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ORIGINAL RESEARCH ARTICLE

Seasonal changes in phytoplankton on the north-eastern shelf of Kangaroo Island (South Australia) in 2012 and 2013[☆]

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Summary This work investigates for the first time the seasonal changes in phytoplankton, bacteria, and photosynthetic picoplankton as well as nutrient concentrations on the North-western shelf of Kangaroo Island, South Australia. Seawater samples were collected off Penneshaw desalination plant, where waters from the Investigator Strait, Gulf Saint Vincent and Backstairs Passage meet. Low nutrient values were measured throughout the period of study (July 2012–July 2013) suggesting the occurrence of oligotrophic conditions on the region. The phytoplankton community was dominated by Bacillariophyceae, Dinoflagellata and Cryptophyta. *Prochlorococcus* Cyanobacteria prevailed among picophytoplankton during most of the period of study (July 2012–July 2013). Previous studies indicate that oligotrophic environments are indeed typically dominated by *Prochlorococcus*. The dominant species found here seem either adapted to grow under low nutrient concentrations, possessing high surface/volume ratios, or have a mixotrophic behaviour allowing them to complement photosynthesis with predation. This study provides base knowledge on the microbial communities north of Kangaroo Island that is needed to sustain the ecosystem and associated economic activities in the future.

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1. Introduction

The South Australian coastal marine ecosystem is one of the most diverse in the world, with a unique endemism rate (O'Hara, 2002). This region supports significant economic activities, notably through aquaculture and most importantly tuna breeding (Prince, 2001). The state of the coastal marine ecosystem in South Australia is then paramount to the sustainability of these economic activities.

Phytoplankton communities, at the bottom of the marine food web, are necessary to the survival of all upper trophic level species (Huertas et al., 2011). Phytoplankton composition fluctuates depending on hydrochemical conditions, such as light, temperature, salinity, nutrients and turbulence (Cloern, 1987; Fisher et al., 1988; Head and Pepin, 2010; Leterme et al., 2005; Sverdrup, 1953). Moreover, the structure of phytoplankton communities is influenced by predation from zooplankton (Landry and Hassett, 1982) and from heterotrophic flagellates (Weisse, 1989), as well as by interactions with bacteria (Azam et al., 1983; Bird and Kalff, 1984) and viruses (Larsen et al., 2001).

Phytoplankton spans across a broad size range, but its smallest fraction, picophytoplankton ($<2\ \mu\text{m}$), accounts for a significant fraction of primary production in seawater, especially in oligotrophic environments (Agawin et al., 2000; Maranon et al., 2001). The prokaryotic component of picophytoplankton mostly includes two cyanobacterial genera, *Prochlorococcus* and *Synechococcus* (Partensky et al., 1999), worldwide. In contrast picoeukaryotes are highly diversified (Vaulot et al., 2008). Oligotrophic environments are typically dominated by *Prochlorococcus* whereas *Synechococcus* and larger sized phytoplankton tend to be more abundant under mesotrophic conditions while picoeukaryotes follow a more complex pattern (Campbell and Vaulot, 1993; Partensky et al., 1996, 1999; Zubkov et al., 2000).

The larger fraction of phytoplankton ($>2\ \mu\text{m}$), which includes nanoplankton ($2\text{--}20\ \mu\text{m}$) and microplankton ($>20\ \mu\text{m}$) is also highly diversified, with Bacillariophyceae and dinoflagellates often being the dominant taxa (Tomas, 1997). Within nanoplankton and microplankton, Haptophyta are more adapted to oligotrophic conditions and Bacillariophyceae tend to dominate under high nutrient concentrations, whereas dinoflagellates and green algae occur mostly under intermediate trophic conditions (Cavender-Bares et al., 2001; Iglesias-Rodriguez et al., 2002; Litchman et al., 2007; Schiebel et al., 2004). However, both cell size and shape are highly variable within each taxonomic group, leading to a variability in the surface/volume ratio of the cells and therefore to different nutrient uptake rates and nutrient requirements for each species. This implies a direct relationship between phytoplankton morphology and nutrient composition of seawater (Alves-de-Souza et al., 2008; Hillebrand et al., 1999; Lewis, 1976; Margalef, 1978).

In coastal ecosystems, the capacity for phytoplankton populations and biomass to fluctuate in response to changing environmental conditions is often highly amplified when compared to the open ocean (Carter et al., 2005; Cloern, 1996). These changes range from temperature and light availability, over naturally occurring nutrient fluctuations caused by upwelling/downwelling-favourable conditions,

to biochemical input from natural and anthropogenic land run-off (Justic et al., 1995).

Previous studies on the Great Australian Bight highlighted the occurrence of a summer upwelling bringing deep waters to the surface (Kampf et al., 2004). This upwelling occurs on the south western shelf of Kangaroo Island and the upwelled water then circulates to the surrounding areas of the Great Australian Bight (McClatchie et al., 2006). However, upwelled waters are not always enriched in nutrients because of an interannual variability of the depth at which upwelling starts (Middleton et al., 2007). This involves an interannual variability in surface picophytoplankton composition, with *Prochlorococcus* dominating under low nutrient concentrations and *Synechococcus* and picoeukaryotes being more abundant under mesotrophic conditions (van Dongen-Vogels et al., 2012). The composition of picophytoplankton has been investigated in detail, both seasonally (Van Dongen-Vogels et al., 2011) and interannually (van Dongen-Vogels et al., 2012) in the Great Australian Bight, whereas the distribution of large phytoplankton ($>2\ \mu\text{m}$) has been assessed in the Gulf Saint Vincent (Leterme et al., 2014). However, despite the importance of phytoplankton to marine ecosystems, no previous study has combined the concentration of nutrients with the abundance and the composition of both picophytoplankton and large phytoplankton during different seasons for South Australian seawater.

The present study investigates the seasonal fluctuations of the phytoplankton communities of the north-eastern shelf of Kangaroo Island, South Australia. Biological and chemical properties of the ecosystem were monitored from July 2012–July 2013 to assess and explain changes in species composition in relation to environmental conditions. This is the first study simultaneously investigating the phytoplankton communities and their environment in this area and is essential to set up the baseline of future studies. This information will indeed allow evaluating the impact of future changes in temperature and seawater pH to the phytoplankton communities in South Australia.

