that I was sufficiently determined to undertake a PhD thesis in Australia. Indeed, it has awakened in me some special interests, though, ironically, considering the title of the present thesis, managing time has always been a struggle.

This present work could not have been achieved without the help of my supervisor Pr. Laurent Seuront and my co–supervisors Ass. Pr. Jim Mitchell and Dr. Justin Seymour each of whom provided me with the valuable little push that I needed at times to get back on track, thank you.

I think the *simplest* way to express my *best* gratitude towards Pr. Laurent Seuront, is to give a fair representation of his words ('The simpler, the better') which I probably heard a thousand times throughout this journey. My most grateful thanks for his support, his interesting views of science, his positivism in life, and his encouragement all along this thesis. I am also thankful to both, Assoc. Pr. Jim Mitchell and Dr. Justin Seymour, for their valuable support, advice, and for changing my own perception with regard to this microscopic world.

I am grateful to Pr. John Middleton, Dr. Charles James, and Dr. John Luick, for all their valuable help in physical oceanography, support, and advice throughout this thesis. Thanks also to Dr. Charles James, Dr. Sophie Leterme, Dr. John Luick, Dr. Carlos Teixeira, and Shaun Byrnes for their great help with sampling, organization, and company during cruises.

I also particularly thank Dr. Paul van Ruth for his precious help and thoughtful advice in biological oceanography all along this thesis, and, Laura Richardson for her encouragements and discussions in regards to the circulations patterns of the Southern Australian shelf waters.

Further thanks go to the crews of the SAZ–SENSE and the SAIMOS/SARDI for the fantastic oceanographic voyages and their responses to my many enquiries about oceanography and to the crews of the RV *Aurora Australis* and RV *Ngerin* for the fantastic job they are doing and valuable help they are providing at sea.

I was pleased to get valuable technical advices from Eugen, Sheree Bailey, and Dr. Peter Macardle with the flow cytometry and I thank them for their time.

Thanks for the administrative and IT staff of Flinders University and SARDI who are always there whenever needed. In particular, I thank Kim, Andrew, Brett, Raphael, and Louise.

Thanks to all Lab Members and in particular the old crew, Mark Doubell, Tom Jeffries, and Trish Lavery for their help in introducing me to Flinders, the

Coorong, and the flow cytometry: to Mark for introducing me to Adelaide's lifestyle, to Trish for her company and help at sea, and to Tom for all his coffee times, discussions, and checking of references, specifically in the last run of our PhD. Here we are finally getting to the end of this journey. Thanks to James Paterson for his valuable help with the cruise organization, and to Kelly Newton, Ben Roudnew, Nardi Cribb, Crystal Beckman, Renee Smith, Paul Rodgers, and Eloise Prime for all the discussion times, science retreat, and field trips we had. Thanks to Coraline Chapperon for her support, and help during the last few days with the formatting of references of this thesis, but also with the packing of my accumulated stuff over this journey.

My special thanks to Coraline and Hamda for their invaluable friendship throughout all the difficult and great times in Adelaide; to my old friend Charlie for remaining his true self and introducing me to his shark world; to my dear friend Patt for sharing his passion, his hospitality, and the superb times we had together, and to my great housemates and friends Shorty, Summa, Guillaume, Fanny, and Sasha for all the many discussions and the shared experience. Thanks to all my mates for all the happy times over my stay in Adelaide.

Finally, I could not have done this PhD without the indefatigable support and inspiration of my dear family and friends from home, and so this present work is dedicated to them.

Declaration

'I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.';

Virginie FM van Dongen–Vogels

1st August 2011

I. General Introduction:

1. Background concepts and the importance of microbial community structure

Global changes and increases in CO_2 into the atmosphere have led scientists in the urge to understand how and up to which degree planktonic communities affect carbon cycle in aquatic systems (IPCC 2007). Oceanic ecosystems are responsible for about half of the Earth's primary production with phytoplankton being a key component of the marine carbon cycle (Behrenfeld et al. 2006). Estimates of global oceanic primary production ranges from 35 to 65 Gt C yr^{-1} (e.g. Field et al. 1998; Morel and Antoine 2002; Carr et al. 2006), while oceanic respiration has been estimated at about 55 to 76 Gt C yr^{-1} (del Giorgio and Duarte 2002). In the ocean, primary production reflects the amount of inorganic carbon fixed by phytoplankton through photosynthesis. As such, production is dominated by phytoplankton and is a major link in the carbon cycle between organic and inorganic stocks, a key regulator of ecological processes, and a major determinant of carbon sinks (Field et al. 1998). Part of this production will then be respired by heterotrophic organisms. This carbon exchange by both photosynthesis and respiration has been referring as the largest biogeochemical cycle in aquatic ecosystems (Brix et al. 2006; Hashimoto et al. 2006). The net production of this whole plankton system, which refer as the net community production (NCP) or metabolism of an ecosystem is then typically evaluated to assess the amount of organic matter available for export to the benthos or to adjacent ecosystems and for transfer to higher trophic levels (Smith and Kemp 1995). The evaluation of this NCP or metabolism has thus a key role in the understanding of oceanic biogeochemical cycles (Falkowski et al. 1998) and in explicitly defining the role of ecosystems as sources or sinks for atmospheric CO_2. Addressing the question of whether the ocean biota locally or globally act as a net source or sink of carbon (Williams 1993, 1998) has thus become a priority research objective in order to understand the role of the oceans in the global carbon cycling and to predict the ocean's response to global climate change (del Giorgio and Duarte 2002; Robinson and Williams 2005).

