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Free-living and particle-associated prokaryote metabolism in giant kelp forests: Implications for carbon flux in a sub-Antarctic coastal area

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Abstract:

Extensive beds of large subtidal kelps are characteristic of many temperate and subpolar coastlines. They provide habitats for a wide range of other species and are sites of high primary production that generate large quantities of water-borne particles and dissolved organic compounds that support distinctive communities of prokaryotes. We measured prokaryotic metabolism along transects from the shore to the outside of three giant kelp forests (*Macrocystis pyrifera*) located in the shelf waters of the Prince Edward Islands (Southern Ocean). Abundance, heterotrophic production (PHP), respiration rates (R-ETS) and growth efficiencies (PGE) were investigated within the particle-associated (PA) and the free-living (FL) communities. Temperature, salinity and inorganic nutrient concentrations indicated distinct hydrological differences among the kelp forests that were related to different levels of freshwater input through island run-off. In contrast, detritus and particulate organic matter concentrations showed a common pattern, decreasing from the near-shore to offshore at all sampling sites, suggesting the retention of organically enriched water masses inshore of the kelp forests. While FL and PA abundances did not differ significantly along transects, FL and PA-PHP and PGE all varied significantly across the kelp forests, following the same pattern across each forest. PA-PGE was significantly higher than FL-PGE in the near-shore waters and farther offshore, while FL-PGE was higher or equal to PA-PGE inside the kelp. This shift can be interpreted in terms of gradients in both the age and origins of organic material across the kelp forests. Higher PA-PGE implies that a larger fraction of organic carbon on colonized particles is converted into prokaryotic biomass and so becomes available to higher trophic levels inshore and offshore of *M. pyrifera* forests than inside the kelp bed. In contrast, low PA-PGE suggests that a large quantity of carbon passes through the PA-community and is mainly respired within the kelp forest. These results suggest the retention of particles within giant kelp forests. In controlling the metabolic activity of PA and FL prokaryotes, this retention will influence overall carbon flux around the archipelago. In particular, the observation of a common pattern across different *M. pyrifera* forests has important implications for the role of this species as an autogenic ecological engineer in coastal environments.

Keywords: prokaryotes; free-living; particle-associated; growth efficiency; kelp; sub-Antarctic island

1. Introduction

Giant kelp (*Macrocystis pyrifera*) forests are among the most productive ecosystems in the world (Mann, 2000). Kelp forests occur along many temperate coasts and scattered islands in the Southern Ocean (Wormersley, 1954; Dayton, 1985), providing habitat, food and refuge for numerous marine organisms (e.g. Foster and Schiel 1985). *M. pyrifera* therefore plays a vital role in coastal environments and is an important ecosystem engineer (*sensu* Dayton, 1985; Jones et al., 1994). More particularly, kelp forests strongly affect flow, reducing water transport from the shore to the outer edge of the kelp and ultimately alter concentrations in flow-derived substances and particles in nearshore coastal waters (e.g. Gaylord et al., 2007; Rosman et al., 2010). While the potential retention of water masses within giant kelp forests has been widely acknowledged (e.g. Pakhomov et al., 2002; Gaylord et al., 2007; Fram et al., 2008), the consequences of this retention on the structure and functioning of organisms at the base of the marine food web remain unclear.

Heterotrophic prokaryotes are critical components of the carbon cycle and food webs in marine ecosystems (e.g. Azam et al., 1983; Williams, 1998; Simo et al., 2002). In particular, the balance between their biomass production and respiration represents a major carbon-flow pathway in these systems (e.g. Azam and Malfatti, 2007). The availability of organic matter (e.g. del Giorgio and Scarborough, 1995; del Giorgio et al., 1997) and inorganic nutrients (Rivkin and Anderson, 1997) is known to control prokaryote metabolic activity tightly. Consequently, the retention of enriched water masses within *M. pyrifera* forests could profoundly affect carbon utilization by prokaryotes and the food web structure of giant kelp forests as well as carbon flux within forests and exchange between them and nearshore waters. Moreover, a substantial amount of suspended debris/particles may accumulate within kelp forests (e.g. Gaylord et al., 2007). Particles are known to be highly active sites of microbial processes (e.g. Grossart and Ploug,

2000; Simon et al., 2002) and elevated enzymatic activity on particles has been shown to release organic and inorganic nutrients into the surrounding water, creating hot spots that greatly extend the volume of intense decomposition processes (Cho and Azam, 1988; Grossart and Ploug, 2001). Therefore, a significant part of overall microbial activity within *M. pyrifera* forests may take place on or in the vicinity of particles.

The Prince Edward Islands comprise Marion and Prince Edward Islands, situated in the Indian sector of the Southern Ocean. The archipelago lies directly in the path of the easterly-flowing Antarctic Circumpolar Current (ACC), giving it a west-east or upstream-downstream axis (Ansorge et al. 1999, Froneman et al. 1999). Like many oceanic islands, the archipelago is seasonally home to up to 5 million breeding pairs of top predators including flying seabirds, penguins and mammals (e.g. Chown and Froneman, 2008). A substantial part of the coastline of the archipelago is occupied by dense *Macrocystis pyrifera* forests, principally in the more sheltered waters of the eastern coast of the larger Marion Island (Attwood et al., 1991). Although the potential for retention of water masses in *M. pyrifera* forests in the near-shore zone around Marion Island has previously been observed (Pakhomov et al., 2002), the consequences for food web structure and carbon flux are still unknown.