2. Material and methods

Seawater samples were collected from a DN280 PE280 intake pipe with a nominal diameter of 0.8 m (Leterme et al., 2014), at Penneshaw desalination plant, located on the north-eastern coast Kangaroo Island, South Australia, where waters from the Investigator Strait, Gulf Saint Vincent and Backstairs Passage meet (Fig. 1). The intake pipe pumps seawater at about 190 m off the north-eastern coast of Kangaroo Island, at a depth of 6 m. The high (typically $8.4\ \text{L s}^{-1}$) and uninterrupted flow of seawater within the pre-treatment system implies a very short residence time of seawater within the intake pipe, preventing particle settling. Seawater from the intake pipe thus reflects the environmental conditions occurring in sub-surface seawater off Kangaroo Island during the sampling period. This method of sampling has been tested previously by Manes et al. (2011) for bacteria and by Sewell and Jury (2011) for phytoplankton and meroplankton and was shown not to cause any bias in observations. Seawater was collected using 1 L polyethylene terephthalate (PET) bottles and samples were stored on ice and transported to the laboratory for immediate processing. For phytoplankton

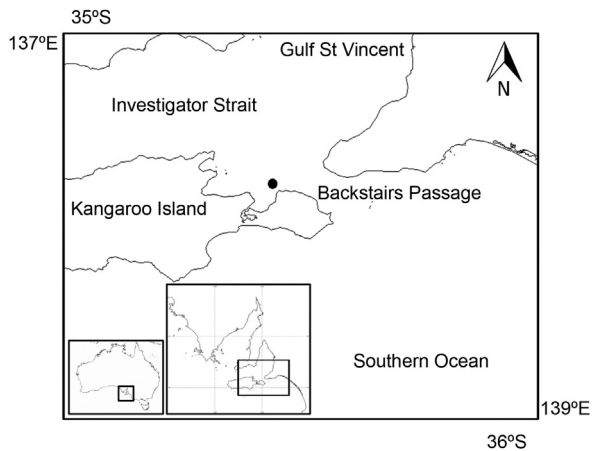


Figure 1 Location of the sampling point on the north-eastern shelf of Kangaroo Island (South Australia).

identification and enumeration PET bottles were pre-filled with 5 mL acidic lugol solution (5 mL; 0.6 M potassium iodide, 0.4 M iodine and 1.5 M acetic acid) for a final 0.5% concentration similar to previous work (Jendyk et al., 2014; Leterme et al., 2014). Samples for phytoplankton identification were stored in the dark for up to 1 month before analyses.

2.1. Nutrients

The concentration of dissolved silica, ammonium, orthophosphate and the combined concentrations of nitrate and nitrite (nitrate/nitrite) were measured simultaneously every two weeks using a Lachat Quickchem Flow Injection Analyser (FIA) and carried out following published methods (Hansen and Koroleff, 2007). Four replicates of seawater (100 mL) were sampled at each site (Fig. 1) and filtered through bonnet syringe Minisart filters (0.45 μm pore size, Sartorius Stedim, Dandenong, Australia) to remove large particles. Filtrates were then stored at -20°C for up to 1 month until further analysis. Prior to analysis, the samples were thawed and mixed before injecting approximately 10 mL of each sample into the FIA in duplicate for a total of 6 replicates per sample. The detection limits were 40 nM for dissolved silica species, 70 nM for ammonium, 30 nM for orthophosphate and 70 nM for nitrate/nitrite. The method was calibrated using standard solutions prepared in 0.6 M sodium chloride, corresponding to typical salinity values in seawater of 35 PSU (practical salinity units).

2.2. Chlorophyll-*a*

The concentration of chlorophyll-*a* (Chl-*a*) was measured every four weeks using methanol extraction and subsequent fluorometric determination (Verity et al., 1999). Seawater (0.5 L) was filtered in triplicate through 47 mm, glass micro-fiber filters (1 μm pore size, Filtech, Fairy Meadow, Australia), using a vacuum pump and a filtration ramp. The filters were then wrapped in aluminium foil and stored at -20°C for up to 24 h before analyses. For analysis the filters were placed in methanol (5 mL) for 24 h at 4°C in the dark and the concentration of the Chl-*a* dissolved in the methanol was determined using a Turner 450 fluorometer previously

calibrated with Chl-*a* extracted from *Anacystis nidulans* (Sigma Chemicals, St Louis, MO, USA).

2.3. Phytoplankton

For phytoplankton identification and enumeration samples containing 1 L seawater fixed with 0.5 mL lugol were filtered through 5 μm pore size Sterlitech mixed cellulose ester membranes (Sterlitech, Kent, USA). The cells on the filters were resuspended in a smaller volume of filtrate from the same sample. This suspension (1 mL) was then pipetted into a Sedgewick Rafter counting chamber and counted using a Zeiss Axiolab upright microscope equipped with bright-field and phase contrast optics (Carl Zeiss Microscopy, Thornwood, USA). The cells were identified up to the genus, or species, level based on their key taxonomic features (Hallegraef et al., 2010; Tomas, 1997) and grouped into 7 different taxa (Bacillariophyceae, Dinoflagellata, Chrysophyceae, Haptophyta, Cryptophyta, Chlorophyta and Euglenophyta).

