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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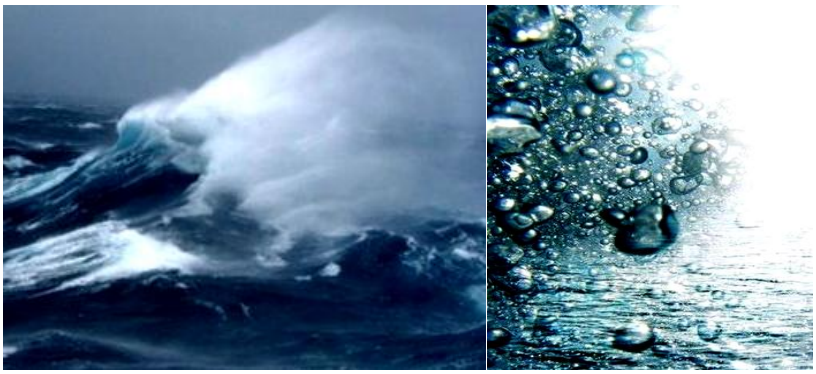
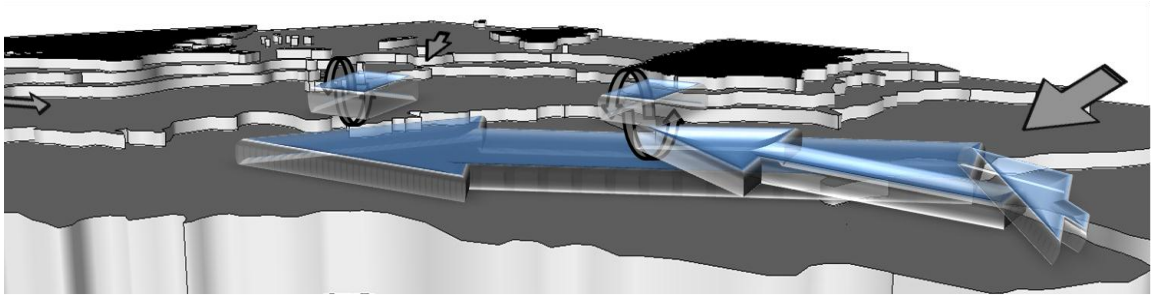


Thesis submitted for the degree of Doctor of Philosophy

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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Table of content:

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.

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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

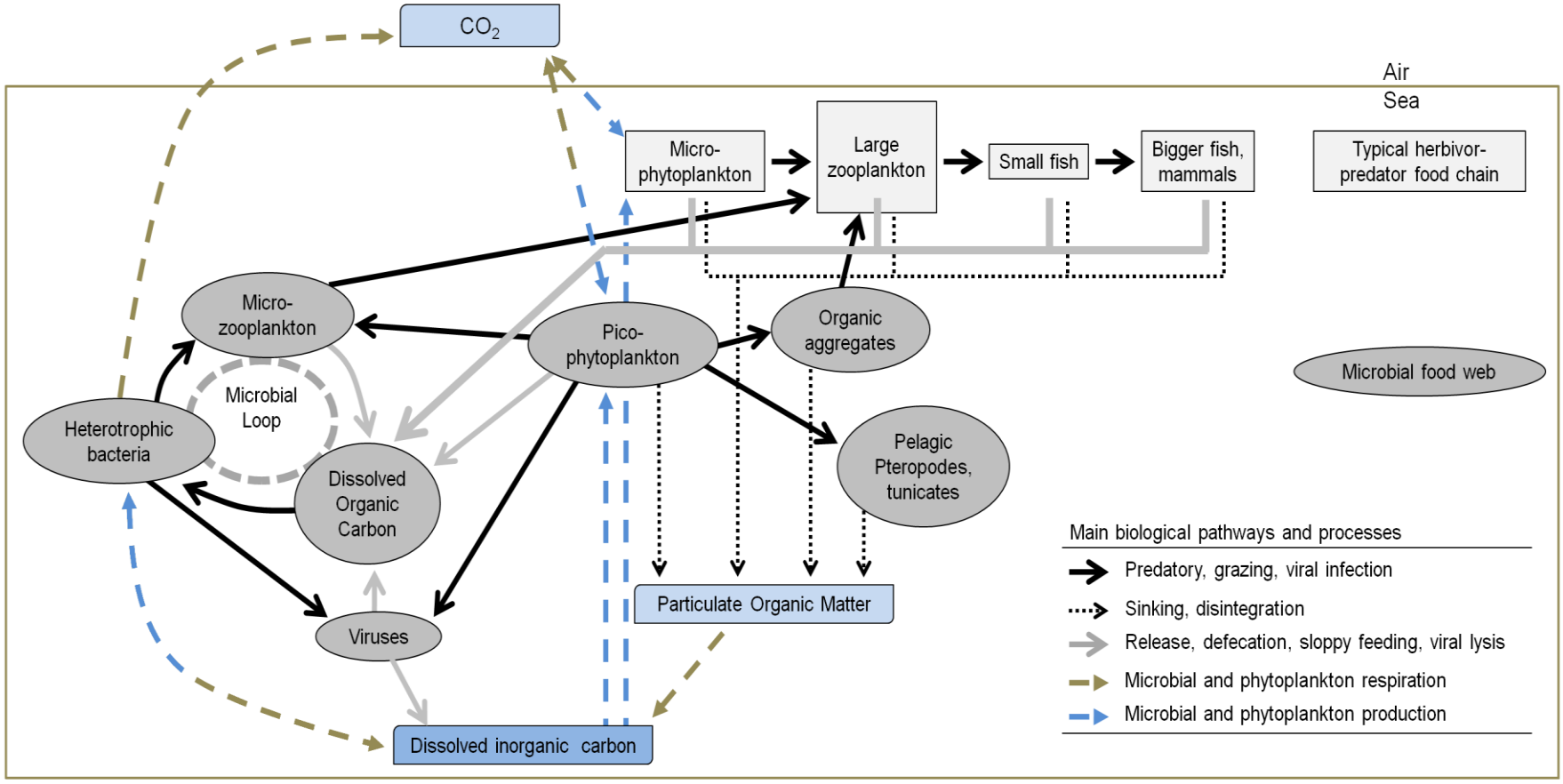

Fig. 1. Schematic microbial food web and the typical herbivor–predator food chain showing the main biological pathways and processes. Adapted from various diagrams (e.g. http://www.nano–reef.com/forums/lofiversion/index.php/t260742.html).

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.

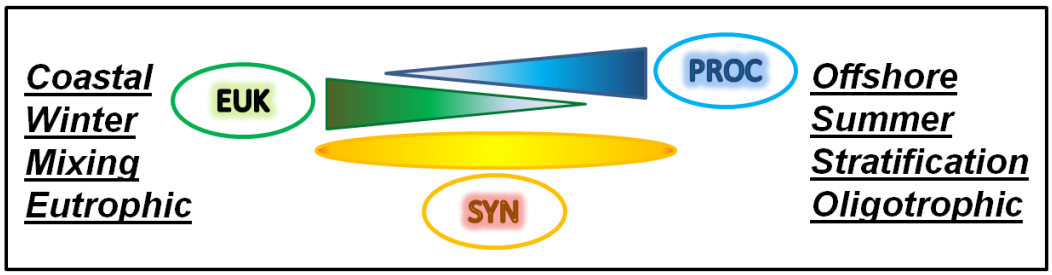

Fig. 2. Schematic old view of the spatial and temporal variability in picophytoplankton community structure. *Prochlorococcus* (PROC) abundances (blue triangle) is often higher in offshore, oligotrophic, warm, and stratified waters, contrasting with picoeukaryotes (EUK) abundances (green triangle) being 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.

- **PEER REFEREED PAPERS**

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- **PAPER TO BE SUBMITTED**

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- **POSTERS:**

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II. Short–term temporal dynamics of heterotrophic bacterial communities within the Polar Frontal Zone (Southern Ocean)

ABSTRACT

Understanding temporal variations in Antarctic waters microbial communities is of particular interest considering the importance of the Southern Ocean to the carbon cycling. In particular, short–term variations in the abundances of bacterial sub–populations provide valuable information on the role of microbes in structuring marine ecosystems. Using flow cytometry, we examined short–term variations in the abundance of the bacterial community and bacterial sub–populations in surface waters (10–20 m) and deep–chlorophyll maximum (DCM 60–80 m) of the Polar Frontal Zone (PFZ). Bacterial communities differed among the two sampled depths in both abundances and short–term patterns, but were similarly consistently made of 4 discrete bacterial sub–populations differing in side–scatter and SYBR–Green fluorescence signals. This structure of the bacterial communities was maintained over 48 hours within the PFZ. These sub–populations similarly increased and decreased in cell abundances in the evening at the surface layer and in the early afternoon at the DCM, respectively. Differences in the magnitude of the observed shifts occurred amongst sub–populations and depths, suggesting their differential contribution to the production and activity of the bacterioplankton in this system.

Keywords: Short–term dynamics, heterotrophic bacteria, Polar Front Zone, Southern Ocean

1. Introduction

Heterotrophic bacteria are critical components of ocean ecosystems (Azam 1998; Arrigo 2005), acting as both remineralisers of organic carbon and trophic mediators (Azam et al. 1983; del Giorgio and Cole 1998). The ecological and biochemical influence of bacteria varies across several space and time scales. Short time scales, such as the diel cycle, are particularly relevant to fluctuations in biomass, abundance, production and losses of heterotrophic bacteria (e.g. Bettarel et al. 2002; Seymour et al. 2005). Previous diel studies have demonstrated a wide–range of temporal dynamics amongst bacterial communities, but no consistent short–term (1 to 2 days) temporal patterns of bacterial abundance have been identified across systems (Table 1).

Most of previous studies, however, have dealt with the heterotrophic bacterial community as a whole, whereas differences in the abundance, activity, and production occur between different populations of a single community (e.g. Gasol et al. 1999; Seymour et al. 2004; Shareck and Latasa 2007). The use of flow cytometry to measure bacterial abundance in aquatic samples has repeatedly revealed the existence of at least two distinct bacterial sub–populations, often containing cells with relatively high (HNA) or low (LNA) levels of nucleic acid content (Gasol et al. 1999). These sub–populations often show divergent spatial and temporal patterns (e.g. Shareck and Latasa 2007), and are typically substantially more variable in abundance than total bacterial counts (Seymour et al. 2004, 2005). Furthermore, as these sub–populations are believed to represent groups with differing metabolic activity (Lebaron et al. 2002) and are differentially impacted by microzooplankton grazing (Gasol et al. 1999), dynamics and patterns of flow cytometrically–defined sub–populations may carry much more ecologically relevant information than total bacteria counts.

The Southern Ocean, and specifically the Polar Frontal Zone (PFZ), is one of the major sink of atmospheric carbon dioxide (e.g. Metzl et al. 1999), although discussions have been recently raised in regards to the weakening of this sink over the last decade (e.g. LeQuéré et al. 2007). Understanding variations in microbial communities in the Southern Ocean is thus of particular interest, but is still relatively limited compared to lower latitudes (e.g. Gasol et al. 1998; Bettarel et al. 2002; Seymour et al. 2005). Largest diel variations in bacterial parameters usually appear in offshore and/or oligortophic waters. However, the short–term temporal variability of bacterial communities in offshore frontal regions such as in the Polar Frontal Zone, where enhance productivity and no coastal influence are found, is yet to be achieved. In this context, the objectives of the present study were (i) to investigate the short–term temporal and diel patterns in the abundances of different bacterial sub–populations as defined cytometrically and (ii) to understand their role in the dynamic of heterotrophic bacterial communities in the surface mixed layer and at the Deep Chlorophyll Maximum in the PFZ of the Southern Ocean.

Table 1. Diel studies of heterotrophic bacteria from diverse environmental trophic conditions. Seawater temperatures (T), chlorophyll *a* concentrations (Chl *a*), bulk bacterial abundances (Bacteria), whether a consistent diel pattern was observed (Diel: Yes or No), and the recorded time period of higher bacterial abundances are given. C: constant, M: morning, A: afternoon, E: evening, N: night. NA: Not available. Note: a: when data were integrated over the water column, a diel pattern was observed with higher bacterial abundances during the night, b: same as 'a' but with higher bacterial abundances observed during the day.

Location	Trophic status	Interval (Total) time (Hours)	Sampled depth	(m)	T (°C)	Chl *a* (µg L^{-1})	Bacteria (× 10^5 mL^{-1})	Diel	Time period of highest abundance	Reference
Mediterranean sea										
Mediterranean Sea	Oligo	6(120)	integrated	0 to150	18.1 to 22.2	14 – 21 (mg m^{-2})				Mével et al.2008
43.25 N 8.00 E			Surface	5 to 40			7.4 to 14.4	N	C	
			below DCM	150 to 1000			0.5 to 1.8	N	C	
Cyprus Eddy	Oligo									Zohary and Robarts 1992
center		4(24)	Surface	9 and 56	27.3	0.02	5.9 to 6.6	N	A	
34.23 N 33.44 E			below DCM	130	16.4	0.09	2.2	N	A/ N	
boundary			Surface	9 and 56	26.2	0.02	2.4 to 4.7	N	N	
33.43 N 35.30 E			below DCM	130	16.3	0.09	1.2	N	A/ N	

Table 1. *Continue*

Location	Trophic status	Interval (Total) time (Hours)	Sampled depth	(m)	T (°C)	Chl *a* (μg L^{-1})	Bacteria (× 10^5 mL^{-1})	Diel	Time period of highest abundance	Reference
Atlantic										
Subtropical Atlantic	Oligo	4.5 or 7.5	Surface	50	26	NA	6.0 to 9.0	Y	M	Kuipers et al. 2000
		(24)	DCM	70 to 140	NA	NA	6.0 to 12.0	Y	M	
Pacific										
East and NorthEast of Taiwan	Oligo (Kuroshio)	3 or 4 (24)	Surface	<0.5	24 to 30	1.1 to 2.6	4.7 to 7.7	N	C	Shiah 1999
Southern California Bight	Meso–oligo		DCM	21 (May)	15.0 ± 0.3	0.4 to 0.8	12.5 to 16.0	Y	Noon	Fuhrman et al.1985
			DCM	9.5 (October)	19.5 ± 0.1	0.3 to 0.5	8.0 to 12.0	Y	M	
Southern Ocean										
South Australian Gulf	Meso–eu (coastal)	0.5 (11)	Surface	0.5	20.7 to 21.3	NA	6.5 to 13.9	Y	E	Seymour et al. 2004
Kergueulen Island plateau	HNLC	3(24)	Surface	0.1	5 to10		1.0 to 9.0	N	Noon/ Midnight	Delille et al.1997
Subtropical Zone	Oligo	2 (24)	Surface	50	17.5 to 18.1	NA	5.0 to 6.5	a	C	Dubreuil et al. 2003
			DCM	75	16.5 to 17.1	NA	3.5 to 6.0	a	N	
			below DCM	125	14.7 to 15.6	NA	1.5 to 3.0	a	M / N	
Convergence AF/STF	HNLC	2 (24)	Surface	50	14.0 to 14.7	NA	4.0 to 9.0	a	M to A	
			DCM	60 to 100	13.0 to 14.0	NA	2.0 to 6.5	a	M / N	
			below DCM	125	11.8 to 12.8	NA	2.0 to 6.0	a	N	
Northern PFZ	HNLC	2 (12)	Surface	50	10.0 to 10.2	NA	4.5 to 5.5	b	C	
			DCM	70	7.0 to 8.5	NA	3.0 to 5.0	b	N	
			below DCM	125	4.6 to 6.5	NA	2.0 to 4.0	b	M	
PFZ	HNLC	6(48)	Surface	10–20	5.3 to 5.4	0.4 to 0.5	3.2 to 6.7	Y	E	This Study
			DCM	60–80	2.2 to 2.9	0.6 to 0.9	2.4 to 5.5	N	C	

2. Materials and Methods

This study was conducted in the Polar Frontal Zone (54°08' S, 146°30' E) of the Australian sector during the SAZ SENSE (http://www.marine.csiro.au/datacentre/saz–sense/) cruise from February 3 to 5, 2007 on board the RV *Aurora Australis*. Seawater samples were collected every 6 h over a 48–h period, from the surface mixed layer (SML) and the deep chlorophyll maximum (DCM) respectively from depths of 10–20 m and 60–80 m. Vertical profiles of temperature and salinity were acquired using a SeaBird SBE9plus CTD attached to a rosette frame supporting 24×10 litres Niskin bottles. Chlorophyll *a* concentrations were estimated by HPLC pigment analysis following Wright and van den Enden (2000). Nutrient (nitrate, phosphate, and ammonium) concentrations were measured on board using a Lachat Quickchem series 8000 FIA.

Triplicate 1 mL samples were taken from each depth every 6 h, fixed with 2% (final concentration) glutaraldehyde and quick frozen in liquid nitrogen. All samples were processed within a month to minimize storage loss (Marchant et al. 2000). Samples were stained with SYBR Green–I and analysed using a FACScanto flow cytometer (Becton Dickinson), using protocols described in detail previously (Seymour et al. 2007). Individual populations of heterotrophic bacteria were separated according to variations in SYBR–Green fluorescence and side light scatter (SSC) (e.g. Marie et al. 1999) using WinMDI 2.8 (® Joseph Trotter) flow cytometry software.

At each depth, the presence of any monotonic trend was tested by calculating Kendall's coefficient of rank correlation between the physical and biological parameters and the x–axis (i.e. time). Potential associations between bacterial populations and environmental parameters were assessed using the Spearman coefficient of rank correlation. Differences between each sampling time period (i.e. night, morning, afternoon, evening) and differences between depths were inferred for each parameter with the Wilcoxon–Mann–Whitney *U*–test (Zar 1996). To examine differences in the diel patterns between all bacterial sub–populations and between depths, the magnitude of the shift in cells abundance between 2 consecutive sampling times were calculated.

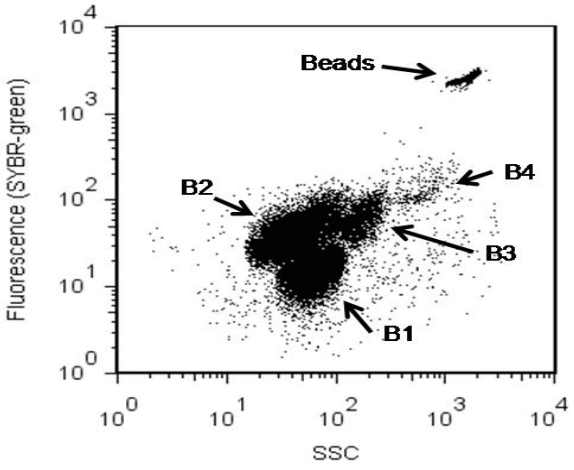

Fig. 1. Dot plot of the fluorescence SYBR–green (apparent nucleic acid content per cell) against 90°C side scatter (SSC, indicative of cell size) typically found over this study. Bacterial populations are discriminated as B1, B2, B3, and B4 according to their differences in SYBR–green fluorescence and SSC signals.

3. Results

The surface mixed layer (SML) and the deep chlorophyll maximum (DCM) differed significantly in temperature, salinity, and nutrients (phosphate, nitrate, and ammonium) and chlorophyll *a* concentrations ($p < 0.05$, Table 2). For both depths, these parameters did not exhibit any significant trend over the duration of the survey ($p > 0.05$).

Based on the SYBR–green fluorescence (or nucleic acid content) levels, two heterotrophic bacterial groups (e.g. Gasol et al. 1999; Marie et al. 1999). The low DNA group was referred here as B1 (Fig. 1). The HDNA bacterial group could be further segregated into different sub–populations of bacteria based on both SYBR–green fluorescence and SSC levels (Seymour et al. 2004; Bouvier et al. 2007; Schapira et al. 2008). These different bacterial sub–populations were referred here as B2, B3, and B4 HDNA sub–populations (Fig. 1).

Abundances of the total bacterial community (B) and of each bacterial sub–population (B1 to B4) are given in Table 2. All abundances were significantly higher ($p < 0.05$) in the surface mixed layer (SML) than at the deep chlorophyll maximum (DCM). However, the same cytometric structure of the bacterial communities was found at both depths. The sub–populations B1, B2, B3 and B4 accounted on average for 41, 47, 11 and 1% of the total bacterial abundance respectively at the SML and for 39, 52, 9, and 0.6% at the DCM. Over the entire survey, at the SML, abundances of the bacterial community (B) and of the sub–populations B1, B2, and B3 showed a significant 1.6–fold increase ($p < 0.05$, Fig. 2), while B4 abundance significantly increased by a factor of 2.4 ($p < 0.05$, Fig. 2). In contrast, no significant trend was found at the DCM ($p > 0.05$, Fig. 2).

Table 2. Environmental and biological characteristics of the surface mixed (SML) and deep chlorophyll maximum (DCM) layers. The range (minimum and maximum), the mean, and standard deviation (S.D.) are given for each variable. N: number of samples taken over the two diel cycles at each layer.

Variables	N	SML (10–20 m)			DCM (60–80 m)		
	/depth	Min.	Max.	Mean ± SD	Min.	Max.	Mean ± SD
T (°C)	9	5.3	5.4	5.4 ± 0.0	2.2	2.9	2.4 ± 0.2
Salinity (psu)	9	33.8	33.8	33.8 ± 0.0	33.9	33.9	33.9 ± 0.0
Fluo (µg L^{-1})	9	0.7	2.34	1.48 ± 0.6	2.8	4.9	3.6 ± 0.7
Chla (µg L^{-1})	6	0.4	0.5	0.4 ± 0.1	0.6	0.9	0.7 ± 0.1
PO4 (µg L^{-1})	9	1.4	1.6	1.6 ± 0.1	1.8	2.1	2.0 ± 0.1
NO3 (µg L^{-1})	9	21.5	24.8	23.9 ± 0.9	25.1	27.4	26.8 ± 0.6
NH4 (µg L^{-1})	9	0.1	0.4	0.2 ± 0.1	0.6	1.1	0.9 ± 0.2
B (×10^5 mL^{-1})	26	3.22	6.73	4.5 ± 0.99	2.4	5.5	3.57 ± 0.85
B1 (×10^5 mL^{-1})	26	1.32	2.94	1.85 ± 0.46	0.91	2.33	1.39 ± 0.39
B2 (×10^5 mL^{-1})	26	1.40	3.22	2.12 ± 0.46	1.27	2.67	1.84 ± 0.40
B3 (×10^5 mL^{-1})	26	0.30	0.78	0.47 ± 0.12	0.20	0.53	0.31 ± 0.09
B4 (×10^5 mL^{-1})	26	0.02	0.13	0.06 ± 0.03	0.01	0.05	0.02 ± 0.01

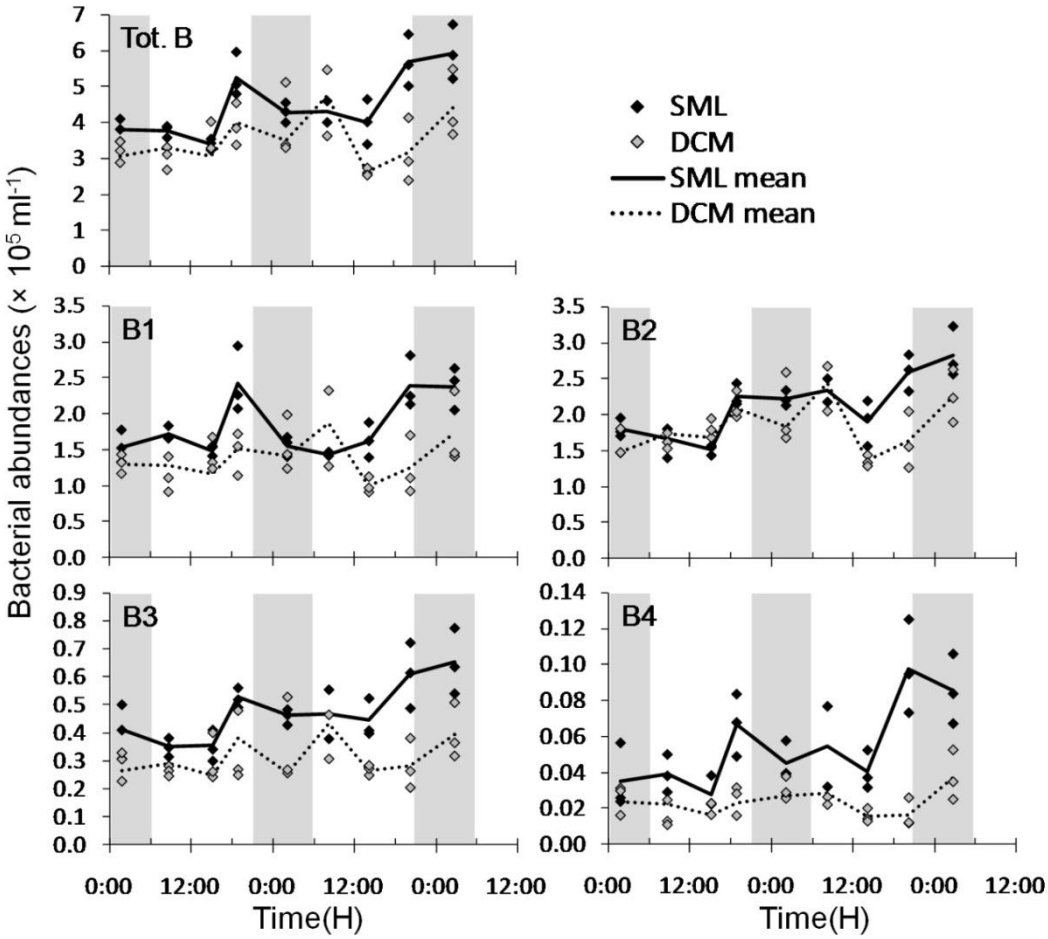

Fig. 2. Short–term dynamics of total heterotrophic bacteria (B), and of each bacterial population (B1, B2, B3, B4) at the surface (SML) and chlorophyll maximum (DCM). The grey and white backround represent the night and day times, respectively.

Over the course of the first 24 hours cycle, a similar pattern in the variation of bacterial abundances was observed for all bacterial sub–populations at both depths. Abundances increased by the evening but remained relatively constant over the other times.

However, the magnitude and significance of this pattern differed between depths and populations (Fig. 3). The increase in cells abundances occurring over the afternoon of the first day was significant only at the SML ($p < 0.05$; Fig. 3), where the shift in B4 was about twice as much as all other sub–populations. Over the following 24 hours cycle, this pattern was recurrently observed at the SML whereas, at the DCM, abundances of all sub–populations significantly decreased (about 3 fold shifts, $p < 0.05$; Fig. 3) by the early afternoon.

The relative contribution of each bacterial population and the cytometric structure of the bacterial communities remained stable over time (Fig. 4). Finally, at both depths, abundances of each bacterial sub–population were significantly positively correlated to each other ($p < 0.05$).

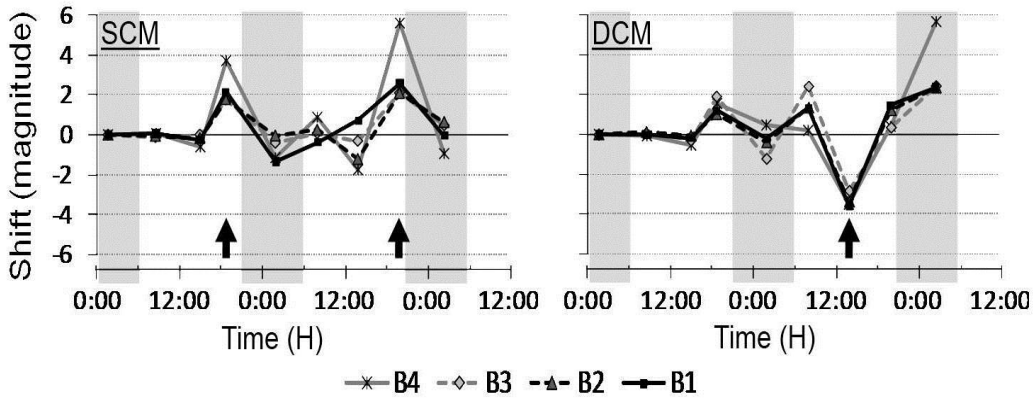

Fig. 3. Magnitude of the shift in cells abundance between two consecutive sampling times, starting from the first time recorded at 1.40am. Positive and negative values represent an increase and decrease in abundance, respectively. Shaded background represents night time. Black arrows point out when the shift occurred to be significant ($p < 0.05$).

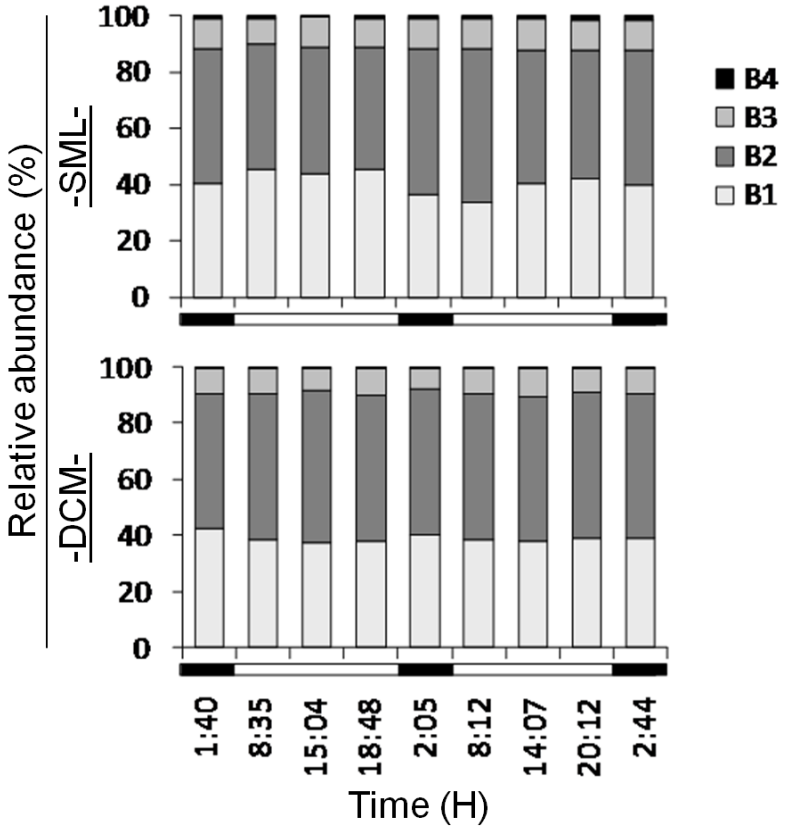

Fig. 4. Short–term dynamics of the relative abundance of each populations (%) at the surface (SML, top) and chlorophyll maximum (DCM, bottom). The black and white thick lines represent night and day times, respectively.

4. Discussion

The abundances of heterotrophic bacteria found in this study were consistent with those reported for the same area (Marchant et al. 2000) and other sectors of the Southern Ocean (Dubreuil et al. 2003). The surface and deep chlorophyll maximum (DCM) differed in their environmental conditions. In addition, similarly to previous studies in the Southern Ocean (e.g. Dubreuil et al. 2003), bacterial abundances significantly differed between these two layer depths, with lower abundances found at the DCM. This decrease in cells numbers with depth reflected a distinct short–term pattern between the bacterial communities of the surface and DCM. At the surface, the increased bacterial abundances in the afternoon suggest a growing community at the short term scale, while bacterial growth may have been counterbalanced by mortality at the DCM. Grazing of bacteria at the DCM was particularly shown by the significant three fold decrease in bacterial abundances in the morning of the second day. It is possible that grazing also occurred at the surface, but growth rates of bacteria were greater than grazing rates. Another possibility could be that bacteria were only secondary preys at the surface, while at the DCM grazers may have favorized heterotrophic bacteria over that of phytoplankton.

At the diel scale, a change in the dynamic patterns of bacterial abundances with depth has also previously been observed in the high nutrients low chlorophyll (HNLC) waters of the Southern Ocean (Dubreuil et al. 2003) and in the Mediterranean Sea (e.g. Zohary and Robarts 1992; Bettarel et al. 2002, Table 1). Due to changes in irradiance regime, phytoplankton physiology and the effect of UV radiation on bacterial production (Van Wambecke et al. 2008) is likely to differ (Sharek and Latasa 2007) between the two sampling depths. UV, and especially UV–B are known to damage the DNA of bacterial cells, hence bacterial production and subsequent nutrient regerneration processes. At the surface, the recurring significant evening peak in bacterial abundances clearly indicated that bacterial growth dominated during the afternoon. Similar patterns in bacterial abundance and production have previously been reported for other coastal and oceanic regions (e.g. Fuhrman et al. 1985; Seymour et al. 2004; Van Wambecke et al. 2008). Previous studies in the Southern Ocean have yet discussed the potential use of phytoplankton DOC released supply by bacterioplankton (e.g. Morán et al. 2007; Ortegua–Retuerta et al. 2008).

The inconsistency in the diel patterns of bacterial abundances observed over the 48–h study at the DCM in contrast to those observed at the surface however suggests the strong dependency of bacterioplankton processes to their local environment at a given time. This dependence of the local environment may also explain the lack of consistency in diel changes in bacterial abundances across a wide range of environments (Table 1), with peaks (or even increase or decrease) in bacterial abundance occurring at distinct periods of a 24 hour time frame (i.e. night, morning, afternoon, evening, Table 1). These discrepancies amongst studies also suggest that short–term temporal changes in biological processes are more important than that in environmental conditions (i.e. coastal *vs* offshore, temperate *vs* tropical *vs* polar systems). Amongst others, the main factors previously reported to affect short–term temporal changes in bacterial abundance, growth, and production are (i) microzooplankton grazing and/or viral lysis (Bettarel et al. 2002), (ii) the limiting nutrients and dissolved organic

carbon (Torréton et al.1994; Kuipers et al. 2000), which ones also may vary with depth (i.e. zooplankton vertical migration, Torréton et al. 1994), (iii) UV radiation (Van Wambecke et al. 2008), and, (iv) turbulence.

These above might also imply that different bacterial sub–populations fluctuate differently at the short–time scale (hours) and may thus contribute differently to carbon fluxes. The present study indicated however a similar short–term (24 to 48 h) pattern in abundance for all sub–populations which reflected those of total bacterioplankton at each depth (Fig. 2, 4). This contrast with those of previous studies dealing with similar bacterial sub–populations in coastal waters of lower latitudes (Seymour et al. 2004; Shareck and Latasa 2007) and reporting distinct diel patterns and distinct cells growth and activity between HDNA and LDNA sub–populations. Indeed, owing to the general theory, these are either physiologically or genetically different (Gasol et al. 1999; Zubkov et al. 2001).