Our main hypothesis was that retention of particles and nutrients within kelp forests may enhance microbial processes and the recycling of carbon and organic matter within the canopy and therefore play a mayor role in carbon cycling and downward flux in nearshore waters. The objectives of this study were to (i) characterize the effect of kelp forests on near-shore water masses, (ii) investigate the variability in free-living (FL) and particle-associated (PA) prokaryote abundances and metabolism across different *M. pyrifera* forests, (iii) characterize the role of particles and retention of water masses by kelp forests on the patterns observed in the prokaryotic

communities, and (iv) explore the potential consequences of these changes for carbon retention and downward flux in the shallow shelf waters of these sub-Antarctic islands.

2. Materials and methods

2.1. Sites and sampling

The Prince Edward Islands (46°38'S-37°57'E) rise from a depth of 3000 m and the two islands (Prince Edward and Marion) are ca. 10 nautical miles apart and separated by a shallow plateau approximately 200 m deep. The archipelago has a hyperoceanic climate (Smith and Steenkamp, 1990) characterized by high precipitation and humidity (e.g. average annual precipitation approximately 1975 mm; le Roux and McGeoch, 2008) so that the near-shore waters of the islands are strongly influenced by freshwater run-off. A substantial part of the coastline of the archipelago is occupied by dense kelp forests; *Durvillaea antarctica* dominates the infra-littoral fringe, while *Macrocystis pyrifera*, formerly *Macrocystis laevis* Hay (see Macaya and Zuccarello, 2010), predominates between the 5 m and 30 m isobaths, particularly in the comparatively sheltered waters of the eastern coast of Marion Island (Attwood et al., 1991; Beckley and Branch, 1992).

Sampling was undertaken in Macaroni and Archway Bays (Fig. 1) during voyage 145 of the research vessel *S.A. Agulhas* in early austral autumn (April/May) 2009, using a small motorized launch (Fig. 1). Macaroni Bay is a relatively large sheltered bay, receiving substantial freshwater input (Fig. 1). Sampling was undertaken at two representative sites in Macaroni Bay: (i) a sheltered site inside the bay (M1) and (ii) a more exposed site located in front of the western cape of the bay (M2) (Fig. 1). In contrast, the smaller Archway Bay receives limited freshwater input and is the site of a large colony of King Penguins (i.e. ~ 1500 breeding adults; Crawford et al., 2009).

126 At each site, samples were collected at 3 stations perpendicular to the coast located (i) in the
127 near-shore kelp-free waters (i.e. < 5m deep), (ii) inside the kelp forest and (iii) offshore of the
128 forest. These stations are hereafter referred to as 'inshore', 'kelp' and 'offshore', respectively
129 (Fig. 1). Temperature and salinity profiles were collected at each sampling station with an XR-
130 620 CTD (conductivity, temperature, depth meter) from the surface to the bottom or a maximum
131 depth of 50 m. Water samples were taken from the sub-surface (1 m) using a 5-L Niskin bottle.
132
133 *2.2. Dissolved inorganic nutrients*
134 For the determination of dissolved inorganic nutrient concentrations (nitrate + nitrite,
135 ammonium and orthophosphate) 20 mL water samples were filtered through glass-fibre filters
136 (Whatman GF/F) and immediately frozen (-20°C). Concentrations were determined in the
137 laboratory with a Lachat Flow Injection auto-analyser, following standard protocols (Grasshoff et
138 al., 1999).
139
140 *2.3. Dissolved organic carbon (DOC) and organic nitrogen (DON)*
141 For the determination of DOC concentrations, 8 mL of seawater was gently filtered through
142 pre-combusted glass-fibre filters (Whatman GF/F), collected in pre-combusted (450°C for 12
143 hours) glass ampoules, acidified with 3-4 drops of 45% H_3PO_4 and stored at -20°C until analysis.
144 DOC analysis was performed using the high temperature combustion method on an elemental Hi-
145 TOC analyser following standard protocols (Clesceri et al., 1998).
146 For DON concentrations, aliquots of 60 mL from each station were gently filtered through
147 pre-combusted glass-fibre filters (Whatman GF/F) in acid-washed polyethylene bottles and stored
148 at -20°C until analysis. Organic and inorganic dissolved nitrogen were determined

149 photometrically following Koroleff's method (1969). DON concentrations were obtained by
150 subtracting the sum of inorganic nitrogen species (i.e. ammonium + nitrite + nitrate) from the
151 corresponding total dissolved N concentrations.
152
153 *2.4. Particulate organic carbon (POC) and organic nitrogen (PON)*
154 Samples for POC and PON (1-1.5 litres) were filtered through pre-combusted (450ºC; 12
155 hours) and pre-weighed glass-fibre filters (Whatman GF/F) and stored at -20°C until analysis. In
156 the laboratory, filters were rinsed with MilliQ water, dried at 60°C for 24 h, and re-weighed to
157 determine the mass of Suspended Particular Matter (SPM) on the filter (Hewson et al., 2001).
158 Analyses were performed on a Thermo Finnigan Delta XP Plus mass spectrometer interfaced
159 with a Conflo III device to a thermo Flash EA 1112 Elemental Analyser.
160
161 *2.5. Phytoplankton biomass*
162 For chlorophyll *a* (Chl *a*) estimates, triplicate 250 mL samples were gently filtered through
163 glass-fibre filters (Whatman GF/F) and immediately extracted in 8 ml of 90% (v/v) acetone for
164 24h at -20°C. Chl *a* concentrations ($\mu g\ L^{-1}$) were then determined fluorometrically following
165 Holm-Hansen and Riemann (1978). Phaeopigment concentrations ($\mu g\ L^{-1}$) were determined after
166 acidification with 1.2M HCl.
167
168 *2.6. Prokaryotic abundance*
169 For the identification and enumeration of prokaryotes, triplicate 1mL samples were collected,
170 fixed with 0.5% (final concentration) glutaraldehyde in the dark at 4°C for 15 minutes, quick
171 frozen in liquid nitrogen and then stored at -80°C until analysis (Brussaard, 2004). Prokaryotes
172 were counted after staining with SYBR Green I solution (1:5000 dilution) using a Beckman

Coulter FC500 flow cytometer, following standard protocols (Brussaard, 2004). Fluorescent beads, 1 μm in diameter (FluoSpheres®) were added to all samples, as an internal size and concentration standard. Prokaryote populations were identified and quantified using the flow cytometry analysis software WinMDI 2.9 (©Joseph Trotter).