2.4. Flow cytometry analyses

Total bacteria, virus-like particles (VLPs), Cyanobacteria (*Prochlorococcus* and *Synechococcus* populations) and photosynthetic picoeukaryotes (hereinafter referred to as picoeukaryotes) were enumerated using flow cytometry (Marie et al., 1997). From each sampling site, triplicates of seawater (1 mL) were fixed with 0.5% glutaraldehyde for bacteria and VLPs and triplicates of seawater (1 mL) were fixed with 2% paraformaldehyde (ProSciTech, Thuringowa, Australia) for Cyanobacteria and picoeukaryotes. The samples were then flash frozen in liquid nitrogen and stored at -80°C until further analysis. For the enumeration of total bacteria and VLPs the samples were diluted 1:10 in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8, National Diagnostics, Atlanta, USA) and DNA in the cells was stained with 2.5% (w/v) SYBR I Green (Invitrogen, Carlsbad, USA). The samples were incubated at 80°C for 10 min and fluorescent marker beads (1 μL , Molecular probes, Eugene, USA) were added to all samples as an internal size and concentration standard prior to analyses which were performed using a FacsCanto (Becton Dickinson, San José, USA). Cyanobacteria and picoeukaryotes were enumerated from the samples based on Chl-*a* autofluorescence, marker beads were added to samples and samples were analyzed on a FacsCanto as described previously.

For each sample forward-angle light scatter, side-angle light scatter (SSC), SYBR I Green (530 nm), red (670 nm), and orange (585 nm) fluorescence were recorded. Total bacteria and VLPs were discriminated based on SSC and SYBR I green fluorescence whereas *Synechococcus*, *Prochlorococcus* and picoeukaryotes were discriminated based on SSC, as well as on natural orange (phycoerythrin) and red (chlorophyll) fluorescence, as described by Marie et al. (1997). After each flow cytometry analysis the concentration of marker beads was estimated by epifluorescence microscopy and used to normalize the abundance of all microbial populations.

2.5. Statistical analyses

To interpret the community composition with respect to the environmental conditions, the relative proportion of the

major phytoplankton taxa identified by microscopy (Cryptophyta, Bacillariophyceae, Dinoflagellata, Haptophyta and Chlorophyta), as well as the microbial populations detected by flow cytometry (total bacteria, VLP, *Prochlorococcus*, *Synechococcus* and picoeukaryotes) were compared to the nutrient concentrations as well as the temperature and the salinity. Spearman rank correlation coefficients (ρ) were calculated using the Vegan package (Legendre and Legendre, 1998) of the R software (<http://www.rproject.org>).

3. Results and discussion

In this study we assessed for the first time microbial communities and nutrient concentrations during different seasons for South Australian waters.

Seawater temperature ranged from 13.1°C (18th July 2012) to 22.8°C (13th March 2013), whereas salinity ranged between 34.4 PSU (17th July 2013) and 35.8 PSU (27th March 2013, Fig. 2). The concentration of Chl-*a* was $\leq 0.05 \mu\text{g L}^{-1}$ from mid-August 2012 until late May 2013 (Fig. 2), then it peaked ($0.32 \pm 0.10 \mu\text{g L}^{-1}$) on the 5th June and then ranged between 0.05 and $0.1 \mu\text{g L}^{-1}$ thereafter. The higher Chl-*a* values measured during the austral winter 2013 suggest higher phytoplankton abundances in this period.

3.1. Nutrients

Low nutrient concentrations were detected throughout the period of study. These data agree with nutrient data from surface seawater off the west coast of Kangaroo Island (Leterme, unpublished) and observed along the South Australian Bight (Van Dongen-Vogels et al., 2011). Nutrient concentrations were particularly low during the late austral winter 2012, with the nitrate/nitrite concentration often below the detection limit of the instrument (70 nM, Fig. 3).

Concentrations of dissolved nitrogen, silica and phosphorus vary spatially and temporally in seawater. The ratio of these elements within phytoplankton cells, and their subsequent nutrient requirements, vary according to the

taxa as well as the physiological state of the cells. While both nitrogen (N) and phosphorus (P) are needed by all taxa, Bacillariophyceae also require silicium for their frustules. Equimolar amounts of dissolved silica and dissolved inorganic nitrogen (DIN) in seawater are considered optimal for diatom growth (Brzezinski, 1985). In the present study the ratio between dissolved silica and DIN was below 1 from August 2012 until July 2013 (Fig. 4), indicating that diatom growth was likely to be limited for most of the period of study. P and N requirements in phytoplankton cells vary according to their physiological state. In particular, during exponential growth the cells are enriched in ribosomes, whereas higher proportion of proteins and chlorophyll occur in cells under slow growing conditions (Klausmeier et al., 2004). High P/DIN ratios in seawater thus promote species adapted to exponential growth whereas slow growing species occur under low P/DIN ratios (Arrigo, 2005; Klausmeier et al., 2004). In the present study the P/DIN ratio varied during the sampling period. Between August 2012 and February 2013 the P/DIN ratio was higher than 1:16, the Redfield ratio for phytoplankton (Redfield, 1958) while it was below 1:16 for the rest of the year (Fig. 4). Exponentially growing phytoplankton groups with high P/DIN requirements were thus promoted between August and February, whereas slow growing species with low P/DIN requirements were likely to occur between March and May 2013 (Arrigo, 2005).

The nutrient concentrations found on the north-eastern shelf of Kangaroo Island are typically low compared to surface water from other coastal environments. Nutrient concentrations in South Australian surface waters were previously measured off Eyre peninsula (van Ruth et al., 2010a, 2010b), on the western coast of Kangaroo Island (Van Dongen-Vogels et al., 2011; van Dongen-Vogels et al., 2012; van Ruth et al., 2010b) and on the shelf of the Gulf Saint Vincent (Leterme et al., 2014). While the nitrate values found here are comparable to those measured on the other regions, the concentrations of both phosphate and dissolved silica (Fig. 3) are lower than those measured in the Great Australian Bight. Higher concentrations of dissolved silica were also measured on the shelf of the Gulf Saint Vincent (Leterme et al., 2014).