While it would be interesting to predict net plankton community production, many studies have, however, encountered issues which highlighted the importance of the temporal and spatial dynamics in plankton community structure (Serret et al. 2001; del Giorgio and Williams 2005; Jouenne et al. 2007). Indeed, phytoplankton community composition has been shown to modify the composition and abundance of higher trophic communities, altering the functional structure of the food webs and ultimately the entire ecosystem (e.g. Karl et al. 2001). However, research has essentially been devoted to coastal regions where productivity are high with the typical view that large phytoplankton cells (>20 µm) mainly dominated in terms of biomass, production, and carbon exports, leaving the ecology of microbes to be overlooked for many decades.

Being less than 3 µm diameter in size, picophytoplankton, heterotrophic bacteria and viruses are all major components of the picoplankton and microbes of aquatic systems. Past studies have for the last decades successively revealed that these can reach abundances of respectively 10^5, 10^6, and 10^8 cells mL^{-1}, and have a key role in marine carbon and energy transfer. The important contribution of autotrophic and heterotrophic microbes to water column production and respiration was first observed in the 70s (Pomeroy 1974; Sieburth et al. 1978). The recognition of the conceptual microbial loop (Azam et al. 1983) and the subsequent discovery of two major groups of cyanobacteria, *Synechococcus* (Johnson and Sieburth 1979; Waterbury et al. 1979) and *Prochlorococcus* (Chisholm et al. 1988), further stressed the importance of distinct trophic pathways (i.e. the microbial food web and the herbivorous–carnivorous food chain; Fig.1). The role of picophytoplankton and heterotrophic bacteria in carbon and energy fluxes in marine ecosystems was further investigated in the early 90s (Griffith et al. 1990; Chisholm et al. 1992; Campbell and Vaulot 1993; Sherr and Sherr 1996; Del Giorgio et al. 1997). These studies showed that heterotrophic bacteria accounted for up to 80% of the respiration in marine systems (Griffith et al. 1990; Sherr and Sherr 1996), and that 30 to 60% of primary production could be processed by autotrophic bacteria in freshwater and marine systems (Del Giorgio et al. 1997), with a large amount of energy and matter likely being directed to the bacterioplankton (Azam et al. 1993). In fact, in contrast to large phytoplankton cells, picophytoplankton show high efficiency in nutrient uptakes due to both their high surface to volume ratio and thin diffusive boundary surface layer (Raven 1998). As such, picophytoplankton are seen as being favoured in low nutrient conditions (e.g. Brink et al. 1995), whereas large chain–forming diatoms are more effective in the uptake of nutrients–rich waters and have faster sinking rates (Hutchings et al. 1995). Hence, the conformist view was that if large diatoms become dominated by smaller and less rapidly sinking picophytoplankton, export could be less efficient despite higher carbon fixation rates in surface waters. However, this view has been questioned and recent studies have shown the existence of distinct indirect and direct pathways for which picophytoplankton could contribute to vertical export by sinking (e.g. Waite et al. 2000; Richardson and Jackson 2007; Stuckel and Landry 2010; Fig. 1). Furthermore, the simple segregation between small and large phytoplankton communities, and in general the distinct plankton components is oversimplifying the ecological responses of phytoplankton to global change in the ocean. For instance, distinct picocyanobacteria strains may respond differently to future CO_2 and temperature increases (Fu et al. 2007). Finally, concurrently to these studies, the ecological importance of viruses in the recycling of organic and inorganic carbon was recognized with their link to the microbial food web (Fig. 1), viruses observed in aquatic environments being mainly bacteriophages (Bergh et al. 1989; Proctor and Fuhrman 1990; Suttle et al. 1990; Thingstad et al. 1993; Fuhrman 1999; Wilhelm and Suttle 1999; Bratbak and Heldal 2000). Both viruses and microzooplankton are known to control picoplankton dynamics allowing for the transfer of energy towards higher trophic levels (Fuhrman 1999; Fig. 1).