Abundance of particle-associated (PA) prokaryotes was estimated in the size fraction greater than 0.8 μm. Immediately after collection, triplicate 5 mL samples were filtered through 0.8 μm pore size polycarbonate membranes (Isopore filters, Millipore), fixed in 5 mL glutaraldehyde (2%) in the dark at 4°C for 15 minutes, quick frozen in liquid nitrogen and then stored at -80°C until analysis. After being gently thawed, filters were sonicated (306 μm amplitude, 50% duty cycle, cooling in water bath) for 2 minutes to disperse prokaryotes from the particles (Velji and Albright, 1993). Attached prokaryotes (i.e. in the fraction >0.8 μm) were identified and counted by flow cytometry after staining with SYBR Green I solution as described above. Free-living (FL) prokaryote abundance was calculated from the difference between total prokaryote abundance in the unfiltered samples and PA prokaryote abundance estimated from the 0.8 μm pore-size filters.

2.7. Prokaryotic heterotrophic production (PHP)

Total heterotrophic production was estimated from ^{3}H-Leucine (Amersham, 139 Ci mmol^{-1}) incorporation rates into proteins as described by Kirchman et al. (1985). ^{3}H-Leucine incorporation rates were measured by incubating triplicate 40 mL samples and 2 formaldehyde killed blanks (2% final concentration), with a saturating 20 nM leucine final concentration (hot:cold = 1:9) in the dark, at *in situ* temperatures. After 5 hours, incubations were terminated by adding formaldehyde (2% final concentration) to the samples. Kinetic experiments conducted during this study showed that leucine incorporation was linear during this time period (data not

shown). The fixed samples and blanks were filtered through 0.22 µm nitro-cellulose membranes (GSWP, Millipore), incubated with 3 ml of 5% ice-cold trichloroacetic acid (TCA) for 15 minutes, rinsed 3 times with 3 ml TCA, dried and stored at -20°C until analysis. After being dried overnight, filters were placed in scintillation vials and dissolved with 1 mL of ethyl acetate for 30 minutes. Scintillation cocktail Ultima Gold XR (10 ml) was added to each vial and after 18 h, samples were analysed using a Beckman 5801 liquid scintillation counter. The mean disintegrations per minute (DPM) of the formaldehyde-killed blanks were subtracted from the mean DPM of the respective samples and the resulting DPM converted into leucine incorporation rates. Prokaryotic carbon biomass production was estimated using the conversion factor of 3.1 kg C mol^{-1} Leu (Kirchman, 1993).

Two sets of samples were incubated at each station: the first set was treated as described to estimate total heterotrophic production and the second set (3 replicates and 2 formalin-killed blanks) was filtered through 0.8 µm filters after incubation to estimate the heterotrophic production by FL prokaryotes (i.e. fraction <0.8 µm). As TCA passes through 0.8-pore size filters during protein extraction (Mével et al., 2008), it was not possible to estimate the production in the >0.8 µm fraction directly, and the heterotrophic production by PA prokaryotes was calculated from the difference between total heterotrophic production and production by FL prokaryotes.

2.8. Respiratory activity of the electron transport system (R-ETS)

The respiratory activity of the electron transport system (ETS) was investigated in both the >0.8 µm (i.e. particle-associated) and the <0.8 µm (i.e. free-living) size fractions. Two-litre samples were filtered through 0.8 µm and subsequently through 0.2 µm cellulose ester membranes (Millipore, AAWP). Filters were folded into cryovials and immediately stored in liquid nitrogen until analysis in the laboratory. ETS activity was measured using the modification

of the tetrazodium reduction technique described in Arístegui and Montero (1995), with minor modifications to increase the sensitivity of the method following Baltar et al. (2009). Rates of oxygen consumption in carbon units (R-ETS) were calculated using the following equation (Arístegui et al., 2002) and assuming a respiratory quotient of 1.

$$Log\ R\text{-}ETS = 0.357 + 0.750 \log ETS \quad (1)$$

2.9. Data analysis

As the normality assumption was not verified with the Shapiro-Wilk's test (Shapiro and Wilk, 1965), non-parametric statistics were used throughout this work (Zar, 1996). Multiple comparisons among stations along transects and among sampling sites, were performed using the Kruskal-Wallis test (KW test hereafter) and subsequently a procedure based on the Tukey test (Zar, 1996) was used to identify different groups of measurements. Comparisons between particle-associated and free-living parameters were conducted using the Wilcoxon-Mann-Whitney *U*-test (*U*-test hereafter; Zar, 1996).

3. Results

3.1. Environmental parameters

Temperature and salinity profiles recorded at Archway Bay and M1, exhibited very similar patterns, with temperature slightly decreasing from 5.62°C inshore to 5.58°C outside the kelp forest and salinity remaining relatively constant along the transect, ranging between 33.70 and 33.90 (Fig. 2). In contrast, at M2, surface temperature and salinity ranged from 5.47°C and 5.60°C and from 33.30 to 33.90 respectively along the transect. Salinity increased from the shore to offshore of the kelp forest and from the surface to the deeper layers (Fig. 2).