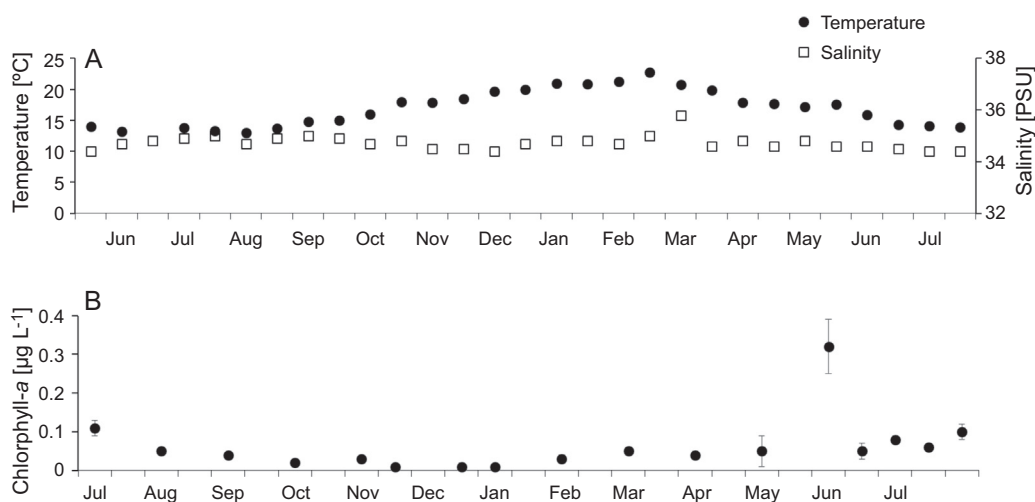


Figure 2 Evolution of (A) temperature [°C] and salinity [PSU] as well as (B) chlorophyll-*a* concentration [$\mu\text{g L}^{-1}$] on the northern shelf of Kangaroo Island during the period of study.

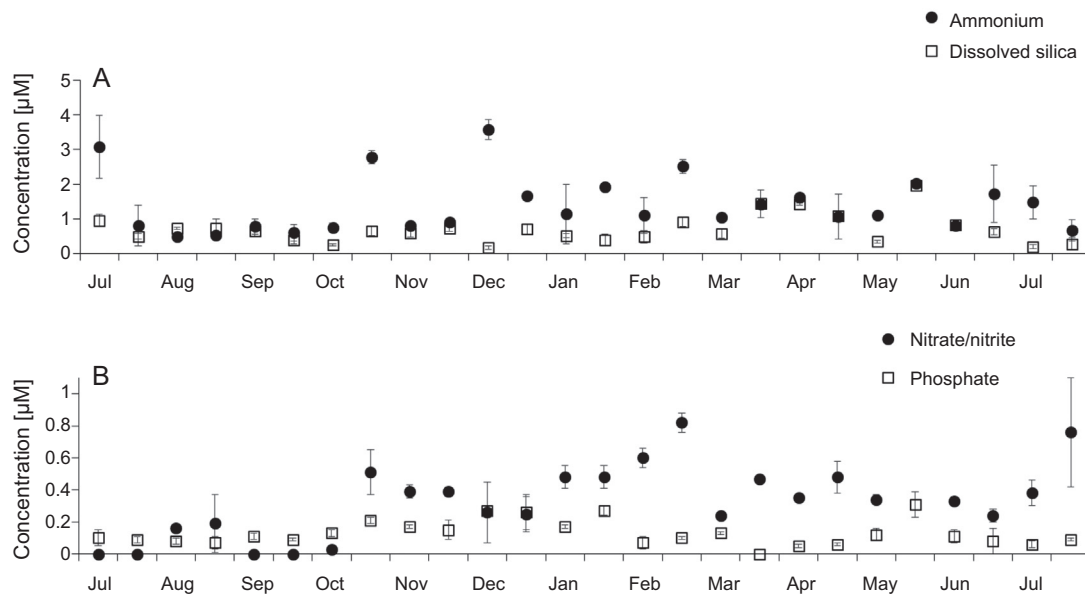


Figure 3 Concentration [μM] of (A) ammonium and dissolved silica as well as (B) nitrate/nitrite and phosphate during the period of study.

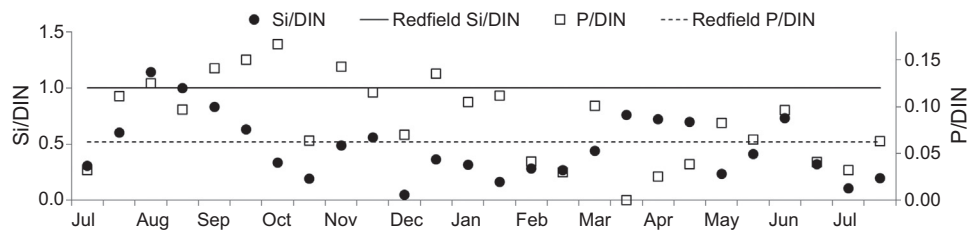


Figure 4 Ratios of the different nutrients measured during the study, in comparison with Redfield values. DIN, dissolved inorganic nitrogen.

3.2. Flow cytometry

Total bacteria varied across 1 order of magnitude i.e., from 2.0×10^5 to 1.2×10^6 over the period of study (Fig. 5). Bacterial abundances were relatively low during winter and early spring 2012, the lowest abundances were then recorded between November and December 2012. In January and February 2013, the bacterial abundances increased again to values similar to those measured in winter and early spring 2012 and then the highest abundances occurred in March and April 2013. In May and June 2013 the abundances decreased again. VLPs ranged from 6.5×10^5 to 1.6×10^7 mL^{-1} over the period of study and VLPs were generally low between June 2012 and March 2013 and their abundance peaked at the end of the bacterial bloom (24th April 2013, Fig. 5).

Photosynthetic picoplankton were generally dominated by the genus *Prochlorococcus*, which was highly variable over the period of study and showed a maximum abundance in June 2013 (3.0×10^5 cells mL^{-1}). *Prochlorococcus* dominated continuously the picophytoplankton community from July to September 2012, and from March to July 2013. Between September 2012 and March 2013 the community was either dominated by *Prochlorococcus* or co-dominated by both *Prochlorococcus* and *Synechococcus*, whereas the abundance of picoeukaryotes was always very low. The

abundance of *Synechococcus* ranged between 2.9×10^3 and 3.5×10^4 cells mL^{-1} and picoeukaryotes ranged between 9.3×10^1 and 1.2×10^4 cells mL^{-1} (Fig. 5).