The differences in the magnitude of the shifts observed amongst sub–populations (Fig. 3) however supports the idea that each bacterial sub–population might contribute differently to the production and activity of the total bacterioplankton in this system. In particular, the B4 sub–population was cytometrically characterized by the biggest cells size (i.e. highest SSC signal) and the highest levels of nucleic acid content (i.e. highest SYBR–green fluorescence signal, Fig. 1), suggesting this sub–population to represent the main 'active' fraction of the community both in terms of growth and production (e.g. Gasol et al. 1999, Lebaron et al. 2002, Morán et al. 2007). In addition, the variability observed in the abundances of the B4 subpopulation was up to twice as much as all of the others. Although recent discussions have been rending sceptic the use of HDNA bacteria as a proxy for estimating bacterial activity and production (Bouvier et al. 2007; Morán et al. 2007; Ortegua–Retuerta et al. 2008), differences amongst sub–populations in terms of magnitude (Fig. 3), suggest a different contribution of each bacterial sub–population to the production and activity of the bacterioplankton in this system. On the other hand, the great plasticity of bacteria, their ability to take advantage of a wide range of dissolved organic carbon supply, and their adaptation to their environment (i.e. psychrophylic bacteria, Bowman et al. 1997), may however question the relative importance of each sub–population to bacterial production within the PFZ. Other approach than flow cytometry (e.g. Denaturing Gradient Gel Electrophoresis (DGGE) which allow detecting dominant members of the microbial population based on individual DNA sequencing) might, however, be needed to complete the present information in regards to the composition of the bacterial communities at the genus or species level.

Overall, a high degree of stability in the structure of the bacterial communities was found in this study area. This was particularly shown by the maintenance of the cytometric signals and of the relative contribution of each of these sub–populations at both depths and over time (Fig. 1, 4). Our results, however, contrast with previous studies in which HDNA and LDNA bacterial sub–populations were not related to each other and show distinct temporal and spatial variations (Corzo et al. 2005; Seymour et al. 2005; Shareck and Latasa 2007). At each depth, the significant positive relationship between the bacterial sub–populations together with the similarity observed in their dynamics thus confirms

the stable ecological structure of the bacterial communities in the PFZ at the short–term temporal scale.

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III. Influence of local physical events on picophytoplankton spatial and temporal dynamics in South Australian continental shelf waters

ABSTRACT

We investigated the space–time dynamics of picophytoplankton in South Australian continental shelf waters from February 2008 to January 2009, focusing on localized physical events. We discriminated six picophytoplankton populations by flow cytometry, including *Synechococcus* (SYN1, SYN2), *Prochlorococcus* (PROC1, PROC2), and small and large picoeukaryotes (EUKS, EUKL). Observed local physical events included downwelling and dense waters outflowing from a nearby gulf in winter–early spring 2008, upwelling in summer and early spring 2008, and, eddy formation in January 2009. Each population responded differently to these events that induced up to four orders of magnitude changes in their abundances. Population–specific hotspots reflected a succession of distinct dominant communities associated to the strength of upwelling events, changes in fluorescence maximum depths, and, local downwelling and mixing processes. The unexpected high abundances and local dominance of *Prochlorococcus* in summer reflected the possible influence of eastward and westward current transports and the presence of a High–Light (PROC1) and Low–Light (PROC2) adapted ecotypes. This study highlights the role of localized physical events to the dominance of all three picophytoplankton groups that may be critical for the high productivity of the present region, and suggests the importance of hydroclimatic forcing to inter–annual changes in picophytoplankton communities.

Keywords: picophytoplankton, spatial and temporal dynamics, coastal upwelling and downwelling, fluorescence maximum, South Australia

1. Introduction

Picophytoplankton (0.2–3 μm in cell size) dominate phytoplankton biomass and productivity in many regions of the ocean (e.g. Chisholm 1992; Agawin et al. 2000; Blanchot et al. 2001) and play a key role in marine carbon and nutrient cycles (Li et al. 1983; Campbell et al. 1994). Both prokaryotes and eukaryotes compose the picophytoplankton community, which includes two major groups of cyanobacteria, *Prochlorococcus* (Chisholm et al. 1988) and *Synechoccocus* (Waterbury et al. 1979), that encompass distinct strains and ecotypes (Rocap et al. 2003; Johnson et al. 2006), and a diverse assemblage of picoeukaryotes (Worden 2006; Shi et al. 2009). The mechanism responsible for the relative distribution of distinct picophytoplankton populations remain poorly understood, particularly in continental shelf waters (Katano et al. 2005; Calvo–Díaz and Morán, 2006; Calvo–Díaz et al. 2008; Mackey et al. 2009). For a given shelf region, the variability in the hydrographical properties and circulation complexities may however explain the current lack of understanding regarding the relative roles of physical and chemical factors on picophytoplankton distribution in these systems (Jiao et al. 2002; Katano et al. 2005). The dependence of picophytoplankton abundances to nutrient supply, light, temperatures, and the general structure of the water column have previously been shown (Glover et al. 1988; Agawin et al. 2000; Duarte et al. 2000, Sommaruga et al. 2005; Bouman et al. 2006; Zinser et al. 2007) but may differ from one system to another and from one strain to another. For instance in the Mediterranean Sea, the spatial distribution of prokaryotes populations have been shown to vary with phosphate concentrations, whereas nitrogen supplies would be more discriminating for picophytoplankton populations in continental shelf regions, particularly if affected by upwelling (Probyn 1985).

The South Australian continental shelf waters of the Kangaroo Island (KI)–Eyre Peninsula (EP) region supports a valuable fishing industry, but the sustainability of this shelf region has remained a long debate because its surface waters show relatively low primary production (<500 mg C m^{-2} d^{-1}) and nutrient and chlorophyll *a* (Chl *a*) concentrations (van Ruth et al. 2010a). Our understandings on the productivity of this region recently increased with the identification of localized hotspots of high primary production (up to 3900 mg C m^{-2} d^{-1}) at deep chlorophyll maximum layers below the surface mixed layer depth over the summer months (van Ruth et al. 2010a, b). These features vary in both space and time in relation to localized summer upwellings, and were related to the fact that 50% of the euphotic zone lie below the surface mixed layer depth (van Ruth et al. 2010a). In contrast, the winter–spring periods during which downwelling processes typically prevail appeared to show low primary productivity (van Ruth 2009).

The wide (i.e. between 60 and 100 km) continental shelf of the Kangaroo Island (KI)–Eyre Peninsula (EP) region shows complex topography with islands, gulfs, and cross–shelf canyons (Fig. 1). These characteristics influence a series of localized physical processes and changes in the structure of the water column that have been recently reviewed (Middleton and Bye 2007). In summer, the Coastal Current (CC) flows westward along the shelf forced by south–easterly winds and Ekman transport. As a result, deep cold nutrient rich waters are upwelled south of KI, potentially through the du Couedic Canyon (Kaempf et al.

2004). These upwelling events occur 3 to 4 times over the austral summer, with each event lasting for a period of 4 to 10 days. Due to the width of the shelf, upwelled waters do not reach the surface; instead, they form a cold (< 16 °C), low saline (< 35.5) pool southwest of KI (McClatchie et al. 2006; Middleton and Bye 2007). This cold pool is subsequently advected northwestward along the 100 m isobath and is responsible for the presence of a deep chlorophyll maximum previously observed above the plume of upwelled waters (McClatchie et al. 2006; Middleton and Bye 2007; van Ruth et al. 2010a). In contrast, in winter, the CC is forced by cool, downwelling favourable winds and flows eastward (Middleton and Bye 2007; Seuront et al. 2010), leading to the potential influence of the tropical waters of the Leeuwin Current on the KI–EP shelf region as it is stronger during that time of the year (Feng et al. 2003; Middleton and Bye 2007). In addition, due to strong summer evaporation and winter cooling processes, Spencer Gulf's waters become denser than shelf waters, resulting in an outflow of salty and cool bottom waters that gravitationally progresses throughout the shelf to south off KI (Lennon et al. 1987; Middleton and Bye 2007). Finally, the KI–EP region is also influenced by a distinct water mass from the Great Australian Bight, the GAB water warm pool, which is warmer year round than the KI–EP shelf waters, except in distinct patches associated to sporadic upwelling events (McClatchie et al. 2006; Middleton and Bye 2007; Richardson et al. 2009; van Ruth et al. 2010a).

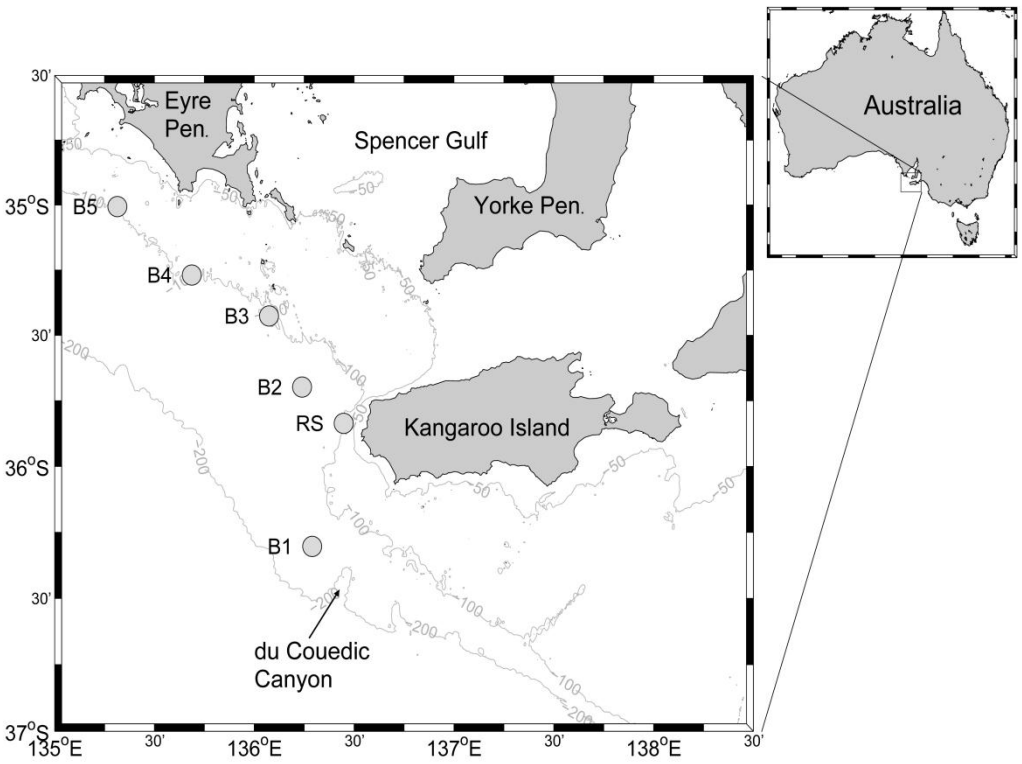

Fig.1. Map of the Kangaroo Island–Eyre Peninsula (KI–EP) region with the different sampled stations (B1, RS, B2, B3, B4, and B5), the du Couedic Canyon, Neptune Island (N.I.), Spencer Gulf, and the two peninsulas (Eyre, Yorke).

Despite the influence of these processes on the microphytoplankton (> 20 µm; e.g. van Ruth et al. 2010a, b), information on the picophytoplankton community within this shelf region remains very limited (Seuront et al. 2010). Particularly, the effect of the complex circulation pattern and physical forcing events, i.e. summer upwelling and winter downwelling (Middleton and Bye 2007; Seuront et al. 2010), on the temporal and spatial distribution of picophytoplankton is yet to be determined. Because primary production appeared particularly important within the fluorescence maximum (FM) layer in South Australian continental shelf waters (van Ruth et al. 2010a), focusing on the dynamic of the picophytoplankton community structure within this particular layer is thus a critical step to increase our understanding of matter and energy pathways in these waters. In addition, given the reported association between picophytoplankton and hydrographic conditions (Jiao et al. 2005; Katano et al. 2005), information on the dynamics of distinct picophytoplankton populations could be particularly relevant in this complex continental shelf system. In this context, the present work aimed to assess the role of local physical events in determining the spatial and temporal dynamics of picophytoplankton community structure of the FM layer. We thus investigated changes in the abundances and cytometric composition of picophytoplankton along the South Australian continental shelf in relation to (i) nutrient availability, (ii) water column stratification and (iii) spatially and temporally localized oceanographic features, such as summer upwelling and winter downwelling.

2. Materials and Methods

2.1. ***Sampling strategy***

Sampling was carried out during cruises conducted on board the *RV Ngerin* in February, March, April, August and October 2008, and in January 2009 as part of the Southern Australian Integrated Marine Observing System (SAIMOS). During each cruise, the physical, chemical and biological properties of the FM layer were investigated (i) at six stations located on the 100 m isobath, i.e. RS (35.50°S, 136.27°E), B2 (35.41°S, 136.14°E), B3 (35.25°S, 136.04°E), B4 (35.16°S, 135.41°E), and B5 (35.00°S, 135.19°E), and (ii) from an offshore station (B1; 36.18°S, 136.17°E) located southwest of Kangaroo Island (Fig. 1). Note that combining the distances between stations (14 to 25 nautical miles), the average component of the current velocity at mid–depth along the shelf (0.01 m s^{-1}), and the average speed of the vessel (i.e. 9 knots) indicates that different waters masses were sampled at each station. Vertical profiles of temperature, salinity, photosynthetically active radiation (PAR), and *in vivo* fluorescence were taken at each station using a pumped Seabird SBE 19*plus* CTD system mounted with a Wet Star fluorometer probe (WetLabs). Processing of the CTD data was done using the SBE Data Processing V7.16a program with 2 db averaged. An inadequate (<40s) surface soak associated with the pump system of our CTD was noticed following the March 2008 cruise. This, however, did not affected temperatures data as these were sufficiently well resolved within the 2db. At each station, *in vivo* fluorescence profiles were used to identify the depth of the fluorescence maximum (FM) from where we collected seawater with Niskin bottles. These were subsequently homogenized prior to subsampling for

nutrients, Chl *a* concentration (Chl *a*), and picophytoplankton analysis. If no FM could be identified, seawater sampling was done in the surface mixed layer at a depth of 15 m that we previously identified by comparing fluorescence and chlorophyll *a* vertical profiles to avoid the effects of photoinhibition.

Seawater samples of 50 mL were filtered through bonnet syringe filters (0.45 µm porosity, Micro Analytix Pty Ltd) and stored at –20°C for nutrients analysis. Nutrient concentrations were not available for October 2008 and for the station RS in March 2008. All other samples were analysed according to the Lachat Quickchem methods for phosphate (PO_4, detection limit; 0.03 µM), nitrate + nitrite (NO_x, detection limit; 0.07 µM), and ammonium (NH_4, detection limit; 0.07 µM) on a QuickChem QC8500 Automated Ion Analyser. Chlorophyll *a* (Chl *a*) concentrations were determined using triplicate 300 mL seawater samples filtered through fibre glass filters (Whatman GF/C, 1.2 µm porosity). The choice of the GF/C filters here allowed us to get comparable data set of Chl a concentrations with that from previous work done in the region (Kaempf et al. 2004; McClatchie et al. 2006; van Ruth et al. 2010a, b). Since GF/C filters might underestimate bulk phytoplankton biomass, we did not attempt to compare values of Chl a concentrations with picophytoplankton data. Filters were stored at –20°C until analysis. Chl *a* was extracted by placing each filter in 5 mL of methanol for 24 hours in the dark at 4°C (Welschmeyer 1994). Chl *a* concentrations in the extracts were determined using a Turner 450 fluorometer previously calibrated with Chl *a* extracted from *Anacystis nidulans* (Sigma Chemicals, St Louis, MO, USA).

2.2. Picophytoplankton populations

Triplicates 1 mL seawater sample were fixed with paraformaldehyde (2% final concentration), frozen in liquid nitrogen, and stored at –80°C. Samples were processed by flow cytometry (FacsCanto Becton Dickinson) within a month following each cruise. Prior to analysis 1 µm fluorescent marker beads (Molecular Probes, Eugene, OR, USA) were added to each sample (Marie et al. 1999) and each sample were run for 5 minutes. For each sample, natural orange fluorescence from phycoerythrin and red fluorescence from chlorophyll, together with forward light scatter (FSC) and side light scatter (SSC) were recorded. All cytograms were then analysed using the software flowJo (TreeStar) following the method described in Marie et al. (1999). The three known major picophytoplankton groups, i.e. *Synechococcus*, *Prochlorococcus*, picoeukaryotes could be easily discriminated by their distinct autofluorescence and light scatter properties relative to the beads. Gates or region around each observed group were drawn such as no adjustment was needed to be done and to maximize cell counts. Since we observed, for most samples, distinct separated cloud of cells of *Prochlorococcus–like* populations, the extension of *Synechococcus* over more than two log decades of the orange fluorescence though at times less clearer and allowing the distinction of different cloud of cells of *Synechococcus–like* populations, and distinct clouds of picoeukaryotes, the same gating procedure achieved for the group level was then performed within each identified group for analysing the population level. Specifically, *Synechococcus* populations differed mainly by their orange fluorescence (Fig. 2) and only SYN1 showed at times slightly higher SSC signals than SYN2 (not shown), whereas *Prochlorococcus* populations presented different red fluorescence (Fig. 2) and overlapping SSC signals (not shown), with PROC2 showing brighter fluorescence and tending to

have higher SSC signals than PROC1. Small (EUKS) and large (EUKL) picoeukaryotes populations were identified by distinct red fluorescence (Fig. 2) and SSC signals (not shown), with EUKL showing higher fluorescence and SSC signals. We did not attempt here to further analyse the spatial and temporal variability of the fluorescence and cell size properties of these populations, which ones will be investigated in more details in another study.

The ratios of the abundance of *Prochlorococcus* and *Synechococcus* (PROC:SYN) and Prokaryotes and Eukaryotes (PROK:EUK) were also calculated and used to quantify changes in the structure of picophytoplankton communities related to changes in environmental conditions. Changes in these ratios have previously been attributed to changes in trophic conditions in the Arabian Sea (Campbell et al. 1998) and in the hydrographic regimes in the southern Bay of Biscay shelf waters (Calvo–Díaz et al. 2008).

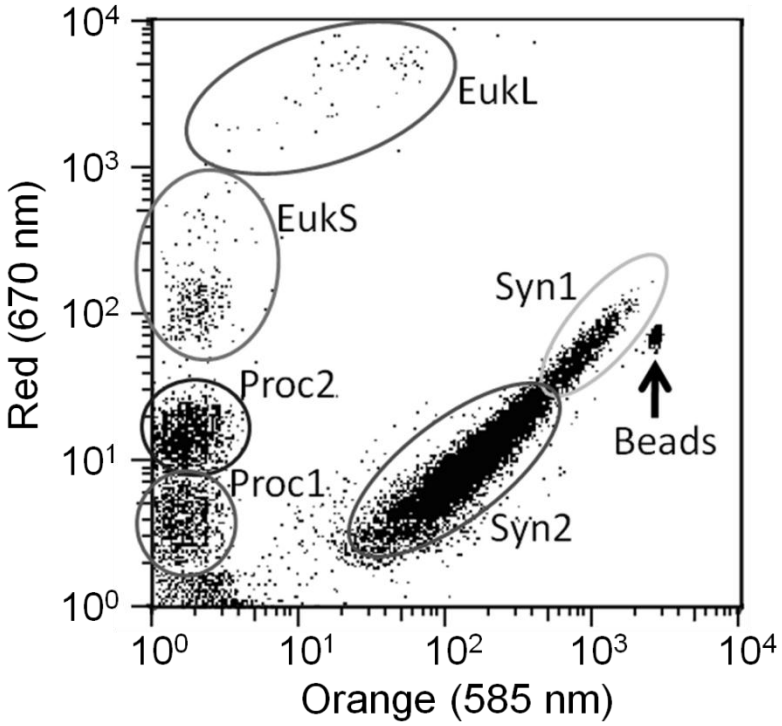

Fig. 2. Example of a cytogram showing the signature of each observed population of *Synechococcus* (SYN1, SYN2), *Prochlorococcus* (PROC1, PROC2), and small (EUKS) and large (EUKL) eukaryotes as identified according to their red and orange fluorescence properties.

2.3. ***Upwelling and downwelling forcing***

The variability in upwelling and downwelling forcing was evaluated from changes in the three daily averaged of the alongshore component of wind stress rotated along 315°T (Middleton and Bye 2007; Middleton et al. 2007). This component represents upwelling favourable wind conditions if positive or downwelling favourable wind conditions if negative, and, is calculated by using the wind data (U) from the Neptune Island weather station (provided by the Australian Bureau of Meterology) and the drag coefficient (C_D) from Gill (1982) into the wind stress (τ) expressed as $\tau = \rho_{air} C_D U|U|$ (Smith, 1980).

2.4. ***Vertical structure of the water column***

The mixed layer depths (Z_m) were calculated following Kara et al. (2000) to be consistent with the work of van Ruth et al. (2010a) performed in the region. Kara et al. (2000) determined that the optimal estimate of turbulent mixing penetration is the first depth at which ΔT equals 0.8 °C from a reference depth of T_{ref} = 10 m. This depth is the reference depth of σ_t or $\sigma_{tref} = T+\Delta T$. From this σ_{tref}, Z_m is defined as the depth at which the σ_t value is greater or smaller than $0.1\Delta\sigma_t$, with $\Delta\sigma_t = \sigma_t(T_{ref}) - \sigma_t(\sigma_{tref})$ (Kara et al. 2000).

A stratification index based on the potential energy anomaly PE_z (J m^{-3}; Simpson 1981; Mann and Lazier 1996) was calculated from available and reliable data of each vertical profile as $PE_z = 1/H \int gz\,(\rho - \bar{\rho})\,dz$, where ρ is the water density, $\bar{\rho}$ the depth–averaged density ($\bar{\rho} = \frac{1}{H}\int_{-H}^{0} \rho dz$), H the water column depth, g the gravitational acceleration, and z the given depth. The potential energy anomaly PE_z reflects the amount of mechanical energy required to completely homogenise a water column that may show differences in density. High PE_z values represent strong and stable density stratification of the water column (Burchard and Hofmeister 2008).

2.5. ***Data analysis***

The extent to which single or combined physical (i.e. temperature, salinity, potential energy of the water column) and chemical (NH_4, NO_x, and PO_4) variables best explain the patterns observed in picophytoplankton abundances was inferred using the BEST procedure (Clarke and Warwick 1998, 2001). This procedure compares a normalized Euclidian distance matrix of a defined set of environmental variables to a Bray–Curtis similarity matrix of biotic variables using rank correlation amongst data samples. For this purpose, we used data collected from all stations across cruises, except those for which nutrients data were not available (i.e., all stations in October 2008 and station RS in March 2008). Prior to analysis, nutrient data and cell abundance data were log(x+1) transformed. Highest rank correlation coefficient was identified as the combination of environmental variables best explaining the variations observed in the picophytoplankton abundances and in Chl *a* concentrations amongst samples. These correlations were then submitted to a permutation procedure (Clarke and Warwick 1998, 2001) to test for their significance at a level of α = 0.05. Analyses were performed using the PRIMER v6 package.

3. Results

3.1. ***Hydrological properties***

The variability observed in the hydrological conditions reflected distinct physical process that occurred locally within the Kangaroo Island–Eire Peninsula (KI–EP) region over the distinct periods of study.

The austral summer of 2008 was characterized by strong easterly upwelling favourable winds (Fig. 3) and upwelling events (Fig. 4) localized southwest of Kangaroo Island (Fig. 1). In February 2008, upwelling was indicated by temperatures and salinities below 15°C and 35.3 along the shelf stations, but at station B5 (Fig. 5). This station showed instead waters influenced by the Great Australian Bigth (GAB) that are warmer and more saline (McClatchie et al. 2006; Middleton and Bye 2007; Richardson et al. 2009). In addition, despites their differences in temperatures and salinities, the conditions of the water column at stations RS and B5 were particularly unstable, contrasting with other stations (Fig. 5). In March 2008, upwelled waters remaining below the warm surface layer (SST > 18°C; McClatchie et al. 2006; Middleton and Bye 2007). Alternatively, a second upwelling pulse likely occurred as indicated by the colder and fresher bottom waters in the eastern stations and (Fig. 4; Middleton and Platov, 2003). This is congruent with the subsequent increase in stratification of the water column (Middleton and Platov 2003) reaching maximal PE_z values at all stations (Fig. 5). Over the summer 2008 upwelling period, FMs were shallower than 45 m depths and below the mixed layer depth, but at the RS and B5 stations in February 2008 (Table 1, Fig. 4). In fact, most of the sampled stations presented an FM appearing like trapped in between the mixed layer depth and the upwelled cold pool (Fig. 4). Upwelled waters of temperatures < 15°C were marked at the FM of station B1 (February 2008), but also of station RS (March 2008) and contrasted with the warmer (> 15°C) FM layer of other stations (Fig 4, 5).

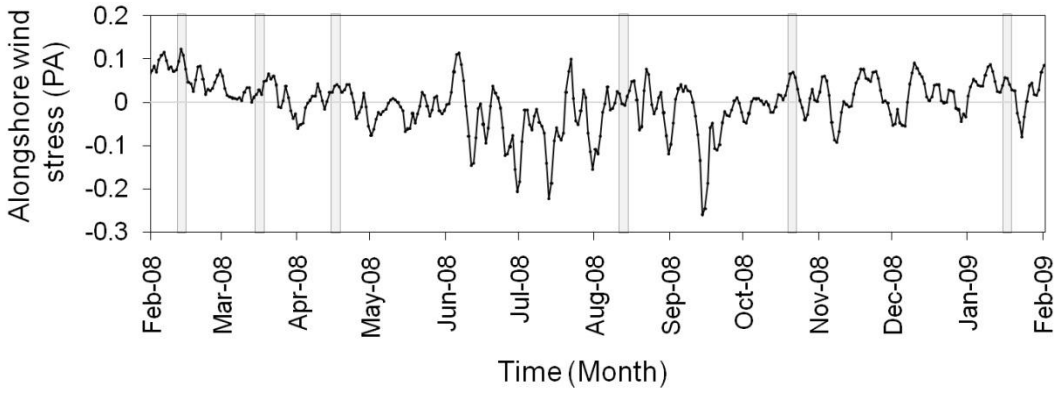

Fig. 3. Time series of the three daily averaged alongshore component of wind stress rotated along 315°T (PA). Positive and negative values indicate upwelling and downwelling favourable winds, respectively.

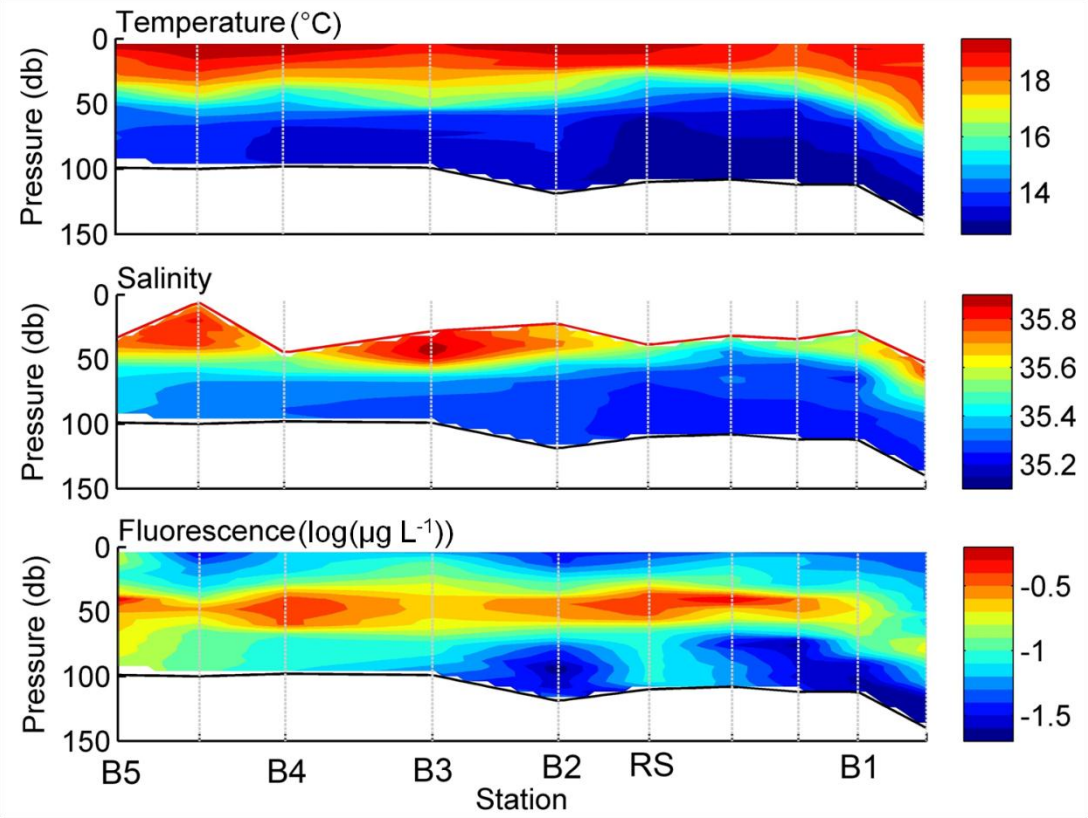

Fig. 4. Vertical section of the temperature, salinity, and fluorescence properties of the water column along the B5 to B1 stations for the March 2008 cruise. Note the upwelled waters marked by temperatures and salinities below 15°C and 35.4.

April 2008 showed the transition period between summer and winter waters in the KI–EP shelf region. The presence of a warm and saline thick surface mixed layer (Fig. 5) suggests the influence of the GAB waters via eastward current transport while cooler summer shelf waters may have remained deeper and at the eastern stations. The destabilisation of the water column (Table 1) deepened the thermocline (Fig. 5) and deep fluorescence maxima (FM < 45 m depth) of low density were commonly observed above the deep mixed layer depth at all sampled shelf stations (Fig. 4, Table 1).

In the following months, downwelling favourable westerly winds dominated (Fig. 3) over the KI–EP region and the FMs were usually found within the well mixed water column or surface mixed layer (Fig. 4, Table 1). As shown in Fig. 6, the late winter (August 2008) was marked by downwelling processes with unstable water column for the western stations (B5, B4, and B3, Figs. 4 and 5), whereas the signature of the dense bottom waters outflowing from Spencer Gulf towards the du Couedic Canyon was clearly visible at station RS, starting to reach station B1 (Fig. 4, Lennon et al. 1987; Middleton and Bye 2007). This likely led to haline stratification at these two stations (Fig. 4) and to a thick surface mixed layer at station RS (Fig. 6, Table 1). Station B2 was however marked by warmer and fresher mixed waters suggesting the potential influence of the Leuuwin Current (LC) (Richardson et al. 2009). The early spring 2008 was marked by the end of a period of strong downwelling favourable winds as

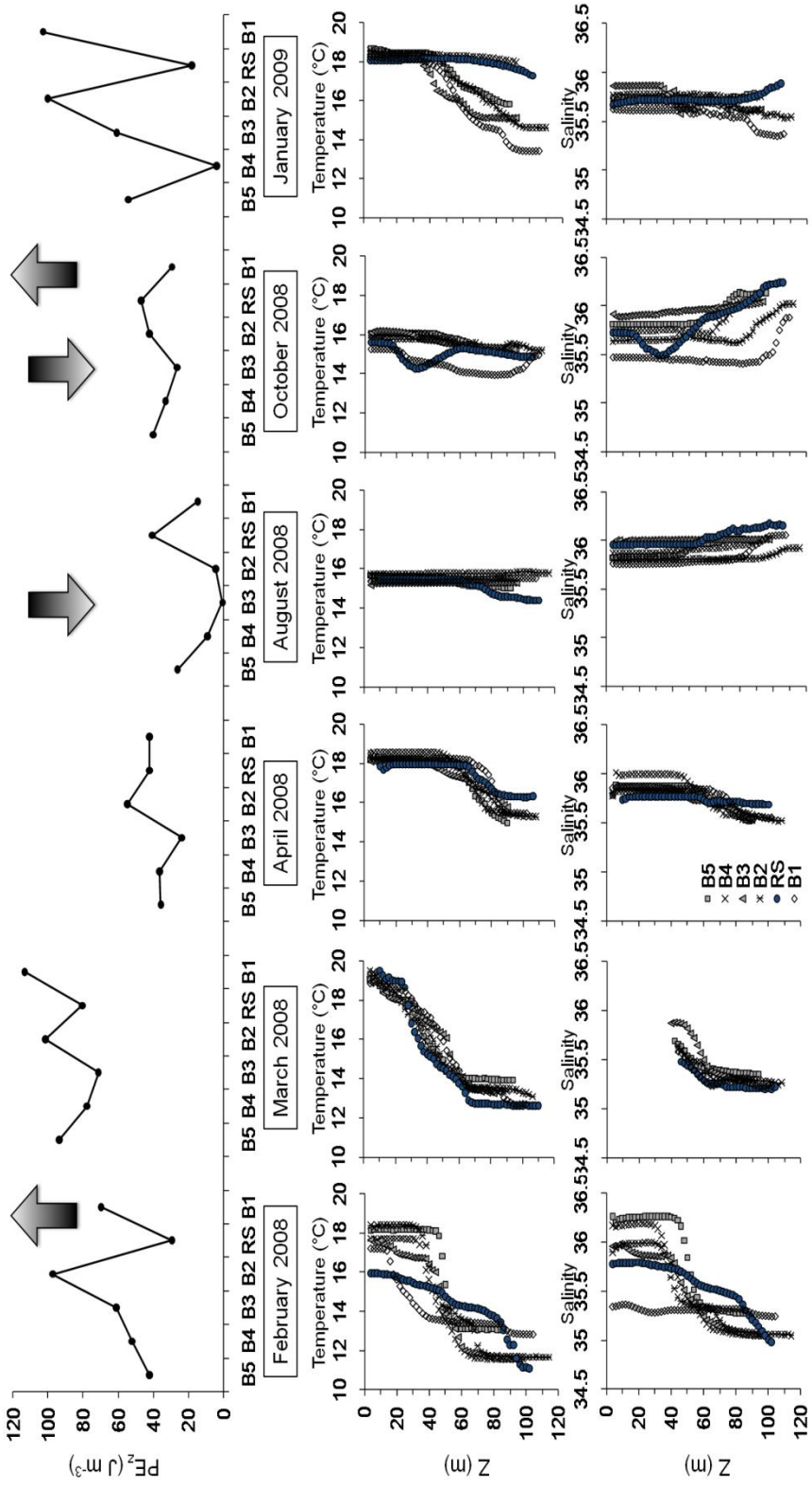

Fig. 5. The upper panel presents the temporal variation in the stratification index (PE_z, J m^{-3}) observed along the shelf. Local upwelling and downwelling events are represented by upward and downward arrows, respectively. The lower panel presents the vertical profiles of temperature (°C) and salinity of each sampled station from February 2008 to January 2009.