Since these discoveries, the physiology, ecology, and genetic of marine microbes have been the topic of increasing investigations concurrently with the development of advanced technologies (i.e. flow cytometry, DNA sequencing) and have been subsequently reviewed over the years (e.g. Stockner 1988; Partensky et al. 1999; Wommack and Colwell 2000; Pernthaler and Amann2005; Legendre and Rivkin 2008; Cermeño et al. 2010). It is now known that

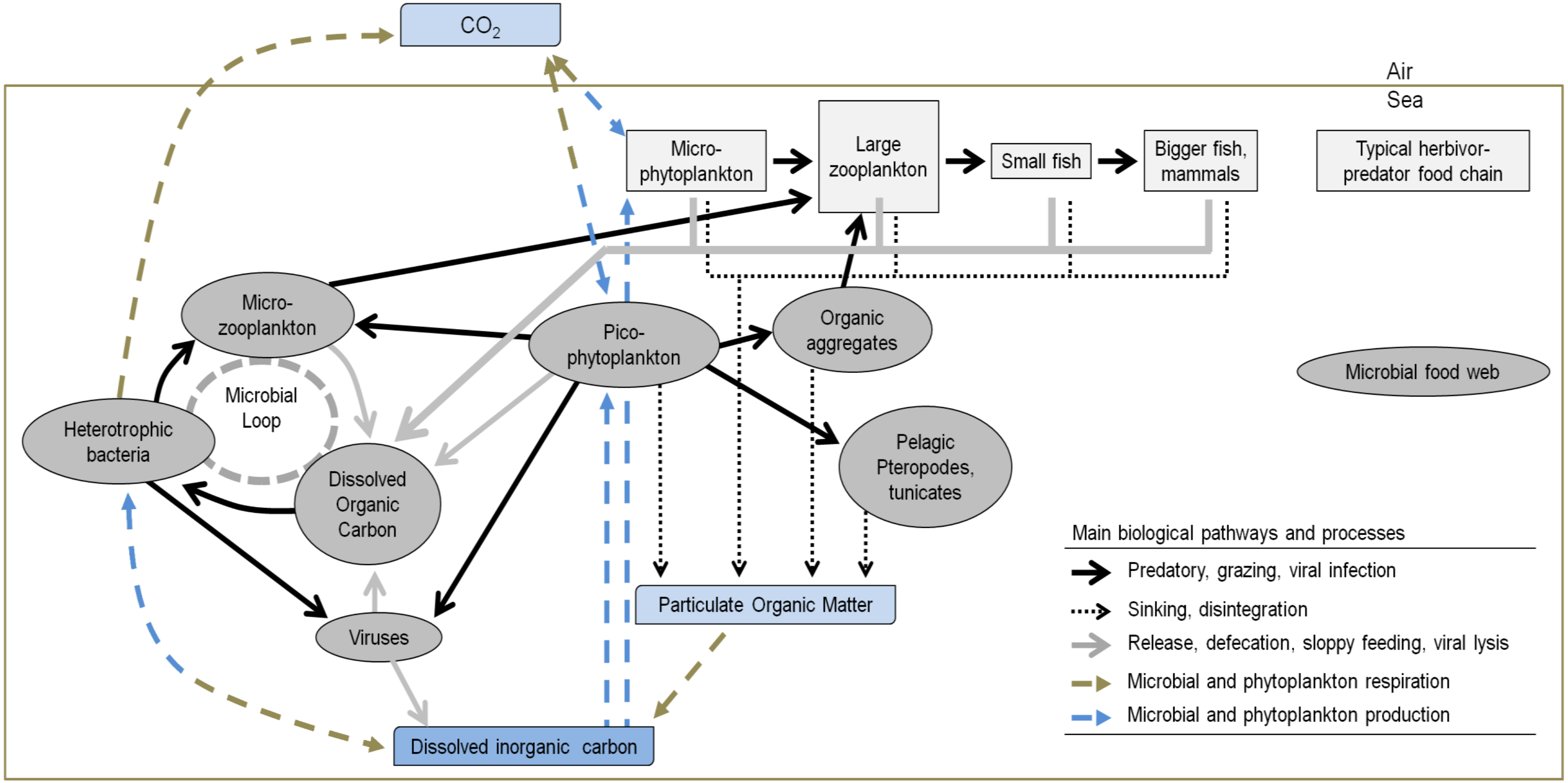

Fig. 1. Schematic microbial food web and the typical herbivor–predator food chain showing the main biological pathways and processes. Adapted from various diagrams (e.g. http://www.nano–reef.com/forums/lofiversion/index.php/t260742.html).