The low nutrient values found in the present study were also likely to promote the dominance of *Prochlorococcus* over *Synechococcus* and picoeukaryotes within small phytoplankton. *Prochlorococcus* are the smallest microbes in seawater, leading to a high surface/volume ratio which allows them to thrive under low nutrient conditions and to dominate small phytoplankton in oligotrophic seawaters (Partensky et al., 1999). Since *Prochlorococcus* dominates low nutrient environments and was shown to prevail over other picoplankton groups in the Great Australian Bight under oligotrophic conditions (van Dongen-Vogels et al., 2012), its dominance in the present study is consistent with the low nutrient values found here.

3.3. Large phytoplankton

Phytoplankton abundance ranged from 1.7×10^4 to 3.5×10^5 cells L^{-1} (Fig. 6) and was particularly high ($>2 \times 10^5$ cells L^{-1}) on the 26th September 2012, 27th March 2013 and 24th April 2013. The Cryptophyta co-dominated the phytoplankton communities along with Bacillariophyceae and to a lesser extent Dinoflagellata. Chlorophyta were also

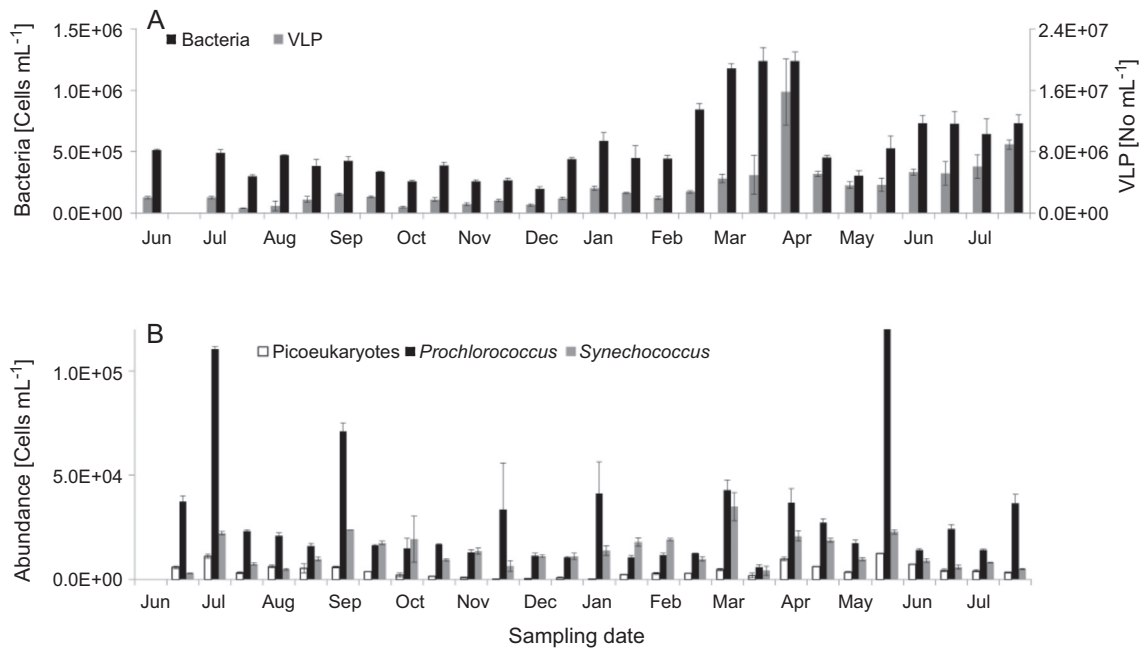


Figure 5 Seasonal abundance of (A) total bacteria [cells mL⁻¹] (left axis) and virus-like particles (VLP) [No mL⁻¹] (right axis) as well as (B) *Synechococcus*, *Prochlorococcus* and picoeukaryotes in seawater [cells mL⁻¹]. Please note that *Prochlorococcus* is off scale for the 5th June as its abundance was 3.0×10^5 cells mL⁻¹.

highly abundant during the period of study with concentrations up to 5.8×10^4 cells L⁻¹ (27th March 2013, Fig. 6), whereas Haptophyta did not account for a significant proportion of the phytoplankton community. The proportion of Cryptophyta to the total community was generally higher during the austral winter and early austral spring 2012 and austral winter 2013, compared to the other sampling dates, where higher proportions of Bacillariophyceae and/or dinoflagellates occurred (Fig. 6).

The phytoplankton community was co-dominated by the three main groups during two of the three abundance maxima (26th September 2012 and 24th April 2013), whereas several Bacillariophyceae species accounted for a higher proportion of the community during the other maximum (27th March 2013). Bacillariophyceae included 68 species, with the most abundant being *Navicula* sp., *Cylindrotheca*, *Nitzschia*, *Chaetoceros* and *Cocconeis* species (Table 1). Cryptophytes accounted for 6–53% of the total phytoplankton abundance and included 11 species the most abundant of which were

Hemiselmis sp., *Plagioselmis prolonga*, *Teleaulax acuta* and *Leucocryptos* sp. (Table 1). Throughout the year dinoflagellates accounted for 10–48% of the total community abundance and were dominated by cf. *Gymnodinium*, *Heterocapsa*, and *Gyrodinium*. Chlorophytes accounted for 1–23% of the total phytoplankton community and were dominated by the genera *Pyraminonas* and *Tetraselmis* (Table 1).