245 Highest inorganic nutrient concentrations were recorded at M2 (Table 1). At Archway Bay,
246 nitrogen and phosphate concentrations followed a similar pattern along the transect, increasing
247 from the inshore to offshore waters (Table 1). At M1 and M2, the kelp forest was characterized
248 by high [NH_4^+] (i.e. >30 µmol L^{-1}) and low [$NO_3^- + NO_2^-$] concentrations (i.e. <13.0 µmol L^{-1})
249 (Table 1). A pattern of decreasing phosphate concentrations was observed at M1 with values
250 ranging from 5.3 µmol L^{-1} inshore to 2.0 µmol L^{-1} outside the kelp, whereas at M2 the lowest
251 concentrations (i.e. 10.7 µmol L^{-1}) were recorded within the kelp forest (Table 1).
252 At Archway Bay and M1, DOC concentrations decreased from the shore to the outside of the
253 kelp forest and varied from 82.1 to 72.5 µmol L^{-1} and from 78.3 to 75.8 µmol L^{-1}, respectively
254 (Table 1). In contrast, at M2, an increasing gradient was observed with values ranging from 75.0
255 inshore to 81.3 µmol L^{-1} offshore (Table 1). DON concentrations ranged between 2.9 and 7.1
256 µmol L^{-1} and followed a similar decreasing pattern from the shore to outside the kelp forest at the
257 3 sampling sites (Table 1). At Archway Bay and M2, the C: N molar ratio of DOM varied from
258 12.8 to 25.4 and from 15.0 to 28.4 respectively along transects. The ratios decreased from the
259 shore to offshore of the forest (Table 1). At M1, C: N molar ratios were low with values
260 remaining below 11.8 along the transect (Table 1).
261 The highest SPM concentrations (32.0 µg L^{-1}) were observed at M2, in the inshore waters
262 (Table 1). At the 3 sampling sites, SPM and PON concentrations exhibited a similar pattern along
263 transects, decreasing from the shore to outside the kelp forest (Table 1). At M1 and M2, highest
264 POC concentrations were observed inshore, at 14.1 µmol L^{-1} and 23.8 µmol L^{-1}, respectively
265 (Table 1). In contrast, at Archway Bay the highest POC concentrations (14.0 µmol L^{-1}) were
266 recorded within the kelp forest (Table 1). At M1 and M2, the C: N molar ratio of POM varied
267 respectively from 9.4 to 7.8 and from 14.8 to 10.4, and decreased from inshore to offshore waters

(Table 1). In contrast, at Archway Bay the C: N molar ratio increased from the shore to outside the kelp forest with values ranging from 5.1 to 11.6 (Table 1).

Total Chl *a* concentrations were consistently lower than 0.3 μg L^{-1} over the 3 studied kelp forests (Fig. 3). At Archway Bay and M1, mean Chl *a* concentrations were significantly ($p < 0.05$) higher offshore than inshore. At M2, total Chl *a* concentrations ranged between 0.11 and 0.13 μg L^{-1} and did not exhibit any significant spatial patterns ($p > 0.05$; Fig. 3). The relative contributions of phaeopigments to total Chl *a* biomass exhibited similar patterns along all three transects (Fig. 3), decreasing significantly from 31.5%-68.6% in the near-shore kelp-free waters to 10.2%-21.8% offshore of the *M. pyrifera* forest ($p < 0.05$; Fig. 3).

3.2. Prokaryote abundances

Free-living (FL) prokaryotes were significantly more abundant than particle-associated (PA) prokaryotes (Mann-Whitney *U*-test, $p < 0.05$), contributing between 88.6% and 99.5% of the total prokaryotic abundance within the 3 kelp forests (Table 2). PA and FL prokaryote abundances did not differ significantly along transects for any of the 3 sampling sites (KW test, $p > 0.05$). PA abundances ranged from 1.53×10^3 to 9.71×10^3 cell mL^{-1} and did not differ significantly among sampling sites (KW test, $p > 0.05$). In contrast, at M1, FL prokaryote abundances ranged between 5.50×10^4 to 7.48×10^4 cell mL^{-1} and were significantly lower than those recorded along transects at M2 and Archway Bay ($p < 0.05$).

3.3. Prokaryotic metabolism

Particle-associated PHP (PA-PHP) ranged from 7.2 to 49.1 μmol C m^{-3} d^{-1} and free-living PHP (FL-PHP) from 0.5 to 20.8 μmol C m^{-3} d^{-1} (Table 3). PA-PHP did not differ significantly

among sampling sites (KW test, $p > 0.05$). In contrast, FL-PHP was significantly lower at M1 than at M2 inshore of the kelp forests ($p < 0.05$; Table 3).

At Archway Bay, PA-PHP was significantly higher inshore (26.6 µmol C m^{-3} d^{-1}) than inside the kelp forest (7.2 µmol C m^{-3} d^{-1}), whereas FL-PHP did not differ significantly along the transect (Table 3). At M1, FL-PHP was significantly higher offshore than inshore, at 12.0 µmol C m^{-3} d^{-1} and 0.5 µmol C m^{-3} d^{-1} respectively ($p < 0.05$), whereas PA-PHP did not differ significantly along the transect (KW test, $p > 0.05$) (Table 3). At M2, PA-PHP and FL-PHP ranged from 9.5 µmol C m^{-3} d^{-1} to 20.8 µmol C m^{-3} d^{-1} and between 11.5 µmol C m^{-3} d^{-1} and 47.2 µmol C m^{-3} d^{-1} respectively, with no significant variation along the transect (KW test, $p > 0.05$).