picophytoplankton include thee major groups of *Synechococcus*, *Prochlorococcus*, and picoeukaryotes which ones have been revealed to be further structured by a variety of clades, ecotypes, and strains differing in their physiological and ecological properties (e.g. Díez et al. 2001, Rocap et al. 2003, Johnson et al. 2006). For instance, two populations of *Prochlorococcus* differing in their amount of chlorophyll content per cell were successively identified using flow cytometry in the central Pacific (Campbell and Vaulot 1993), the eastern Atlantic (Partensky et al. 1996), and the western tropical Pacific Ocean (Blanchot and Rodier 1996). These appeared at distinct depths of the water column, being able to adapt to low or high light levels. Their isolation further revealed the genetic division of distinct Low–Light (LL) and High–Light (HL) ecotypes of *Prochlorococcus*. Further ecological studies have shown that these ecotypes also differed in their nutrient regime, revealing the potential of *Prochlorococcus* to carbon export (Johnson and Lin 2009). Heterotrophic bacteria are also known to present a great diversity with populations of distinct activity levels, and strains (Middelboe et al. 2001; Pernthaler and Amann 2005). As such, the relative importance of distinct microbial populations could be particularly important to the fate of organic matter and effectiveness of remineralisation processes throughout the water column (Gattuso et al. 2002; Pernthaler and Amann 2005; del Giorgio and Williams 2005; Brix et al. 2006; Jouenne et al. 2007), affecting the direction and efficiency of oceanic carbon and nutrients fluxes and ultimately atmospheric CO_2 concentration and climate (e.g. Arrigo et al. 1999; Chavez et al. 2003; Cloern and Dufford 2005; Brix et al. 2006). Hence, understanding the functioning of an ecosystem is above all to understand the dynamics of microbial community structure both spatially and temporally.

Size fractionations studies have shown their interest in the succession dynamics of distinct phytoplankton cells size. Some studies have thus suggested that phytoplankton size structure had an important consequence in the magnitude of 1998, Bell and Kalff 2001, Cermeño et al. 2006). In contrast, others (Brown et al. 2004, Lopèz–Urrutía et al. 2006, Jouenne et al. 2007) have shown that because when referred to carbon–use efficiency, net primary production does not depend on cells size (see e.g. the metabolic theory of ecology by Brown et al. 2004). More recently, phytoplankton cells size distribution was shown to only partially explain variations in primary production (Jouenne et al. 2007). High Performance Liquid Chromatography (HPLC) has also shown its value to discriminate between distinct phytoplankton populations of distinct physiological characteristics and genetic and metagenomic methods are of great interest to get to know the distribution of microbes (Cermeño et al. 2010), though these techniques do not account for cells densities and/ or are often expensive. Flow cytometry have been shown to be effective in getting rapidly reliable numbers of microbial cells and to allow for the identification of discrete populations of distinct physiological properties (Campbell and Vaulot 1993, Gasol et al. 1998, Marie et al. 1999, Lebaron et al. 2001, Brussaard 2004, Seymour et al. 2005). This latter method will be thus carried throughout the present work in order to identified and enumerate the different picoplankton populations in their environment.

2. Temporal variability in microbial community structure

Picophytoplankton and heterotrophic bacterioplankton are ubiquitous in the pelagic realm of both freshwater and marine ecosystems (Johnson and Sieburth 1979; Stockner et al. 2000), but distinct populations have been shown to be segregated, overlap or succeed to each other both spatially and temporally (e.g. Olson et al. 1990; Partensky et al. 1996; Campbell et al. 1997; Partenky et al. 1999; Rocap et al. 2003; Johnson et al.2006; Calvo–Díaz and Morán 2006). Hence, these distributions and dynamics of distinct populations suggested that these are controlled by different environmental factors (e.g. Partensky et al. 1996) with distinct relative contributions of top–down and bottom–up processes (e.g. Pace and Cole 1994).