Table 1. The mixed layer depths (Z_m) and the nutrient (NH_4, NO_x, PO_4) and chlorophyll *a* (Chl *a*) concentrations, and PROC:SYN and PROK:EUK ratios of the sampled depths (Z) are given for each cruise and sampling station.

Cruises	*Stations*	Z (m)	Z_m (m)	NH_4 (µM)	NO_x (µM)	PO_4 (µM)	Chl *a* (µg L^{-1})	PROC: SYN	PROK: EUK
Feb–08	*B5*	20	44	0.1	0.4	0	0.2	0.3	6.3
	B4	40	37	0	1.6	0	0.3	0.2	3.1
	B3	45	39	0.4	0.2	0	0.4	0.4	1
	B2	40	36	2	2.3	0.3	0.2	0.3	17.5
	RS	28	48	0.1	1.3	0	1.3	1.1	0.8
	B1	35	15	0.1	1.4	0.2	0.2	2.1	8.3
Mar–08	*B5*	40	31	0	0.5	0.1	0.3	0.3	3.1
	B4	45	20	0	2	0.2	0.2	0.5	14
	B3	40	19	0.4	0.3	0.1	0.2	0.2	4.1
	B2	40	23	0	0.1	0.1	0.3	0.1	54.5
	RS	45	20	2.1	5.2	10.9	0.2	0.3	14.4
	B1	42	31	0.3	0.1	0.1	0.1	0.4	21.9
Apr–08	*B5*	60	65	0.1	0.8	0.1	0.2	6.8	31.2
	B4	55	63	0.3	0.1	0	0.2	1.6	62.1
	B3	45	54	0.4	0.3	0	0.1	1.2	40.6
	B2	55	61	0.3	0.4	0	0.1	1.4	23.7
	RS	65	68	0.4	0.9	0.9	0.1	2.1	6.4
	B1	45	78	0.2	0	0	0.1	2.1	38.2
Aug–08	*B5*	40	–	0.1	0.1	0	0.3	0.5	5.3
	B4	30	–	0.1	0.1	0	0.2	0.5	5.1
	B3	15	–	0.1	0.2	0.1	0.2	0.3	2.5
	B2	20	–	0.1	0.4	0.1	0.2	0.5	7.7
	RS	15	82	0.2	0.5	0.1	0.5	0.4	3.9
	B1	40	–	0.1	0.6	0.1	0.2	0.6	3
Oct–08	*B5*	65	–	–	–	–	0.1	2	8.5
	B4	60	–	–	–	–	0.1	0.5	7.2
	B3	70	80	–	–	–	0.1	0.7	2.3
	B2	80	–	–	–	–	0.2	2.2	4.6
	RS	33	24	–	–	–	0.1	0.3	4
	B1	40	55	–	–	–	0.1	0.2	60.1
Jan–09	*B5*	70	49	0.1	0.1	0.1	0.1	5.3	11.4
	B4	65	–	0.1	1.4	0.2	0.1	4.9	5.4
	B3	65	40	0.1	1.4	0.2	0.2	4	2.1
	B2	85	50	0.3	0.1	0.1	0.2	6.6	18.9
	RS	48	103	0.1	1	0.1	0	1.3	28.4
	B1	65	52	0.1	0.4	0.1	0.1	2.6	5.1

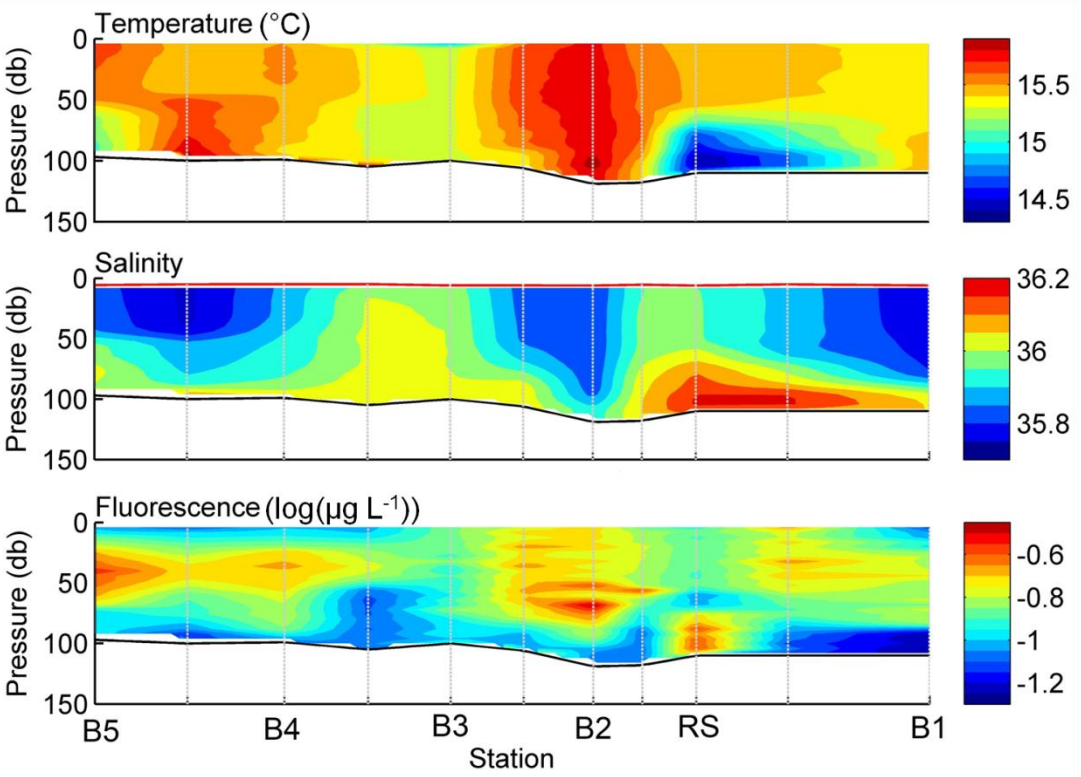

Fig. 6. Vertical section of the temperature, salinity, and fluorescence properties of the water column along the B5 to B1 stations for the August 2008 cruise. Note the downwelled waters in the western stations (B5–B3) and the signature of the dense bottom outflow from Spencer Gulf at stations RS and B1.

indicated by the remaining of saline bottom waters (Fig. 5), while upwelling favourable winds conditions dominated subsequently during the cruise (Fig. 3).

Such conditions likely induced processes of subduction (Szymanska and Tomczak 1994) around stations B1 and RS that showed distinct physical structure, i.e. low temperature and salinity, strong haline stratification (Fig. 5) and shallow FM layer (Table 1). The barrier effect of the dense bottom water outflow likely promoted, together with the alongshore upwelling favourable winds, the uplift of deep oceanic waters towards the surface.

Finally, in January 2009, southeasterly winds were weaker than in summer 2008 (Fig, 3) and as indicated by relatively high bottom temperatures (> 15°C) and salinities (> 35.5), upwelling event may have been weaker such as only B1 and B2 presented temperatures of < 15°C below 67 and 89 m depth, respectively (Fig. 5). In contrast, temperatures and salinities of > 17°C and > 35.7, and low potential energy found at stations RS and B4 (Fig. 5) were potentially related to the presence of local eddies on the shelf (Middleton and Bye 2007). Stable thermal stratification was apparent at the other stations (Fig. 5). All shelf stations presented deep FM layers that were found below the mixed layer depth, but in RS and B4, with the deepest FM observed at 85 m at station B2 (Table 1).

3.2. ***Chlorophyll a and nutrient concentrations***

Chlorophyll *a* concentrations were consistently low at the sampled depths, ranging between 0.1 μg L^{-1} in summer 2009 and 1.2 μg L^{-1} in summer 2008 (Table 1), respectively in the absence and presence of an upwelling event. Ammonium, nitrate, and phosphate concentrations ranged from undetectable levels to 2.0, 2.3, and 0.9 μM, respectively (Table 1). Peaks in ammonium concentrations of > 1.0 μM coincided with the stratified water column conditions of some stations in summer 2008 (B2, B3, RS, Table 1) and at most stations (RS, B2, B3, B4) in early autumn (April 2008). In contrast, ammonium concentrations were generally low or undetectable in August 2008 and in January 2009 (Table 1). Although some local peaks, concentrations of phosphate and nitrate were generally lower in April and August 2008 and in January 2009 than over the summer 2008 upwelling event (Table1).

3.3. ***Spatial and temporal dynamics of picophytoplankton abundances***

The co–occurrence of distinct picophytoplankton populations marked by differences in their red and orange fluorescence properties (Fig. 2) was observed within the FM layers most of the time and at most stations. This shows that each of these populations likely differed in their amount of chlorophyll and phycoerythrein cell content. PROC2 was, however, absent in October 2008 and EUKL was absent at stations B5 and B3 in March 2008 and at station RS in January 2009.

The spatial and temporal variability in the abundance of these different picophytoplankton populations was characterized by population–specific hotspots with shifts in abundances of up to more than two orders of magnitude occurring at different times of the year and at different stations (Fig. 7). In addition, the abundance of the picophytoplankton groups identified here generally varied by, and differed between each other by up to one order of magnitude. Specifically, SYN1 (0.2×10^4 to 11.0×10^4 cells mL^{-1}; Fig. 7A), PROC1 (0.2×10^4 to 11.0×10^4 cells mL^{-1}; Fig. 7C), and EUKS (0.2×10^4 to11.0×10^4 cells mL^{-1}; Fig. 7E) abundances were up to over one order of magnitude higher than those of SYN2 (0.2×10^3 to 18.1×10^4 cells mL^{-1}; Fig. 7B), PROC2 (0.2×10^3 to 18.1×10^4 cells mL^{-1}; Fig. 7D), and EUKL (0.2×10^3 to 18.1×10^4 cells mL^{-1}; Fig. 7F), respectively. However, SYN2, PROC2, and distribution of PROC1, PROC2, and SYN1 was characterized by peaks at sites B1, B2, and B5, while SYN2 concentrations remained relatively high at station B3. In late winter 2008, the abundance of all picophytoplankton groups varied by less than 50% along the shelf, with most exhibiting lowest abundances in station B3 (Fig. 7B, C, E, F). In contrast to prokaryotes, picoeukaryotes were less abundant, particularly at the offshore station B1, and peaked periodically on the shelf (Fig. 7E and F). EUKL showed higher abundances than SYN1 locally in summer and early spring 2008, PROC1 in early winter 2008, and EUKS locally in early spring 2008 (Fig. 7). PROC1 and SYN1 abundances exhibited relative similar spatial and temporal patterns (Fig. 7A and C). Over the summer–autumn 2008 periods, the spatial distribution of PROC1, PROC2, and SYN1 was characterized by peaks at sites B1, B2, and B5, while SYN2 concentrations remained relatively high at station B3. In late winter 2008, the abundance of all picophytoplankton groups varied by less than 50% along the shelf, with most

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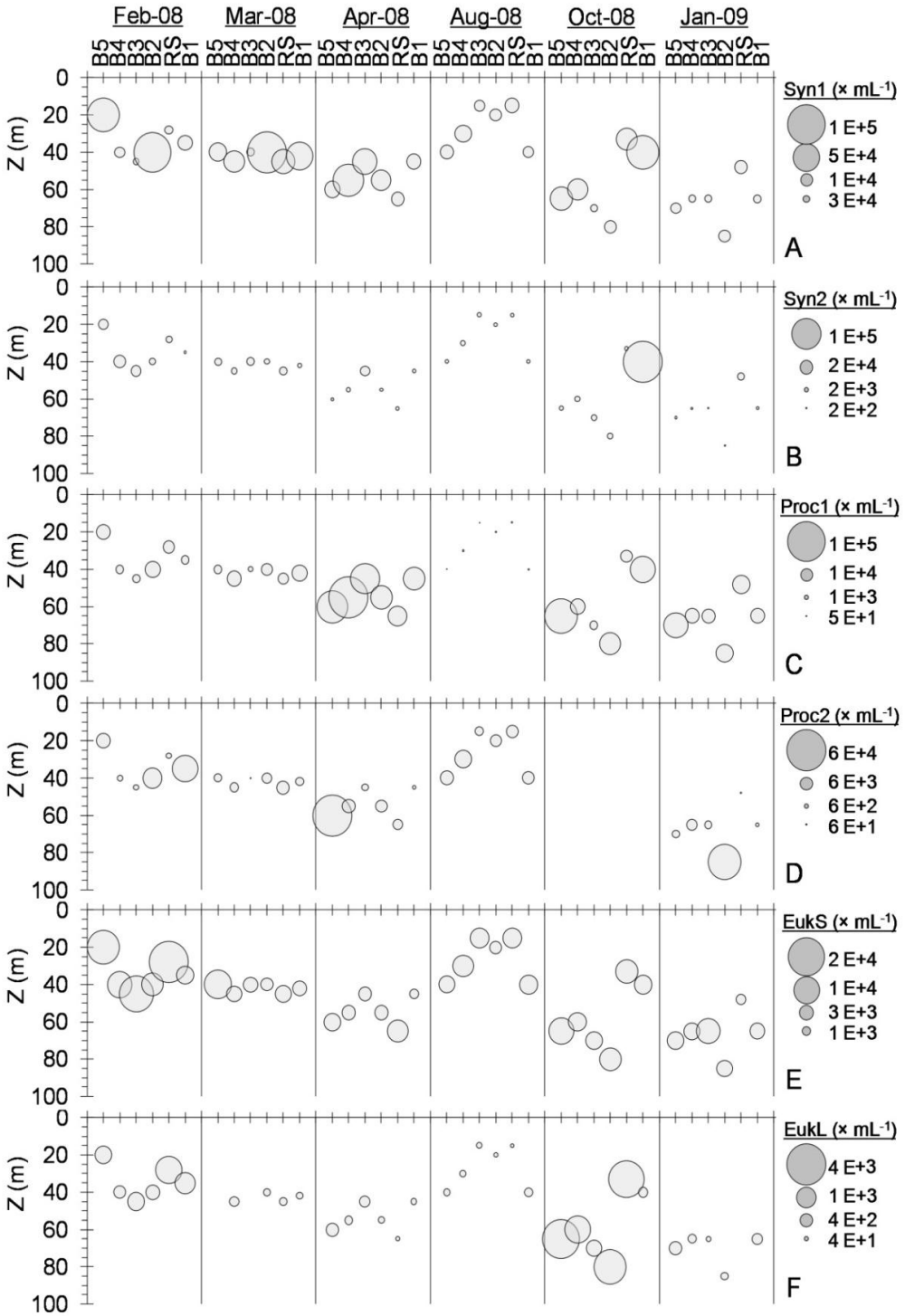

Fig. 7. Dynamics in the abundances of each population of *Synechococcus* (SYN1, SYN2), *Prochlorococcus* (PROC1, PROC2) and eukaryotes (EUKS, EUKL) of the fluorescence maximum (FM) layer at each station from February 2008 to January 2009.

3.4. ***Picophytoplankton abundances and environmental variables***

The BEST analysis revealed that the spatial and temporal patterns of SYN2 and of PROC1 abundances were significantly related to changes in concentrations of NO_x and a combination of the potential energy anomalies, temperature and ammonium concentrations respectively (Table 2). The environmental variables considered in the BEST analysis were not found to be significantly responsible for the spatial and temporal dynamics of any of the other picophytoplankton populations (Table 2).

3.5. ***Local physical events and picophytoplankton community structure***

The relative contribution of each group and population to the total picophytoplankton community abundance changed spatially along the shelf samples and temporally during the course of this study (Fig. 8). We observed distinct population–dominated communities that were associated with changes in the physical structure of the water column and localized physical events. Shifts between *Synechococcus*–dominated communities and *Prochlorococcus*–dominated communities were particularly apparent over time (Fig. 8). *Synechococcus*–dominated communities mainly occurred during (i) periods of strong stratification due to temperature gradients preceded by the occurrence of an upwelling event such as in the late summer of 2008 and (ii) periods of downwelling such as in the winter of 2008 Both periods were characterized by shallow FM layers and low PROC:SYN ratios (Table 1). In contrast, *Prochlorococcus*–dominated communities mainly occurred at.deep FM layers during the summer–winter transition period (April 2008) and when weaker upwelling forcing events were recorded (January 2009; Table 1, Fig. 5). These FM layers typically showed highest temperatures and lowest densities and

Table 2. The BEST analysis ran for each picophytoplankton population gave distinct environmental variables that were responsible for the space–time dynamics observed in the abundances of each population.

Populations	n	*ρ*	*P–value*	k	Environmental variables
SYN1	28	0.090	0.18	3	PE_z, T, S
SYN2	28	0.192	0.01*	1	NO_x
PROC1	28	0.252	0.01*	3	PE_z, T, NH_4
PROC2	28	0.089	0.11	2	T, NH_4
EUKS	28	0.058	0.19	3	T, S, PO_4
EUKL	28	0.040	0.28	2	S, NH_4

n, number of sample; ρ, Spearman rank correlation coefficient; *P*–value, levels of significance with significant results being < 0.05 (*); k, number of significant environmental variables ordered according to their significant contribution level.

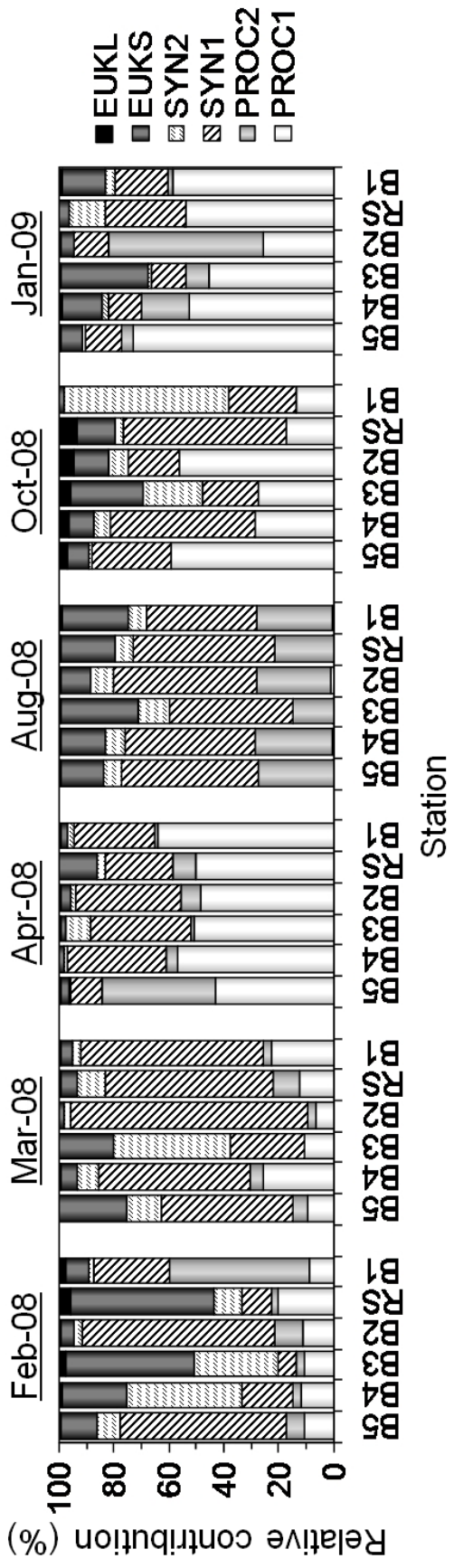

Fig. 8. Temporal succession in the relative contribution of each population of *Synechococcus*, *Prochlorococcus*, and eukaryotes to the total picophytoplankton at each sampling station.

were.characterized by high PROC:SYN and PROK:EUK ratios (Table 1). *Synechococcus* abundance was generally dominated by SYN1 which contribute up to 86% of the picophytoplankton in late summer 2008 (March 2008; Fig. 8), whereas SYN2 contributed to 42 and 60% of total picophytoplankton abundance at stations B3 and B1 in summer and early spring 2008, respectively. On the other hand, PROC1 accounted for up to 74% of the total picophytoplankton in early autumn 2008. In contrast, PROC2 only occasionally significantly contributed to the picophytoplankton community such at stations B1 and B2 in summer 2008 and 2009, respectively (Fig. 8).

Finally, shifts between dominant populations were particularly apparent along the shelf stations in February and October 2008 (Fig. 8). During these two periods, each station was characterized by a different population–dominated community, whereas for the other cruises a single group–dominated community occurred across all stations (Fig. 8). Secondly, the contribution of SYN2 and eukaryotes to the picophytoplankton communities were both higher for these two particular periods. For instance, a greater than 50% contribution of eukaryotes to the community was observed during the February 2008 upwelling event, and a greater than 20–30% contribution was observed during the downwelling event in August 2008 and, in October 2008 following a strong downwelling period (Figs. 2, 5 and 8). This led to a decrease in the PROK:EUK ratio during these periods (Table 1).

4. Discussion

4.1. ***Spatial and temporal variability in picophytoplankton abundances***

All three common groups of picophytoplankton (i.e. *Synechococcus*, *Prochlorococcus* and eukaryotes) were observed and enumerated for the first time in South Australian continental shelf waters, specifically at the *in vivo* fluorescence maximum (FM) layers. Abundances of each group varied spatially and temporally, and were within the concentration range reported for other temperate coastal shelf and oligotrophic oceanic waters (Collier and Palenik 2003; Sherr et al. 2005; Calvo–Díaz et al. 2008; Echevarría et al. 2009; Mitbakvar et al. 2009; Moisan et al. 2010). This high spatial and temporal variability in the abundances of each picophytoplankton population was comparable to that observed seasonally in the Bay of Biscay (Calvo–Díaz et al. 2008) and spatially across the Atlantic (Zubkov et al. 2000) and the Mediterranean Sea (Lasternas et al. 2010). Such variability in picophytoplankton abundances within the FM layer (e.g. Veldhuis and Kraay 2004) may be explained by the fact that *Synechococcus*, *Prochlorococcus* and picoeukaryotes may accumulate at different layer depths within the water column due to their different regime in light, nutrients, and temperatures. As such, peaks in the abundances of all picophytoplankton groups may not always correspond to the FM layer as seen from *in situ* fluorescence vertical profiles, particularly in the presence of larger phytoplankton cells, i.e. diatoms, with higher C/Chl *a* ratios. The high spatial and temporal dynamics observed in picophytoplankton abundances within the FM layers at the group level was, in this case, reflected by

that observed at the population level, which is hence congruent with previous observations in these layers (e.g. Veldhuis and Kraay 2004).

Synechococcus, *Prochlorococcus*, and picoeukaryotes are known to encompass distinct strains and/ or ecotypes of different physiological and ecological characteristics (e.g. Rocap et al. 2003; Johnson et al. 2006; Zinser et al. 2007). The observed local disappearance of large eukaryotes but not small ones suggest indeed rapid changing picoeukaryotes community composition (Massana et al. 2004). In addition, the co–occurrence of a 'bright' and a 'dim' population of *Synechococcus* and *Prochlorococcus* within single samples taken from the FM shows that these two flow cytometrically identified populations of *Synechococcus* and *Prochlorococcus* were not the result of photoacclimation (Falkowski and LaRoche 1991; Lasternas et al. 2010), but possibly of photoadaptation of each population. The potential horizontal and vertical transport of distinct prokaryotes populations may also have occurred on the shelf region. Because of the observed physiological (i.e. fluorescence and SSC; Fig. 2) and ecological (i.e. response to physical forcing events; Figs. 7 and 8) differences between two different populations of each of these groups, this study suggests the existence of distinct picophytoplankton ecotypes (or strains) in South Australian shelf waters. The observation of PROC1 and PROC2 hence suggest that these two populations, respectively, represent High–Light (HL) and Low–Light (LL) adapted ecotypes. The distinct ability to harvest light (e.g. Moore et al. 1995) and the distinct nitrogen type regime (e.g. Moore et al. 2002; Johnson et al. 2006) between PROC1 and PROC2 are supported by the BEST analysis (Table 2) and flow cytometric signatures of each population (Fig. 2). Further work is, however, needed to assess the role of vertical variability in the genetic structure of the picophytoplankton community.

4.2. ***Upwelling and downwelling control the structure of the picophytoplankton community***

We found that distinct populations of all three major picophytoplankton groups alternatively dominated in coastal shelf waters affected by upwelling and downwelling events. Upwelling and downwelling events may build up distinct constraints to picophytoplankton in South Australian continental shelf waters due to the association between stratification and upwelled waters, the transparency of the water column, and the spatial and temporal variability of these events. Mixing and stratification processes have been shown to shape picophytoplankton community structure in other oceanic waters (Bouman et al. 2006, 2011). In the present study, the higher contribution of both SYN2 and picoeukaryotes during the upwelling (i.e. 79%) and downwelling (i.e. 56%) periods (Fig. 5) were likely related to local hydrophysical shifts as shown by the destabilisation of the water column due to upwelling and downwelling (Fig. 2). However, the dominance of SYN1 at all stations in the contrasted prevailing physical events of March 2008 and August 2008 clearly shows the great capacity of adaptation of SYN1 and suggests that variations in the structure of the water column may not always control all picophytoplankton populations (Bouman et al. 2011).

During summer, when the southeasterlies are strong enough and remains for a long time period, a first upwelling event may change the structure of the water column and nutrients availabilities as the upwelled waters reached the 100 m isobath of the shelf, remaining still well below the surface mixed layer. This

upwelled plume is slowly (ca. 0.1 m s^{-1}) advected northwestward which might lead to a slow succession of stratification along the shelf allowing the growth of cyanobacteria over that of larger picoeukaryotes (Raven 1998). This upwelled plume encounters the distinct topographic features (islands, peninsula), which may lead to a mixing with surface water, hence promote the growth of picoeukaryotes. This is consistent with the occurrence of picophytoplankton communities dominated by a different population at each station of the B1–B5 transect in our snapshot of February 2008 (Fig. 8). In addition, the sporadic dominance of PROC2 associated to upwelled waters in February 2008 in B1 or in January 2009 in B2 and that of SYN2 may be due to vertical shear processes, leading to the uplift and the growth of PROC2 and SYN2. This is consistent with previous studies that showed that the Low–Light adapted ecotypes also differed from the High–Light ecotypes in their capacity to uptake nitrate (Rocap et al. 2003; Johnson et al. 2006). In addition, SYN2 have also been reported to be characteristic of deeper waters (Lasternas et al. 2010) and exhibited significant correlation with NO_x concentrations.

Furthermore, if upwelling pulses are close enough in time, when a second upwelling pulse occurs, it is likely that the already settled stratified condition along the shelf may not produce spatial variability in picophytoplankton community structure. However, the new input of nutrients might promote the growth of the adapted population (i.e. *Synechococcus*, March 2008; Fig. 8). Our results hence agree with the dominance of *Synechococcus* reported in other upwelling regions (Zubkov et al. 2000; Sherr et al. 2005; Masquelier and Vaulot 2008; Echevarría et al. 2009). However, while most of these studies reported the picophytoplankton to develop seaward or after nitrate depletion, the offshore upwelled waters, the subsequent advection towards the coast, and the tight coupling between stratification and mixing processes observed in the present work, stress the uniqueness of the South Australian coastal upwelling system leading to a great diversity of dominant picophytoplankton populations.

During winter, local mixing processes involving reduced light irradiance and increased NO_x availabilities might have led to the increase in EUKS abundances and the decline of more than three orders of magnitude in PROC1 abundances (August 2008; Figs. 7 and 8). On the other hand, downwelling processes suggest that the potential for picophytoplankton to remain at the surface would be very low as shown by their lower abundances in August 2008, and the observation of deep FM layers following the downwelling season (October 2008; Fig. 7). Indeed, in October 2008, the transparency of the water column and the period of strong downwelling events prior to the cruise may have caused (i) the dominance of both SYN1 and PROC1 at depths, and, (ii) the absence of PROC2 within the chlorophyll FM layer. SYN1 and PROC1 may have then reached a depth of 80 m, whereas PROC2 may have been present either below this layer or disappeared to deeper depths following to the downwelling season. This agrees with PROC2 being previously observed in deep well mixed waters in contrast to PROC1 (Zinser et al. 2007). Alternatively, the low abundances of PROC1 and disappearance of PROC2 over the winter and spring periods (e.g., Worden et al. 2004; Calvo–Díaz et al. 2008; Linacre et al. 2010) suggest the influence of the oligotrophic surface GAB warm waters to be important for the growth of *Prochlorococcus* as indicated by their highest abundances in April 2008 (Fig. 7).

Finally, the similar spatial and temporal patterns observed between PROC1 and SYN1 abundances agreed with previous studies that reported both *Prochlorococcus* and *Synechococcus* to dominate and co–occur in oligotrophic environments (Crosbie and Furnas 2001; Lasternas et al. 2010). As such, shifts in the PROC:SYN and PROK:EUK ratios that followed the upwelling and downwelling periods (Table 1) do not support a change in the trophic state of the studied region as previously suggested elsewhere (Campbell et al.1998; Pan et al. 2005; Calvo–Díaz et al. 2008; Echevarría et al. 2009). However, the decrease in the PROC:SYN ratio with the depth of the FM agreed with the deeper distribution of *Prochlorococcus* (e.g. Partensky et al. 1999; Zubkov et al. 2000; Calvo–Díaz et al. 2008) and was likely controlled by the occurrence of PROC2 and SYN2, both showing contrasting patterns with that of PROC1 and SYN1.

4.3. ***Local circulation patterns shape the spatial distribution of picophytoplankton***

During summer and early spring 2008, the relative contribution of EUKS and SYN2 increased from the B1 and B5 stations towards B3, the closest station to Spencer Gulf, while that of *Prochlorococcus* and SYN1 decreased over the same periods. The occurrence of this offshore–to–Gulf spatial feature during the upwelling and following the downwelling season show the importance of the local circulation dynamics around B3 and suggests the influence of the seasonal exchange between Spencer Gulf and the shelf waters (Middleton and Bye 2007) on the distribution of picophytoplankton populations. In summer 2008, higher evaporation rates towards Spencer Gulf related to mixing with the cold upwelled pool or the front separating the shelf waters and those of Spencer Gulf (Middleton and Bye 2007) might have promoted picoeukaryotes over that of *Prochlorococcus*. In contrast, in the early spring 2008, the exchanges between Spencer Gulf and the shelf waters associated to processes of downwelling and mixing might have led to the decline in the abundances of most picophytoplankton populations (Fig. 7). These potential above explanations are consistent with the decrease in potential energy anomalies from stations B2 to B3 (Fig. 5).

4.4. ***Potential transport of Prochlorococcus to the Southern Australian shelves***

In other coastal upwelling systems, *Prochlorococcus* is particularly low in abundance or even absent (Hall and Vincent 1990; Zubkov et al. 2000; Sherr et al. 2005; Masquelier and Vaulot 2008; Echevarría et al. 2009). This contrasts with the relatively high abundance ($> 10^4$ cells mL^{-1}) of *Prochlorococcus* over the summer upwelling season in the present region. The only study referring to *Prochlorococcus* south of Australia is from Alvain et al. (2008) who by using satellite models at the global scale have shown an isolated patch of *Prochlorococcus* off Southern Australian shelves in summer but not in winter. Their modelling work is consistent with our *in situ* observations. However, caution here is needed due to the lack of the resolution at the regional scale and the potential inability of Alvain et al. (2008)'s results to differentiate *Synechococcus* to *Prochlorococcus*. On the other hand, the Kangaroo Island–Eire Peninsula (KI–EP) region would be close to the southern boundary distribution limit of *Prochlorococcus* (i.e. 40ºS; Partensky et al. 1999), with observations of *Prochlorococcus* at the subtropical front of the Atlantic sector

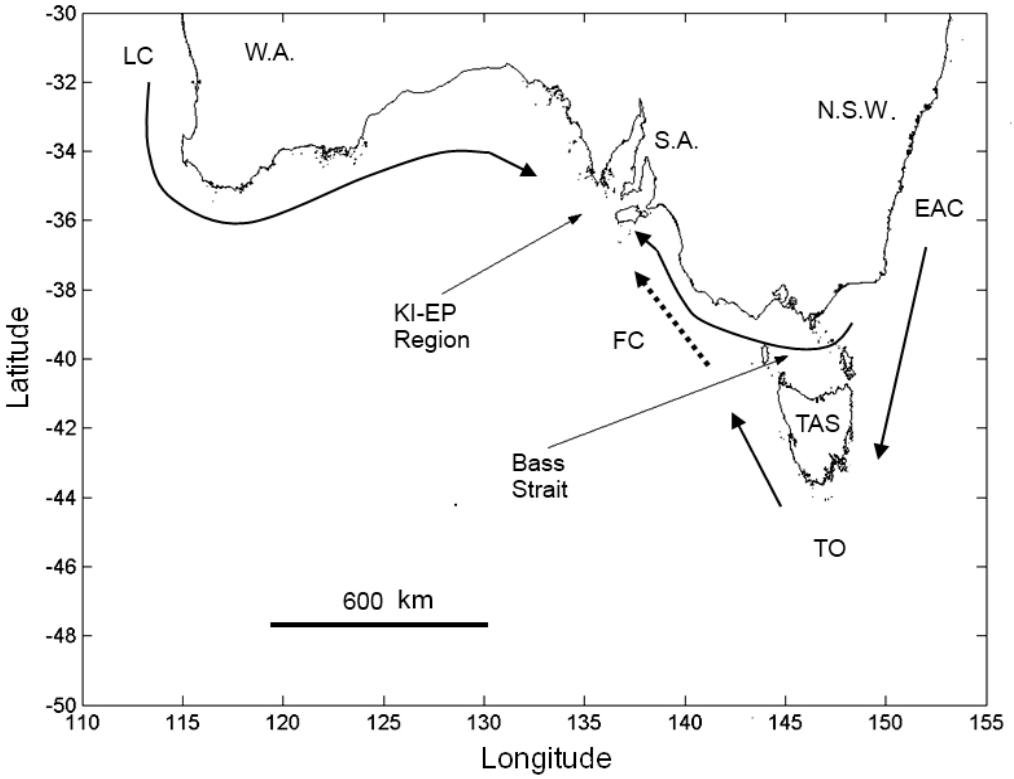

Fig. 9. The Southern Australian region and possible paths of *Prochlorococcus* to the Kangaroo Island–Eyre Peninsula (KI–EP) region. The paths include a) the Leuuwin Current (LC), b) the East Australian Current (EAC) via the Tasman Outflow (TO) and sub–surface Flinders Current (FC) and c) the EAC via Bas Strait. The thick black curve denotes a distance of 600 km. The states: Western Australia (W.A.), New South Wales (N.S.W.), South Australia (S.A.) and Tasmania (TAS) are indicated.

with temperatures as low as 10.7°C (Doolittle et al. 2008). The precise reasons for the disappearance of *Prochlorococcus* below that limit remain unknown, but low temperature have widely been reported as a main control factor (Partensky et al. 1999; Agawin et al. 2000; Doolittle et al. 2008). In the present case, the lowest temperatures recorded at sampled depths were 13.9°C at station B1 in February 2008 when *Prochlorococcus* dominated the picophytoplankton. This questions whether *Prochlorococcus* is advected to or able to growth in South Australian continental shelf waters. Around Australia, *Prochlorococcus* is known to occur in the northern tropical Eastern and Western Australian waters (Crosbie and Furnas 2001; Hanson et al. 2009). We thus here suggest three possible oceanic pathways that might transport *Prochlorococcus* from tropical waters to Australia's southern shelves:

(i) a transport by the tropical LC (Fig. 9), being likely responsible for the presence of *Prochlorococcus* in Western Australian shelf waters. The extension of this current into the present shelf region typically occurs during the austral winter months (June–August) but may also happen earlier during years of stronger LC (i.e. La Niña years; Feng et al. 2003).