Particle-associated and free-living R-ETS were highly variable, ranging from 1.1 to 106.7 µmol C m^{-3} d^{-1} and 1.1 to 86.3 µmol m^{-3} d^{-1}, respectively (Table 3). At M1, particle-associated R-ETS varied from 1.1 to 81.9 µmol C m^{-3} d^{-1} along the transect, increasing from the shore to outside the kelp (Table 3). At M2, the highest particle-associated R-ETS were observed within the kelp forest, at 43.8 µmol C m^{-3} d^{-1} (Table 3). Lowest free-living R-ETS values were recorded within the kelp at Archway Bay and M1. At M2 R-ETS decreased from 38.9 µmol C m^{-3} d^{-1} inshore to 19.3 µmol C m^{-3} d^{-1} offshore of the kelp forest (Table 3).

3.4. Prokaryotic growth efficiency (PGE)

Prokaryotic growth efficiency (PGE) within the FL and PA communities ranged from 0.01 to 0.87 and from 0.18 to 0.97 respectively and differed significantly among sampling sites (KW test, $p < 0.05$; Fig. 4). Inshore, PA-PGE was significantly higher at M1 than at Archway Bay at 0.97 and 0.19 respectively ($p < 0.05$), whereas FL-PGE did not differ significantly among inshore sites ($p > 0.05$). In contrast, FL-PGE measured within the kelp and offshore significantly differed

among sampling sites (KW test $p < 0.5$; Fig. 4). Inside the kelp forest highest FL-PGE was observed at M1whereas highest offshore values were recorded at M2 ($p < 0.05$).

At Archway Bay, PA-PGE ranged from 0.19 to 0.21 (Fig. 4A). FL-PGE was significantly higher within the kelp forest than offshore ($p < 0.05$) at 0.13 and 0.05, respectively (Fig. 4A). At M1, PA-PGE varied from 0.97 to 0.21, decreasing significantly ($p < 0.05$) from the shore to outside the kelp forest (Fig. 4B) and highest FL-PGE (i.e. 0.84) was observed within the kelp forest (Fig. 4B). At M2, PA-PGE was significantly higher offshore than inside the kelp ($p < 0.05$) at 0.82 and 0.18 respectively (Fig. 4C). In contrast, FL-PGE ranged between 0.07 and 0.50 did not differ significantly along transect (KW test, $p > 0.05$).

Inshore PA-PGE was significantly higher than FL-PGE at all 3 sampling sites (*U*-test, $p < 0.05$). No significant difference was observed between FL-PGE and PA-PGE within the kelp forest at M2 (*U*-test, $p > 0.05$), whereas FL-PGE was significantly higher than PA-PGE in the kelp forest at M1 (*U*-test, $p < 0.05$). Offshore of the kelp forest, PA-PGE was significantly higher than FL-PGE at Archway Bay and M2 (*U*-test, $p < 0.05$) whereas no significant difference was observed between the two fractions offshore at M1 (*U*-test, $p > 0.05$).

4. Discussion

4.1. M. pyrifera kelp forests and near-shore water masses

Temperature and salinity profiles indicated different hydrological conditions at the 3 sampling sites (Fig. 2). Surface water of reduced salinity and temperature extended into the kelp forest at M2, suggesting a significant impact of freshwater run-off from the island during sampling (Fig. 2). This is consistent with the high ammonium, phosphate and SPM concentrations observed along the transect (Table 1). High concentrations of reduced nitrogen and phosphate have previously been observed in the shallow shelf waters of the island and have been shown to be

largely derived from the guano, dung, urine and animal remains that are carried off the island to the shelf waters *via* freshwater run-off (Allanson et al., 1985; Ducombe Rae, 1989). In contrast, temperature and salinity profiles recorded at M1 and Archway did not suggest any significant influence of freshwater run-off (Fig. 1). Moreover, despite the high density of top predators on the shore at Archway Bay, inorganic nutrient and SPM concentrations were lower than in Macaroni Bay (Fig. 1; Table 1). These results confirm the importance of freshwater run-off to inorganic nutrient and SPM inputs in the near shore waters.

In addition, the differences observed within Macaroni Bay between M1 and M2 demonstrate the restricted nature of the influence of freshwater input, leading to high spatial variability in nutrient and SPM concentrations along the shore. This could have large implications for prokaryote dynamics in the shallow shelf waters of Marion Island. Furthermore, as nutrient availability and light penetration exert a major control on *Macrocystis pyrifera* growth (Dayton et al., 1992, 1999), this small scale variability in nutrient and SPM inputs may also influence the density and extent of the kelp itself (e.g. Fram et al., 2008) and ultimately the retention capacities of the kelp forests (Gaylord et al., 2007; Rosman et al., 2010).