While Bacillariophyceae and dinoflagellates often dominate marine phytoplankton, Cryptophyta usually account for a minor proportion of the community. Although Cryptophyta are often observed in seawater they do not account for a high proportion of phytoplankton (Not et al., 2012). In spite of their low contribution worldwide Cryptophyta can occasionally account for major proportion of phytoplankton communities in the Southern Ocean (Buma et al., 1992; Detmer and Bathmann, 1997; Rodriguez et al., 2002) and the South Pacific (di Tullio et al., 2003), as found by high performance liquid chromatography (HPLC) pigment analyses and/or microscopy techniques. *Hemiselmis* sp., *Plagioselmis prolonga*, and

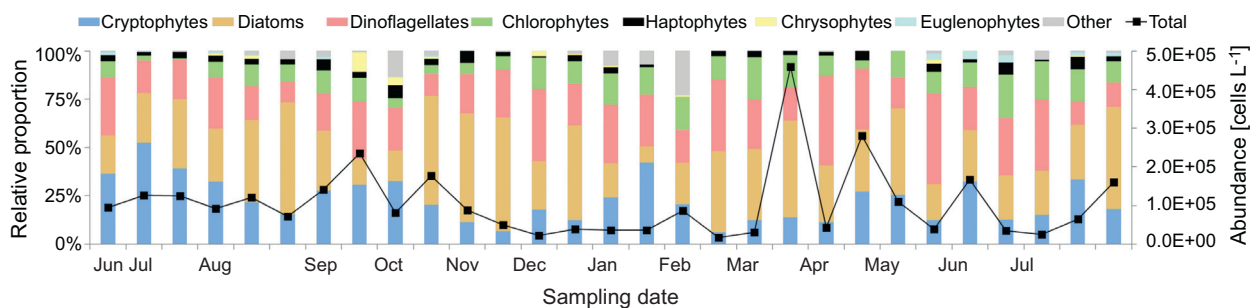


Figure 6 Relative proportion [%] of different taxa (left axis) and total abundance [cells L⁻¹] (right axis) for the large (>2 μm) phytoplankton during the period of study.

Table 1 List of algal species identified during the period of study.

Species	Taxon	Average ^a	n ^b	Max
<i>Actinoptychus</i> sp.	Bacillariophyceae	19	1	500
<i>Amphora</i> sp.	Bacillariophyceae	763	17	4500
<i>Anaulus australis</i>	Bacillariophyceae	37	1	1000
<i>Asteromphalus sarcophagus</i>	Bacillariophyceae	28	2	500
<i>Bacillaria paxillifera</i>	Bacillariophyceae	287	3	4500
<i>Bacteriastrum elegans</i>	Bacillariophyceae	67	2	1500
<i>Cerataulina</i> sp.	Bacillariophyceae	9	1	250
<i>Chaetoceros</i> spp.	Bacillariophyceae	2757	23	17,500
<i>Climacodium</i> sp.	Bacillariophyceae	37	1	1000
<i>Cocconeis</i> spp.	Bacillariophyceae	2252	12	10,000
<i>Coscinodiscus</i> spp.	Bacillariophyceae	102	3	1500
<i>Cyclotella</i> spp.	Bacillariophyceae	2033	17	10,000
<i>Cylindrotheca closterium</i>	Bacillariophyceae	7615	27	56,000
<i>Dactyliosolen antarcticus</i>	Bacillariophyceae	65	2	1500
<i>Dactyliosolen fragilissimus</i>	Bacillariophyceae	565	6	8000
<i>Diploneis</i> sp.	Bacillariophyceae	250	9	2500
<i>Entomoneis</i> sp.	Bacillariophyceae	348	10	3000
<i>Eucampia zodiacus</i>	Bacillariophyceae	9	1	250
<i>Fragilaria</i> sp.	Bacillariophyceae	687	17	3000
<i>Fragilariopsis</i> sp.	Bacillariophyceae	267	5	4500
<i>Grammotophora serpenti</i>	Bacillariophyceae	324	9	2500
<i>Guinardia flaccida</i>	Bacillariophyceae	31	2	630
<i>Guinardia striata</i>	Bacillariophyceae	581	10	5000
<i>Gyrosigma</i> spp.	Bacillariophyceae	135	3	2500
<i>Helicotheca tamesis</i>	Bacillariophyceae	56	1	1500
<i>Hemiaulus</i> sp.	Bacillariophyceae	37	1	1000
<i>Leptocylindrus danicus</i>	Bacillariophyceae	1381	10	15,000
<i>Leptocylindrus minimus</i>	Bacillariophyceae	37	2	500
<i>Licmophora</i> sp.	Bacillariophyceae	439	14	2000
<i>Lioloma</i> sp.	Bacillariophyceae	50	3	1000
<i>Meunieria membracea</i>	Bacillariophyceae	6	1	150
<i>Minidiscus trioculatus</i>	Bacillariophyceae	1528	6	37,500
<i>Navicula</i> sp.	Bacillariophyceae	5137	27	27,500
<i>Nitzschia</i> spp.	Bacillariophyceae	3096	12	12,000
<i>Paralia sulcata</i>	Bacillariophyceae	37	1	1000
<i>Pleurosigma</i> sp.	Bacillariophyceae	487	16	2000
<i>Porosira</i> sp.	Bacillariophyceae	22	2	500
<i>Pseudo-nitzschia delicatissima</i>	Bacillariophyceae	156	11	750
<i>Pseudo-nitzschia fraudulenta/australis</i>	Bacillariophyceae	26	2	400
<i>Pseudo-nitzschia multistriata</i>	Bacillariophyceae	37	3	500
<i>Pseudo-nitzschia turgidula</i>	Bacillariophyceae	15	1	400
<i>Rhizosolenia imbricata</i>	Bacillariophyceae	19	1	500
<i>Rhizosolenia setigera</i>	Bacillariophyceae	37	1	1000
<i>Rhizosolenia</i> spp.	Bacillariophyceae	307	10	3000
<i>Skeletonema costatum/pseudocostatum</i>	Bacillariophyceae	346	4	7500
<i>Synedra</i> sp.	Bacillariophyceae	31	2	450
<i>Thalassionema</i> sp.	Bacillariophyceae	185	2	3000
<i>Thalassiosira</i> cf. <i>mala</i>	Bacillariophyceae	1072	10	11,000
<i>Thalassiosira</i> sp.	Bacillariophyceae	333	7	4000
<i>Cryptomonas pyrenoidifera</i>	Cryptophyta	15	1	400
<i>Hemiselmis</i> sp.	Cryptophyta	12,581	27	56,000
<i>Leucocryptos</i> sp.	Cryptophyta	1063	14	5000
<i>Plagioselmis prolonga</i>	Cryptophyta	5694	26	28,000
<i>Rhodomonas salina</i>	Cryptophyta	56	2	1000
<i>Teleaulax acuta</i>	Cryptophyta	2863	14	22,000
<i>Akashiwo sanguinea</i>	Dinoflagellata	7	3	130
<i>Alexandrium</i> sp.	Dinoflagellata	1	1	25
<i>Amphidinium</i> sp.	Dinoflagellata	102	5	1000