(ii) a transport by the East Australian Current (EAC) and via its extension, the Tasman Outflow at the southern tip of Tasmania (Fig. 9). The Tasman Outflow water is downwelled to depths of 500–800 m and in part acts to drive the Flinders Current (FC) to the north–west and into the KI–EP region (Middleton and Cirano 2002). Recent isotopic analyses (Barker 2004; Richardson, personal communication, 2011) do show that Tasman Outflow water is upwelled in the KI–EP region during summer. The current is relatively weak (10–15 cm/s) with a maximum at depths of 600 m or so with temperatures of 9°C (Barker 2004). From the distance scale shown in Fig. 9, it would take two months for this water to be transported to the KI–EP region.

(iii) an aestival transport by the EAC but via Bass Strait and the south–eastern shelves of South Australia (Fig. 9). EAC water could be mixed (through shear dispersion by the strong tides) and/ or transported into Bass Strait and out onto the south–eastern shelves of S.A. as indicated in Fig 9, shelf currents here can be quite strong (40 cm/s) and directed to the north–west during summer upwelling events (Schahinger 1987).

4.5. ***Hotspots of picophytoplankton and primary productivity of the shelf region***

FM layers were mainly located below the base of the mixed layer over the summer upwelling season and showed chlorophyll a concentrations that were similar to that previously recorded in the region (van Ruth et al. 2010a, b). Hotspots of primary productivity were associated to upwelled waters that were observed around southwest of KI in February and March 2005 and south of EP in February and March 2006 (van Ruth et al. 2010a). Hence, primary productivity showed high spatial and temporal variability during the summer upwelling season, whereas lower primary productivities were observed over the winter downwelling season (van Ruth 2009). Picophytoplankton have been shown to contribute up to 75% of the primary productivities in shelf waters (e.g. Morán 2007). In this study, the observed population–specific hotspots of abundance over time and along the shelves, and, the overall higher and lower total picophytoplankton abundances in summer and winter, respectively, yet suggest that picophytoplankton may largely contribute to the primary productivity of the region. Nevertheless, both hotspots of primary production (van Ruth et al. 2010a) and of picophytoplankton abundances (this study) further suggest that such hotspots are a typical response of the phytoplankton communities of the KI–EP region affected by upwelling events. Our work indicates that these pulses of productivity may be linked to shifts in community structure.

5. Conclusion

The spatial and temporal patterns identified in the six distinct picophytoplankton populations observed here provide new information on the occurrences of distinct picophytoplankton populations in South Australian continental shelf waters. The patterns observed in the abundances of these distinct populations are complicated due to (i) the influence of different water masses (Spencer Gulf, FC, GAB, Leeuwin Current, and extensions of the Eastern Australian Current) over time, (ii)

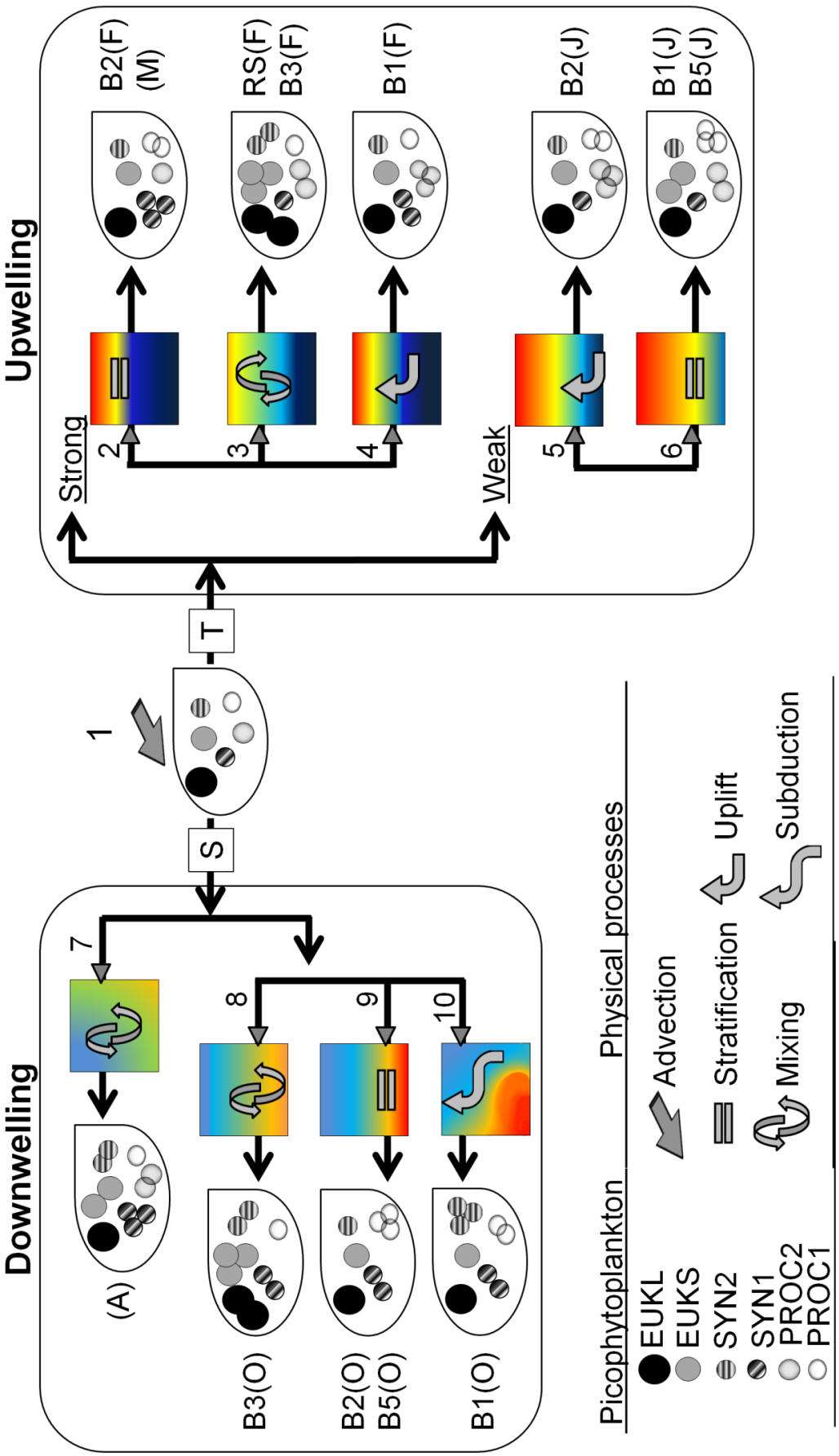
Upwelling
Strong
Weak
B2(F) (M)
RS(F) B3(F)
B1(F)
B2(J)
B1(J) B5(J)
2
3
4
5
6
T
S
1
Downwelling
(A)
B3(O)
B2(O) B5(O)
B1(O)
7
8
9
10
Physical processes
Advection
Stratification
Uplift
Mixing
Subduction
Picophytoplankton
EUKL
EUKS
SYN2
SYN1
PROC2
PROC1

Fig. 10. Schematic illustration of the mechanistic links between physical events (upwelling and downwelling) and the responses of the picophytoplankton community in South Australian continental shelf waters. In these waters dominated by advective processes (1), the dynamics of picophytoplankton community are forced by temperature (T) and salinity (S) respectively during upwelling and downwelling events, and by a range of physical processes (grey arrows). Specifically, under conditions of upwelling, the relative abundance of picophytoplankton groups (circles) are forced by the intensity of (2, 6) the water column thermal stratification, (3) vertical mixing, and (4, 5) uplift. Similarly, under conditions of downwelling, the relative abundance of picophytoplankton groups are forced by first (7) a combination of mixing and downwelling, then the intensity of (8) vertical mixing, (9) water column haline stratification and (10) subduction processes. The stations characterized by the processes described above are shown, together with the months were they occurred (J: January; F: February; M: March; A: August; O: October).

the spatial variability in the topography of the shelf system and (iii) the temporal variability in the occurrence and strength of upwelling events. Our results suggest the importance of advective transport which may have brought *Prochlorococcus* within the KI–EP region (Fig. 9), and, a series of distinct mechanistic links between physical processes and the biological responses of the picophytoplankton community (Fig. 10). Further work involving a greater spatial sampling resolution or a lagrangian investigation of the plume of upwelled water is, however, needed to untangle the details of the mechanisms controlling the growth of picophytoplankon populations. The marked differences in picophytoplankton communities found between summer 2008 and 2009 in strong and weak upwelling conditions also stress the need to relate changes in community composition to the strength of the identified local physical events that are similarly controlled on a larger time scale by El Niño and La Niña (Middleton et al. 2007). In addition, following downelling events, the potential of sinking flux of organic material could be particularly important for the biogeochemical cycling of the South Australian shelf waters. As a consequence to these physical events and hydrographic dynamism, the continental shelf waters of South Australia show high variations in their picophytoplankton communities which may be of importance to evaluate their carrying capacity and their effects on the sustainability of the region's biodiversity and fisheries industry.

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IV. Shifts in picophytoplankton community structure influenced by changing upwelling conditions

ABSTRACT

The influence of upwelling events on the structure of picophytoplankton communities was assessed at the annual scale from a station within the South Australian shelf region. In this region, local (wind) and global (La Niña/El Niño–Southern Oscillation) hydroclimatic conditions affect the development of upwelling over the austral summer. Using flow cytometry, changes in picophytoplankton community structure were investigated in relation to the properties of the water column when the nature and strength of upwelling event differed for the upwelling seasons of 2008, 2009, and 2010. In 2008, strong upwelling favorable southeasterlies were responsible for extensive upwelling and the dominance of picoeukaryotes. Alternatively, in 2009, the observed dominance of *Prochlorococcus* reflected the presence of oligotrophic conditions whilst south–easterlies were replaced by down–welling favorable north–westerlies that likely prohibited the full development of upwelling. In 2010, whilst southeasterlies remained relatively weak, particularly cold and low saline upwelled waters indicated enhanced upwelling events. This weak local wind field together with the occurrence of El Niño explained the observation of shallow upwelled waters below the warm surface layer and subsequent enhanced stratification. These conditions led to the dominance of *Synechococcus* in surface and fluorescence maximum depths, but of *Prochlorococcus* in bottom upwelled waters. The tight association between upwelling and stratification, i.e. whether upwelled waters reach shallower depths and/ or mix with those of the surface as a result of variable climatic conditions, was suggested as the process driving the vertical heterogeneity of picophytoplankton populations. This study brings valuable information for changing picophytoplankton community structure with potential future changing hydroclimatic forcing.

Keywords: picoplankton, *Prochlorococcus*, continental shelves, upwelling, El Niño phenomena, South Australia

1. Introduction

Picophytoplankton (i.e. photoautrophic cells less than 3 µm in diameter) largely contribute to total phytoplankton abundance, biomass, and production in the ocean (Chisholm 1992; Vaquer et al. 1996; Partensky et al. 1999; Agawin et al. 2000; Marañon et al. 2001; Morán 2007). They include three major groups that are *Prochlorococcus*, *Synechococcus*, and picoeukaryotes, which can in turn be subdivided into distinct ecotypes or strains (Rocap et al. 2002; Rodríguez et al. 2005; Worden and Not 2008). Because each of these groups are characterized by distinct physiological and ecological properties (Partensky et al. 1999; Veldhuis et al. 2005), understanding the variability in the relative importance of *Prochlorococcus*, *Synechococcus*, and picoeukaryotes is crucial to understand the fate of carbon and energy fluxes within and between ecosystems.

Current knowledge on picophytoplankton community structure in coastal upwelling systems is sparse (Hall and Vincent 1990; Partensky et al. 1996; Sherr et al. 2005; Reul et al. 2006; Echevarría et al. 2009; Linacre et al. 2010; Daneri et al. 2011; van Dongen–Vogels et al. 2011) largely due to the fact that these nutrient–enriched systems are typically seen as larger phytoplankton (> 20 µm) dominated systems (e.g. Herrera and Escribano 2006; Teixeira et al. 2011). Various studies have previously reported changes in the relative contribution of distinct phytoplankton size classes in terms of both abundances and primary production for coastal upwelling systems (e.g. Iriarte and González 2004; Herrera and Escribano 2006; Cermeño et al. 2006). Despites this lack of information on the dynamic of picophytoplankton communities, picophytoplankton could be important for nutrient and carbon transfer to higher trophic levels in such dynamic regions (e.g. Vargas et al. 2007; Morales and Anabalon 2011). Past and recent studies have nevertheless recorded the dominance of *Synechococcus*, or picoeukaryotes, in surface waters of coastal regions influenced by upwelling, although more typically being reported to growth within the diluted upwelled surface waters advected offshore (Hall and Vincent 1990; Partensky et al. 1996; Sherr et al. 2005; Echevarría et al. 2009). Upwelling coastal waters involving the uplift of deep cold nutrient rich waters towards the surface would be hostile to *Prochlorococcus*, known to dominate in warm (> 20°C) oligotrophic open oceanic waters, having limited growth below 12°C (Partensky et al. 1999). Recent studies have however showed that distinct ecotypes of *Prochlorococcus* have their optimal growth rates at distinct temperatures with relatively higher cell abundances (c.a. 10^4 cells mL^{-1}) of low light adapted ecotypes in relatively cold environments (< 15°C) in contrasted to that of high light–adapted ecotypes being more restricted to warm oligotrophic waters (e.g. South East Atlantic, Zwirglmaier et al. 2008; Jameson et al. 2010, North West Atlantic, Zinser et al. 2007). The abundances dynamic of diverse picophytoplankton populations has only been recently investigated in South Australian continental shelf waters where their high spatial and temporal variability in abundances reflected the complex hydrodynamic of the shelf region, involving localized upwelling and downwelling events (van Dongen–Vogels et al. 2011). Taken together these patterns indicate that varying upwelling conditions are likely to influence picophytoplankton community structure.

Summer upwelling events within the South Australian continental shelf waters could be important to sustain the productivity of the highly valuable fisheries of the region (Ward et al. 2006; van Ruth et al. 2010). During summer, southeasterly upwelling favorable winds and Ekman transport force cold (< 16°C) and low saline (< 35.7) waters of the northern boundary current, the Flinders Current, onto the shelf through the du Couedic Canyon, located south off Kangaroo Island (KI, Fig. 1). The physical dynamic of the summer upwelling season has previously been reviewed by Middleton and Bye (2007) and involve the occurrence of single to multiple upwelling events by pulse, each event potentially followed by intermittent periods of relaxation, mixing, or downwelling processes (Middleton and Platov 2003; Kaempf et al. 2004; Middleton and Bye 2007; van Ruth et al. 2010). Due to the width of the shelf, upwelled waters typically remain below the surface and have previously been observed to slowly (c.a. 0.1 cm s^{-1}) advected northwestward along the 100m isobath of the shelf region (Middleton and Bye 2007; van Dongen–Vogels et al. 2011). The prevailing hydroclimatic conditions such as the alongshore wind and La Niña/El Niño Southern Oscillation (ENSO) may, however, strongly affect the depth at which deep waters are upwelled and the structure of the water column (Middleton and Bye 2007; Middleton et al. 2007). The high inter–annual variability in phytoplankton community structure and production previously

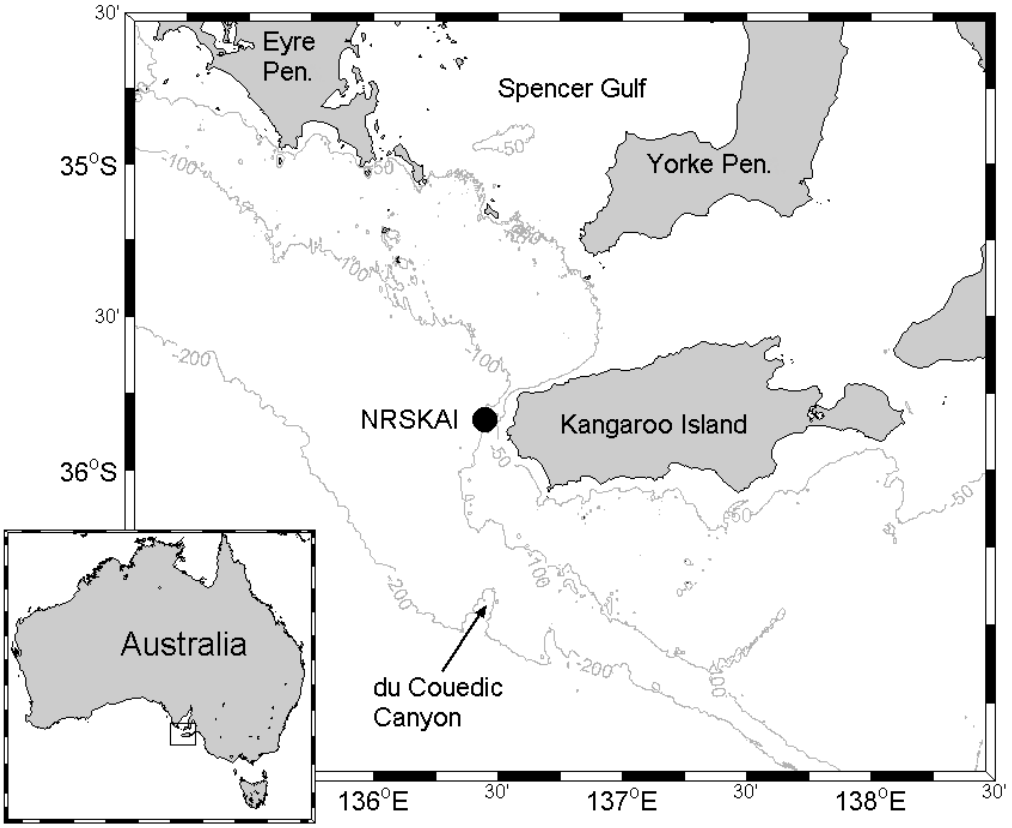

Fig. 1. Map of the South Australian continental shelf region showing the national reference station located off Kangaroo Island (NRSKAI) and the du Couedic Canyon from which waters of the Flinders Current are upwelled over the austral summer.

observed may hence be ultimately controlled by local and large scale changes in hydroclimatic forcing (van Ruth et al. 2010; van Dongen–Vogels et al. 2011).

In this context, the present study focuses on changes in picophytoplankton community structure at a single station located directly on the path of the upwelled plume for three distinct upwelling events that occurred in the summer of 2008, 2009, and 2010. Changing picophytoplankton communities at the fluorescence maxima, but also above and below these depths, yet previously unavailable, was then analysed in relation to changes in the water column structure as a result of varying upwelling conditions. This will improve the current knowledge on how picophytoplankton community structure relate to upwelling–driven processes in South Australian shelf waters where the microbial food web is likely to be important.

2. Materials and Methods

Every one to three months from February 2008 to July 2010, 18 research cruises were performed aboard the *RV Ngerin* at the NRSKAI station (35°50 S, 136°26 E, Fig. 1), a national reference station located west of Kangaroo Island (KI) on the path of upwelling waters advected from its source, the du Couedic Canyon (Kaempf et al. 2004; Middleton and Bye 2007). Collected hydrographic data from each cruise and hydroclimatic data were first explored to quantify changes in upwelling conditions observed over the upwelling season of 2008, 2009, and 2010. To track changes in physical, biological and chemical parameters due to upwelled waters, we particularly focused on the surface mixed layer (15 m), at the fluorescence maximum (FM; identified as the maximum *in vivo* fluorescence from vertical profiles), and in the bottom layer (100 m). To determine a potential mechanism between upwelling and picophytoplankton community structure we analyzed the bulk conditions of the water column and picophytoplankton abundances from the February cruise of each upwelling season, i.e. when the presence/absence and intensity of upwelling conditions differed.

2.1. ***Hydroclimatic parameters***

El Niño and La Niña conditions were evaluated using the Southern Oscillation Index (SOI) given by the Australian Bureau of Meteorology. The method used by the Australian Bureau of Meteorology is the Troup SOI, which refers to the standardized anomaly of the Mean Sea Level Pressure difference between Tahiti and Darwin (http://www.bom.gov.au/climate/glossary/soi.shtml).

Changes in upwelling and downwelling favorable wind conditions were evaluated from changes in alongshore wind stress according to Middleton et al. (2007) and van Ruth et al. (2010). Wind data were automatically recorded at the Neptune Island weather station and were provided by the Australian Bureau of Meterology. Monthly averages were considered here to be consistent with the SOI data.

2.2. ***Biological and Chemical parameters***

Seawater was collected in the surface mixed layer (15 m), at the chlorophyll fluorescence maximum, and in the bottom layer using 5 litre–Niskin bottles from which, three subsamples of (i) 50 mL of seawater were filtered through bonnet

syringe filters (Micro Analytix Pty Ltd) and stored at –20°C for nutrient analysis, (ii) 300 mL of seawater were filtered through fibre glass filters (Whatman GF/C) and stored at –20°C for chlorophyll *a* analysis, and, (iii) 1 mL of seawater were fixed with paraformaldehyde (2% final concentration), frozen in liquid nitrogen, and stored at –80°C for picophytoplankton analysis.

Nutrient analysis was performed according to the Lachat Quickchem methods for phosphate (PO_4, detection limit; 0.03 µM), nitrate + nitrite (NO_x, detection limit; 0.07 µM), and ammonium (NH_4, detection limit; 0.07 µM) on a QuickChem QC8500 Automated Ion Analyser. Chlorophyll *a* was extracted by placing each filter in 5 mL of methanol for 24 hours in the dark at 4°C (Welschmeyer 1994) and chlorophyll *a* (Chl *a*) concentration was determined using a Turner 450 fluorometer previously calibrated with chlorophyll *a* extracted from *Anacystis nidulans* (Sigma Chemicals, St Louis). The chlorophyll concentrations determined from GF/C filters were used here as a standard for environmental parameter for microphytoplankton pigment concentration, allowing comparison with previous investigations in the region (e.g. McClatchie et al. 2006; van Ruth et al. 2010; van Dongen–Vogels et al. 2011). Abundances and fluorescence properties of distinct picophytoplankton populations were analyzed by running each sample for 5 minutes on a FacsCanto flow cytometer from Becton Dickinson (Marie et al. 1999; van Dongen–Vogels et al. 2011). *Synechococcus*, *Prochlorococcus*, and picoeukaryotes were identified based on their autofluorescence and light scatter properties using the software FlowJo (TreeStar). Although distinct populations of picoeukaryotes, *Prochlorococcus* and *Synechococcus* were recently reported within the South Australian shelf waters (van Dongen–Vogels et al. 2011), only two distinct *Prochlorococcus*–like populations (Proc1, Proc2) could be consistently characterized by distinct SSC and Fl3 signals along the present study (Fig. 2). Mean red fluorescence per cell was reported as an indication of the amount or conditions of chlorophyll pigment per picophytoplankton cell (e.g. Moore et al. 1995; Blanchot et al. 2001).

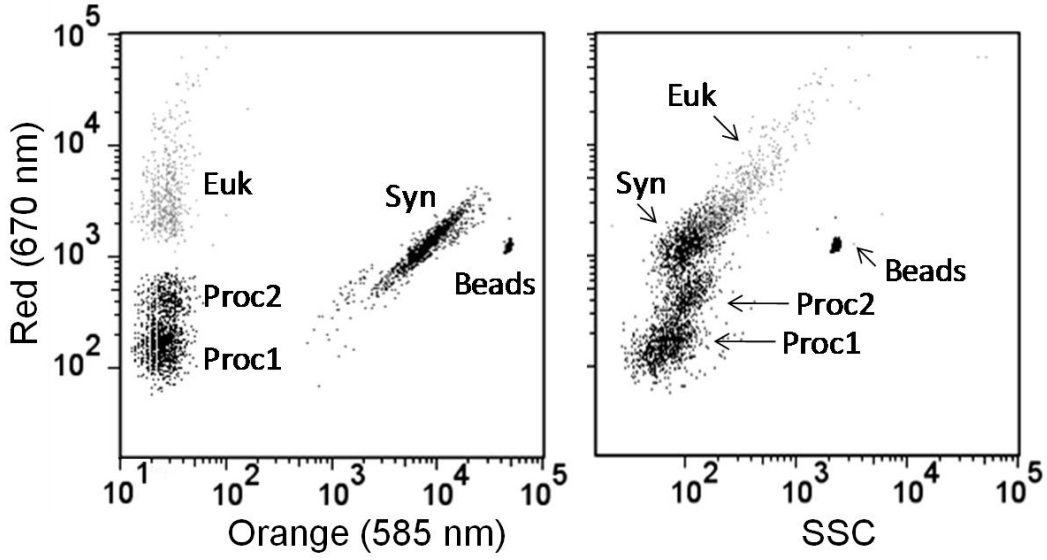

Fig. 2. Example of a cytogram showing the distinct analysed picophytoplankton populations characterized by their pigment cell content (red and orange auto–fluorescence) and cell size (side scatter, SSC).

2.3. *Physical parameters*

For each cruise, vertical profiles were performed with a Seabird SBE 19*plus* CTD system mounted with a biospherical PAR sensor and a Wet Star fluorometer probe (WetLabs). Pressure, conductivity, temperature, light irradiance, fluorescence concentrations, and derived salinity, depth, and density of the water column were then processed using Seabird SBE data processing win32 software. Pressure, temperature, salinity and density data were then used to calculate a stratification index based on the potential energy anomaly PEA_z (J m^{-3}; Simpson, 1981; Mann and Lazier, 1996) defined as:

$$PEA_z = 1/H \int gz\,(\rho - \bar{\rho})\,dz \qquad (1)$$

where ρ is the water density, $\bar{\rho}$ the depth–averaged density ($\bar{\rho} = \frac{1}{H}\int_{-H}^{0} \rho dz$), H the water column depth, g the gravitational acceleration, and z is a given depth. High PEA_z values represent strong and stable density stratification of the water column, whereas low PEA_z values represent unstable water column due to mixing and downwelling process (Mann and Lazier 1996). Given the dependence of the stability of the water column upon the distribution of heat and salt in the ocean, the vertical variation of the potential density, ρ, was further considered as a function of temperature (θ) and salinity (S). Given equation (1), the effect of the variations in temperature and salinity separately to the stabilization of the water column was thus defined as follow:

$$PEA_z(T) = 1/H \int gz\,(\rho\,(\theta, \bar{S}) - \bar{\rho})\,dz \qquad (2)$$

$$PEA_z(S) = 1/H \int gz\,(\rho\,(S, \bar{\theta}) - \bar{\rho})\,dz \qquad (3)$$

where $\bar{\theta}$ and $\bar{S}$ are the average temperature and salinity of the water column, respectively. Finally, the difference between equations (2) and (3), $PEA_z(T)$–$PEA_z(S)$, allowed for a distinction between stratification due to (i) cold and low saline upwelled waters, and (ii) temperature gradients alone which is typical for the summer of temperate systems. This latter consideration was based on the earlier works of Budeus et al. (1989) and Czistrom (1982) who used the ratio between density and salinity gradients to differentiate the contribution of salinity gradients alone from that of temperature to total density gradients.

3. Results and Discussion

3.1. *Hydroclimatic conditions and upwelling strength*

As indicated by the Southern Oscillation Index (SOI) and alongshore wind stress, the nature, intensity and duration of upwelling favorable conditions varied markedly between the three years studied (Fig. 3a). In the long–term, the monthly averaged alongshore wind stress showed stronger upwelling favorable winds (positive alongshore wind stress values) over the 2008 upwelling season than over that of the year 2009 or 2010 (Fig. 3a). Fluctuation in SOI indicated the alternance of La Niña (positive SOI) and El Niño (negative SOI) events over the

study period (Fig. 3a). Although a seemingly positive link between SOI and wind data is noticed during El Niño (Fig. 3a), this relationship is clearly uncertain at such short time scales. Nevertheless, the present observed long–term trend agreed with past investigations showing the absence of long–term relationship

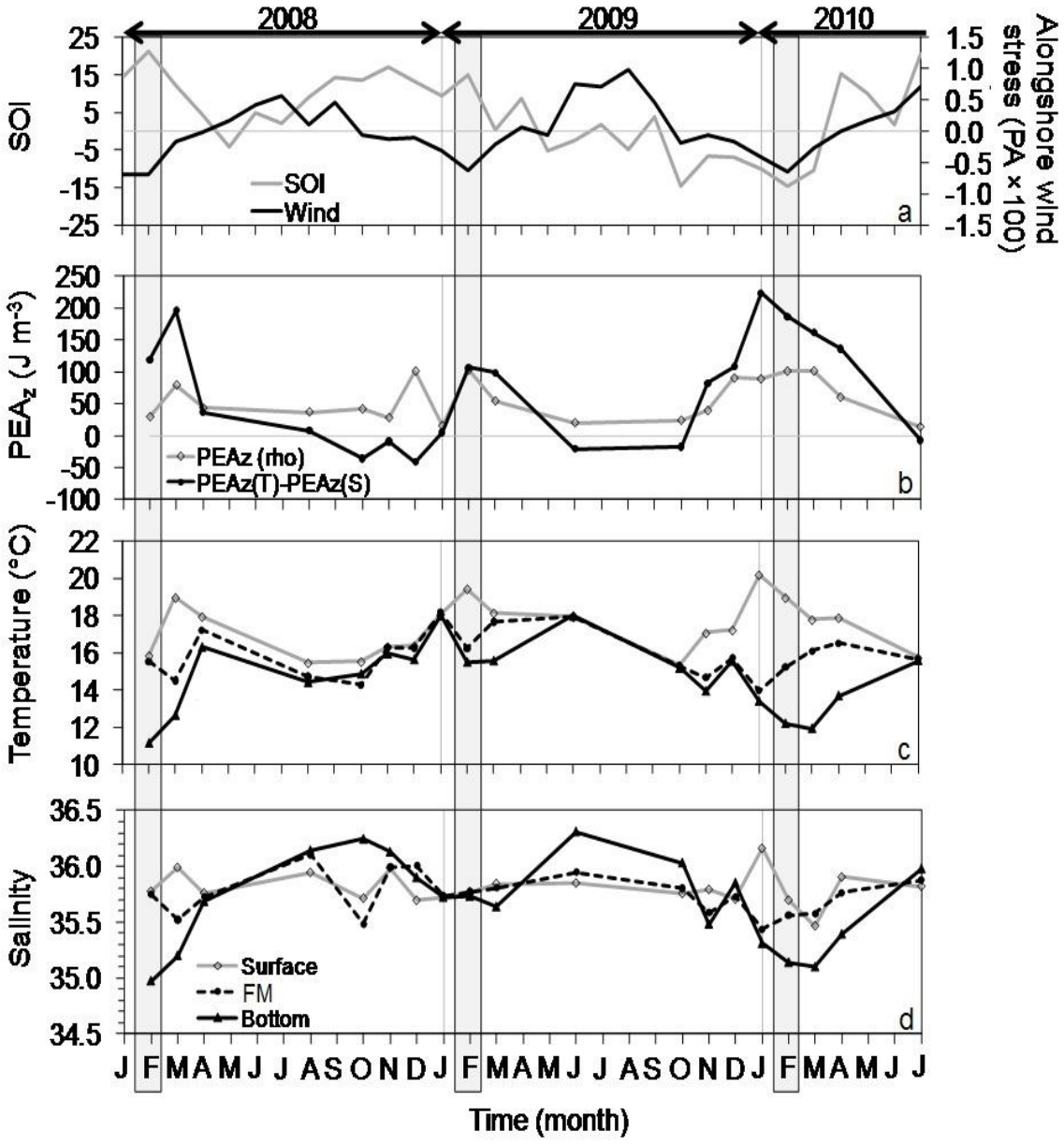

Fig. 3. Temporal changes in hydroclimatic forcing (a) from February 2008 to July 2010 are shown by the Southern Oscillation Index (SOI) with positive values indicative of La Niña conditions and negative values for El Niño conditions, and the alongshore wind stress being upwelling favorable when negative and downwelling when positive. Temporal changes in stratification (PEA_z and $PEA_z(T–S)$), b) and in temperatures and salinities of the sampled depths (c, d). Selected sampling cruises for this study are hached in grey.

between ENSO and local wind field fluctuations (Middleton et al. 2007). The physical effect of El Niño on the upwelling season of the South Australian continental shelf region hence differ from that observed in the Eastern Pacific upwelling coastal regions, where shorter and less intense wind–driven upwelling events are related to El Niño (e.g. Montecinos and Gomez 2010).

Differences observed between upwelling seasons in the hydroclimatic conditions were reflected in the potential energy anomalies of the water column which showed, for instance, more persistent stable stratification (Fig. 3b) over the 2010 upwelling season but relatively unstable stratified water column over the 2008 and 2009 ones (Fig. 3b). This variation in the intensity of stratification over the upwelling season is perceptible from the differences between the potential energy anomalies expressed as a function of temperature and salinity (Fig. 3b). The presence of upwelled waters influenced both salinity and temperature gradients, but temperature gradients also result from the summer warming of surface waters. Upwelled waters were characterized by temperature and salinities below 15ºC and 35.5 (Fig. 3c, d, McClatchie et al. 2006; Middleton and Bye 2007). Depending on the frequency of upwelling pulses, a greater difference in temperature and salinity between the sampled depths suggests that multiple upwelling events successively occurred as deeper waters may have accumulated on the continental shelf slope (Kaempf et al. 2004; McClatchie et al. 2006; Middleton and Bye 2007). In contrast, a smaller difference would reflect a discrete upwelling event. The observed fluctuations in stratification intensities and in the differences between temperature and salinity recorded at the distinct depths (Fig. 3b–d) hence suggest the occurrence of single (e.g. February 2009) or multiple (e.g. February to March 2008, November 2009 to March 2010) upwelling events that may have been intermitted by mixing or downwelling periods (i.e. drop in temperature and/ or salinity differences between sampled depths, e.g. December 2009; Fig. 3c–d).