Because both current and vertical mixing are reduced within kelp forests, *M. pyrifera* has been shown to potentially limit nutrient fluxes and transport of particles from the shore to the outer edge of the forest (e.g. Pakhomov et al., 2002; Rosman et al., 2010). Changes in water masses properties were observed from the shore to the outer edge of the kelp forest at the three sampling sites (Fig. 2; Table 1). However, these changes were sometime small and spatial patterns across kelp beds were not consistent among sampling sites. We are not able to estimate the residence times or flushing rates of water from our data, but as residence times within kelp beds may be high (Graham et al., 2007; Fram et al., 2008), biological activity of both *M. pyrifera* and its

362 associated fauna is likely to have significantly influenced the patterns of inorganic nutrient and
363 organic matter observed.
364 While phytoplankton biomass did not change consistently through the kelp forests, the
365 contribution of phaeopigments to total phytoplankton biomass decreased significantly from
366 inshore to offshore stations at all three locations (Fig. 3), indicating the retention of detritus by
367 the kelp forests (Pakhomov et al., 2002). This is also seen in the offshore decrease in particular
368 and dissolved organic matter (see Table 1). To our knowledge, our results are the first report of
369 POM and DOM enriched waters in near-shore waters and their potential retention within kelp
370 forests. As organic matter supply influences prokaryote growth strongly (e.g. del Giorgio and
371 Cole, 1998), this could have important implications for prokaryote metabolism in other kelp
372 forests.
373 Total prokaryote abundances varied between 6.2×10^4 to 3.6×10^5 cell ml^{-1} over the study area
374 (Table 1). While these abundances are congruent with those observed elsewhere in the Southern
375 Ocean (Pedrós-Alió et al., 2002; Vaqué et al, 2002; Granéli et al., 2004; Corzo et al., 2005;
376 Ortega-Retuerta et al., 2008; Obernosterer et al., 2008), they are within the lower range of values
377 observed offshore of the archipelago in the same months of April/May 2009 (i.e. 3.3×10^5 cell ml^{-1}
378 to 5.5×10^5 cell ml^{-1}; Schapira et al., 2012). Free-living and particle-associated prokaryote
379 abundances did not vary along transects, but prokaryotic metabolism was highly variable among
380 sampling sites and across the *M. pyrifera* forests. This suggests strong modifications to carbon
381 utilization by prokaryotes along the shore and from the near-shore kelp-free waters to offshore of
382 *M. pyrifera* forests. This emphasizes that information on prokaryote abundances alone is not
383 enough to evaluate their ecological and functional role in carbon cycling within the plankton, it is
384 necessary to understand their metabolic state as well.
385

386 *4.2 Variability in prokaryote metabolism along the shore of Marion Island*

387 No significant differences in PA prokaryote abundances were observed, but FL prokaryotes
388 were significantly less abundant at M1 than at M2 and Archway Bay (Table 2). While the supply
389 of dissolved organic matter (DOM) did not exhibit any significant differences among the three
390 sampling sites, C:N ratios at M1 were lower than along transects at M2 and Archway Bay (Table
391 2). The lower C:N ratios were accompanied by significantly lower free-living production rates
392 (Table 3). As organic matter supply and stoichiometry (i.e. C: N ratios) influence prokaryote
393 growth strongly (e.g. del Giorgio and Cole, 1998), the lower FL abundances observed at M1could
394 be explained by a lower quality of DOM at this site. Moreover, this also suggests that despite
395 similar supplies, the organic matter pool is likely to exhibit different degrees of lability and
396 energetic quality at the different locations along the shore, with significant consequences for
397 prokaryote growth and standing stocks. Other factors such as inorganic nutrient concentrations
398 (e.g. Rivkin and Anderson, 1997) and temperature (e.g. Kirchman and Rich, 1997; Rivkin and
399 Legendre, 2001) are strongly affected by freshwater input, and could also have played a role in
400 establishing the differences observed among the different kelp forests. Since multiple factors may
401 interact to control prokaryote production (Church et al., 2000; Smith and Kemp, 2003;
402 Obernosterer et al., 2008; Mills et al., 2008; Martínez-García et al., 2010), specific experiments
403 are needed to understand fully the limitation of heterotrophic free-living prokaryote production in
404 these shallow shelf waters.

405 One of the main features of the spatial dynamics of prokaryotic metabolism was the high
406 variability in FL and PA prokaryote growth efficiencies (PGE) among sampling sites (Fig. 4).
407 The PGE values reported in each of the three sampling zones (i.e. inshore, kelp and offshore)
408 varied greatly among the different kelp forests. Since PGE has been shown to vary along
409 gradients of environmental conditions (e.g. Cotner and Biddanda, 2002; Carlson et al., 2007), this

suggests that FL and PA prokaryotes were subject to more or less favorable conditions inshore, inside the kelp and offshore depending on the intrinsic properties of the kelp forest considered (e.g. freshwater input, nutrient dynamics, size of the forest and perhaps the physiological state of the *M. pyrifera* itself). Since the allocation of energy in prokaryotes depends on many factors (Carlson et al., 2007), it is difficult to identify a single factor responsible for the high variability in PGE observed among kelp forests. Instead we highlight the great variability of free-living and particle-associated PGE that occurred at these small spatial scales. This suggests that the role of kelp forests in shaping the interactions within the microbial loop is strongly dependent on the water in which it is immersed.

4.3 Spatial variability in prokaryote metabolism across kelp forests

The contribution of particle-associated prokaryotes to total prokaryote abundances is generally between 5 and 10% (e.g. Cho and Azam, 1988; Simon et al., 2002; Mével et al., 2008). This is consistent with the low contribution of PA prokaryotes (i.e. <11%) to the total abundance observed during this study. While the relative abundances of these two fractions of prokaryotes did not vary significantly along transects, their relative contributions to overall carbon flux did.