Short–term temporal variability in picoplankton are mainly dictated by the daily variations in sunlight levels and their high turn–over rates typically let them to show a rapid response to environmental variations. The scale of the diel cycle is thus relevant to fluctuations in biomass, abundance, production and losses of microbes (Jacquet et al. 2002; Seymour et al. 2005; Hewson et al. 2006). Most diel studies related to microbial community structure have, to our knowledge, been conducted either under controlled conditions or in temperate and tropical coastal waters (e.g. Gasol et al. 1998; Shiah 1999, Bettarel et al. 2002; Seymour et al. 2005). Furthermore, microbial community structure has shown weak to strong seasonal dynamics which have appeared to be recurrent over years in some region such as in the continental shelf waters of the Bay of Biscay (Calvo–Díaz et al. 2008) or at the station BATS (Malmstrom et al. 2010). On the other hand, *Prochlorococcus* have been observed to dominate the picophytoplankton communities most of the year in oligotrophic oceanic waters such as in the tropical and subtropical Atlantic Ocean where its abundances can be greater than 10^5 cells mL^{-1} (Chisholm et al. 1988; Campbell et al. 1997; DuRand et al. 2001). However, the dominance of *Prochlorococcus* does not seem to be restricted to tropical oceanic waters. For instance, in the western tropical Pacific Ocean, the relative contribution of each picophytoplankton groups was found to be up to 62%, 51%, and 20% for picoeukaryotes, *Prochlorococcus*, and *Synechococcus*, respectively (Blanchot and Rodier 1996). In contrast to *Synechococcus* and picoeukaryotes, *Prochlorococcus* seems to be bounded by latitudes of about 40 °N and 40 °S (Partensky et al. 1999) and has been shown to disappear during the winter–spring periods in continental shelf waters (e.g. Worden et al. 2004; Calvo–Díaz and Morán 2006, 2008). The vertical variability in picophytoplankton groups is thought to be caused by the differences in light sensitivity and/or adaptation related to the pigment content of each group (e.g. Campbell and Vaulot 1993; Veldhuis and Kraay, 1993), the depth of the nitracline and/or the mixed layer depth, and temperature variations (Partensky et al. 1999; Agawin et al. 2000). Distinct phytoplankton communities forming deep chlorophyll maximum layers within the water column have previously been documented (e.g. Brunet et al. 2006). Deep chlorophyll maxima have also been shown to be dominated by picophytoplankton, particularly by *Prochlorococcus* in warm oligotrophic waters (e.g. Kuipers and Witte 2000; Brunet et al. 2006). The causes of seasonal changes in phytoplankton community structure of deep chlorophyll maxima remains however difficult due to the various physical and biological factors affecting these layers (e.g. Kuipers and Witte 2000; Brunet et al. 2006). For instance, deep chlorophyll maxima may appear as a boundary layer between the nutrient–poor surface layer and nutrient–rich deep layer (e.g. Kuipers and Witte 2000) such as nutrients of the deep chlorophyll maximum depends on mixing and diffusion processes with seasonal changes in the relative position of the thermocline. Deep chlorophyll maxima are also found at the bottom of the euphotic zone such as light irradiance as low as 0.1% could be more favorable to *Prochlorococcus* than picoeukaryotes (e.g. Kirk 1983; Kuipers and Witte 2000). Finally, studies on the long–term (decadal) variability in picoplankton community structure have remained restricted to local systems where ocean observing systems have been put into places (e.g. Malmstrom et al. 2010). These recent studies have provided information on both the resilience and shifts in picophytoplankton communities.

3. Role of physical forcing in picophytoplankton community structure

Decades of efforts have been devoted to the importance of physical processes to the dynamic of large phytoplankton and the seasonal succession of distinct phytoplankton cells size has probably been one of the most studied phenomena (e.g. Margalef 1967; Longhurst 1995, 1998; Estrada and Berdalet 1997). However, it is only over the last decade that distinct picophytoplankton communities have been shown to respond to the varying physical environment through the interplay of temperature, light, vertical mixing, advection, stratification, and nutrient supply. Heterotrophic bacteria, while also responding to physical forcing, will in turn be mainly constrained by the magnitude and timing of this primary production and the relative importance of allochthonous and autochthonous dissolved organic matter.

Stratification has been observed to affect both plankton community composition and production (e.g. Pitcher et al. 1991; Olesen et al.1999; Le Quéré et al. 2003; Field et al. 2004; Salihoglu 2005) by restricting the exchange of nutrients between deep and surface layers (e.g. Moncoiffé et al. 2000; Carmack et al. 2006; Strom et al. 2006). In contrast, the seasonal or episodic mixing events generated by density gradients, winds, and currents modify the physical and chemical environment of planktonic communities (Jin et al. 2006). By affecting the irradiance levels and/or the amount of nutrients of the water column, different mixing regimes (intensity of mixing) can result in differences in plankonic community composition and physiological activity (Lizon et al. 1995). Vertical mixing usually result in the enrichment of nutrients of the euphotic zone by the deepening of the surface mixed layer. While vertical mixing may offset the effect of nutrient limitation (Olesen et al. 1999; Szeligiewicz 1999; Jin et al. 2006), light conditions may become unfavourable to phytoplankton growth. Hence, the temporal variability in mixing and stratification processes has been observed to affect picophytoplankon community structure, with the old view that picoeukaryotes dominate bulk abundances during winter mixing, whereas the dominance of *Prochlorococcus* occur during summer stratification (e.g. Partensky et al. 1999; Fig. 2). *Prochlorococcus* have indeed been reported to be often absent from mixed waters (Chisholm et al. 1988; Veldhuis et al. 1993; Lindell and Post 1995). However, reports of *Prochlorococcus* in mixed waters in e.g. the Mediterranean and Sargasso Seas (Vaulot and Partensky 1992; Goericke and Welschmeyer 1993) suggest that mixing may not always be a constraint to the growth of *Prochlorococcus*. Changes in stratification intensity have recently been shown to influence the distribution of distinct ecotypes of cyanobacteria, specifically in the subtropical Pacific and Atlantic oceans (Bouman et al. 2006, 2011). Whether this latter is also true for continental shelf system remains however poorly quantified.