The weak monthly averaged upwelling favorable winds over both the 2009 and 2010 upwelling seasons could not, however, explain alone the differences in temperature and salinity and stratification observed between these two seasons. Peaks of strong downwelling favorable winds recorded over the 2009 upwelling season (not shown) most likely reduced the potential full development of upwelling. On the other hand, enhanced stratification and upwelling events have been shown to result from an input of a distinct cold water source to the Flinders Current during El Niño event (Middleton et al. 2007). Specifically, El Niño promotes the poleward propagation of a cold coastal trapped waveguide along the western side of Australia which is then further directed along the southern side of Australia. The encountering of the coastal trapped waveguide to the Flinders Current at the shelf break off South Australia may have helped the accumulation and uplift of colder waters (Middleton et al. 2007). These upwelled waters do not, however, reach the surface because of the relatively wide shelf in this region and its specific topography. As such, when (i) upwelled waters reach shallower depths, (ii) are colder or less saline or (iii) sea surface temperatures are higher, stratification intensifies. The observed changes in upwelling conditions and stratification intensities (Fig. 3b–d) could have been hence ultimately driven by the combined local wind field, the occurrence of El Niño, and shelf topography.

3.2. ***Upwelling strength and changes in the water column properties***

Upwelled waters of temperatures and densities respectively below 15.5°C and above 26 kg m^{-3} laid below 70 m in February 2008, were absent in February 2009, and reached a depth of 40 m in February 2010 (Fig. 4). In February 2008, the mixed layer was relatively cold for the summer. The long lasting southeasterlies favored vertical mixing between the cold upwelled waters and the warm upper the surface mixed layer, with subsequent cooling and nutrient enrichment, and, the development of a thick and strong subsurface fluorescence maximum (FM, Fig. 4). In contrast, enhanced stratification due to El Niño

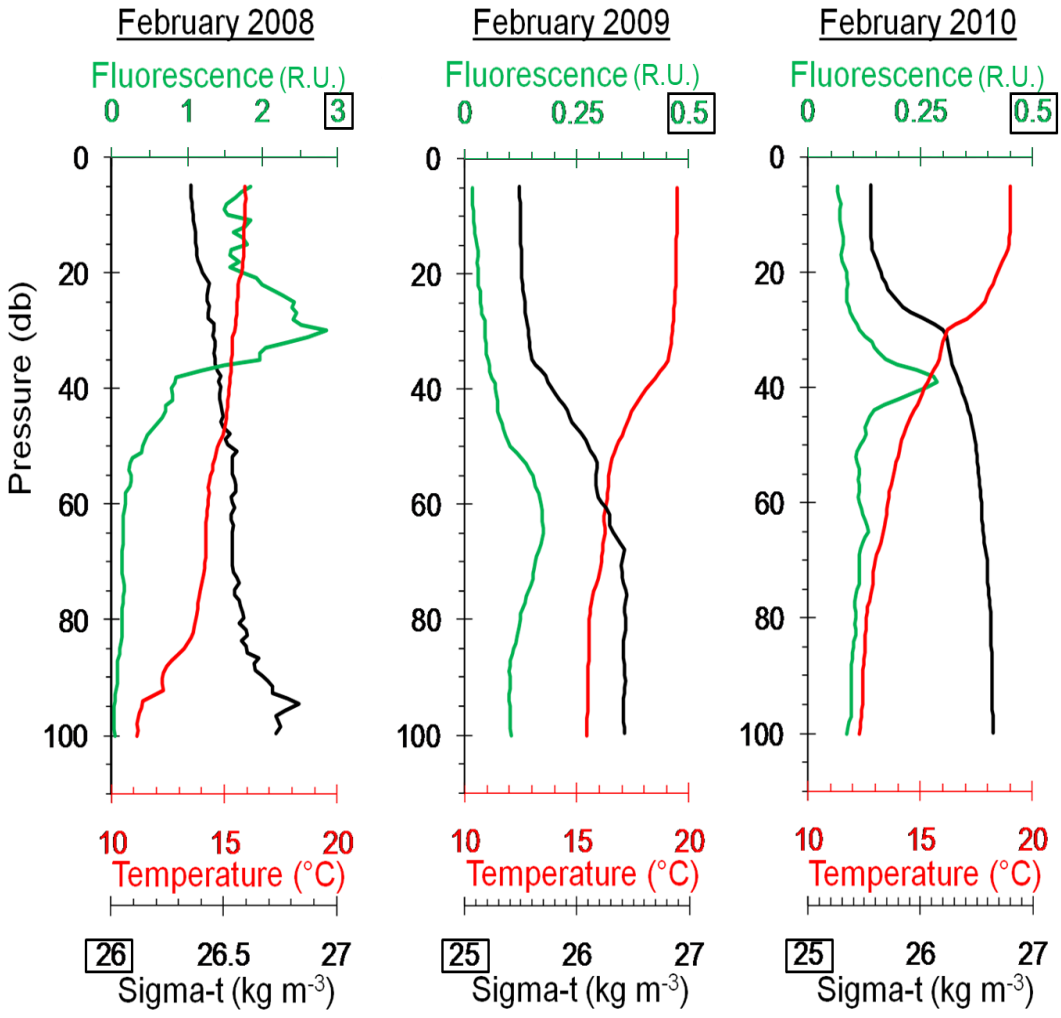

Fig. 4. Vertical structure of temperature, sigma–t, and fluorescence of the NRSKAI station in February 2008, 2009, and 2010. As indicated by temperatures and densities below 15°C and above 26.5 kg m^{-3}, the signature of upwelled waters was observed in February 2008 and 2010, but not in 2009. Note the difference in the scale of the fluorescence and sigma–t between February 2008 and those of 2009 and 2010, as well as in the relative position of the subsurface fluorescence maximum depth to the mixed layer depth between years.

conditions in February 2010 led to relatively low nutrient concentrations the upper depths and strong shallow thermocline and pycnocline. The thin and weak subsurface FM observed in February 2010 contrasted with that observed in 2008. Such differences likely reflected a greater C/Chl *a* ratio due to the presence of larger phytoplankton cells in 2008 in contrasted to that observed in 2010. Although the location of the FM relative to the thermocline and nutrient concentrations differed between upwelling events of 2008 and 2010, chl *a* concentrations greater than 1 µg l^{-1} were recorded for both years (Table 1). Similar chl *a* concentrations were previously recorded for the region during the 2004 wind–driven upwelling season (van Ruth et al. 2010), but were about four times lower than that of the major upwelling event recorded in March1998 (Kaempf et al. 2004), an El Niño upwelling season. Although Kaempf et al. (2004) were not aware of the potential influence of El Niño to upwelling activity, the stronger upwelling favorable winds that occurred during their study may have explained the differences observed between the present results and those of Kaempf et al. (2004). On the other hand, the vertical structure of the water column in February 2009 presented a weak fluorescence maximum below a deep thermocline(Fig. 4) with chl *a* concentrations not exceeding 0.1 µg L^{-1} and nutrient concentrations increasing below the thermocline (Table 1), reflecting oligotrophic conditions (e.g. Marañon et al. 2001).

Table 1. Nutrients (µM) and chlorophyll *a* (µg L^{-1}) concentrations, picophytoplankton abundances (× 10^3 mL^{-1}), and the relative contribution of red fluorescence (%) of each population observed at the surface, fluorescence maximum (FM), and bottom sampled depths (m) for each February of the 2008, 2009, and 2010 upwelling season. Note: '<':lower than limit of detection, '–':absent.

	Surface		FM			Bottom	
	Feb–09	Feb–10	Feb–08	Feb–09	Feb–10	Feb–09	Feb–10
Depth	15	15	28	62	30	92	103
NH_4	<	<	0.1	<	<	0.1	<
NO_x	<	0.4	1.3	0.2	0.0	2.0	8.8
PO_4	<	<	0.1	0.1	<	0.2	0.5
Chl *a*	0 to 0.1	0.5 to 0.8	1.3	0.1	1 to 1.1	0.1	0.4 to 0.8
Abundance							
Syn	35.1 to 53.4	35.4 to 113.4	7.9 to 9.5	1.8 to 2.2	57.8 to 137.5	2.6 to 4.2	1.0 to 1.4
Proc1	44.4 to 59.7	15.1 to 45.8	6.4 to 6.8	4.7 to 5.2	27.4 to 56.5	1.1 to 1.5	0.4 to 0.5
Proc2	-	-	0.1 to 0.6	1.9 to 2.3	-	7.6 to 11.2	1.3 to 1.6
Euk	0.9 to 1.8	1.7 to 5.3	21.3 to 32.9	0.9 to 2.1	2.7 to 3.3	3.4 to 4.7	0.5 to 0.6
Relative mean red fluorescence							
Syn	47.6 to 60.4	63.3 to 63.6	3.3 to 7.4	18.4 to 22.8	77.0 to 84.2	13.4 to 14.3	33.1 to 36.2
Proc1	14.8 to 22.5	8.0 to 9.1	0.2 to 0.5	4.6 to 8.7	6.9 to 7.3	0.3 to 0.4	0.5
Proc2	-	-	0 to 0.1	5.6 to 8.8	-	10.8 to 12.7	7.6 to 8.7
Euk	17.1 to 37.6	27.0 to 28.7	92.1 to 96.4	59.6 to 71.5	8.8 to 15.7	73.5 to 74.6	55.8 to 57.7

3.3. ***Upwelling strength and changes in picophytoplankton community structure***

Picophytoplankton abundances (Table 1) were between those previously observed in eutrophic and oligotrophic waters (e.g. Campbell et al. 1997; Partensky et al. 1999; DuRand et al. 2001; Sherr et al. 2005; Zwirglmaier et al. 2008; Echevarría et al. 2009) and agreed with those recently reported for the present region (van Dongen–Vogels et al. 2011). Each picophytoplankton group showed a maximum abundance value specific to each observed upwelling condition (i.e. fluorescence maximum (FM) in February 2010 for *Synechococcus*, surface in February 2009 for *Prochlorococcus*, and FM in February 2008 for picoeukaryotes) while lowest cell abundances were reported for all three groups in bottom cold nutrient rich upwelled waters recorded in February 2010 (Table 1).

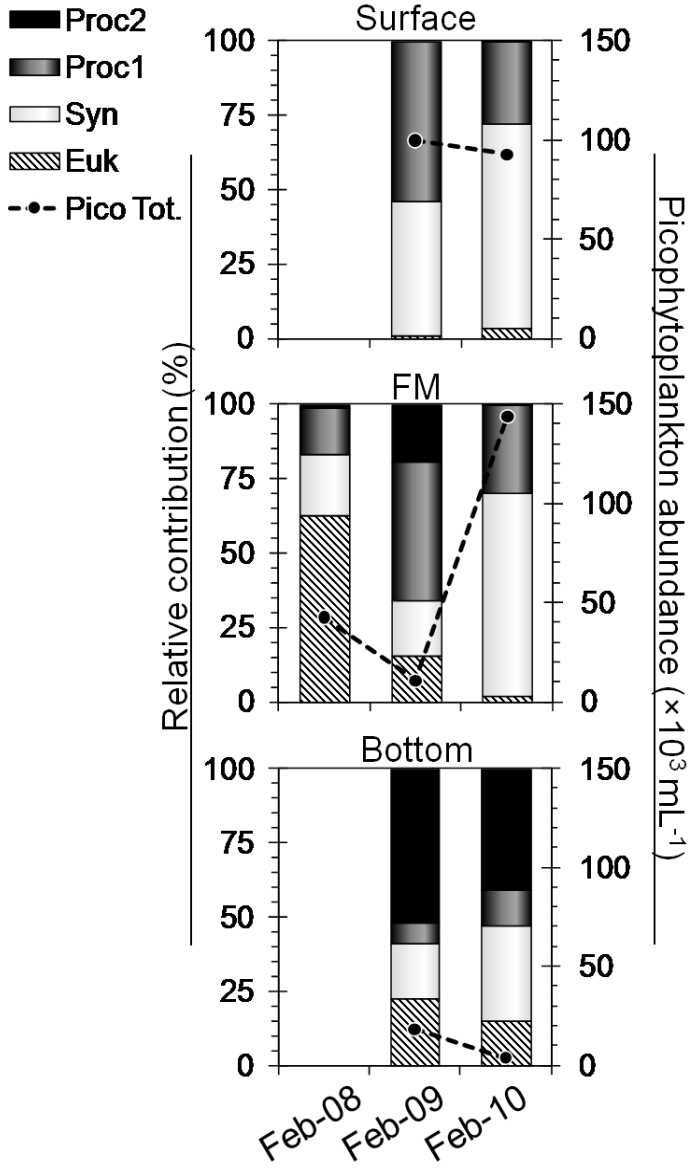

Fig. 5. Vertical and annual changes in the relative contribution of *Prochlorococcus*, *Synechococcus* and picoeukaryotes (bars) to total picophytoplankton abundances (dashed line) at the surface, fluorescence maximum (FM), and bottom layer depths for February 2008, 2009, and 2010.

In surface waters, *Synechococcus* mainly dominated bulk picophytoplankton abundances, although differences between *Synechococcus* and *Prochlorococcus* absolute abundance values were two times lower in February 2009 than in 2010, *Prochlorococcus* abundances being lower in 2010 (Fig. 5, Table 1). In contrast, picoeukaryotes absolute and relative abundances were higher in February 2010. These observed differences in the abundances of each picophytoplankton group between years (Fig. 5, Table 1) might be explained by their difference in surface to volume ratio (e.g. Raven 1998; Veldhuis et al. 2005) together with (i) a potential load of nutrients from upwelled waters through small shear processes prior to sampling in February 2010, and, (ii) the shallower upwelled waters observed in February 2010 (Fig. 4). Shifts in picophytoplankton community structure between picophytoplankton community structure reflected the inter–annual years were particularly marked at the fluorescence maxima (FM, Fig. 5). There, picoeukaryotes dominated bulk picophytoplankton abundances in 2008, *Prochlorococcus* in 2009, and *Synechococcus* in 2010. Changes in variability in stratification intensity associated with upwelling conditions and in the location of the FM depths relative to those of the thermocline and pycnocline (Table 1, Fig. 4). In February 2009 and 2010, the vertical variability in picophytoplankton communities was marked by the inverse relative contribution of two distinct populations of *Prochlorococcus* (Proc1, Proc2) with depths (Fig. 5). This vertical

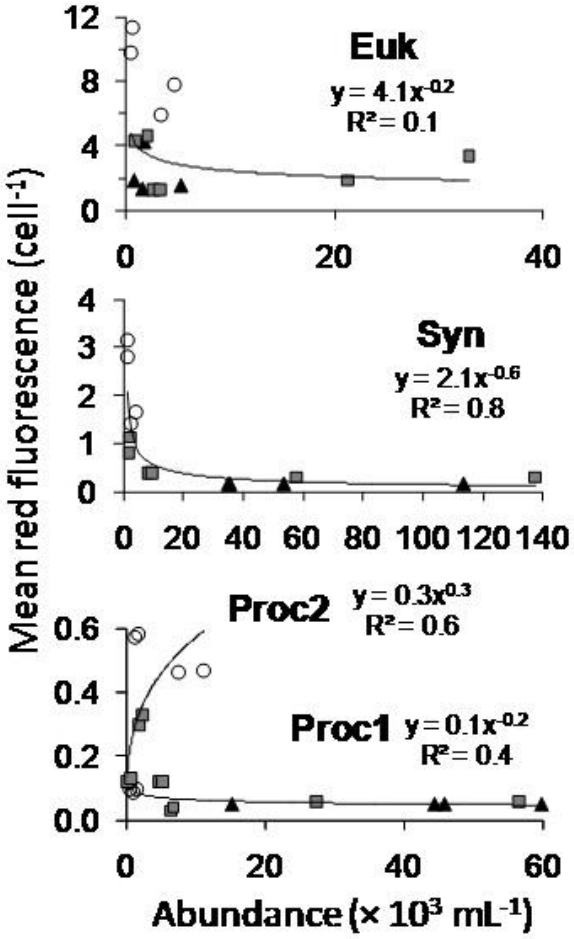

Fig. 6. Mean red fluorescence as a function of abundances for eukaryotes (Euk), Synechococcus (Syn) and Prochlorococcus (Proc1, Proc2) at the surface (black diamonds), fluorescence maximum (grey squares), and bottom (open circles) depths

segregation between Proc2 and Proc1 suggests that Proc1 may represent one high–light and Proc2 one low–light adapted ecotypes of the *Prochlococcus* genera (West and Scalan 1999; Johnson et al. 2006). Because of the low vertical resolution of our sampling strategy, their niche partitioning was not apparent. However, Proc2 was absent in the surface depths and accounted for less than 30% of the total *Prochlorococcus* abundances at the FM. In contrast, at the bottom, Proc2 accounted for 77 and 92% of the total *Prochlorococcus* and for 41 and 56% of the total picophytoplankton in 2009 and 2010, respectively.

Synechococcus contributed the most to bulk picophytoplankton chlorophyll in surface waters and only in FM waters in February 2010, whereas picoeukaryotes made the most of picophytoplankton chlorophyll at the FM and bottom depths (Table 1). These dominances, both in terms of chlorophyll content and abundance, of picoeukaryotes and *Synechococcus* in the presence of upwelled waters conformed to those previously reported in the surface waters of other coastal regions affected by upwelling (Hall and Vincent 1990; Sherr et al. 2005; Echevarría et al. 2009). The relationship between the amount of chlorophyll content per cell and abundances also differed between picophytoplankton groups (Fig. 6), likely reflecting their differential capacity of physiological adaptation to changing light levels (e.g. Veldhuis and Kraay 1990; Moore et al. 1995; Dusenberry et al. 2000). For instance, the significant inverse relationship between cell abundances and red fluorescence for *Synechococcus* showedimportant photoacclimation processes. This was also shown by its increasing chlorophyll content per cell but decreasing cell abundances with depth (Fig. 6). In contrast, the absence of significant relationship between cell abundances and red fluorescence for picoeukaryotes suggests the presence of diverse picoeukaryotes with distinct potential of photoacclimation (Fig. 6). Further insights into the nature of the distinct populations identified here through flow cytometry could be provided by molecular approaches. The molecular characterization of the picoeukaryotic populations remains too rudimentary to currently provide a complete characterization of the community, only recently being achieved (Massana et al. 2004, 2011).

Besides changing light levels, the low temperatures of upwelled waters might limit the growth of picophytoplankton, and especially *Prochlorococcus*. Low temperatures have previously explained their reduced cells abundance or absence in polar or coastal regions affected by seasonal changes in the ocean hydrodynamic, including coastal upwelling systems. The relatively high abundance of *Prochlorococcus* observed here (up to 10^4 cells mL^{-1}) however fall within the upper ranges of past recorded *Prochlorococcus* abundances found elsewhere at temperatures below 15°C (Table 2). In summer, surface temperatures above 15°C may however be critical for the growth of *Synechococcus* and *Prochlorococus* at the upper depths of the sampled station, both groups being able to thrive in nutrients depleted waters (DuRand et al. 2001; Tai and Palenik 2009). At the bottom, abundances of all picophytoplankton groups were largely reduced in cold (c.a. 12°C) and high nitrate upwelled waters (i.e. February 2010, Table 1, Fig. 5) when compared with warmer and lower NO_x conditions (i.e. February 2009, Table 1, Fig. 5). Nevertheless, the present finding that *Prochlorococcus* (potentially a LL–adapted ecotype) can also dominate bulk picophytoplankton abundances in relatively deep coastal nutrient–rich upwelled waters as cold as 12°C (Fig. 4–6, Table 1)

Table 2. *Prochlorococcus* abundances (Abs) recorded in diverse cold (c.a. < 15°C) environments. LL: Low–Ligth.

Region	T (°C)	Abs (× 10^3 mL^{-1})	References	Notes
Meditteranean Sea				
NW Mediterranean Sea	< 14	40	Vaulot et al. 1990	winter surface waters
Atlantic				
North Atlantic Ocean	<15	<10	Buck et al. 1996	
	10.2	8		
	<15	6.8 +/- 12.8		
SE to NE Atlantic	7 to 19	few to >0.1	Johnson et al. 2006	LL with higher abs at T=13°C passed 40°S than passed 40°N
SE Atlantic	12 to 13	15 to 51	Zwirglmaier et al. 2007	LL genotypes found throughout the mixed water column
Pacific				
Southern California Bight 32.53°N, 117.15°W	13 to 15	absent to 40	Worden et al. 2004	Proc dominated bulk picophytoplankton when T=15°C
East China Sea	6 to 17.1	absent to 6	Jiao et al. 2005	winter waters
(transitional waters)	15.6	0.3		lowest winter T for Proc to be present
	14.3	4		summer stratified cold waters
SE Pacific	< 20	< 0.1	Zwirglmaier et al. 2008	LL ecotype observed deep down in mixed waters only detectable south of 60°N
Southern Ocean				
34. 8 to 55.0°S at about	< 10.64	absent	Doolittle et al. 2008	only detectable north of 38°S
10°W to 10°E	14.3 to 15.7	10 to >100		Proc dominated bulk picophytoplankton when T>14°C

was in line with previous studies showing relatively abundant LL–adapted ecotypes in cold waters (Table 2) and the great potential microdiversity of various *Prochlorococcus* ecotypes being to grow in diverse ecological niches (e.g. Johnson et al. 2006; Zinser et al. 2007; Zwirglmaier et al. 2007; Martiny et al. 2009; Bouman et al. 2011).

Overall, the observed inter–annual change in picophytoplankton community structure was associated to changes in the physical properties of the water column reflecting the importance of stratification and upwelling processes. Under relatively weak or absence of upwelling event (i.e. easterly upwelling favorable winds, El Niño condition, temperature of < 15.5ºC, salinity > 35.5, February 2009), *Prochlorococcus* is likely to dominate the picophytoplankton standing stock because of the development of deep stratification leading to oligotrophic conditions which may have reduced the development of picoeukaryotes and *Synechococcus*. The presence of two distinct *Prochlorococcus*–like ecotypes showing distinct niche partitioning and physiological adaptation (e.g. Fig. 2, 5–6; Zinser et al. 2007) also likely allowed for the extended dominance of this group on the vertical. Under relatively strong upwelling event, the dominance of picoeukaryotes and *Synechococcus* to picophytoplankton standing stocks are both likely to take place at the FM, but the former could be dependent on the occurrence of mixing processes between the nutrient–rich upwelled and nutrient–depleted upper mixed waters (i.e. February 2008 *vs* 2010). This is consistent with the faster growth rates of larger phytoplankton cells under mixing conditions (e.g. Raven 1998; Partensky et al. 1999) and the increasing absolute and relative abundances of picoeukaryotes below the thermocline in the presence of higher nutrients concentrations in the bottom layer (Fig. 5, Table 1). In contrast, upwelling activity and enhanced stratification likely allowed the dominance of *Synechococcus* during El Niño. Although the physical processes involved in the effect of El Niño on upwelling events observed here might differ from that of the Eastern Pacific (e.g. Chavez et al. 2002; Massoti et al. 2011) and Western Tropical Pacific regions (e.g. Blanchot and Rodier 1996), the high *Synechococcus* abundances (> 10^4 cells mL^{-1}) observed at the surface and FM depths were similar to those reported in these above regions of the Pacific, being in all cases the result of an increase in abundances between the early to later stages of El Niño (van Dongen–Vogels, unpublished data; Blanchot and Rodier 1996; Chavez et al. 2002; Masotti et al. 2011). Such coincidence between distinct geographical environments calls for further work to enable to depict the potential mechanistic linkages between *Synechococcus* and El Niño events, but yet question its ecological meaning.

Finally, the succession of distinct dominant picophytoplankton populations possibly also occurred following upwelling due to intermittent mixing and downwelling in between upwelling pulses and the subsequent redistribution of nutrients in the water column (Fig. 2, Table 1, McClatchie et al. 2006; Middleton and Bye 2007). The better adapted population to changing local water conditions will have then rapidly increased in abundances. The differential dependence of temperatures, light availabilities, and nitrate concentrations to the growth and abundances of distinct picophytoplankton groups, ecotypes, or strains together with changes in stratification intensity associated to varying upwelling conditions likely explained the depth heterogeneity in picophytoplankton community structure observed here.

4. Conclusions

Changing wind conditions were recently suggested to affect the productivity and community structure of the Concepción upwelling area (Daneri et al. 2011). Here, variable upwelling conditions associated to climatic forcing (wind field and El Niño) was suggested as the process influencing vertical and annual changes in picophytoplankton community structure, abundances, and fluorescence properties within the continental shelf of South Australia. Wind–driven upwelling event could be expected to result in rather homogeneous distribution of phytoplankton populations through mixing. However, depending on the strength of upwelling favorable winds and the occurrence of El Niño, the depth reach by upwelled waters might differ, resulting in varying mixing and stratification intensity. The local topography characterized by a relatively wide continental shelf and the presence of islands might also have affected mixing between upwelled and surface waters around the sampled station (i.e. Figs. 3 and 4). The strong patterns observed here indicate that the hydroclimatic variability observed in this location may exert a strong influence on the microbial ecology of this region. Further studies investigating the influence of these processes on other groups of the prokaryotic and eukaryotic plankton will be useful for better integrating microbial food–web processes into temporal hydroclimatic variability.

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V. Distinct temporal dynamics in the abundances of picophytoplankton, heterotrophic bacteria, and viruses revealed a vertical decoupling of viruses and bacteria during upwelling of an El Niño.

ABSTRACT

The effect of hydroclimatic forcing events on the temporal dynamics of picophytoplankton, heterotrophic bacteria, and viruses abundances was investigated from 2008 to 2010 from the surface, fluorescence maximum, and bottom waters of a station located in South Australian continental shelf waters. The location of this station was chosen to assess the effect of local (wind) and global (El Niño/La Niña) climatic forcing on upwelling and downwelling events characterizing these waters. Using flow cytometry, we enumerated and discriminated picophytoplankton, including populations of bright and dim *Prochlorococcus*, *Synechococcus*, and picoeukaryotes, as well as heterotrophic bacteria and viruses. Viruses showed a significant long term decrease in abundance, while heterotrophic bacteria remained relatively constant up to October 2009. The seasonality in picophytoplankton abundances was marked at the surface, where each group present distinct temporal patterns with peaks consistently observed for *Prochlorococcus* and picoeukaryotes, and to a lesser extent for *Synechococcus*. Significant linkages among groups were found at the bottom. There, up to one order of magnitude higher picophytoplankton abundances than the upper depths occurred in October 2009, following the downwelling season. As a result to these distinct temporal patterns, we found significant relationships between microbial groups and viruses. Correlations analyses suggest a vertical decoupling between heterotrophic bacteria and viruses during upwelling under El Niño conditions and weak southeasterlies, when viruses significantly correlated to only cyanobacteria at the upper depths. The Southern Oscillation Index significantly explained the variability in heterotrophic bacterial abundances and prokaryotes:eukaryotes ratios. The remaining changes in microbial abundances were likely attributed to the local variability in the wind field. Our findings reflected the differential effect of temporal and vertical temperature and salinity gradients associated to changing upwelling and downwelling conditions. These results have critical implications for the long–term understanding of the ecology and biogeochemical cycling of

the still poorly understood South Australian continental shelf waters and brings further insights on the effect of hydroclimatic forcing events on picophytoplankton, heterotrophic bacteria and viruses.

Keywords: microbial dynamics, picophytoplankton, heterotrophic bacteria, virus, upwelling, downwelling, El Niño, South Australia

1. Introduction

The efficiency of carbon and energy transfer within aquatic systems depends upon the time and space scales at which the distinct planktonic components respond to any given physical forcing (Legendre and Demers 1984). Picophytoplankton and heterotrophic bacterioplankton are responsible for the production and regeneration of organic and inorganic matter within the microbial food chain (Azam et al. 1983; Cho and Azam 1988; Chisholm 1992). The contribution of the microbial loop to biogenic flux is believed to increase with decreasing productivity levels of a system (Cotner and Biddanda 2002). Hence, in contrast to coastal upwelling regions, in the oligotrophic ocean, picophytoplankton often dominate the phytoplankton community both in terms of biomass and productivity (Agawin et al. 2000). However, recent studies have also shown picophytoplankton to provide up to 75% of the primary productions in coastal waters (Morán 2007), perhaps due to increasing of sea surface temperatures (Morán et al. 2010). Both picophytoplankton and heterotrophic bacteria have been shown to be controlled by temperatures (e.g. Campbell et al. 1997; Li 1998; Agawin et al. 2000) but are also subject to grazing and viral infection (Proctor and Fuhrman 1990; Bratbak et al. 1992; Suttle and Chan 1994; Weinbauer and Peduzzi 1995; Furhman 1999; Mann 2003). In return, the release of dissolved organic and inorganic products from grazing and viral lysis is used by bacteria and picophytoplankton for growth and production (Proctor and Fuhrman 1990; Weinbauer 2004; Weinbauer et al. 2011). As such, viruses also play a key role in carbon and energy transfers and could be important in sustaining regenerated production in marine systems (Bratbak et al. 1994; Furhman 1999; Bettarel et al. 2011).

Prochlorococcus, *Synechococcus*, and picoeukaryotes are well known picophytoplankton groups of pelagic systems and each have been shown to present distinct ecological and physiological characteristics as well as encompassing distinct ecotypes and strains (Chisholm et al. 1992; Partensky et al. 1999; Rocap et al. 2003; Veldhuis 2005; Johnson et al. 2006; Shi et al. 2009). For instance, the recurrence observation of a bright and a dim *Prochlorococcus* population identified by flow cytometry, led to the founding of Low–Light (LL) and High–Light (HL) ecotypes of *Prochlorococcus*, respectively (Campbell and Vaulot 1993; Partensky et al. 1999). These distinct populations or ecotypes have been shown to be vertically segregated within the water column due to their differences in light harvesting properties, and have also shown distinct capacity in nitrogen uptake (West and Scanlan 1999; Rocap et al. 2003; Johnson et al. 2006). Because of the specific capacity of adaptation of each picophytoplankton group to changing conditions (Campbell and Vaulot 1993; Veldhuis and Kraay 1993), mechanisms relating changes in picophytoplankton community structure to hydrographic conditions remain poorly understood in continental shelf waters. However, the relative importance of each group may lead to distinct carbon and energy pathways and question the existence of distinct relationships among the dynamics of distinct picophytoplankton, heterotrophic bacteria and viruses (Cotner and Biddanda 2002; Clasen et al. 2008) associated to temporal variability in hydroclimatic forcing events.

Seasonal to annual variability in microbial abundances and community structure have largely been reported for diverse environment (Campbell et al. 1997;

Zubkov et al. 2000; Calvo–Díaz et al. 2008) and have been related to changes in hydrographic conditions (Jiao et al. 2002; Linacre et al. 2010), light irradiances, the depth of the nitracline and mixed layer and water column stability and mixing (Campbell et al. 1997; Partensky et al. 1999; Bouman et al. 2011). Variability in viral abundances mainly reflects that of their cell hosts, depending however on their life cycle strategies (i.e. lytic and lysogenic) which have also been shown to change with environmental conditions (Weinbauer and Suttle 1999; Williamson et al. 2002; Bettarel et al. 2011). Viral abundances are also affected by UV radiations (Suttle and Chen 1992; Weinbauer et al. 1997), and have been reported to vary with salinity and temperature gradients, depths, and productivity levels (e.g. Brussaard et al. 2008; Schapira et al. 2009; Weinbauer et al. 2011).

At the annual scale, phenomena such as El Niño/La Niña are known to induce perturbations to the thermocline with subsequent important changes in the phytoplankton community structure and productivity (Karl et al. 1995; Blanchot and Rodier 1996; Campbell et al. 1997; Masotti et al. 2011). In the equatorial and western Pacific, recent and past studies on picophytoplankton and El Niño Southern Oscillation (ENSO) events have reported a decrease in *Synechoccocus* abundances in the early stage of El Niño while increasing subsequently at later stage of El Niño (Blanchot and Rodier 1996; Masotti et al. 2011). On the other hand, enhanced upwelling conditions during La Niña years were recently reported to be responsible for the decline in picophytoplankton abundances in the western coastal Pacific (Linacre et al. 2010).

The South Australian continental shelf region presents seasonal upwelling and downwelling events and exchanges between shelf and adjacent gulf waters (Middleton and Bye 2007) which have been shown to be affected by changes in wind duration and intensities and El Niño/La Niña cycles at the annual scale (Middleton and Bye 2007; Middleton et al. 2007). In the summer, upwelling occurs by pulses from the du Couedic Canyon southwest of Kangaroo Island (Middleton and Platov 2003; Kaempf et al. 2004; Middleton and Bye 2007). Due to the width of the shelf, cold and low saline upwelled waters remain below the surface and are advected northwestward along the shelves (Middleton and Bye 2007). Downwelling occurs in the winter during which the cooling of the summer warm and high saline waters of Spencer Gulf lead to the gravitational outflow of dense bottom waters towards the du Couedic Canyon (Fig. 1; Lennon et al. 1987; Middleton and Bye 2007). While the present shelf waters typically show oligotrophic conditions, they hold one of Australia's largest fishing industry, likely as a consequence of upwelling events (Kaempf et al. 2004; McClatchie et al. 2006; Middleton and Bye 2007, van Ruth et al. 2010). Hotspots of phytoplankton production and abundances have recently been reported in the region, but the mechanism underlying these observations remains poorly understood (Seuront et al. 2010; van Ruth et al. 2010a, b). Yet knowledge on picophytoplankton, and specifically heterotrophic bacteria and viruses are sparse for this system (Seuront et al. 2010), the efficiency of the microbial food chain in these waters could be greatly affected by the inter–annual variability in the relative contribution of the local wind field and the ENSO–driven upwelling and downwelling events.

As part of the Intergrated Marine Observation System (IMOS), a Southern Australian national reference station, NRSKAI (Fig. 1), was implemented on the

South Australian continental shelf in February 2008. Specifically, this station is located on the path of upwelled and downwelled (i.e. outflow of dense bottom) waters west of Kangaroo Island (KI) (Lennon et al. 1987; Middleton and Bye2007; Fig. 1). This station thus offered a unique location to investigate the long–term impact of these physical events on the ecology and biogeochemical cycling of this system. In this context, the goal of this study was to examine the temporal variability in picophytoplankton, heterotrophic bacterial and viral abundances within the surface, fluorescence maximum, and bottom depths of the NRSKAI station from the 19 sampling cruises performed from February 2008 to July 2010. Specifically, our aims were (i) to determine the temporal variability in the abundances of distinct picophytoplankton populations, heterotrophic bacteria and viruses and quantify their relationships within each depth, (ii) to infer potential mechanistic links with temporal changes in the chemical and physical properties, and (iii) to quantify how the observed temporal patterns in microbial community structure are linked to inter–annual variability in local and global hydroclimatic forcing.