Inshore of *M. pyrifera* forests, the highest PGE were observed within the particle-associated community (Fig. 4). Since PGE typically increase as conditions become optimal (e.g. Cotner and Biddanda, 2002; Carlson et al., 2007), this suggests that environmental conditions in the near shore waters were more favourable for particle-associated than free-living prokaryotes. The near-shore kelp-free waters showed high concentrations of detritus (SPM) and particulate organic matter with low C: N ratios (Table 1), that are characteristic of freshly produced organic matter (e.g. Ogawa et al., 1999; Carlson et al., 2000). High PGE has previously been reported on freshly colonized particles and related to low respiration and/or high production rates associated with

434 highly labile organic matter (Grossart and Ploug, 2000; Azam and Long, 2001). The high
435 particle-associated PGE observed in this zone may thus be related to the colonization of freshly
436 introduced particles in the inshore kelp-free area.
437 In contrast, free-living PGE were higher than or similar to particle-associated PGE in the kelp,
438 suggesting that environmental conditions were less favourable for particle-associated or more
439 favourable for free-living prokaryotes inside the kelp forest (Fig. 4). As aggregates and particles
440 are likely to be transported from inshore toward the kelp forest interior (e.g. Gaylord et al., 2007),
441 the metabolic activity of attached prokaryotes might have led to a progressive depletion of labile
442 compounds, resulting in more refractory particles within the kelp and ultimately lower particle-
443 associated PGE (Fig. 4). Furthermore, enzymatic activities and uptake rates are largely decoupled
444 on aged particles, leading to the release of labile compounds in the surrounding media and
445 resulting in the formation of hot spots or plumes of organic and inorganic nutrients around
446 particles (Cho and Azam, 1988; Grossart and Ploug, 2001). Free-living prokaryotes may have
447 clustered in this chemosphere, exploiting the locally high levels of organic and inorganic
448 nutrients (Cho and Azam, 1988; Azam and Malfatti, 2007), leading to high growth efficiencies.
449 However, organic matter is likely to originate from different sources (i.e. terrestrial run-off, kelp-
450 derived material and phytoplankton) and therefore to have different degrees of lability and
451 energetic quality in the different regions of the forest. In particular, phytoplankton may have
452 contributed to a larger fraction of the DOM and POM pool offshore of the forest where
453 phytoplankton biomass was high (Fig. 3). Changes in organic matter lability and/or the
454 colonization of new particles produced offshore of *M. pyrifera* forest could favour particle-
455 associated over free-living prokaryotes, leading to higher PGE within the particle-associated
456 community offshore (Fig. 4).

457 The allocation of energy in prokaryotes depends on many other factors, making it difficult to
458 identify a single factor controlling the variability in PGE (Carlson et al., 2007). Abiotic factors
459 such as solar radiation (particularly UV-B) and osmotic shock can contribute to an increase in
460 cell respiration (Koch, 1997) and may have played a role particularly in the shallow near-shore
461 kelp-free waters. In addition, viral infection has been demonstrated to increase cell respiration
462 rates in prokaryotes (Bonilla-Findji et al., 2008) and may have been important. Nevertheless,
463 shifts in the predominance of different fractions of the prokaryotic community can be interpreted
464 in terms of gradients in both the age and origins of organic material across the kelp forests.
465
466 *4.4. Kelp forests, turbulence and prokaryote dynamics*
467 One of the main characteristic of *M. pyrifera* is that it significantly alters the local flow
468 environment (e.g. Gaylord et al., 2007; Rosman et al., 2010). Depth averaged currents in kelp
469 forests can be reduced by a factor of 1.5 to 5 relative to nearby kelp-free areas (Jackson, 1998;
470 Gaylord et al., 2007; Rosman et al., 2007). Consequently, colonized particles within forest are
471 likely to be subject to less turbulent conditions than inshore or offshore. As the fluid flow around
472 aggregates plays a critical role in the chemical micro-environment and growth conditions of
473 particle-associated prokaryotes (e.g. Ploug et al., 1997; Ploug, 2001; Simon et al., 2002),
474 modification of turbulence conditions within the kelp forest is likely to influence prokaryotic
475 metabolism. In addition, turbulence intensity at small scales can control nutrient patchiness, with
476 significantly more heterogeneous/patchy distributions under low turbulence conditions (Seuront
477 et al., 2002; Seuront, 2008). At scales relevant to prokaryotes, changes in turbulent conditions
478 across kelp forests may have important consequences to the micro-environment experienced by
479 free-living and particle-associated prokaryotes and may therefore influence their relative
480 contribution to carbon flux.

481

482 *4.5. Implications for carbon flux and food web structure across kelp forests*

483 Regardless of which combination of factors produced the observed pattern, the variability in
484 the growth efficiency of prokaryotes across kelp forests could have significant implications for
485 the fate of organic carbon in these nearshore waters. The significance of higher growth efficiency
486 within the particle-attached community inshore and offshore of *M. pyrifera* forests, is that a
487 larger fraction of organic carbon was converted into prokaryotic biomass and was therefore
488 available to higher trophic levels on colonized particles. Since particle-attached prokaryotes are
489 less susceptible to grazing than their free-living counterparts (Jürgens and Güde 1994; Pernthaler
490 2005), this difference could have significant consequences for food web structure and
491 biogeochemical cycling in the inshore kelp-free area and offshore of the kelp forests.

492 In contrast, particles-associated PGE was lower or equal to the free-living PGE inside the kelp
493 forest. The significance of low particle-associated PGE is that a large quantity of carbon passes
494 through the prokaryote community and is mainly respired inside the kelp forest, so that it is not
495 available to higher trophic levels (Legendre and Rassoulzadegan 1995). Intense remineralisation
496 processes on particles and within their surrounding micro-environment, has important
497 implications for vertical carbon flux within the kelp forest. These findings provide new insights
498 into the role of *M. pyrifera* forests as a bioengineer species in coastal ecosystems.