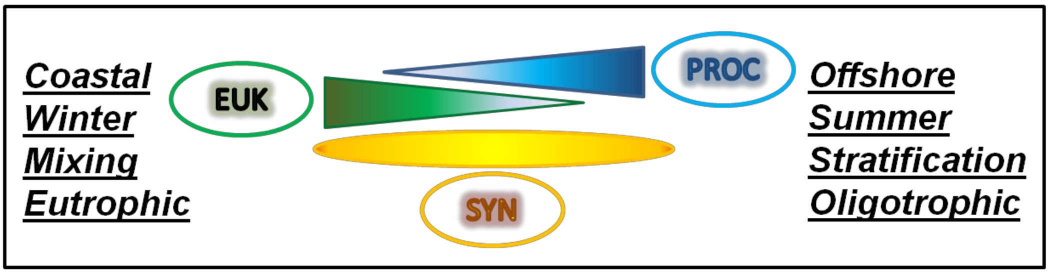

Fig. 2. Schematic old view of the spatial and temporal variability in picophytoplankton community structure. *Prochlorococcus* (PROC) abundances (blue triangle) is often higher in offshore, oligotrophic, warm, and stratified waters, contrasting with picoeukaryotes (EUK) abundances (green triangle) being often higher in coastal, eutrophic, cold, and mixed waters, while *Synechococcus* (SYN) abundances (yellow ellipse) has been reported to be high in either situation.

Besides, recent molecular approaches have also revealed the large picoeukaryotes diversity which has let to question their role and function in diverse marine systems (e.g. Díez et al. 2004, Massana et al. 2004, 2011). The diversity of picoeukaryotes may hence reveal various adaptation processes and change the old view presented in Fig.2.

The effect of stratification on phytoplankton community structure of an ecosystem may not only depend on stratification intensity but also on the type, frequency, timing, and duration of stratification, as well as on the environmental conditions preceding the stratification period (e.g. Strom et al. 2006). Advection transports and water mass intrusion have also been shown to affect picoplankton community structure over the winter–spring period (e.g. Calvo–Díaz et al. 2004; Worden et al. 2004; Mitbavkar et al. 2009). For instance, the intrusion of warm Kuroshio waters in Sagami Bay, have been shown to be responsible for increasing cyanobacterial abundances, but low abundances of picoeukaryotes and heterotrophic bacteria (Mitbavkar et al. 2009).

Furthermore, coastal and equatorial upwelling events bring deep nutrient–rich waters up to the surface as a result of the Coriolis Effect and the Ekman transport of the surface layer offshore or the divergence of surface water away from the equator, respectively. Such nutrient pulses are usually followed by changes in plankonic community composition and enhanced primary production of the euphotic zone. Variations in upwelling conditions have been observed to vary geographically and can occur seasonally or over a cross–shelf axis or a given shelf domain. Shelf basin water properties exchange may also be enhanced by upwelling in canyons (e.g. Carmack and Kulikov 1998). Coastal upwelling in the ocean's eastern boundary currents are well known to sustain high productivity and fisheries (e.g. Barber and Smith 1981; Bakun 1996). As a result, the importance of picoplankton community structure and the microbial food web has been less studied in such systems (e.g. Hall and Vincent 1990; Sherr et al. 2005; Echevarría et al. 2009; Linacre et al. 2010), and thus imbalanced with the greater current knowledge on physical–biological coupling processes implying the typical herbivorous food chain.

Finally, changes in the duration and intensity of stratification (e.g. Arrigo et al. 1999; Bopp et al. 2001), as well as of upwelling events (Bakun 1990, 2010) could be important with global changes. This may imply major shifts in picoplankton community structure with consequences to carbon fluxes in the oceans and potential catastrophic feedbacks on global warming (e.g. Sarmiento et al. 1998; Arrigo et al. 1999; Bopp et al. 2001; Behrenfeld et al. 2006; Huisman et al. 2006; Arrigo et al. 2008). This further stresses the need to understand the relationships between plankton community structure and functioning in future ocean and coastal systems. Finally, increasing investigations are made to further understand the importance of viruses to regulate both picophytoplankton and heterotrophic bacteria in diverse environments, though few studies have shown the potential influence of physical forcing to the relationship between viruses and distinct picoplankton populations. In fact, very few studies have investigated the role of viruses in coastal upwelling regions (He et al. 2009).