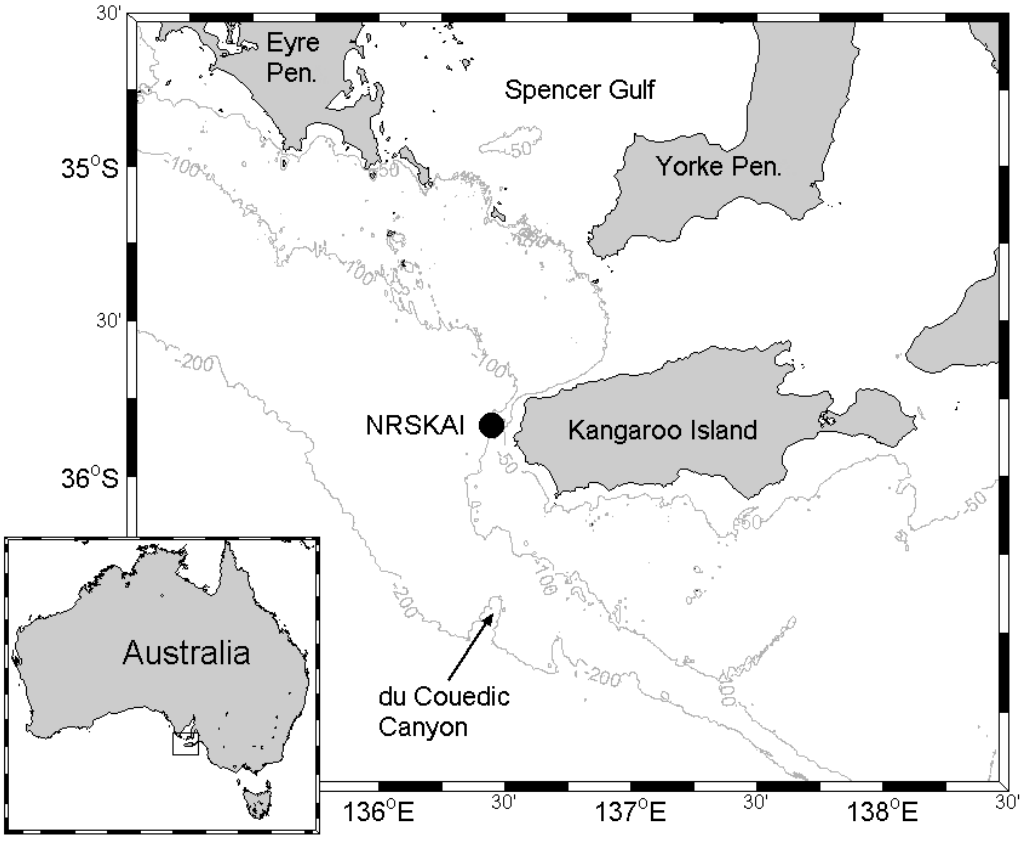

Fig. 1. Map of the Kangaroo Island–Eyre Peninsula region showing the location of the NRSKAI station, the national reference station of the Southern Australian shelf waters, and the location of the du Couedic Canyon from which upwelled and downwelled waters enter and exit the shelf region.

2. Materials and Methods

2.1. *Sampling strategy*

Sampling was carried out on board the *RV Ngerin* at the national reference station located southwest of Kangaroo Island (NRSKAI; 35°50 S, 136°26 E; Fig. 1) on the South Australian continental shelves on 19 occasions; once a month between February and April 2008, October 2008 and March 2009, October 2009 and April 2010, and in August 2008, June 2009, and July 2010. During each cruise, hydrographic data were collected through vertical profiles conducted with a Seabird SBE 19*plus* CTD system mounted with distinct probes including PAR (Photosynthetically Active Radiation) and chlorophyll fluorescence (WetLabs). However, no PAR data were available in March and December 2009, February and March 2010. All measurements taken from CTD casts were processed and binned averaged to 1 db interval using Seabird SBE Data Processing win32 software. Derived density and salinity were also computed from pressure, temperature, and conductivity during data processing. Water samples were taken using 5–litre–Niskin bottles at the surface or 15 m depth (August 2008 to July 2010), (ii) the maximum fluorescence determined by the *in vivo* fluorescence profile (February 2008 to July 2010), and (iii) the bottom or below 75 m depth (October 2008 to July 2010 cruises). From Niskin bottles, seawater subsamples were taken for analysis of nutrient concentrations (NH_4, NO_x, PO_4) and microbial abundances. For nutrients, triplicates of 50 mL of seawater were filtered through 0.45 µm bonnet syringe filters (Micro Analytix Pty Ltd) and kept at –20°C until analysis. For picophytoplankton, triplicates of 1 mL of seawater were fixed with paraformaldehyde (0.2%, final concentrations), and for heterotrophic bacteria and viruses, triplicates of 1 mL of seawater were fixed with glutaraldehyde (0.2%, final concentrations) and left in the dark for 15 minutes. These samples were frozen in liquid nitrogen, and stored at –80°C until analysis (Marie et al. 1999; Brussaard 2004).

2.2. *Nutrient concentrations*

Subsamples were analyzed according to the Lachat Quickchem methods for phosphate (PO_4, detection limit; 0.03 µM), nitrate + nitrite (NO_x, detection limit; 0.07 µM), and ammonium (NH_4, detection limit; 0.07 µM) concentrations on a QuickChem QC8500 Automated Ion Analyser at the South Australian Research and Development Institute (SARDI, South Australia).

2.3. *Microbial abundances*

All samples were processed on a Becton Dickinson FacsCanto flow cytometer fitted with a 488 nm argon laser. One µm yellow–green fluorescent marker beads (Molecular Probes, Eugene, Oregon) were used as an internal control to normalize each recorded flow cytometric parameters (Campbell and Vaulot 1993; Marie et al. 1999; Sherr et al. 2005).

Prochlorococcus (Proc), *Synechococcus* (Syn), picoeukaryotes (Euk) – Twenty µL of beads solution was added to each subsample prior processing them through the flow cytometer. Orange fluorescence from phycoerythrin (585 nm), red fluorescence from chlorophyll (670 nm), side light scatter (SSC), and forward light scatter (FSC) were recorded for five minutes. *Synechococcus*, *Prochlorococcus*, and eukaryotes populations were identified according to their

respective autofluorescence and light scatter properties (Marie et al. 1999) using the software FlowJo (TreeStar). Although two distinct sub–populations of *Synechococcus* and picoeukaryotes could previously be discriminated, the lack of a consistent separation between clouds of these sub–populations, led us to only deal here with the group level. In contrast, we were able to consistently distinguish two *Prochlorococcus* sub–populations (Proc1, Proc2) which were characterized by distinct SSC and red signals with Proc1 showing lower SSC and red signals than Proc2.

Heterotrophic bacteria (HB) and virus–like particles (VLP) – Seawater subsamples were stained with SYBR–Green according to Brussaard (2004) and ten µL of beads solution were added in each subsample prior analysis. SYBR–green fluorescence (530 nm), SSC, and FSC parameters were recorded for two minutes. To get an overall estimate of the bulk HB and VLP abundances, we only focused on defining the whole HB and VLP communities. These communities were easily identified by their differences in SYBR–green fluorescence and SSC signals, VLP showing lower SYBR–green fluorescence and SSC than HB (Marie et al. 1999; Brussaard 2004).

2.4. *Meteorological and oceanographic conditions*

Southern Oscillation Index – El Niño and La Niña conditions were evaluated using the Southern Oscillation Index (SOI) provided by the Australian Bureau of Meteorology (http://www.bom.gov.au/climate/glossary/soi.shtml).

Upwelling and downwelling – Changes in upwelling and downwelling intensities over time were evaluated on three days averaged of the alongshore wind stress ($\tau = \rho_{air} C_D U|U|$, Middleton et al. 2007, van Ruth et al. 2010). This latter was calculated with three hourly wind data (U) automatically recorded at the Neptune Island weather station and provided by the Australian Bureau of Meteorology. The drag coefficient, C_D, was taken from Gill (1982).

Evaluations of mixing and stratification – Mixing *vs* stratification intensities were evaluated from distinct computations to address stratification from sampling depths as well as the whole water column and to differentiate stratification due to density, salinity, and temperature gradients. Stratification at sampling depths was evaluated using the Richardson number, Ri, a dimensionless number, which is defined by the ratio between the buoyancy or Brunt–Vaisälä frequency ($N^2 = -g/\rho\ (d\rho/dz)$) of the density gradient ($d\rho/dz$) and the square of the shear velocity gradient ($d\mu/dz$) and is formulated as (Mann and Lazier 1996):

$$Ri = N^2/(du/dz)^2 \qquad (1)$$

where ρ is the water density, g the gravitational acceleration, u the horizontal current velocity. Horizontal current velocity data were provided from continuous recorded measurements of a moored acoustic Doppler current profiler (ADCP) put at the bottom of the NRSKAI station. A Richardson number less than 0.25 represents unstable waters and mixing is likely to occur. Inversely, a Richardson number of above the 0.25 value represent more stable conditions (Mann and Lazier 1996). Stratification of the whole water column was evaluated from the potential energy anomaly, PEA (J m^{-3}; Simpson 1981; Mann and Lazier 1996), defined as:

$PEA_{z(\rho)} = 1/H \int gz\,(\rho - \bar{\rho})\,dz$ (2)

where ρ is the water density, $\bar{\rho}$ the depth–averaged density ($\bar{\rho} = \frac{1}{H}\int_{-H}^{0} \rho dz$), H the water column depth, g the gravitational acceleration, and z is a given depth. High and low PEA values are stable and unstable density stratification of the water column, respectively (Mann and Lazier 1996). The development of stratification and the stability of the water column depending on the distribution of heat and salt in the ocean, we also considered the difference of the potential energy anomaly as a function of temperatures and salinities, $PEA_{z(T–S)} = PEA_{z(T)} – PEA_{z(S)}$ by substituting temperatures and salinities from density in the formula described above. This allowed for a distinction between stratification due to (i) cold and low saline upwelled waters, and (ii) temperature gradients mainly influencing the summer stratification of temperate systems. This latter consideration was based on the ratio between density and salinity gradients used by Budeus et al. (1989) from the earlier work of Czitrom (1982), to differentiate the contribution of salinity gradients alone from that of temperature to total density gradients.

Euphotic zone and surface mixed layer – The base of the euphotic zone (Z_{eu}) was calculated by substituting the coefficient of downwelled irradiance, K_d, into the Beer–Lambert equation ($Z=\ln(E_z/E_0)/K_d$, Kirk 1994) following van Ruth et al. (2010). Due to the occurrence of strong salinity gradients observed over the downwelling season (i.e. high saline waters from Spencer Gulf at the bottom), we considered here the base of the surface mixed layer as the depth (Z_m) at which $d\sigma_t = 0.125$ from a 10 m depth referential value (Bouman et al. 2011).

2.5. ***Data analysis***

The presence of a long–term trend in the abundances of viruses, heterotrophic bacteria, and picophytoplankton was assessed for each sampled layer through linear regression analysis with sampling times taken as the dependent variable. Because the distribution in the abundances of most microbial populations were not significantly normal (Kolmogorov–Smirnov test, $p < 0.05$), the non parametric, Spearman's rank correlation coefficient, ρ, was carried out throughout this study, using PASW18 software.

3. Results

3.1. ***Temporal variations in meteorological and oceanographic conditions***

At the annual scale, the South Australian continental shelf region can be separated into two distinct seasons based on the seasonal alternation between prevailing easterlies and westerlies that are upwelling and downwelling favourable, respectively (Fig. 2). From February 2008 to July 2010, we observed that these local climatic conditions also vary in intensity and durations between years (Fig. 2). For instance, upwelling favourable winds of higher magnitudes were recorded over January–February 2008 than in 2010, but the strongest peak was recorded in March 2010. On the other hand, downwelling favourable winds were clearly stronger in2009 than 2008 (Fig. 2). Vertical profiles of temperatures

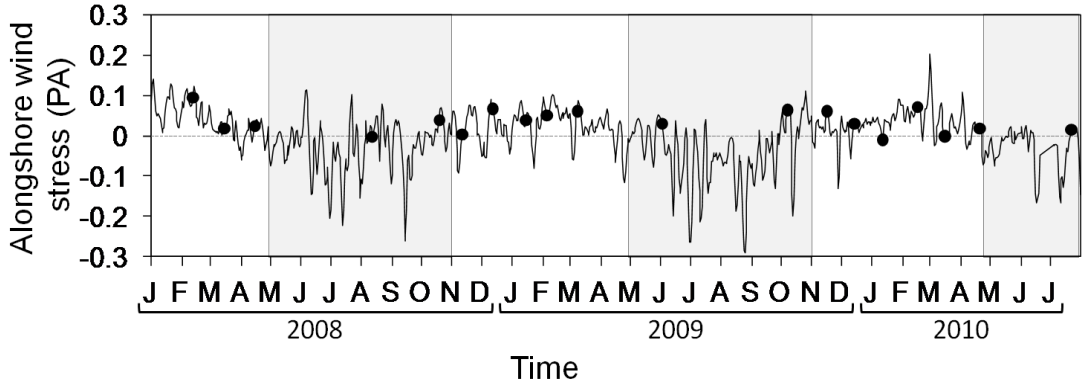

Fig. 2. Time series of the three daily averaged alongshore wind stress component rotated on 315T. From February 2008 to July 2010, the two distinct seasons are shown by the alternance of positive and negative values corresponding to upwelling (unshaded) and downwelling (shaded) favourable winds, respectively. Sampling times are shown by filled black circles.

and salinities showed the occurrence of upwelling events for each sampled upwelling season. They differed, however, between years in numbers and in intensity as indicated by the extension of temperature and salinities less than 15°C and 35.4 in the water column and the sampled times (Fig. 3A, B).

As such, the year 2009 showed a weaker upwelling season than the other two years. The period of relaxation observed in December 2009 also shows that multiple upwelling events may have occurred and likely forced the buildup of deeper upwelled waters off south Kangaroo Island (Middleton and Platov 2003; Kaempf et al. 2004). The presence of El Niño that year may have also forced upwelled waters to reach shallower depth as indicated by a strong thermocline (Middleton et al. 2007). Cooler and fresher surface waters indicated mixing between upwelled and summer warm (> 17°C) surface waters (i.e. February 2008, April 2010; Fig. 3B), while warm and saline surface waters, likely indicated summer evaporation (i.e. March 2008, January 2010; Fig. 3B). In contrast, the downwelling season of each year was particularly marked by saline bottom waters greater than 36, indicative of dense bottom waters that outflowed from Spencer Gulf towards the du Couedic Canyon (Lennon et al. 1987; Middleton and Bye 2007). This event was, however, cooler in 2008 and 2010 than in 2009. The warmer water column and saline bottom waters of June 2009 were likely due to important evaporation in the Gulf during the previous summer. The April–May and October–November months hence appeared more like a period of transition being modulated by the hydroclimatic conditions of each year, such as upwelling or downwelling events can be observed in the November or April months (Fig. 2; Middleton and Platov 2003). Consequently to these physical forcing events, differences in the nature and intensity of mixing and stratification of the water column were marked both at the seasonal and annual scale. As indicated by the differences in the potential energy anomalies between temperatures and salinities $PEA_{z(T–S)}$, enhanced stratification of the water column and thin surface mixed layers over the 2010 upwelling season agreed with the occurrence of El Niño from June 2009 to March 2010, while La Niña prevailed at other times (Fig. 3C, Table 1). Richardson number, when available, showed that mixing was specifically

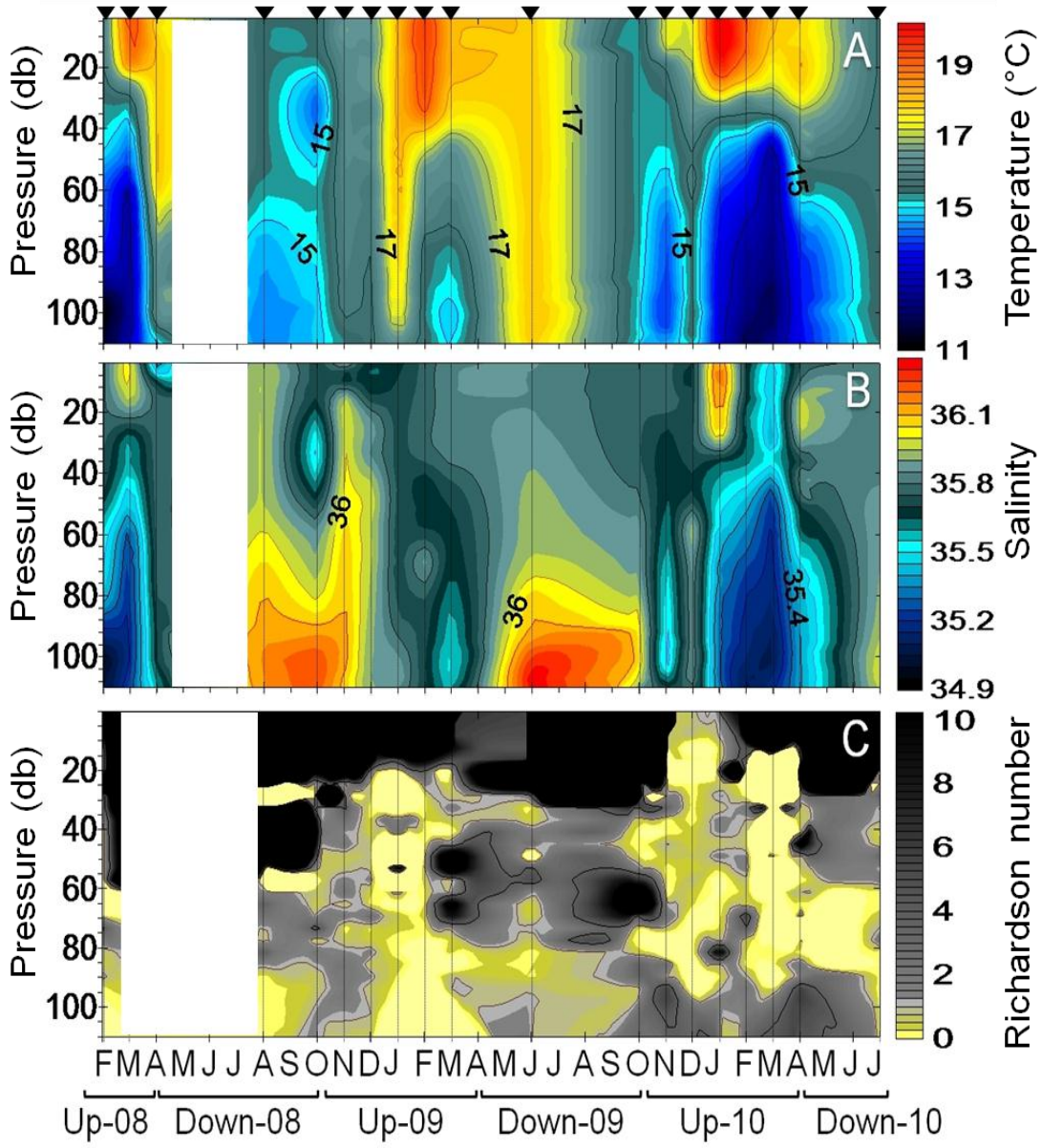

Fig. 3. Vertical sections of temperature (A), salinity (B), and the Richardson number (C) over the distinct upwelling and downwelling seasons from February 2008 to July 2010 at the NRSKAI station. Sampling cruises are noted by dotted lines and dark filled triangles. Krigging method was used to extrapolate temperature, salinity, and Richardson number data (Surfer v.7). Note that data for computing the Richardson number were not always available for depths above 24 m and below 98 m and no data were available in March and April 2008.

occurring at the fluorescence maximum depths, and to a lesser extent at the bottom layer, depending on the sampling cruises (Fig. 3C). Overall, the base of the euphotic zone followed that of the base of the surface mixed layer (r^2=0.45, $p < 0.05$), agreeing with previous reports assessed during past upwelling seasons (van Ruth et al. 2010). However, the relative position of the base of the euphotic zone to the pycnocline (Z_m) differed between sampling times. Specifically, the base of the euphotic zone was closer to the pycnocline when the water column was unstable (i.e. February 2008, April 2008 and 2010, January 2009), and was above it for the 2009 and 2010 downwelling seasons, but below it when upwelled waters reached shallow depths and in August 2008 (Table 1). This may have led to the difference observed in the relative position of the fluorescence maximum to the pycnocline (Z_m), being e.g. below the base of the mixed layer when high stratification occur over the upwelling season (Table 1).

Table 1. Variability in the physical properties of the structure of the water column over the sampling cruises at the NRSKAI station. K_d: coefficient of downwelled irradiance, r^2: pearson correlation coefficient between K_d and depth, Z_{eu}: euphotic depth, Z_m: the base of the mixed layer, and, $PEA_{z(\rho)}$: potential energy anomalies based on densities, $PEA_{z(T-S)}$: differences between PEA(T) and PEA(S).

Cruises (dates)	K_d (m^{-1})	r^2	Z_{eu} (m)	Z_m (m)	$PEA_{z(\rho)}$ (J m^{-3})	$PEA_{z(T-S)}$ (J m^{-3})
12–Feb–08	0.09	0.94	53	50	29.4	119.1
15–Mar–08	0.08	0.99	60	22	79.9	196.3
15–Apr–08	0.07	0.96	63	61	43.2	36.6
12–Aug–08	0.05	1.00	96	65	36.9	7.8
19–Oct–08	0.06	1.00	75	42	41.7	–35.4
11–Nov–08	0.07	0.98	62	–	28.0	–9.1
12–Dec–08	0.08	0.93	55	20	102.0	–41.4
15–Jan–09	0.05	0.98	92	88	15.1	5.2
05–Feb–09	0.05	0.97	86	38	103.6	106.8
08–Mar–09	–	–	–	40	54.5	98.4
02–Jun–09	0.07	0.95	65	77	19.7	–21.0
07–Oct–09	0.07	1.00	64	76	23.6	–17.2
16–Nov–09	0.11	0.96	43	16	39.3	82.4
14–Dec–09	–	–	–	23	91.0	108.0
12–Jan–10	0.09	0.94	50	21	89.6	223.8
16–Feb–10	–	–	–	21	101.9	186.4
16–Mar–10	–	–	–	23	102.2	161.1
20–Apr–10	0.12	0.92	39	31	60.4	136.2
22–Jul–10	0.14	0.95	33	91	13.2	–6.9

3.2. *Temporal variability in nutrients concentrations*

Ammonium (NH_4), nitrate+nitrite (NO_x) and phosphate (PO_4) concentrations were at times below detection limits mainly at the surface and FM layers (Table2). Both NO_x and PO_4 concentrations were lower during the 2009

Table. 2. Nutrient (ammonium (NH_4), nitrate+nitrite (NO_x), phosphate (PO_4)) concentrations, light irradiance level (PAR), fluorescence (Fluo), and microbial ratios (VBR, Prok:Euk, Proc:Syn, Proc2:Proc1) of related depths (Z) sampled within the surface, fluorescence maximum (FM), and bottom for each cruise.

Cruises	Z (m)	NH_4 (µM)	NO_x (µM)	PO_4 (µM)	PAR	Fluo (RU)	VBR	Prok: Euk	Proc: Syn	Proc2: Proc1
Surface										
Aug–08	15	0.3	0.5	0.1	23.5	0.18	3.0	4.2	0.4	0.3
Oct–08	15				419.5	0.03	5.5	13.6	0.1	0.0
Nov–08	15	0.1	0.0	0.1	13.3	0.06		17.6	0.1	0.5
Dec–08	15				0.8	0.06		4.6	1.6	4.3
Jan–09	15	0.1	1.2	0.1	53.7	0.04		8.0	0.3	5.4
Feb–09	15	0.0	0.0	0.0	245.6	0.03	2.3	86.1	1.2	0.0
Mar–09	15	0.0	1.7	0.0	0.0	0.08	2.0	21.6	0.8	0.0
Jun–09	15	0.2	0.5	0.0	3.9	0.16	2.8	12.0	0.8	0.0
Oct–09	15	0.4	0.4	0.1	22.5	0.10	5.0	3.1	0.7	0.1
Nov–09	15	0.3	0.0	0.0	14.1	0.08	2.1	12.9	0.1	0.1
Dec–09	15	0.2	0.0	0.0	6.6	0.09	3.5	8.7	0.6	0.2
Jan–10	15	0.3	0.0	0.7	7.6	0.06		9.5	0.0	4.3
Feb–10	15	0.0	0.4	0.0		0.08	3.1	28.7	0.4	0.0
Mar–10	15	0.5	0.1	0.1		0.05	0.6	6.0	0.0	4.6
Apr–10	15	0.1	0.2	0.0	0.2	0.11	3.3	6.8	0.1	0.1
Jul–10	15				0.1	0.14	3.1	3.1	0.2	0.2
FM										
Feb–08	28	0.1	1.3	0.0	0.8	2.41	8.4	0.6	0.8	6.3
Mar–08	45				13.2	0.36		8.5	0.3	799.4
Apr–08	65	0.4	0.9	0.9	0.8	0.19	8.7	7.6	2.2	33.3
Aug–08	75	0.2	0.5	0.1	1.4	0.26	4.9	1.5	0.2	0.1
Oct–08	33				118.9	0.13	5.9	4.2	0.3	0.0
Nov–08	20	0.0	0.1	0.2	9.6	0.07		3.3	0.1	0.6
Dec–08	50				0.1	0.15		9.9	1.4	11.5
Jan–09	48	0.0	1.0	0.1	8.7	0.34		14.4	1.7	0.0
Feb–09	62	0.0	0.2	0.0	27.0	0.17	3.0	6.4	3.6	41.6
Mar–09	35	0.3	0.9	0.1	0.0	0.17	1.7	8.4	1.0	48.9
Jun–09	65	0.2	2.0	0.0	0.2	0.11	2.3	6.5	0.6	23.0
Oct–09	48	0.6	0.5	0.1	2.0	0.18	4.8	17.2	3.9	70.9
Nov–09	60	0.2	0.8	0.1	0.2	0.78	1.4	7.0	0.2	0.2
Dec–09	50	0.2	1.2	0.1	0/2	0.30	3.3	6.2	0.3	13.4
Jan–10	60	0.4	1.0	2.6	8.3	0.47	2.2	44.6	2.6	168.2
Feb–10	40	0.0	0.0	0.0		0.26	3.1	48.3	0.4	0.1
Mar–10	35	0.5	1.0	0.2		0.09	0.7	5.6	0.2	20.2
Apr–10	38	0.3	2.4	0.6	0.0	0.24	3.0	2.3	0.2	214.0
Jul–10	50				0.0	0.13	3.0	3.5	0.1	0.0

Table. 2. *Continued.*

Cruises	Z (m)	NH_4 (µM)	NO_x (µM)	PO_4 (µM)	PAR	Fluo (RU)	VBR	Prok: Euk	Proc Syn	Proc2: Proc1
Bottom										
Oct–08	105				1.4	0.11	12.2	2.6	0.5	10.5
Nov–08	100	0.1	1.0	0.2	0.0	0.22		2.2	0.3	1490.6
Dec–08	100				0.0	0.14		2.7	1.8	107.4
Jan–09	75	0.1	2.4	0.3	1.1	0.14		6.4	9.6	204.3
Feb–09	92	0.1	2.0	0.2	4.1	0.10	2.9	3.9	3.0	921.5
Mar–09	75	0.1	2.9	0.3	0.0	0.10	1.0	5.8	2.6	353.4
Jun–09	105	0.3	0.9	0.0	0.0	0.07	3.4	2.4	0.5	1.0
Oct–09	92	0.6	0.5	0.1	0.1	0.05	3.9	17.2	0.6	0.0
Nov–09	94	0.2	3.1	0.2	0.0	0.10	2.0	2.7	0.1	2.0
Dec–09	100	0.3	1.5	0.1	0.0	0.07	4.0	5.8	0.4	0.0
Jan–10	100	0.2	4.7	0.6	7.9	0.09	3.8	6.3	2.5	614.6
Feb–10	103	0.0	8.8	0.5		0.08	3.6	5.7	1.7	339.3
Mar–10	103	0.3	9.7	0.7		0.09	1.6	3.2	1.6	1467.9
Apr–10	105	0.2	8.3	0.5	0.0	0.07	2.4	2.0	0.2	172.0
Jul–10	100				0.0	0.09	3.1	2.3	0.2	0.0

downwelling season and showed highest values over the upwelling season of 2010 (Table 2). A maximum of 9.7 µM of NO_x was observed at the bottom layer in March 2010, whereas values > 0.7 µM of PO_4 were observed at the surface and FM layers in April 2008 and January 2010. Ammonium (NH_4) concentrations showed values greater than 0.3 µM in April 2008 and January 2010 at the FM and in March 2010, reaching up to 0.6 µM at the bottom and FM depths in October 2009 (Table 2).

3.3. ***Temporal variability in picophytoplankton abundances***

Overall, abundances of *Synechococcus* (Syn), *Prochlorococcus* (ProcT) including its two distinct sub–populations (Proc1 and Proc2), and picoeukaryotes (Euk) respectively ranged from 10^3 to 10^5, 10^2 to 10^5 and 10^2 to 10^4 cells mL^{-1}, and did not exhibit any significant long–term trend in their abundances ($p > 0.05$, Fig. 4). The highest abundances were observed at the bottom in October 2009 with abundances of picoeukaryotes, *Synechococcus*, and *Prochlorococcus* reaching, respectively, $3.3 \pm 0.6 \times 10^4$, $3.7 \pm 1.3 \times 10^5$, $2.1 \pm 0.6 \times 10^5$ mL^{-1} (Fig. 4). These maxima coincided with the highest ammonium concentrations and segregated two distinct periods of low abundances for all groups. The temporal pattern of Proc2 abundances was, however, markedly different from that of Proc1 but both significantly contributed to ProcT abundances (Fig.4, Table 3). In contrast, their minima in February and April2010 coincided with high nitrate+nitrite concentrations (Fig. 4, Table 2).

At the surface, abundances of *Synechococcus* (Syn), *Prochlorococcus* (ProcT), and picoeukaryotes (Euk) showed a minimum in October 2009, March 2010, and February 2009, respectively; all groups exhibited an abundance peak during

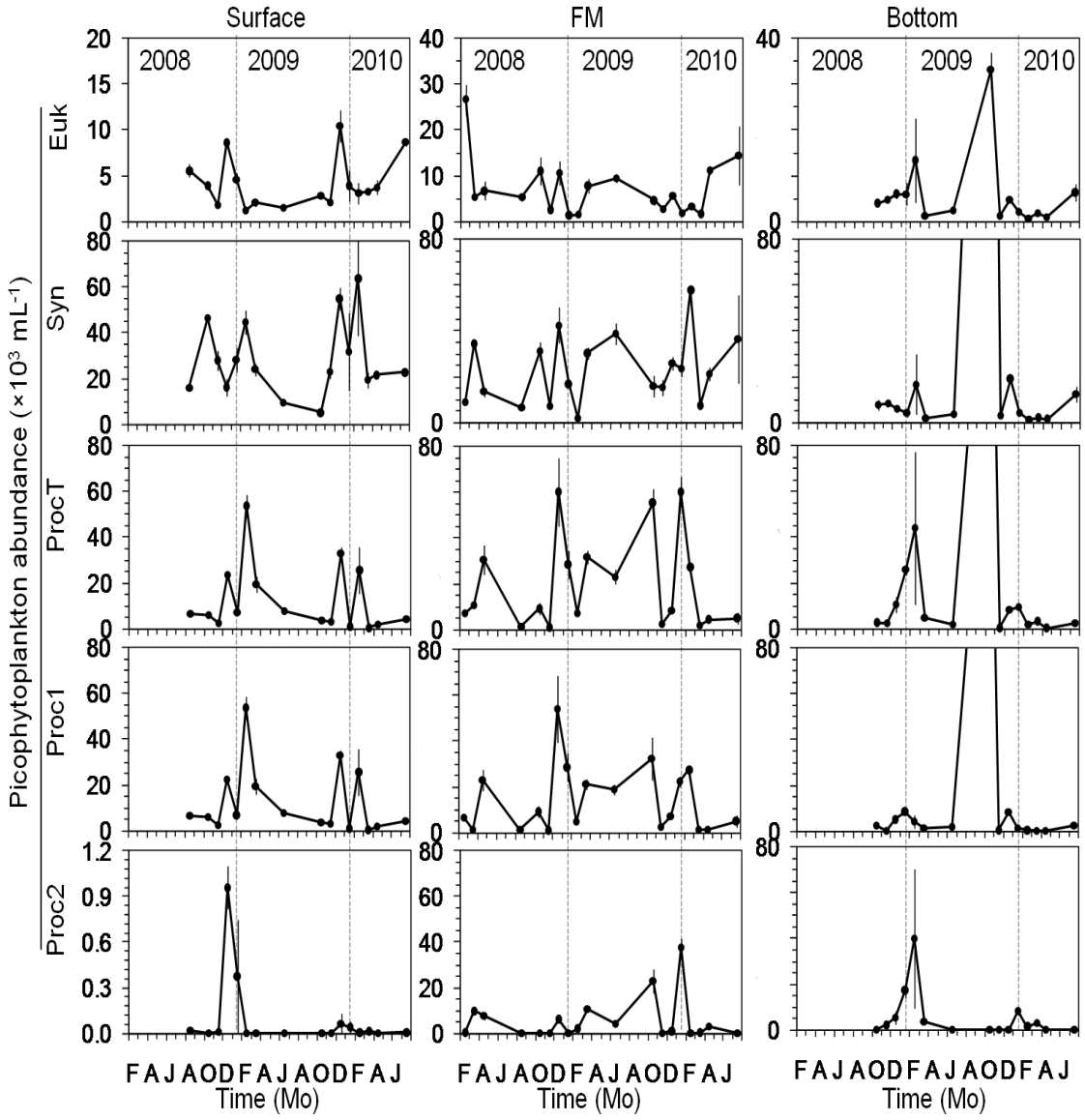

Fig. 4. Temporal variations in the abundances of *Synechococcus* (Syn), *Prochloroccus* (ProcT) including its populations (Proc1 and Proc2), and picoeukaryotes (Euk) from February 2008 to July 2010 at the surface, fluorescence maximum (FM), and bottom of the NRSKAI station.

the relaxation time of December 2009 (Fig. 4). Peaks in the abundances of ProcT and Euk were remarkably consistent between years, and picoeukaryotes also exhibited high abundances during the downwelling season following the 2008 and 2010 upwelling seasons (Fig. 4). As indicated by their similar patterns, Proc1 significantly contributed to ProcT (Table 3), while Proc2 was mainly absent except in the summer (Fig. 4). In contrast, no clear seasonality was observed in Syn abundances.

At the fluorescence maxima (FM), variations in picophytoplankton abundances were marked with delays and inconsistencies between years (Fig. 4). Peaks in

ProcT abundances were associated to a temporal segregation of the relative contribution of Proc1 and Proc2 to ProcT (Fig. 4), and all significantly positively correlated to each other (Table 3).

Proc2 and Euk abundances were significantly positively related to each other at the surface, while Syn abundances were significantly positively correlated to Proc1 abundances at both surface and FM (Table 3). At the bottom, picophytoplankton groups were significantly positively related to each other (Table 3).