Table 1 (Continued)

Species	Taxon	Average ^a	n ^b	Max
<i>Ceratium lineatum</i>	Dinoflagellata	1	1	25
<i>Cochlodinium</i> spp.	Dinoflagellata	9	2	130
<i>Dinophysis acuminata</i>	Dinoflagellata	8	5	75
<i>Dinophysis caudata</i>	Dinoflagellata	6	5	50
<i>Gymnodinium</i> spp.	Dinoflagellata	12,115	27	52,500
<i>Gyrodinium</i> spp.	Dinoflagellata	1753	16	21,000
<i>Heterocapsa rotundata</i>	Dinoflagellata	5850	27	41,000
<i>Karenia mikimotoi</i>	Dinoflagellata	2	1	50
<i>Karenia papillionaea</i>	Dinoflagellata	28	1	750
<i>Karenia</i> spp.	Dinoflagellata	6	1	150
<i>Katodinium</i> sp.	Dinoflagellata	56	2	1000
<i>Oscillatoria</i> sp.	Cyanobacteria	56	1	1500
<i>Ostreopsis</i> sp.	Dinoflagellata	1	1	25
<i>Peridinium</i> sp.	Dinoflagellata	185	5	2000
<i>Prorocentrum cordatum</i>	Dinoflagellata	74	1	2000
<i>Prorocentrum gracile</i>	Dinoflagellata	28	3	300
<i>Prorocentrum lima</i>	Dinoflagellata	1	1	25
<i>Prorocentrum micans</i>	Dinoflagellata	50	4	500
<i>Prorocentrum rhathymum</i>	Dinoflagellata	17	2	250
<i>Prorocentrum triestinum</i>	Dinoflagellata	141	8	1000
<i>Protoperidinium</i> spp.	Dinoflagellata	119	6	1000
<i>Scrippsiella</i> spp.	Dinoflagellata	269	4	5000
<i>Takayama pulchella</i>	Dinoflagellata	7	4	75
<i>Torodinium</i> sp.	Dinoflagellata	37	2	500
<i>Triceratium</i> spp.	Dinoflagellata	9	1	250
<i>Nephroselmis</i> sp.	Chlorophyta	296	5	3000
<i>Pterosperma</i> sp.	Chlorophyta	74	2	1000
<i>Pyramimonas</i> spp.	Chlorophyta	8070	26	55,000
<i>Tetraselmis</i> spp.	Chlorophyta	1493	19	8000
<i>Eutreptiella</i> spp.	Euglenophyta	633	9	7000
Unidentified bodonids	Euglenophyta	422	2	9400
<i>Chrysochromulina</i> spp.	Haptophyta	2789	23	9000
<i>Emiliana huxleyi</i>	Haptophyta	287	8	2000
<i>Gephyrocapsa oceanica</i>	Haptophyta	9	1	250
<i>Pleurochrysis</i> sp.	Haptophyta	37	1	1000
<i>Umbilicosphaera</i> sp.	Haptophyta	28	2	500
<i>Meringiosphaera mediterranea</i>	Xantophyceae	19	1	500
<i>Dinobryon</i> sp.	Chrysophyceae	102	3	2000
<i>Ochromonas</i> spp.	Chrysophyceae	1181	8	24,000
<i>Mesodinium rubrum</i>	Ciliata	11	1	300
<i>Apedinella spinifera</i>	Dictyochophyceae	130	3	2000
<i>Dictyocha fibula</i>	Dictyochophyceae	79	7	1000
<i>Oocystis</i> sp.	Cyanobacteria	67	1	1800
<i>Phormidium</i> sp.	Cyanobacteria	6	1	150
<i>Trichodesmium erythraeum</i>	Cyanobacteria	19	1	500
Heterotrophic flagellates	Unknown	685	5	11,000
Unidentified amoeba	Unknown	102	4	1000
Unidentified flagellates	Unknown	443	8	2800

^a Average species abundance over the period of study.

^b Number of samples where the species was counted.

Teleaulax acuta are the most representative Cryptophyta in other oceans. *Plagioselmis prolonga* and *Teleaulax acuta* were previously found in the north-western Mediterranean Sea (Percopo et al., 2011), and *Plagioselmis* and *Hemiselmis* species were the dominant Cryptophyta in the western Mediterranean Sea (Novarino, 2005) and the Gulf of Naples

(Cerino and Zingone, 2006). Moreover 18S rRNA gene sequences from *Teleaulax* sp. were recovered from the English Channel (Romari and Vaulot, 2004) and plastidial 16S rRNA gene sequences from *Hemiselmis* sp. and *Plagioselmis* sp. were frequently recovered from the Gulf of Naples (McDonald et al., 2007).

Besides Cryptophyta, other abundant species included the dinoflagellates *Gymnodinium* spp. and *Heterocapsa rotundata*, the green alga *Pyramimonas* spp. and the Bacillariophyceae *Cylindrotheca closterium*, *Navicula* sp. and *Nitzschia* sp. The genus *Gymnodinium* is highly diverse consisting of 297 species to date occurring worldwide (www.algaebase.org). *Heterocapsa* also occurs worldwide and was recently reported to bloom in New South Wales coastal waters (Ajani et al., 2011). The green alga *Pyramimonas* was also abundant in the north-eastern shelf of Kangaroo Island. It is a highly diverse genus which has been frequently reported in polar waters (Balzano et al., 2012; Bell and Laybourn-Parry, 2003; Moro et al., 2002) but was also previously observed in south-eastern Australia (McFadden et al., 1986) and in the Gulf Saint Vincent (Leterme et al., 2014).