4. Relevance of temporal dynamics in microbial community structure

The lack of consensus on the factors influencing the temporal distribution of the planktonic community structure certainly prevent any possibility to predict its changes forced by environmental fluctuations resulting from short–term to seasonal, inter–annual, and global changes (e.g. Herrera and Escribano 2006). The observed temporal patterns in total picophytoplankton abundances might reflect that of the diverse picophytoplankton groups which in turn might be the reflection of distinct populations (ecotypes, strains) such as patterns initially observed at the community level exhibit complex behavior when viewed at the group or even greater at the population level (Johnson et al. 2006). Because of this complexity, the temporal patterns in picophytoplankton community structure remains relatively poorly understood, and have appeared to be system dependent (Katano et al. 2005). This is particularly true in continental shelf waters where the hydrological forcing could be particularly important to the temporal variability in picoplankton communities (Jiao et al. 2002; Katano et al. 2005; Calvo–Díaz and Morán 2006).

The temporal dynamics in picoplankton community structure remains poorly understood, particularly in polar environments due to cold and remote conditions and in temperate continental shelf waters due to high dynamics in local hydrographic forcing and circulation patterns. This contrast with observations conducted in oligotrophic subtropical and tropical oceanic waters, where relatively high temperatures and low nutrient conditions typically favor the microbial food web over that of the herbivorous food chain. In addition, picoplankton community structure have shown little variability over short–time scales, and seemed to be relatively resilient over the long–term. Furthermore, both polar and temperate continental shelf waters could be directly and indirectly impacted by the effect of global changes (i.e. sea surface temperatures and sea level rise), which may have tremendous consequences on plankton community structure and functioning. Indeed, the microbial food web is likely to play an important role in the future of these systems (Behrenfeld 2011). Assessing the temporal dynamics of heterotrophic bacteria and picophytoplankton communities would thus greatly improve our ability to understand, and ultimately predict (Anderson et al. 2008), net community productions and the direction of carbon fluxes in oceanic systems.

The importance of temporal variations in the physical, biological, and chemical properties of the water column via oceanographic (e.g. mixing, stratification, advection, mesoscale features) and meteorological, e.g. wind regimes, El Niño Southern Oscillation (ENSO), forcing may have a major impact on the functioning of planktonic communities and pelagic ecosystems (e.g. Williams 1998; Gonzáles et al. 2002; Montero et al. 2007). Time is considered as one of the key variables in the control of microbial processes (Smith and Hollibaugh 1997) which may consequently affect the whole functioning of the ocean (e.g. del Giorgio and Williams 2005; Duarte et al. 2005; Karl 2007). Finally, inter–annual variations stressed by processes linked to global changes also point the importance of investigating the mechanisms driving temporal variations in the picoplankton community structure that are ultimately responsible for the metabolic balance of the considered system. Indeed, questions have recently been raised about the importance of the microbial food web to future oceanic and coastal aquatic systems (Morán 2007; Morán et al. 2010; Behrenfeld 2011). In addition, changes in picophytoplankton abundances and community structure have been seen as the reflection of environmental conditions (Fenchel 1982), and are thus of particular interest for understanding future global changes scenario.

5. Major goal, questions, and outcomes of the present research

The major goal of the present research was to further improve our understanding on the temporal dynamics in picoplankton community structure with specific emphasis on physical forcing. This will help to further our understanding of the functioning of distinct ecosystems in relation to global changes. Each chapter hence addressed distinct questions which all seek to bridge the current gaps in the temporal dynamics of picoplankton community structure. The following sections briefly state the idea behind each question and the sequential relevance of the present research.

Do discrete heterotrophic bacterial populations respond differently to local short–term environmental fluctuations in a remote frontal system of the Southern Ocean?

The idea behind this question was to further elucidate the lack of consensus in the short–term dynamics of bacterioplankton abundances and the gap existing in the temporal studies in bacterioplankton community structure within a remote polar location such as the Polar Frontal Zone of the Southern Ocean. Hence, in contrast to coastal waters where allochthonous dissolved organic and inorganic carbon are likely to influence abundances of bacterioplankton communities, the bacterioplankton communities of the Polar Frontal Zone of the Southern Ocean would mainly depend upon the dynamic of phytoplankton. By sampling at the surface and deep chlorophyll maximum we would also expect changes in the bacterioplankton community structure at the short–term. In addition, the Polar Frontal Zone of the Southern Ocean being one of the major sink of atmospheric CO_2, the above–mentioned question is hence of interest for future investigations of the importance of bacterioplankton community structure in the functioning of this system with global changes.

Does the temporal dynamics in picophytoplankton community structure of the fluorescence maxima are influenced by local seasonal physical forcing along the continental shelf waters of South Australia?