3.4. ***Temporal variability in heterotrophic bacterial and viral abundances***

Abundances of heterotrophic bacteria (HB) were one order of magnitude higher than those observed for picophytoplankton and showed relatively little variability when compared to that of picophytoplankton. HB abundances ranged between a minimum of 1.8×10^5 cells mL^{-1} in February 2010, coinciding with that of picophytoplankton, and a maximum of 1.1×10^6 cells mL^{-1} in November 2009, following that of the picophytoplankton and coinciding with the onset of the 2010 upwelling season (Fig. 5). In contrast to HB and picophytoplankton

Table. 3. Spearman's rank correlation coefficient, ρ, between picophytoplankton, heterotrophic bacteria, and viruses for each depth over time. Significant relationships ($\alpha = 0.05$) are underlined.

	N	HB	ProcT	Euk	Syn	Proc1	Proc2
Surface							
VLP	36	.25	.24	.06	.27	.24	.01
HB	36		–.04	–.33	.19	–.04	–.04
ProcT	48			.12	.38	1.00	–.07
Euk	48				.19	.12	.32
Syn	48					.38	–.07
Proc1	48						–.07
FM							
VLP	45	.19	.33	.14	–.08	.38	.05
HB	45		.14	–.57	–.08	.19	.16
ProcT	57			.14	.56	.90	.65
Euk	57				.44	.13	.10
Syn	57					.49	.25
Proc1	57						.38
Bottom							
VLP	36	.60	.31	.60	.66	.67	–.46
HB	36		.24	.42	.50	.51	–.40
ProcT	45			.67	.49	.75	.43
Euk	45				.90	.71	–.09
Syn	45					.63	–.29
Proc1	45						–.08

abundances, viral (VLP) abundances showed a significant negative long–term trend at the FM and bottom layers ($p < 0.05$), but not at the surface ($p > 0.05$). VLP abundances ranged between minima of 3×10^5 cells mL^{-1} at the bottom in March 2009 and 2010 and a maximum of 5.5×10^6 cells mL^{-1} in April 2008 (Fig. 5). VLP showed high abundances in the October month of each year (Fig. 5), coinciding with that of picophytoplankton abundances, with some distinction, however, between picophytoplankton groups in the year and depth (Fig. 4). For instance, only *Synechococcus* showed a peak in abundances at the surface in 2008, whereas in 2009, only *Prochlorococcus* abundances peaked at the fluorescence maximum. In addition, at the bottom, all three groups showed a peak in their abundances only in 2009, which also coincided with an increase in HB abundances (Fig. 4, 5).

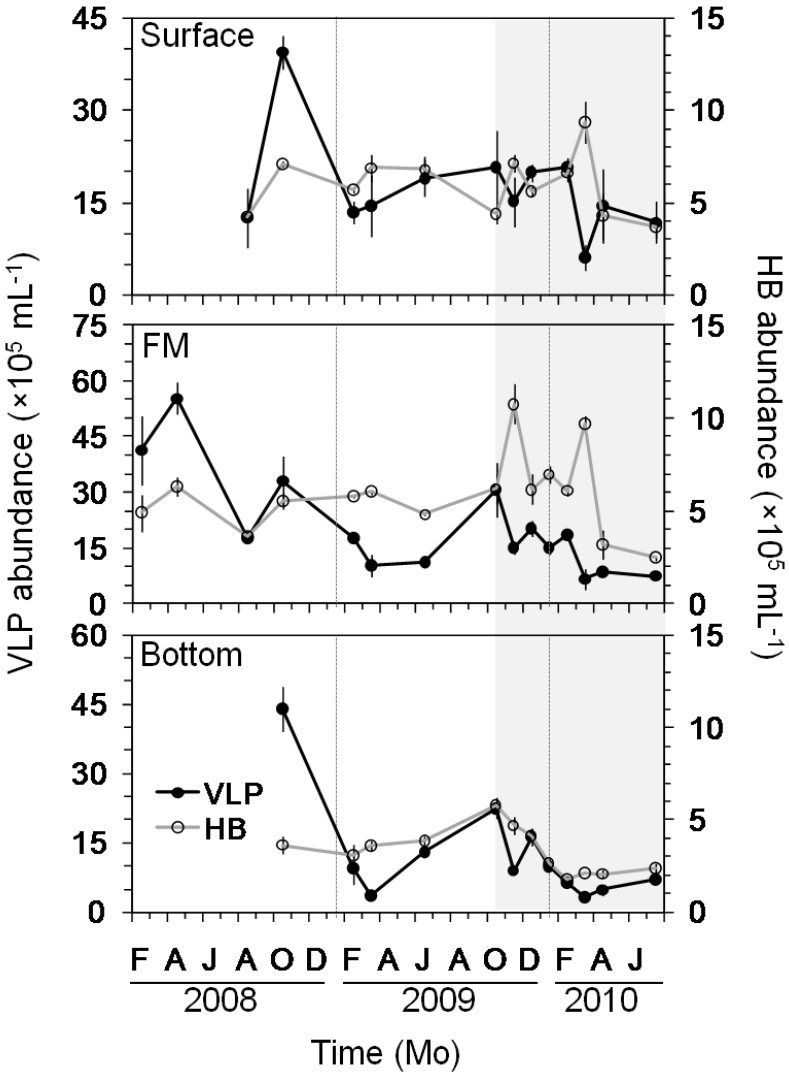

Fig. 5. Temporal variations in the abundances of viruses (VLP) and heterotrophic bacteria (HB) from February 2008 to July 2010 at the surface, fluorescence maximum (FM), and bottom of the NRSKAI station. Note the distinct temporal patterns before (unshaded) and after (shaded) the October 2009 month.

Table. 4. Spearman's rank correlation coefficient, ρ, between picophytoplankton, heterotrophic bacteria, and viruses before/ after October 2009 and during upwelling/ downwelling seasons. Significant relationships (α = 0.05) are underlined.

	N	HB	Euk	Syn	Proc 1	Proc 2	N	HB	Euk	Syn	Proc 1	Proc 2
	Before Oct09						**After Oct09**					
Surface												
VLP	15	.60	.14	.33	–.46	.05	18	.08	.04	.56	.49	.08
HB	15		–.15	.19	.01	–.46	18		–.53	–.20	–.36	.24
Euk	24			–.13	–.06	.33	21			.50	.45	.31
Syn	24				.00	–.34	21				.77	.19
Proc1	24					–.04	21					–.06
FM												
VLP	21	.36	.24	–.21	–.10	–.30	21	.24	.03	.49	.66	.14
HB	21		–.02	.13	.38	.42	21		–.69	–.38	.02	–.10
Euk	33			.52	.32	.27	21			.57	.02	–.08
Syn	33				.47	.49	21				.74	.00
Proc1	33					.30	21					.08
Bottom												
VLP	12	.35	.62	.60	.46	–.56	21	.58	.42	.65	.68	–.33
HB	12		–.06	–.06	.01	–.45	21		.36	.56	.36	–.45
Euk	21			.76	.32	.26	21			.89	.73	–.39
Syn	21				.12	–.05	21				.85	–.50
Proc1	21					.41	21					–.42
	Downwelling						**Upwelling**					
Surface												
VLP	15	.53	–.33	.30	.10	–.15	21	.08	.22	.57	.34	.10
HB	15		–.63	.10	.46	–.46	21		–.05	–.38	–.50	.41
Euk	18			.28	.06	.02	30			.16	.13	.47
Syn	18				–.12	.05	30				.59	–.13
Proc1	18					–.37	30					–.05
FM												
VLP	15	.84	–.24	–.13	.33	.25	30	–.10	.29	–.04	.46	–.06
HB	15		–.10	.23	.62	.46	30		–.57	–.11	–.09	–.28
Euk	18			.72	.46	–.20	39			.35	.07	.30
Syn	18				.67	.13	39				.39	.29
Proc1	18					.63	39					.07
Bottom												
VLP	12	.53	.26	.12	.37	.21	24	.46	.47	.63	.52	–.25
HB	12		.34	.31	.49	.24	24		.41	.52	.44	–.26
Euk	15			.88	.54	.00	30			.85	.80	.38
Syn	15				.57	–.11	30				.72	.15
Proc1	15					–.55	30					.38

Overall, HB abundances significantly negatively correlated to both Euk and Syn at the fluorescence maximum, but only to Euk at the surface. In contrast, at the bottom, HB abundances significantly positively correlated to all picophytoplankton abundances (Table 3). Viral abundances were significantly positively related to that of HB at the bottom, but to that of Syn and ProcT at the FM layers (Table 3). In contrast, no significant correlation was found at the surface, although we noted a higher coefficient between Syn and VLP abundances. Our results thus suggest a potential decoupling between viruses and heterotrophic bacteria.

3.5. ***Coupling vs decoupling between viruses, heteroptrophic bacteria, and picophytoplankton***

HB abundances showed a clear shift in October 2009, while remaining relatively constant before that time, specifically at the surface and fluorescence maximum (FM) layers (Fig. 5). A shift in picophytoplankton abundances was observed, particularly at the bottom, but mainly at the FM and surface layers for picoeukaryotes abundances (Fig. 4). This time coincided with subsequent changes in the structure of the water column such as salinity, temperature, nutrients levels (i.e. ammonium concentrations), as well as the depth of the mixed layer (Fig. 3, Table 1). We noticed that following October 2009, while VLP abundances decreased at the bottom and FM layers, HB abundances increased at the FM layer (Fig. 5), but decreased at the bottom. We thus separated our data set of microbial abundances and looked whether relationships between abundances of VLP, HB, and picophytoplankton occurred to be significant before and/ or after this October 2009 time. As shown in Table 4, results confirmed the occurrence of a decoupling between viruses and heterotrophic bacteria both vertically and temporally, and, the significant relationship between distinct picophytoplankton populations and viruses before and after October 2009. Analysis also showed an increase in the coefficients of the correlations between Syn and Euk, and, distinct relationships between HB and Proc1 and Euk at the FM layer before and after October 2009, respectively (Table 4). We further investigate whether this decoupling was specific of the 2010 upwelling event by separating again our data set between upwelling and downwelling season (Table 4). Combining both results, we found that this decoupling between abundances of viruses and heterotrophic bacteria occurred during upwelling events following the 2009 downwelling season between the bottom and the two upper depths (Table 4). Finally, we noted distinct significant combinations of the microbial communities between downwelling and upwelling seasons (Table 4).

3.6. ***Vertically segregated temporal variability in microbial community structure***

Viral abundances (VLP) were at times lower than that of heterotrophic bacteria which resulted into low virus to bacteria ratios (VBR). The VBR ranged between 0.7 in March 2010 at the surface and FM layers and 12.2 at the bottom layer in October 2008 (Table 2). For each three depth, VLP were found responsible for the temporal variability in the VBR ($p < 0.05$). In contrast to VBR, the PROK:EUK and PROC2:PROC1 ratios respectively decreased and increased with depth (Table 2). At the surface, the PROK:EUK ratios consistently peaked in November and February of each year but was lower in 2010 than in 2009,

whereas at the FM depths, peaks in the PROK:EUK ratios occurred in March 2008, then in January of 2009 and 2010 and increased in magnitude with time (Table 2). *Synechococcus* and picoeukaryotes were significantly positively and negatively correlated to the PROK:EUK ratios at the surface ($p < 0.05$), whereas only *Prochlorococcus* significantly positively contributed to the variability of the PROK:EUK ratios at the FM and bottom depths ($p < 0.05$). At the bottom, peaks in the PROK:EUK ratios occurred in October 2009. Peaks in the PROC2:PROC1 ratios occurred consistently over the upwelling season of each year (Table 2) and reflected changes in Proc2 abundances ($p < 0.05$). Finally, the PROC:SYN ratio was more variable and significantly positively correlated to *Prochlorococcus* sub–populations, i.e. Proc1 at the surface and FM depths and Proc2 at the FM and bottom depths.

3.7. ***Link to local and global environmental factors***

The significant relationships between abundances of picophytoplankton populations, viruses, and heterotrophic bacteria, and their abiotic environments were often specific to the sampled layer (Table 5). We will thus relate the main significant relationships found between biotic and abiotic variables for each depth separately as shown in Table 5.

At the surface, Syn significantly inversely contributed to the bulk fluorescence, whereas the negative relationships between HB and fluorescence likely reflected an inverse relationship between HB and large phytoplankton cells. This agreed with the inverse relationship between HB and Euk abundances (Table 3). Furthermore, Proc1 and Syn were significantly inversely related to NH_4 concentrations, suggesting the growth of these populations on ammonium. Proc1 abundances were also found to increase with decreasing PO_4 concentrations, but with increased local stabilities and upwelling favorable winds. This latter also explained the increase abundances of Proc1 at the fluorescence maximum depths. Finally, the significant positive relationship between the alongshore wind stress and VLP, likely reflected the higher VLP abundances during the 2008 upwelling season and/ or explained their significant positive relationship with Proc1 during upwelling.

At the fluorescence maximum, the significant positive relationship between $PEA_{(T)}$ and the PROC2:PROC1 ratios reflected the dominance of Proc2 over that of Proc1 during summer. Proc2 and Euk significantly positively correlated to ammonium and nitrate+nitrate concentrations, likely reflecting the differences in their respective preference for distinct form of nitrogen supplies. In addition, we found that HB abundances were significantly affected by shifts in the prevailing La Niña/El Niño conditions but also by changes in salinities. This confirmed our observations of a shift in the dynamics of HB abundances following October 2009, coinciding with the 2010 upwelling season when El Niño prevailed (Fig. 5). SOI values also significantly negatively correlated to the PROK:EUK ratios. Hence, these above relationships showed that while abundances of prokaryotes increased with prevailing El Niño conditions, abundances of picoeukaryotes were shown to increase with La Niña conditions. In fact, as indicated by the significant inverse relationship between $PEA_{(T)}$ and Euk and the significant positive relationship between SOI and Euk, this latter likely reflected the high abundances of picoeukaryotes during the 2008 upwelling season, when temperature gradients were important and La Niña conditions prevailed.

Table 5. Spearman's rank correlation coefficient, ρ, indicating the strength of the relationship between microbial and viral abundances and environmental variables over the long term at the surface, fluorescence maximum (FM), and bottom depths. Significant relationships ($\alpha = 0.05$) are underlined.

	N	VLP	HB	VBR	N	Euk	Proc2	Proc1	Syn	Prok: Euk	Proc: Syn	Proc2: Proc1
Surface												
SOI	12	–.42	–.39	.01	16	.10	–.18	.03	.04	.05	.00	–.16
Wind	12	.69	.27	.16	16	–.19	–.20	.59	.13	.26	.62	–.33
T	12	–.07	.27	–.45	16	–.32	–.10	.23	.46	.59	.01	–.10
S	12	–.31	–.47	–.12	16	–.26	–.21	–.36	–.18	.07	–.22	–.16
$PEA_{(\rho)}$	12	–.06	.36	–.22	16	–.05	.00	.22	.35	.33	.07	–.06
$PEA_{(T-S)}$	12	–.25	.09	–.30	16	–.04	.01	–.15	.42	.25	–.41	.01
$PEA_{(S)}$	12	.24	–.17	.41	16	.10	.09	.19	–.38	–.28	.44	.05
$PEA_{(T)}$	12	–.18	.17	–.29	16	–.02	.15	.01	.43	.28	–.21	.12
Z_{eu}	8	.12	.19	–.12	12	–.13	.06	.59	.10	.20	.48	.03
PAR	10	.32	.21	.36	14	–.16	–.01	.05	.31	.18	–.06	.04
Ri					6	.37	.26	.94	–.26	–.03	.94	–.09
NH_4	10	–.19	.14	–.04	13	.27	.36	–.59	–.63	–.75	–.38	.51
NO_x	10	.10	–.07	–.06	13	.10	–.10	.23	–.34	–.16	.34	–.16
PO_4	10	–.28	.03	–.17	13	.26	.50	–.62	–.47	–.57	–.38	.69
Fluo	12	–.20	–.63	.14	16	.18	–.03	–.04	–.56	–.49	.14	–.15
FM												
SOI	15	.01	–.72	.31	19	.50	–.33	–.39	–.14	–.63	–.31	–.21
Wind	15	.43	.09	.25	19	.27	.09	.57	.24	.29	.41	–.09
T	15	–.28	–.23	–.13	19	.32	.08	.17	–.05	–.05	.18	.08
S	15	–.24	–.63	.04	19	.23	–.09	–.07	–.13	–.34	–.11	–.17
$PEA_{(\rho)}$	15	–.09	.42	–.21	19	–.51	.34	.02	.02	.22	.22	.39
$PEA_{(T-S)}$	15	–.19	.33	–.30	19	–.36	.26	–.25	–.11	.11	.03	.44
$PEA_{(S)}$	15	.20	–.26	.32	19	.20	–.28	.20	.02	–.11	–.03	–.43
$PEA_{(T)}$	15	–.16	.44	–.32	19	–.49	.37	–.09	–.05	.28	.18	.47
Z_{eu}	11	.50	.05	.43	15	–.13	–.22	.13	–.35	.06	.34	–.26
PAR	13	.62	.30	.42	17	–.40	–.12	–.18	–.40	.11	.22	–.04
Ri	12	–.10	–.31	.06	13	.23	–.36	.09	.51	–.04	–.35	–.44
NH_4	13	–.11	.39	–.08	15	–.09	.56	.01	.00	.04	.15	.58
NO_x	13	–.29	–.26	–.13	15	.56	.28	–.08	.31	–.32	–.01	.34
PO_4	13	–.14	.45	–.15	15	–.19	.19	–.20	–.22	.00	–.12	.33
Fluo	15	.45	.23	.26	19	.07	.10	.09	–.06	.22	.21	.10
Bottom												
SOI	12	–.12	–.19	–.24	15	.23	.11	–.15	.18	–.68	–.25	.08
Wind	12	.13	.32	.06	15	.06	.04	.39	–.04	.36	.29	–.23
T	12	.32	.48	.07	15	.52	.05	.43	.40	–.09	.11	–.22
S	12	.73	.64	.48	15	.60	–.32	.44	.64	–.28	–.25	–.44
$PEA_{(\rho)}$	12	–.31	–.45	–.13	15	–.21	.41	–.15	–.19	.14	.35	.44
$PEA_{(T-S)}$	12	–.53	–.64	–.22	15	–.53	.18	–.42	–.44	.26	.16	.40
$PEA_{(S)}$	12	.66	.64	.39	15	.70	–.15	.53	.64	–.19	–.11	–.35
$PEA_{(T)}$	12	–.41	–.53	–.14	15	–.42	.39	–.29	–.37	.36	.35	.50
Z_{eu}	8	.71	.43	.43	11	.36	.56	.58	.31	.49	.71	.31
PAR	10	.54	–.02	.46	13	.26	.56	.26	.27	.37	.55	.47
Ri	10	.08	.12	–.02	13	–.54	–.29	–.55	–.42	–.25	–.37	.06
NH_4	10	.42	.52	.27	12	.23	–.56	.15	.29	.07	–.40	–.41
NO_x	10	–.81	–.82	–.45	12	–.76	.30	–.59	–.78	–.16	.08	.38
PO_4	10	–.73	–.72	–.43	12	–.59	.48	–.52	–.64	.01	.23	.53
Fluo	12	–.15	–.04	–.33	15	.12	.57	–.07	.01	–.18	.20	.47

N: number of sampling cruises. Wind: alongshore wind stress, T: temperature, S: salinity, Fluo: Fluorescence, Z_{eu}: euphotic zone. Stratification evaluations include: Ri: Richardson number, PEA: Potential energy anomalies based on densities, $PEA_{(S)}$: Potential energy anomalies based on salinities, $PEA_{(T)}$: Potential energy anomalies based on temperatures, $PEA_{z(T-S)}$: differences between $PEA_{(T)}$ and $PEA_{(S)}$.

At the bottom, the relationship between SOI and the PROK:EUK ratios showed the pronounced influences of the shift in La Niña/El Niño conditions on picophytoplankton community structure. However, the local significant relationships between biotic and abiotic variables showed that processes involved at the bottom differed from those occurring at the fluorescence maximum. Indeed, we found that VLP, HB, Euk, and Syn abundances were all related significantly negatively to NO_x and PO_4 concentrations but positively to salinity. These relationships together with our observations might reflect an input of all these biotic variables via the high saline bottom waters from Spencer's Gulf, but a decrease of all of them with theoccurrence of low saline upwelled waters. Curiously, only Euk were found significantly positively related to temperatures, suggesting the warmer the high saline bottom outflow is, the higher abundances of picoeukaryotes, but also, the colder the low saline upwelled water is, the lower abundances of picoeukaryotes. In fact, the tight relationships between biotic and abiotic variables at the bottom make the interpretation of these correlations difficult, but showed that salinity gradients were here particularly important to the dynamics of most picophytoplankton groups, hetertrophic bacteria, and viruses. In contrast, Proc2 abundances significantly positively correlated to PAR and fluorescence, suggesting Proc2 to significantly contribute to the increase of the bulk fluorescence at the bottom and that its growth was likely limited by light irradiances. This latter and the significant positive relationship between the depth of the euphotic zone (Z_{eu}) and the PROC:SYN ratios also agreed with the significant contribution of Proc2 to the PROC:SYN ratio at these depths.

Finally, VLP abundances significantly positively correlated to PAR at the fluorescence maximum and to the depth of the euphotic zone (Z_{eu}) at the bottom, which may suggest here that changes in light attenuations with depth also contributed to the dynamics of viruses at these depths. Alternatively, these reflected the relationships of viruses and *Synechococcus* and *Prochlorococcus* at these depths (Table 3).

3.8. ***Link with La Niña/ El Niño***

We further explore the link between SOI values and microbial abundances at the fluorescence maxima to understand how shifts in heterotrophic bacteria and picophytoplankton community structure related to the prevailing La Niña/El Niño conditions. First, changes in SOI values explained most of the variability in $PEA_{z(T-S)}$ ($p < 0.05$, Fig. 6A) when excluding the likely local effect of the wind field (i.e. June and October 2009 and upwelling events due to strong southeasterlies, i.e. February and March 2008), agreeing with the distinct effect of El Niño and La Niña previously reported for the region (Middleton et al. 2007). Secondly, we observed that SOI values also explained most of the increased in ammonium concentrations of the water column over time ($p < 0.05$, Fig. 6B), suggesting that regenerated production (Fernández et al. 2009) might increase with upwelling and downwelling events associated to El Niño. Indeed, the highest ammonium concentrations which coincided with the highest SOI value occurred in October 2009 (Table 2), which time also show the signature of the warm and high saline bottom waters (Fig. 3B). Finally, we observed that the significant negative relationships of SOI and HB abundances and the PROK:EUK ratios were both non linear with absolute changes of similar magnitudes, but of opposite direction (Fig. 6C). This agreed with the significant inverse relationship of

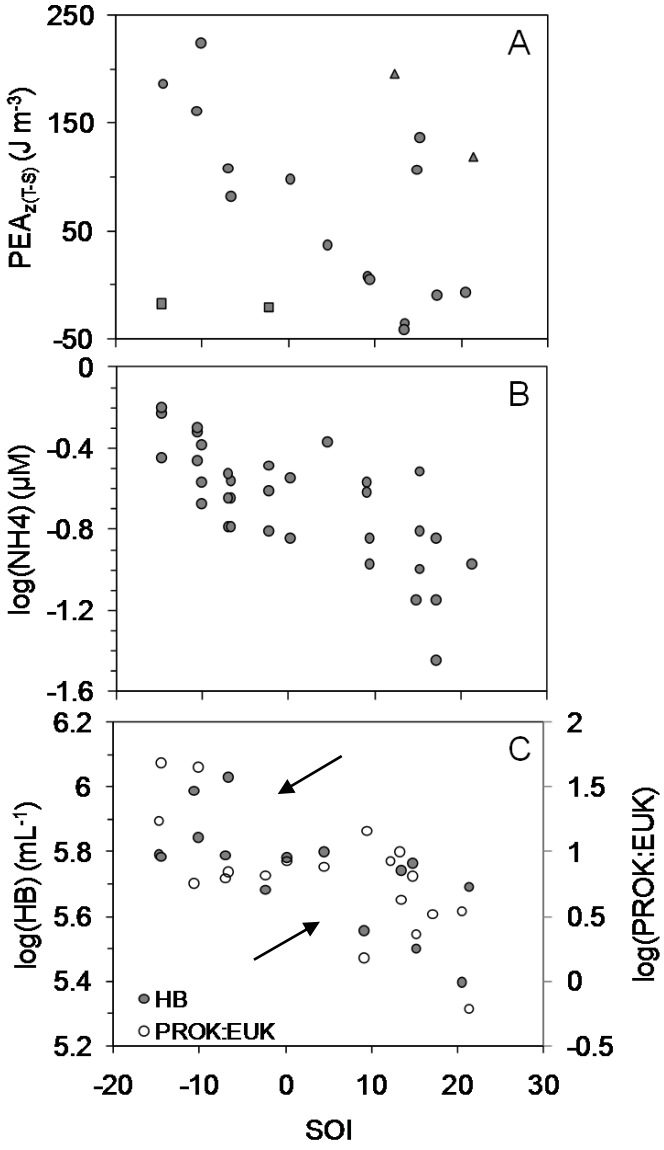

Fig. 6. Relationship between the Southern Oscillation Index (SOI) and stratification ($PEA_{z(T–S)}$, A), NH_4 concentrations of all three layers (B), heterotrophic bacterial abundances (HB) and the PROK:EUK ratios of the fluorescence maximum (C). Note: graph A: squares are June and October 2009 and, triangles are February and March 2008, graph C: arrows represent potential effect from other local variables (i.e. wind field). Negative and positive SOI values are El Niño and La Niña, respectively.

picoeukaryotes and HB abundances (Table5), while *Prochlorococcus* significantly contributed to the PROK:EUK ratios. We noted that the effect of the local wind–driven physical forcing events, which ones appeared to dismantle the linearity of these relationships, were likely responsible for the shape observed here (Fig. 6A, C), agreeing with the significant positive relationship between PROK:EUK and $PEA_{z(T–S)}$ (Table 5), and the significant relationship of Proc1 abundances and the wind stress (Table 5).

4. Discussion

4.1. *Depth–related temporal variability in microbial abundances*

The temporal variability observed in picophytoplankton populations and heterotrophic bacteria is consistent with observations conducted in other coastal and oligotrophic oceanic waters showing that abundances of heterotrophic bacteria showed less variability than that of picophytoplankton (Partensky et al. 1996; Campbell et al. 1997; Zubkov et al. 2000; Jiao et al. 2005; Calvo–Díaz et al. 2008). These studies have often related to seasonality in microbial abundances, and to changes in temperatures, light irradiance, nutrient availabilities, stratification and mixing processes encountered in the water column. However, these environmental variables are often related with depth integrated data and factors responsible for the variability in microbial abundances also depend on the specificity of the system. Here, the seasonality in picophytoplankton abundances was marked at the surface, where each group present distinct temporal patterns with consistent peaks observed for *Prochlorococcus* and picoeukaryotes, and to a lesser extent for *Synechococcus* (Fig. 4). However, recurrences in their seasonal patterns decreased with depth, whereas their similarity increased (Fig. 4). These observations showed that over time the factors controlling the dynamics of picophytoplankton abundances differed according to their location in the water column, agreeing with our correlation analysis (Table 5). Indeed, the sinking of particles decreased with cell size, and as indicated here picophytoplankton such as *Prochlorococcus* are known to present distinct ecotypes or strain that show distinct temporal and vertical niches (Rocap et al. 2003). These results may also agree with systems exhibiting long periods of stratification, implying little exchanges within different water layers, hence the local adaptation of microbial communities within the water column. Exception may however occur when picophytoplankton are trapped within small zooplankton fecal pellets (Richardson and Jackson 2007) or like in the present case, during mixing intermittence over the upwelling and downwelling seasons. In this regards, our results also indicate that most groups tend to behave similarly (December 2009; Fig. 4), suggesting a redistribution of the microbial communities and nutrients within the water column. Overall, the differences in these temporal patterns observed between sampled depths clearly showed the importance of the different nature of stratification and mixing processes and reflected the differential effect of temporal and vertical temperature and salinity gradients associated to upwelling and downwelling conditions.

4.2. *Sporadic existence of high picophytoplankton abundances at the bottom caused by downwelling*

At the end of the 2009 downwelling season, in October 2009, we found at the bottom up to one order of magnitude higher picophytoplankton abundances than that of the upper two depths, and this for most picophytoplankton populations, but for Proc2. This may be quite unexpected for *Synechococcus* which one is particularly sensitive to the attenuation of light irradiance with depths (Partensky et al. 1999, Sommaruga et al. 2005). While the higher abundances of picoeukaryotes at depths is congruent with higher nutrient abundances during the upwelling season, higher *Synechococcus*, picoeukaryotes, and Proc1 abundances

were also observed in November 2008, though at lower magnitude than that observed in October 2009. We will suggest here distinct possible explanations, which may not be exclusive to each other, that may support this sporadic presence of bottom maxima in picophytoplankton abundances at the end of the downwelling season.

First, the stronger downwelling favorable winds that occurred in 2009 agree that downwelling processes over the downelling season may have concentrate picophytoplankton at the bottom, suggesting an important flux of organic matter towards the bottom. With the exception of Proc2, picophytoplankton abundances at the bottom were not found significantly limited by light such as their growth may have also occurred at the bottom, agreeing with the high transparency of the water column for most of the year and the low Proc2 abundances at that time. High picophytoplankton abundances have also been observed in the Gulf of Eilat down to 100 m where light less than 0.1% irradiance from that of the surface was recorded following mixing processes (Stambler 2006). This is also a common feature of many deep chlorophyll maximum layers where light irradiance is often at the level or under 0.1% of surface light irradiance and competition is high. Stambler (2006) also explained their presence by the fact that mixing may have occurred at rates faster than the acclimation rate. In the present case, bottom maxima also coincided with highest (0.6 µM) ammonium concentrations which only slightly decreased towards the surface, where ammonium was shown to be the main source of nitrogen for *Synechococcus* and Proc1 (Table 5). Hence, following downwelling, ammonium uptake may have subsequently support the growth of picophytoplankton at the bottom. *Prochlorococcus* is indeed known to be more dependent on regenerated form of nitrogen supplies (Moore et al. 2002, Matsumoto et al. 2004). In addition, although some studies contrast in their minimum requested value of ammonium concentrations for ammonium inhibition of nitrate uptake to occur (Probyn 1985; Dortch 1990; Wheeler and Kokkinakis 1990; Price et al. 1994), ammonium concentration of 0.15 µM was reported to reduce by about 50% nitrate uptake rates of phytoplankton in the equatorial Pacific (Price et al. 1994).

Secondly, the importance of salinity gradients for picoeukaryotes and *Synechococcus* abundances (Table 5) and the presence of the high saline bottom waters which were warmer in 2009 than in 2008 and 2010 (Fig. 3) suggest the potential transport of picoeukaryotes and *Synechococcus* from Spencer Gulf. While this may also imply the occurrence of Proc1 in the gulf, it suggests that warmer Gulf waters may have increased picophytoplankton abundances prior to this bottom circulation, agreeing with the general view of the importance of temperatures to picophytoplankton growth.

Finally, it is also possible that the viral lysis of heterotrophic bacteria may have supported the growth of picophytoplankton at the bottom (Table 5; Weinbauer et al. 2011). Indeed, Weinbauer et al. (2011) have recently shown that *Synechococcus* growth may depend upon viral lysis of heterotrophic bacteria even if infected. In the present study, we observed that viral abundances increased consistently in October and coincided with peaks in picophytoplankton abundances (Fig. 4, 5) and showed that overall, viruses significantly positively correlated with picophytoplankton and heterotrophic bacteria at the bottom (Table 3). However, the significance of these relationships was not observed during downwelling conditions (Table 4). Nevertheless, while the relatively high

coefficient of correlation may suggest that the lack of data may have resulted in the absence of significance for that season, the absence of significance found before October 2009 when removing the October peak (Table 4) and our observations (Fig. 4, 5) do support the recent work of Weinbauer et al. (2011).

4.3. ***Existence of temporal and vertical decoupling of viruses and bacteria due to upwelling under El Niño***

The relationship between abundances of viruses and heterotrophic bacteria is typically reported as the virus to bacteria ratio (VBR), with values reflecting the strength of this relationship. However, distinct controversies have appeared in that regards. For instance, Bratbak and Heldal (1995) suggested that VBR values may increase with the diversity of the bacterioplankton, while others reported that a decrease in the VBR would be indicative of the productivity of the system (e.g. Danovaro et al. 2002; He et al. 2009). In the present case, variations in VBR values mainly reflected that of viral abundances, and, the VB ratios below one at the surface and FM depths where we found a significant correlation between viruses and picophytoplankton and not heterotrophic bacteria suggest again a distinct related mechanism.

While a few studies have reported differences in the relationships of viruses and heterotrophic bacteria in distinct environmental conditions (Brussaard et al. 2008; Clasen et al. 2008), this study is the first, to our knowledge, to report a significant decoupling between viruses and heterorophic bacteria in the marine environment. This decoupling was observed by a marked vertical shift in the temporal dynamics of viral and bacterial abundances that occurred beyond October 2009, coinciding to the onset of the 2010 upwelling season (Fig. 5), and, was further confirmed by a temporal and vertical shift in the significance of the correlations between picophytoplankton, heterotrophic bacteria, and viruses (Table 4). It is difficult at this stage to unambiguously elucidate the reasons for this decoupling. We suggest, however, distinct scenarii that are not mutually exclusive:

(i) a vertical shift in viral–host communities (Table 4; Mann 2003; Weinbauer 2004),

(ii) a shift in viral and bacterial community structure linked to different sourced waters or water conditions,

(iii) a vertical shift in viral life strategies due to enhanced oligotrophic conditions in surface waters (Weinbauer and Suttle 1999; Williamson et al. 2002), and,

(iv) a potential inhibiting effect of UV radiation on viruses in surface waters.

Prochlorococcus phages have been shown to help the growth of *Prochlorococcus* in the oligotrophic environments (Sullivan et al. 2005), which may thus also helped the growth of *Synechococcus* (Bettarel et al. 2011). The existence of distinct biological controls for viral abundances amongst distinct aquatic environmental conditions has previously been discussed (Clasen et al. 2008). In their study, they showed a difference in the relationships of viruses and bacteria between freshwater lakes and the marine oceanic environments and

suggested that phytoplankton might be the main viral–host in freshwater lakes, while heterotrophic bacteria remained the main viral host in the marine environment. Although, one striking difference in environmental conditions between lakes and marine systems would be salinity, Clasen et al. (2008) did not relate their founding with abiotic factors. Increasing VLP abundances with salinity and the importance of salinity gradients to changes in viral abundances and their relationships to heterotrophic and autotrophic bacterial host communities have also been reported along continuous salinity gradients (c.a. 35 to >100, Schapira et al. 2009, 2010; Bettarel et al. 2011). Overall, salinity gradients here (c.a. 2) were certainly not as high as these distinct systems such a complete comparison here may not be realistic. However, the upwelling and downwelling seasons separated the occurrence of distinct sourced waters. This was clearly noted at the bottom (Fig. 3), but the circulation of the surface waters involves the influence of the oligotrophic GAB warm waters and the seasonal exchanges between gulf and shelf waters (McClatchie et al. 2006; Middleton and Bye 2007). One may hence also suggest that these distinct origins may have carried out distinct populations of bacteria and viruses, similarly to picophytoplankton. Yet advective transport of viral and heterotrophic bacterial communities has, to our knowledge, never been addressed in past studies, this latter may agree with changes in viruses and heterotrophic bacterial communities in distinct environments (Corzo et al. 2005; Shareck and Latasa 2007; Schapira et al. 2009).