3.4. Relationships between nutrients and phytoplankton

Bacillariophyceae dominated the community along with Cryptophyta and Dinoflagellata and the low silica concentrations found here (Fig. 3) might have limited the dominance of Bacillariophyceae in surface waters of the north-eastern shelf of Kangaroo Island promoting both Cryptophyta and Dinoflagellata.

Spearman rank correlation coefficients were used to investigate relationships between environmental and biological variables. Temperature and salinity were not correlated with any phytoplankton taxa or FCM population and similarly no significant relationship was found between the biological variables and both the concentration of dissolved silica (Table 2) and nutrient ratios (data not shown). Positive correlations were found between ammonium and dinoflagellates ($\rho = 0.56$, $p < 0.01$), phosphate and picoeukaryotes ($\rho = 0.50$, $p < 0.01$), whereas nitrate/nitrite was negatively correlated with Haptophyta ($\rho = 0.51$, $p < 0.01$) and Cryptophyta ($\rho = -0.50$, $p < 0.01$). The positive relationship between dinoflagellates and ammonium, and the negative relationship between Cryptophyta and nitrate/nitrite (Table 2) suggest that the high ammonium to nitrate/

nitrite values found here might have promoted the dominance of Cryptophyta and Dinoflagellata (Fig. 5). The correlation between ammonium and dinoflagellates might be due to mixotrophy or heterotrophy from their dominant component, Gymnodiniales. Phytoplankton can assimilate nitrogen in the form of either nitrate, nitrite or ammonium and whether specific classes preferentially assimilate one of these three forms is not clear (Dortch, 1990; Flynn et al., 1997).

The Bacillariophyceae found here were able to cope with the low silica concentrations measured. Silica concentrations were not only low but also the Si/DIN ratio was below the Redfield values almost for the entire period of study (Fig. 4). The most abundant species found here were either pennate Bacillariophyceae such as *Cylindrotheca closterium*, *Navicula* sp., or *Nitzschia* spp. or centric Bacillariophyceae provided with setae such as *Chaetoceros*. These Bacillariophyceae all have a high surface-to-volume ratio allowing cells to grow under low nutrient conditions. Two genera of Bacillariophyceae abundant here, *Cylindrotheca* and *Leptocylindrus* were found to occur under lower silica to nitrate ratio in Southern Chile (Alves-de-Souza et al., 2008). In contrast the high abundance values found for centric Bacillariophyceae with low surface-to-volume ratios such as *Cocconeis* spp. and *Cyclotella* spp. seem difficult to explain under such low silica concentrations. Although the concentration of dissolved silica was not correlated with any biological parameters, it is likely that the low values found here promoted the dominance of Cryptophyta and Dinoflagellata along with Bacillariophyceae. More data points, especially with higher silica concentrations, are needed to further investigate the occurrence of any relationship between dissolved silica and diatom abundance and composition.

The presence of some of the species found here under low nutrient concentrations might be explained by a mixotrophic behaviour, in which microalgae can rely on photosynthesis but also feed on bacteria and smaller flagellates. Mixotrophy and heterotrophy frequently occur in Dinoflagellata (Sherr and Sherr, 2007) and Chlorophyta (Shi et al., 2000). In particular, the ability to feed on smaller prey has been previously demonstrated for the dominant Dinoflagellata

Table 2 Spearman rank correlation coefficients (ρ) and p -values between the main taxonomic groups and environmental variables.

Correlation ^a	Dissolved silica		Ammonium		Phosphate		Nitrate/nitrite	
	ρ	p -value	ρ	p -value	ρ	p -value	ρ	p -value
Total phytoplankton >5 μ m	-0.08	0.71	0.48	0.01	-0.12	0.55	-0.45	0.02
Cryptophyta	-0.06	0.76	0.21	0.30	-0.03	0.89	-0.50	0.01
Bacillariophyceae	0.04	0.84	0.37	0.07	-0.32	0.11	-0.32	0.11
Dinoflagellates	-0.13	0.51	0.54	<0.01	0.04	0.83	-0.40	0.04
Chlorophyta	0.16	0.42	0.26	0.19	-0.15	0.47	-0.18	0.37
Haptophyta	-0.41	0.04	0.24	0.23	-0.03	0.89	-0.51	0.01
VLP	0.17	0.40	-0.29	0.15	-0.16	0.42	0.24	0.23
Bacteria	0.27	0.18	0.05	0.82	-0.47	0.01	0.04	0.84
<i>Prochlorococcus</i>	-0.01	0.97	0.14	0.49	0.03	0.88	-0.22	0.28
<i>Synechococcus</i>	-0.13	0.53	0.25	0.22	0.24	0.24	-0.25	0.21
Picoeukaryotes	0.23	0.25	-0.04	0.86	-0.50	0.01	-0.30	0.14

^a Significant values are in bold for both the Spearman rank correlation coefficients ($\rho > 0.5$, $\rho < -0.5$) and the p -values ($p < 0.02$).

and Chlorophyta species found here such as *Gymnodinium* (Bjornsen and Kuparinen, 1991; Bockstahler and Coats, 1993), *Heterocapsa* (Legrand et al., 1998), *Gyrodinium* (Uchida et al., 1997), *Pyramimonas* (Bell and Laybourn-Parry, 2003) and *Tetraselmis* (Azma et al., 2011).

4. Conclusion

Low concentrations of nutrients were measured on the surface waters of the north-eastern Shelf of Kangaroo Island between July 2012 and July 2013. Subsequently, the picoplankton community was dominated by *Prochlorococcus* Cyanobacteria which are smaller in size, and have a higher surface-to-volume ratio, compared to other picoplankton. Moreover, the concentration of dissolved silica was lower than that of inorganic nitrogen throughout the year, likely promoting high abundances of dinoflagellates and Cryptophyta. The dominant species found here seem either adapted to grow under low nutrient concentrations or have a mixotrophic behaviour allowing them to complement photosynthesis with predation. This study provides base knowledge on the microbial communities north of Kangaroo Island that is needed to sustain the ecosystem and associated economic activities in the future.

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