The idea behind this question was first to assess the picophytoplankton populations of the South Australian continental shelf waters since knowledge on picophytoplankton was extremely limited for the region (Seuront et al. 2010; van Ruth et al. 2010). The Southern Australian continental shelf waters exhibits seasonal upwelling events and harbours valuable fisheries and seafood industries, but nothing is known yet on its microbial food web (van Ruth et al.2010), which could be of great importance to the Southern Australian shelf ecosystem (Waite and Suthers 2007). This question is thus a first step into the understanding on the temporal dynamics of picophytoplankton community structure of this system. Secondly, the poor understanding of the role of the hydrological properties to the dynamics of picophytoplankton in continental shelf waters (Jiao et al. 2002; Katano et al. 2005; Calvo–Díaz and Morán 2006) led us to further question the role of localized physical forcing events to the temporal variability in picophytoplankton community structure. This question is of interest for further understanding the role of physical forcing in the functioning of South Australian continental shelf waters. Picophytoplankton communities may highly vary in the dynamic systems of the South Australian continental shelf region typically showing seasonal circulation patterns and upwelling and downwelling events (Middleton and Bye 2007). For this purpose, the temporal dynamics of picophytoplankton populations were investigated for six distinct stations along the continental shelve. In addition, the importance of fluorescence maxima to processes of primary production which were previously related to upwelling events was thus of particular focus for the present investigation (van Ruth et al. 2010).

Does annual variability in picophytoplankton community structure depend on upwelling conditions?

The idea behind this question was to further elucidate the role of upwelling events to the observed annual shift in picophytoplankton community structure. Upwelling events occurring typically during the summer are known to be influenced by local (wind field) and large–scale (El Niño/La Niña) hydroclimatic forcings; the dynamics of picophytoplankton communities may thus be largely influenced by the distinct nature of upwelling events in the continental shelf waters of South Australia. Changing upwelling conditions should affect light, nutrients, and stability properties of the water column, hence picophytoplankton community structure and physiological responses. Typically we would rather expect two distinct situations of upwelling conditions. Strong upwelling might enhanced the homogeneity of picophytoplankton communities over the water column through mixing processes, which may quickly reduce light with depth but enhance nutrient conditions. On the other hand, weak upwelling might result into rather vertical heterogeneity of picophytoplankton communities through stratification processes, which may reduce nutrient concentrations at the surface but light levels reaching deeper layers. However, changing upwelling conditions is not straightforward and instead many distinct situations may be observed, depending on the climatic forcing controlling upwelling events. To adddress this issue, we focused on one station and analysed the structure of the picophytoplankton communities and fluorescence properties of each population inhabiting the surface, fluorescence maxima, and bottom layers, and, compared it with changes in the physical profiles of the water column for three distinct upwelling seasons. This contributes to increase our understanding of the role of upwelling in the South Australian continental shelf waters to picophytoplankton dynamics. In addition, this may improve our perception on the role of distinct picophytoplankton communities under future influence of global changes, specifically for shelf regions affected by upwelling.

Do picophytoplankton, heterotrophic bacteria, and viruses respond differently to local long–term dynamics in hydrophysical forcing? Are their relationships affected by hydroclimatic forcing?

The idea behind these two final questions was to assess the relative importance of local (wind) and large–scale (El Niño/La Niña) temporal variability in climatic forcing on the microbial food web at distinct depths (surface waters, fluorescence maximum, and bottom waters). This may allow for the determination on the local processes directly linked to both upwelling and downwelling conditions subjected to inter–annual variability in intensity. Indeed, the use of vertically–integrated data has often been accounted for the compensation of imbalances over the water column (Williams 1998), but global climate change should lead to a more stratified ocean. Evaluating the effect of stratification on the dynamics of picoplankton communities could hence be of great interest and may help to adequately account for the factor influencing the variability of distinct picophytoplankton populations within the water column and with implications to future biogeochemical models and understanding of the functioning of the South Australian shelf waters. For this purpose, the national reference station of the Southern Australian Integrated Marine Observing System (SAIMOS) was the most relevant location to address this question, as it is on the path of both upwelled and downwelled waters and being sampled every one to three months from February 2008 to July 2010.

The major outcomes of the present thesis have been the submission of three out of the four present chapters to peer reviewers for publication to distinct scientific international journals, the presentation of the present work in its evolution to three distinct international conferences with for one of them the reception of the Ron Kenny award for best student poster and research presentation from the Australian Marine Science Association (AMSA), and the significant contribution of the present work to future research of interdisciplinary research programs. The fourth chapter will be also considered for publication in the near future. Please note that for avoiding recurrent references across chapters, references have all been placed at the end of the present PhD research thesis.

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