Another potential explanation for this decoupling is a shift in viral life cycle strategies with environmental conditions. Indeed, lysogenic viruses have been shown to prevail in oligotrophic conditions (Weinbauer and Suttle 1999; Williamson et al. 2002). This release of viral pressure for heterotrophic bacteria may have led to the increased bacterial abundances at the surface and fluorescence maxima. This potential lysogenic virus may then question the relationship of viruses and cyanobacteria found here. However, it is possible that following October 2009, the successive increase and decrease in viral and bacterial abundances might have led to rapid shift in lytic *vs* lysogenic viruses due to intermittent mixing in between upwelling pulses, which ones could not however been observed here. Finally, the relatively high irradiances at the surface in periods of weak southeasterlies and El Niño conditions may have led to the decay of viruses. While this contrast with our results (Table 5), the shallower fluorescence maxima in the 2009 and 2010 upwelling seasons do not rule out such hypothesis. Indeed, this latter is consistent with the high viral abundances in February 2008 when mixed upwelled and surface layers likely occurred and in April 2008 when fluorescence maxima reached deeper depths. High VLP abundances (ca. 10^6 mL^{-1}) here were however one order of magnitudes lower than the high (ca. 10^7 mL^{-1}) viral abundances recently reported in the coastal upwelling system off Valparaiso Bay (Kuznar et al. 2009) and off Concepción (Eissler 2010). In addition, in this latter, heterotrophic bacteria and viruses were found significantly related to each other, stressing the present decoupling of viruses and bacteria to reflect the unusual oligotrophic conditions of the 2010 upwelling season under El Niño conditions.

4.4. *The relative importance of wind vs La Niña/ El Niño to microbial and viral dynamics*

On the physical point of view, the impact that El Niño Southern Oscillation events generate in the present shelf waters is known to differ from that observed around the Pacific due to the absence of relationships between the alongshore wind stress and El Niño/La Niña cycles and the occurrence of upwelling events during El Niño (Middleton et al. 2007). However, the response of the picophytoplankton communities to prevailing El Niño conditions appeared to be very similar to that of the equatorial and western Pacific (e.g. Blanchot and Rodier 1996; Masotti et al. 2011), which hence question the ecological meaning of the relationship between SOI values and microbial community structure and/or may suggest a similar underlying mechanism.

In the present case, changes in the seasonal upwelling conditions over time were associated to increasing stratification due to, first, the decrease in the prevailing wind intensities and durations and then, in the Southern Oscillation Index, leading to prevailing weak upwelling favorable wind and El Niño conditions in the 2010 upwelling season. As a result, changes in the picophytoplankton community structure occurred between each upwelling season within the upper depths, the dominance of picoeukaryotes in 2008 to *Prochloroccoccus* in 2009 and *Synechococcus* in 2010, while Proc2 remained the dominant population at the bottom (Fig. 4). Note that *Synechococcus* also dominated in March 2008. In addition, viral abundances significantly decreased with increasing oligotrophic conditions at the surface and fluorescence maximum and decreasing bacteria at the bottom (Fig. 5). Hence, while heterotrophic bacteria remain relatively constant at the surface and fluorescence maximum (Fig. 5), the release of viral pressure may have increased heterotrophic bacteria at the upper depths (Table 4, Fig. 5).

At the surface, our results suggest that on the long term, regenerated production regulated by picophytoplankton (mainly Proc1 and Syn) and heterotrophic bacteria are likely to increase with increasing stratification associated to enhanced upwelling events and weaker southeasterlies, but also the release of viral pressure. Furthermore, light was not limiting below the mixed layer depth where shear processes close to the upwelled waters and stability allowed the growth and accumulation of picophytoplankton at the fluorescence maxima (Table 1, 2, 5, Fig. 3C, Fig. 4). There, nitrate uptake by picoeukaryotes (and larger phytoplankton) was likely responsible for new production (Table 5) which may have feed the pool of dissolved organic and inorganic matter following grazing. This may have increased ambient ammonium concentration (Table 2) and allowed the growth of heterotrophic bacteria (Table 5), but also Proc2 (Table 5). *Synechococcus* and Proc1 likely beneficiated of high NO_x and PO_4 concentrations from upwelled waters, respectively (Table 2, 5), especially in 2010 (Table 2). In the case of 2009 and 2010, viruses may have affected cyanobacteria (Table 3), whereas in 2008, viruses may have affected heterotrophic bacteria (Table 3). In contrast, Proc2 consistently dominated the picophytoplankton communities (Fig. 4) at the bottom where we found tight linkages among picophytoplankton, viruses, and heterotrophic bacteria (Table 4). Hence, our findings clearly stress here the potential effect of viruses on the microbial food chain and biogeochemical cycling of the system linked with the relative importance of wind *vs* La Niña/ El Niño upwelling conditions. In

addition, because the temporal variability in the PROK:EUK ratios was significantly controlled by the abundances of *Prochlorococcus* and to a lesser extent *Synechococcus*, one may suggest that both groups may sustain the productivity of these shelf waters during upwelling conditions, particularly in prevailing El Niño conditions.

5. Conclusion

This study shows that hydroclimatic forcing conditions are differently influencing the long–term temporal variability in microbial and viral abundances inhabiting the surface, fluorescence maximum, and bottom depths of the NRSKAI station of the Southern Australian continental shelf waters. The microbial communities are thus susceptible to differently respond to processes resulting from upwelling and downwelling depending on their location within the water column. As a result, we found the existence of a vertical decoupling of viruses and heterotrophic bacteria, but further work is needed to elucidate the mechanisms behind it. These results were related to the saline bottom waters flowing out of Spencer's Gulf, downwelling and mixing processes, and the occurrence of upwelling events influenced by the prevailing wind intensities and El Niño/La Niña conditions. As such, the inter–annual variability in the microbial and viral communities of the present system reflected the differential effect of temporal and vertical temperature and salinity gradients associated to changing upwelling and downwelling conditions. Our findings are critical for the long–term understanding in the ecology and biogeochemical cycling of the present system and bring further insights on the effect of hydroclimatic forcing events on picophytoplankton, heterotrophic bacteria and viruses.

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VI. General Discussion:

1. Summary of the results

This work has taken on the opening of two distinct interdisciplinary research projects to develop further understanding on temporal and spatial changes in the abundances of microbial communities in relation to physical patterns and processes. This is of major importance to assess the relationship between changes in picoplankton community structure and the functioning of distinct ecosystems with global changes.

First, the short–term temporal fluctuation of distinct heterotrophic bacterial populations was carried out within the Polar Frontal Zone during the international research project of the sensitivity of Sub–Antarctic Zone waters to global changes. Results showed the importance of depth and the determination of discrete microbial population to the short–term dynamics of heterotrophic bacterioplankton and an overall stability in bacterial community structure.

Secondly, emphasis was given on the dynamics of the picophytoplankton community structure at the seasonal and inter–annual scale carried out within the South Australian continental shelf waters during cruises undertook under the Southern Australian Integrated Marine Observing System (SAIMOS). Key results showed the importance of physical forcing events (upwelling, downwelling, shelf circulation) to the temporal and spatial dynamics of picophytoplankton communities encompassing distinct populations of *Prochlorococcus*, *Synechococcus* and picoeukaryotes. As such, the potential existence of diverse picophytoplankton ecotypes or strain was for the first time reported for the region and, specifically, an unexpected southern extension of *Prochlorococcus* in an environment that was thought to be relatively hostile due to the hydrodynamic of the shelf waters, such as upwelling events in the summer. It was then suggested that the occurrence of *Prochlorococcus* in South Australian shelf waters could be due to advective transports. The linkage between the dominance of distinct picophytoplankton populations and the vertical structure of the water column showed that both local (wind field) and global (El Niño/La Niña) hydroclimatic forcing modified the structure of picophytoplankton communities over distinct upwelling conditions.

Finally, the temporal dynamics of picophytoplankton, heterotrophic bacteria, and viruses at the surface, fluorescence maximum, and bottom depths was further analyzed at the national reference station of the Southern Australian continental shelf waters. There, particular attention was also given to changes in the relationships between these three components associated to local and global hydroclimatic forcing events. Results showed that processes controlling the temporal dynamics of picoplankton communities differed between depths and, that the strength of the relationships between picophytoplankton, heterotrophic bacteria, and viruses differed both vertically and over time. El Niño and the wind field both were likely responsible for the shift in picophytoplankton community structure and the decoupling of viruses and bacteria during upwelling. These results indicated that physical events of distinct nature differentially influenced various nodes of the microbial food web.

2. Constant *vs* sporadic variability in microbial community structure

The concept of scales is important in ecology to picture the observed dynamics of a given individual, population, or community in relation to its environment. In marine systems, this concept has received increasing attention at the end of the last century (Schneider 2001) and is still today even greater with the rapid development of observational technologies and modelling systems to get answers to observed patterns and to be able to forecast the future (e.g. Sarmiento et al. 1998; Vallino 2000; Karl et al. 2001). Throughout this work, we have analyzed the dynamics of microbial communities at distinct temporal scales of the order of hours, months, and years, at the meter–scale on the vertical and the kilometer–scale on the horizontal. Overall, the present results indicated a diffentialstructure of heterotrophic bacterioplankton and picophytoplanktonover time, depending upon the sampled location. As such, the constant or sporadic behavior of any given picoplankton community may not depend on the scale considered. Throughout this work, the most abundant population or group often presented relatively little variability over time, but the least abundant population varied the most. For instance, considering the population level, the more abundant populations showed relatively little variability over a 48 hours time frame in the Polar Front of the Southern Ocean, but the least abundant population showed a much higher variability (Chapter II). Furthermore, in the South Australian continental shelf waters, and at the seasonal scale, Syn2 and Proc2 populations occurred sporadically at the fluorescence maxima, whereas Syn1 and Proc1 varied less (Chapter III). Considering the group level, abundances of heterotrophic bacteria were up to one order of magnitude higher than those of picophytoplankton, these varied less (coefficient of variation, CV < 40%) in abundance than picophytoplankton (CV > 56%) from the considered sampling times (Chapter V). The higher variability observed in the abundances of cyanobacteria compared to that of heterotrophic bacteria and picoeukaryotes has been also reported at the seasonal scale in other continental shelf region (e.g. Jiao et al. 2002, 2005; Calvo–Díaz et al. 2008). It thus appeared that for different levels of organization, the abundance dynamics among populations or groups reflect their relative constant *vs* sporadic behavior over distinct time periods. While such observation may reflect the generalist or opportunistic behavior of microbes, this dichotomy could be of particular interest if one may seek to understand the long–term variability in picoplankton community structure and the related processes in a given system.

While intrinsic forcing might cause temporal variations in the abundances of a given microbial population, this work specifically focused on external forcing. The relevance of short time scales (i.e. hours) has previously been discussed in relation to the short generation time of microbes such as their abundances fluctuate in the environment in the form of patches and hotspots (e.g. Seymour et al. 2005; Hewson et al. 2006). Our results also showed that the structure of bacterial communities can be relatively constant over 48 hours, which agreed with the lack of consistent responses of the bacterial abundances for diverse systems (Chapter II). At the seasonal and annual scale, hotspots of abundances specific to distinct picophytoplankton populations were again observed (Chapter III), but on the long term, both picophytoplankton and heterotrophic bacteria did not significantly increased or decreased in abundances (Chapters V). Reasons for

these patterns would be the direct regulations of both top–down (i.e. activity of predators and viruses) and bottom–up (i.e. nutrients availabilities) processes (Pace and Cole 1994) which act at distinct time and space scales such as their phasing may generated discrete or continuous patterns of microbial dynamics, depending on the system properties.

The processes generating the observed behaviour of picophytoplankton might be related to the scale considered. The recurrent nature of external physical forcing across both time and space scales may however suggests a recurrent behaviour of microbial communities in response to these external forcing. Indeed, the ecological responses of marine microbes are directly or indirectly dependent on oceanographic and climatic forcings that occur at various time scales, from e.g. the daily sunlight patterns and turbulent shear processes through seasonal variations in sunlight declination and water circulation to inter–annual and longer term variations in climatic (e.g. ice age) and hydrologic (e.g. conveyor belt) forcing. These cyclic variations may, however, encounter natural sporadic events over a wide range of scales such as nutrient patches, upwelling, water mass intrusion, cyclones and volcanic eruption. Anthropogenic disturbance such as the breakdown of an oil ship and increasing atmospheric compounds may also affect the dynamic in microbial communities, such as delays or cascading responses may all affect microbial behaviors across distinct time scales. These microbial communities are however quite resourceful to respond to such events through their great diversity and capacity of adaptation to changing environmental conditions (e.g. Masotti et al. 2011). Variations in microbial abundances also occur spatially such as geographically distinct environments either being a coastal or an oceanic ecosystems, variability occurs as each have their own biotic and abiotic properties.

3. Physical forcing and the long term ecology of picophytoplankton communities

One of the main implications of the present research is to bring further information on the responses of picophytoplankton populations to physical forcing events and to ultimately improve our understanding in the dynamics of the metabolic status of an ecosystem and the factors that regulates it. Indeed, global increase in sea surface temperature (SST) on the functioning of oceanic systems has been stressed with ocean acidification and increasing heterotrophy (Bopp et al. 2001; IPCC 2001; Arrigo et al. 2005; Behrenfeld et al. 2006; Lopèz–Urrutía et al. 2006; IPCC 2007; Hare et al. 2007; Donney et al. 2009), though the ‘how’ and ‘by how much’ remain uncertain. The importance of the microbial food web and picophytoplankton production in surface oligotrophic oceanic waters, but also in coastal shelf waters (Morán 2007; Morán et al. 2010), could thus become particularly important with global warming (Lopèz–Urrutía et al. 2006, Hare et al. 2007).

While we have accumulated an increased literature dealing with the impact of global changes and anthropogenic disturbances we are still far from being able to generate a clear answer of what will be the response of the picophytoplankton dynamics in future oceanic and coastal waters. Time series studies have, however, proved for the last decades to be particularly relevant and shown shifts

in primary production, phytoplankton community structure, and up to a complete regime shift of the system (e.g. Karl et al. 1995, 2001; Andersen et al. 2008). Mesoscale processes such as upwelling, downwelling, fronts and eddies all have the potential to act on the trophic status of an ecosystem (e.g. Montero et al. 2007; Chen et al. 2008) and has often explained the enhancement of phytoplankton growth and production (e.g. Hense et al. 2000; Arrigo et al. 2008), though their role and consequences on carbon fluxes remain uncertain (Arístegui et al. 2004). Besides, a critical question yet to be addressed is to assess if the effects of a given process observed in the past would still be similar in the future within the context of global change.

Yet, in contrast to the sporadic blooming of large phytoplankton cells, such as diatoms, picophytoplankton have been suggested to show a relatively steady–state over time, being controlled by top down processes, but having the ability to thrive in nutrient depleted habitat (Raven 1998; Baraber and Hiscok 2006). The difficulty to observe any clear seasonal patterns in bulk phytoplankton biomass have nevertheless been suggested to be due to the different timing in the inter–annual cycles of distinct picophytoplankton groups (Campbell et al. 1997). In terms of trophic status, any perturbation (i.e. physical forcing such as an upwelling) is expected to take an ecosystem away from its steady–state (the system should be expected to be imbalanced), leading to export of a significant amount of organic carbon (Serret et al. 1999). Brix et al. (2006) have thus suggested that a steady–state may be reached when considering, however, a sufficient time scale. It is consequently tempting to suggest that a balanced system might simply reflect the contrasting behavior of distinct dominated phytoplankton communities over time.

The important sporadic variability in distinct dominant picophytoplankton groups observed in the present work could also be of interest to other potential future scenario involving physical forcing events. If assuming being within a period of transition, the occurrence of sporadic events are likely to increase in both frequency and intensity and distinct phytoplankton blooming events may hence increase in both frequency and intensity in relations to these events. For instance, one may want to question the impact of a cyclone (i.e. Katrina 2005 (US), Larry 2006 (AU), Yasi 2011 (AU)), tsunami (i.e. Thailand 2006, Japan 2011), volcanic eruption (i.e. Iceland 2010, Chile 2011), etc. on the responses of plankton communities and the potential cumulative effect of such events on plankton dynamics over time. Hence, despites the potential resilient feature of picophytoplankton communities to physical disturbances (Masotti et al. 2011), we may question some potential cascading effect from such events on longer term trends.

Global climate change should lead to a more stratified ocean and enhanced upwelling events (e.g. Bakun 2010; Behrenfeld 2011), though depth–integrated measurements are often found in the literature (e.g. DuRand et al. 2001; Li and Harrison 2001; Brix et al. 2006). The present study offers a distinct approach by directly understanding the local effect of upwelling and downwelling to the long–term variability in picophytoplankton community structure. This led to show the importance of the differential effect of temperature and salinity gradients to distinct picophytoplankton populations of three distinct layer depths. Such approach may hence have important implications in the use of vertically integrated data of gross primary production and community respiration when

evaluating the metabolism of ecosystems. In fact, while integrated data has been accounted for the compensation of imbalances over the water column (Williams 1998), the need to assess both gross plankton community production and respiration in order to adequately describe the trophic status of a system has previously been stressed (e.g. Waku and Furuya 1998, Serret et al. 1999). More recently, Claustre et al. (2007) have discussed the importance of the role of the deep layer, below the euphotic layer, in contributing to carbon fixation, particularly when surface irradiance is high such as in the South Pacific Gyre in absence of cloud cover. Finally, Serret et al. (1999) and Nicholson et al. (2008) have demonstrated the strong influence of physical forcing (i.e. mixing, stratification, and mesoscale features such as Rossby waves and eddies) in the deep euphotic zone which was not extended into the mixed layer. Such results therefore stress the importance to further investigate the role of different layers associated to different forcing to the community metabolism of an ecosystem.

4. Relevance of the microbial food web for the South Australian shelf waters

The present approach was to further understand the seasonal and inter–annual dynamics of picoplankton communities in relation to natural physical forcing within the Southern Australian continental shelf waters. Very little is known in regards to how the valuable fisheries and overall productivity of these shelf waters are sustained. This, despites increasing focus on the physical and biological processes associated to the Kangaroo Island upwelling system undertaken for nearly 10 years (Kaempf et al. 2004; Ward et al. 2004; McClatchie et al. 2006; Middleton and Bye 2007; van Ruth 2009; van Ruth et al. 2010). Yet, the microbial food webs of this region remained to be examined. The present work on the space–time dynamics of picophytoplankton, bulk bacterial and viral communities (Chapters III–V) in relation to seasonal upwelling and downwelling events and circulation patterns, hence brings crucial implications in the understanding of the functioning of the Kangaroo Island–Eyre Peninsula (KI–EP) system and in the light of biogeochemical models of the KI–EP region.

The present research allowed for the temporal and spatial capture of distinct snapshots of the picoplankton communities, and in particular the picophytoplankton communities of the KI–EP region in relation to physical forcing events. The close examination of picophytoplankton community structure in relation to the water column, and, the dynamics of picophytoplankton, heterotrophic bacteria, and viruses over three years were also useful to understand the consequences of El Niño Southern Oscillation (ENSO) events on planktonic food web in the South Australian continental shelf waters.

In the Peru upwelling system, regenerated production contributed up to 50% of the total nitrogen production within the euphotic zone (Metzler et al. 1997). Past studies have also explained the higher primary production of the Pacific eastern boundaries compared to that of the Atlantic, by their higher nitrate concentrations in upwelled sourced waters and mixing processes (Metzler et al. 1997). In South Australian continental shelf waters, upwelled waters were shown to remain as a nitrate rich cold pool, with limited nutrient fluxes between upwelled and the surface mixed waters, due to stratification processes

(McClatchie et al.2006; Middleton and Bye 2007; van Ruth et al.2010). Primary production was nevertheless shown to reach values as high as those of the eastern boundaries in some localized spots in the region such as southwest of KI (van Ruth et al. 2010). van Ruth (2009) however suggested that the lack of iron associated to the oligotrophic waters of the GAB warm pool could be influencing phytoplankton growth and production. Phytoplankton iron limitation has been reported in upwelling systems such as the eastern boundaries (Hutchins et al. 1998, 2002). In regards to picophytoplankton, their high variability in abundances and high abundances of various populations during the upwelling seasons (Chapter III) may not however support this hypothesis. This would agree with previously observed increasing growth rates of picophytoplankton in response to iron enrichments (Barber and Hiscock 2006), although some controversies have appeared due to the differential physiological responses of distinct cyanobacterial ecotypes to iron limitation (Scalan et al. 2009). In addition, at the bottom, a specific picophytoplankton group (Proc2) consistently dominated the picophytoplankton communities during the upwelling season (Chapters IV and V), and tight couplings were observed between picophytoplankton, viruses, and heterotrophic bacterial communities (Chapter V). A lack of iron may have enhanced the importance of ammonium, in contrast to nitrate, to phytoplankton growth (Armstrong 1999) within the KI–EP system. Fernández et al. (2009) have shown that in the Peru upwelling system, ammonium regeneration may contribute up to 50% of NH_4 assimilated in surface waters and nitrification up to 16% of nitrate. In South Australian shelf waters, nutrient from the upwelled pool and bottom resuspension of dissolved organic matter may be advected to shallower waters, sustaining regeneration processes in relatively warmer conditions via the microbial food web over the upwelling season. The significant importance of ammonium concentrations to picophytoplankton and heterotrophic bacteria shown in Chapter V does support the potential importance of picophytoplankton to regenerated productions processes. This regenerated production may be enhanced under El Niño conditions, due to the increase in NO_x concentrations in upwelled waters and stratification (Chapter V). Iriarte and González (2004) observed a high contribution of picophytoplankton (> 50%) to chlorophyll *a* and primary production during the El Niño event of 1997–1998 in coastal waters off Chile. Their results were explained by the shoaling of the thermocline which resulted into oligotrophic conditions allowing picophytoplankton growth to overtake that of microphytoplankton. In the present case, the relation between the depth of the fluorescence maximum and the surface mixed layer depth was shown to be important to the dominance of distinct picophytoplankton populations under distinct stratification intensities (Chapter IV) and to changes in the strength of the correlation between viruses and bacteria (Chapter V). These results derived from distinct upwelling conditions suggested that the dominance of picoeukaryotes might occur only at certain circumstances such as during periods of relaxations or nearby islands, yet spatially following stratification and the dominance of cyanobacteria (Chapters III and IV). This agrees with the rising tide hypothesis recently suggested by Barber and Hiscock (2006). In their work, both diatoms and picophytoplankton growth rates increased under favourable conditions, but the larger biomass of diatoms and strong top–down control of picophytoplankton by heterotrophic nanoflagellates may restrain the dominance of picophytoplankton. They further suggested that; in contrast to previous

believes (Margalef 1967), blooms of diatoms succeed to that of picophytoplankton which are further sustained following the decline of diatoms. In the present case, the findings of a significant positive relationship between picophytoplankton and viruses at the surface and FM depths also suggest the regulation of picophytoplankton abundances by viruses. On the other hand, the release of viral control on heterotrophic bacteria may increase regenerations and remineralisation processes, particularly during upwelling under El Niño conditions (or in a lesser extent, under weak southeasterlies and La Niña).

Finally, inter–annual variations in picophytoplankton community structure and heterotrophic bacterial abundances have also been noted as a results of El Niño in distinct long–term observing and monitoring systems, i.e. SEATS (Liu et al. 2007), ALOHA (Karl et al. 1995; Campbell et al. 1997), central California (Chavez et al. 2002). Masotti et al. (2011) have recently reported large–scale shifts in productivity and phytoplankton community composition in the Equatorial Pacific. Both Blanchot and Rodier (1996) and Landry et al. (2006) noted a decrease in *Synechococcus* during the early stage of El Niño or weak El Niño conditions (SOI=–0.4), but high *Synechococcus* abundances in the mature stage of El Niño (SOI=–3.5). In the present case, the decrease in *Synechococcus* abundances occurred with the onset of El Niño (June 2009) and was followed by an increase of one order of magnitude in *Synechococcus* abundances at the bottom in October 2009, when El Niño was strongest (SOI=-14). During the mature stages of El Niño (i.e. from November to March 2010), an increase in *Synechococcus* was also observed but only at the surface and fluorescence maximum depths. The exact ecological meaning of the relationship between SOI and the PROK:EUK ratios was however questioned in Chapter V. Indeed, coastal–trapped waves generated from El Niño Southern Oscillation (ENSO,Church and Freeland 1987) may take a few weeks or months to travel up to Southern Australian shelves (Middleton et al. 2007 and references therein). Increasing sea level anomaly of the KI–EP shelf waters is also likely to be associated to El Niño coastal–trapped waves (Middleton et al. 2007). The strong local downwelling events was likely responsible for the patterns in picoplankton abundances observed in 2009, while strong El Niño together with the relatively weak southeasterlies led to enhanced stratification of the water column and the subsequent dominance of *Synechococcus* during the 2010 upwelling event (Chapters IV and V). Variations in stratification intensities migth thus explain the effect of El Niño on picoplankton community structure in both the present case and in the equatorial and eastern Pacific. The present results showing the decoupling between viruses and bacteria during the 2010 upwelling season also suggest a disturbance of the microbial food web and associated carbon and nutrient fluxes due to the relative intensity of the local wind field and El Niño.

Future studies for the South Australian continental shelf waters should particularly focus on the role of the microbial food webs to the functioning of the system and evaluate the relative contribution of picophytoplankton to bulk phytoplankton biomass and production associated to upwelling and downwelling events. In particular, recommendation would be the use of 0.2 µm porosity filters (e.g. nucleopore) to evaluate bulk chlorophyll *a* concentrations, GF/F filters having a porosity around 0.7–0.8 µm (e.g. Dickson and Wheeler 1993). Furthermore, the suggested reasons (see Chapter V) of the decoupling of viruses and bacteria should be further evaluated, particularly in relation to changes in

upwelling conditions. The determination of distinct populations of picophytoplankton, bacterioplankton, and virioplankton should be further assessed in relation to the circulation patterns of the distinct waters masses influencing the KI–EP region. The genetic determination of these populations should also bring further inside in the potential importance of the diversity of these groups within the South Australian continental shelf waters.

5. Future research directions and the need to think both locally and globally

First, it is clear that observed variations in the dynamics of microbes are often specific to the studied system. As a consequence, a generalization to a global level is surely not appropriate without clear evidences of the linkages between local to global observed patterns. Indeed, as stated by Polis et al. (1998), the absolute and relative strength of a factor varies temporally and spatially, and factors may be important continually or sporadically, concurrently or sequentially. As such, multifactorial and integrated approaches tending to generalized conceptual relationships must be taken with care, and a thorough examination of how factors interact and vary in both space and time must be assessed. In addition, multiyear studies conducted at several spatial scales should be achieved to detect temporal variability and examine the interplay between spatial and temporal variability (Polis et al. 1998). Indeed, the significance effect of a factor may become a general trend despites potential exception. For instance, the effect of temperature on microbial dynamics is today well established (e.g. Shiah and Ducklow 1994; Agawin et al. 2000), though exceptions are still reported. It is thus important to distinguish the direct and indirect effect of temperatures on microbial communities (Beveridge et al. 2010) which may differ for distinct temporal and spatial scales. In the present case, physical forcing events associated, however, to changes in temperatures but also salinities, were more important than temperatures in the regulations of (i) microbial abundances, (ii) the relationships between viruses, heterotrophic bacteria, and picophytoplankton, and (iii) picophytoplankton community structure (i.e. PROK:EUK ratio) over the long–term (Chapter V). These three points were in turn all related to large–scale physical forcing (El Niño Southern Oscillation, Chapter V). Nevertheless, the decrease in microbial abundances at the bottom during the 2010 upwelling season was likely due to temperatures alone (Chapter V). In fact, temperatures directly affect the metabolism of microbes with increasing temperature leading to increasing picoplankton growth rates (Falkowski and Raven 2007). However, because metabolic reactions have different kinetics and different species respond differently to temperature variations, there is no simple relationship between photosynthesis or respiration rates and temperature (Falkowski and Raven 2007). In addition, temperature can interact with other factors such as variations in dissolved inorganic and organic carbon or light availability, or other mechanistic processes may hide the direct effects of temperature (Falkowski and Raven 2007; Fu et al. 2007). This direct temperature effect should thus be particularly clear at short–term time scale and small spatial scales relevant to the picoplankton. However, significant relationships between temperatures and picophytoplankton abundances are often reported in studies performed at large time (season to years (i.e. global climate

changes); Calvo–Díaz and Morán 2006; Calvo–Díaz et al. 2008; Bopp et al. 2001; Finkel et al. 2005; Behrenfeld et al. 2006) and spatial scales (kilometers to basin scale; Partensky et al. 1999; Matsumoto et al. 2004; Lasternas et al. 2010), which ones should be considered as indirect and reflect the effect of temperature gradients with distinct related processes from that of changes in temperatures alone. As a result, shifts in community structure occur with the smaller surface and high volume prokaryotes cells thriving in such conditions (Raven 1998; Karl et al. 2001). Recently, Morán et al. (2010) has further addressed a theory behind these observed patterns of phytoplankton size structure related to global changes. In their work, shifts in phytoplankton cell size were largely affected by changes in seawater temperatures alone, explaining that this temperature effect may occur regardless of the trophic status of the system or nutrients availabilities. Such metabolic theory was also questioned earlier by Lopèz–Urrutía et al. (2006) and Lopèz–Urrutía (2008). Nevertheless, the various potential direct and indirect effects of increasing sea surface temperatures due to global climate change (see Finkel et al. 2010 for review), may act together and subsequently lead to a boost up of the microbial loop into future oceanic and coastal waters (Behrenfeld 2011). Hence, the results of the present study (Chapter V), in addition to the potential intensification of coastal upwelling with global changes (e.g. Bakun 2010; except for the western coastal waters where increasing stratification are likely to prevent upwelling events), may agree with these above recent work and review. However, results from the present work further question the role of viruses in this future warmer stratified oceanic and coastal waters, specifically knowing that viral infection increase with temperatures (Kilpatrick et al. 2008) and may potentially shift host–cells communities or life cycle strategies. On the other hand, while viruses are able to infect all types of both eukaryotic and prokaryotic plankton, the short generation time of microbes and the probability of mutations due to viral infection in the ocean could also be essential in regards to the evolution of microbes (Weinbauer and Rassoulzadegan 2004; Sullivan et al. 2005). This, together with the great dynamics of distinct picoplankton communities, certainly call for further research on the interactions between microbes, phytoplankton, and viruses with changing environmental conditions to understand future trends in carbon and energy transfer in relation to global changes.

Finally, wind–driven surface oceanic currents are carrying both nutrients and plankton communities. The upper oceanic layers (i.e. where light are still available for photosynthesis) move at a certain velocity which can affect planktonic populations of different composition and the fate of inorganic and organic carbon in the ocean (e.g. Copin–Montégut 2000). Oceanic currents and water masses are often characterized by temperature–salinity diagrams. However, both temperatures and salinities have been shown to affect picoplankton abundances (e.g. Shiah and Ducklow 1994; Agawin et al. 2000; Hirose et al. 2008; Schapira et al. 2010). Hence, characterizing oceanic currents in terms of picoplankton properties may bring some valuable insights in terms of the connectivity between isolated systems and future changes. Indeed, while the last decades has made a huge amount of progress in terms of understanding the different geographical ecosystems of the marine environment and their biological, physical, and chemical properties, one may want to know about the connectivity between these systems in terms of microbial community structure and functioning. Few studies performed in continental shelf waters have noted

changes in community structure and suggested the role of advective currents for carrying distinct groups of picophytoplankton to specific location (e.g. Katano et al. 2005; Calvo–Díaz and Morán 2006; Hirose et al. 2008; Chapter III). While these studies have mainly questioned the role of advective transport to explain the occurrence of *Prochlorococcus* (e.g. Calvo–Díaz and Morán 2006), to our knowledge, no such suggestion has been made for heterotrophic bacteria and viruses (Chapter V). In fact, no real attempt has been made to fully understand the role of oceanic and coastal currents in the variability of microbial community structure and their subsequent impact on the functioning of these geographical systems.

Furthermore, we are facing a current increasing knowledge on the huge amount of diverse ecotypes and strains encompassing picoplankton communities and have the ability to genetically characterize them further in conjunction with their marine environment. Hence, it is evident that the genetic, metagenomic, and environmental variables will be next compiled to bring further insights on the diverse dynamics of these strains for each geographic locations, but also distinct microhabitats (e.g. Zwirglmaier et al. 2008; Scalan et al. 2009, Cermeño et al. 2010). However, understanding the mechanisms behind this spatial variability, specifically the connectivity between systems is needed to understand further their interactions and relationships. Temporal studies conducted at appropriate timescales allowing the determination of the phasing of photosynthesis and respiration are still rare (Blight et al. 1995; Serret et al. 1999, Williams 2000) and physical processes such as advection transport of water masses may greatly affect any of these observations. The efficiency of the biological pump has been reported to be controlled by complex processes that related to the interaction between water masses circulation, vertical mixing, geochemical reactions and biological responses of food webs and microbial communities (e.g. Quéguiner et al. 2011). Amongst others, physical forcing such as wind–driven mixing events or advection processes (Olesen et al. 1999) have also been noted for the potential discrepancies between in situ and in vitro methods to estimate the trophic status of a given system.

In fact, it is often a challenge to track water masses in tidal and ocean currents (e.g. d'Avanzo et al. 1996; Vallino et al. 2005), especially in systems with time varying transport characteristics, such as in coastal and estuarine systems and other vertically well mixed systems. However, this should be of particular interest to establish the metabolic connectivity between ecosystems at the global scale, but also at smaller spatial scales (e.g. connection between coastal *vs* open ocean). In this regards, it has been stressed that we should investigate on integrative approaches that account for exchanges between (ecological) compartments often studied in isolation (Duarte and Prairie 2005). Hence, the dispersion of microbial species across oceanic and coastal systems and understanding the interactions between the planktonic communities themselves are certainly interesting questions. However, characterizing the role of oceanic currents on these interactions and the microbial properties and functioning of oceanic currents would also be of interest to understand the role of global changes to the carbon and energy transfer within and across systems being affected by both local and global physical forcing.

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