499

500 **5. Conclusion**

501 Metabolic rates of both free-living and particle-associated prokaryotes were highly variable
502 within and among kelp beds, suggesting that the quantity of carbon processed by prokaryotes was
503 strongly influenced by the intrinsic characteristics of the forests (e.g. physiological state of *M.*
504 *pyrifera*, size of the forest etc.) and by the water in which they were immersed (e.g. freshwater

505 inputs, sources of organic matter). It is difficult to untangle these various effects, but spatial
506 patterns in prokaryote PGE are interpretable in terms of the age and sources of organic material
507 in the water. Importantly, the variability in prokaryotic growth efficiency has implications for
508 carbon flux within kelp forests.
509
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521
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720 **Table legends**

721 **Table 1.** Ammonium [NH_4^+], Nitrite + nitrate [$NO_2^- + NO_3^-$], orthophosphate [PO_4^{3-}], dissolved
722 organic carbon (DOC), dissolved organic nitrogen (DON), suspended particular matter (SPM),
723 particular organic carbon (POC) and particular organic nitrogen (PON) concentrations (µmol l^{-1}),
724 and carbon to nitrogen molar ratios of the dissolved organic matter (C: N DOM) and of the
725 particular organic matter (C: N POM) observed at observed (i) inshore, (ii) within the kelp and
726 (iii) offshore of the kelp forest at the 3 sampling sites: Archway Bay, Macaroni Bay 1 and
727 Macaroni Bay 2. Confidence levels (µmol l^{-1}): ammonium = 0.02; nitrite + nitrate = 0.01;
728 orthophosphate = 0.07; DOC = 0.2; DON = 0.1; POC = 0.2; PON = 0.2. ×: no data available
729
730 **Table 2.** Average (±SE) free-living (FL) and particle-associated (PA) prokaryotes abundances
731 (cell ml^{-1}) observed (i) inshore, (ii) within the kelp and (iii) offshore of the kelp forest at the 3
732 sampling sites: Archway Bay, Macaroni Bay 1 and Macaroni Bay 2.
733
734 **Table 3.** Prokaryotic heterotrophic production (PHP; µmol C m^{-3} d^{-1}) and potential respiration
735 estimated via ETS measurements (R-ETS; µmol C m^{-3} d^{-1}) of free-living (FL) and particle-
736 associated prokaryotes (PA) observed (i) inshore, (ii) within the kelp and (iii) offshore of the kelp
737 forest at the 3 sampling sites: Archway Bay, Macaroni Bay 1 and Macaroni Bay 2.
738
739 **Figure legends**
740 **Fig. 1.** Location of sampling sites in the vicinity of Prince Edward Islands. Transects were
741 conducted in 3 kelp forests (*Macrocystis pyrifera*) located along the east coast of Marion Island:
742 Archway Bay, Macaroni Bay 1 (M1) and Macaroni Bay 2 (M2). Dark grey area between Marion

743 Island coastline and the 30 m isobaths shows the kelp forest. At each site, samples were collected
744 at 3 stations (black stars) perpendicular to the coast: in the near-shore kelp-free waters (i.e. depth
745 <5 m), within the forest and offshore. SAF: sub-Antarctic Front. APF: Antarctic Polar Front.
746 *ACC*: easterly-flowing Antarctic Circumpolar Current.
747
748 **Fig. 2.** Temperature (°C) and salinity profiles recorded along transects at Archway Bay (A and
749 B), Macaroni Bay 1 (C and D) and Macaroni Bay 2 (E and F). Inshore (black marks), inside the
750 kelp forest (white marks) and offshore (grey marks).
751
752 **Fig. 3.** Average chlorophyll *a* concentrations ([Chl *a*] μg L^{-1}) in black bars (A, B and C) and
753 relative concentration of phaeopigments to total chlorophyll *a* (% phaeopigments) in grey bars
754 (D, E and F) along transects (inshore, kelp and offshore) at Archway Bay (A and D), Macaroni
755 Bay 1 (M1; B and E) and Macaroni Bay 2 (M2; C and F). The error bars are standard errors. *
756 Significant differences (Tukey test; $p<0.05$)
757
758 **Fig. 4.** Average prokaryotic growth efficiency PGE of free-living (FL: in grey) and of particle-
759 associated prokaryotes (PA: in black) along transects (inshore, kelp and offshore) at Archway
760 Bay (A), Macaroni Bay 1 (M1; B) and Macaroni Bay 2 (M2; C). Particle-associated PGE within
761 the kelp at Archway Bay was not calculated as the respirations rates were not available at this
762 station. The error bars are standard errors. * Significant differences between FL-PGE and PA-
763 PGE (Wilconxon-Mann-Whitney *U*-test; $p<0.05$).

Table 1. Ammonium $[NH_4^+]$, nitrite + nitrate $[NO_2^- + NO_3^-]$, orthophosphate $[PO_4^{3-}]$, dissolved organic carbo (DOC), dissolved organic nitrogen (DON), suspended particular matter (SPM), particulate organic carbon (POC) an particular organic nitrogen (PON) concentrations (µmol l^{-1}), and carbon to nitrogen molar ratios of the dissolved organi matter (C: N DOM) and of the particular organic matter (C: N POM) observed at observed (i) inshore, (ii) within the kelp an (iii) offshore of the kelp forest at the 3 sampling sites: Archway Bay, Macaroni Bay 1 and Macaroni Bay 2. Confidence level (µmol L^{-1}): ammonium = 0.02; nitrite + nitrate = 0.01; orthophosphate = 0.07; DOC = 0.2; DON = 0.1; POC = 0.2; PON = 0.2> no data available.

	Archway bay			Macaroni bay 1			Macaroni bay 2		
	Inshore	Kelp	Offshore	Inshore	Kelp	Offshore	Inshore	Kelp	Offshore
$[NH_4^+]$ (µmol L^{-1})	5.3	8.1	9.3	11.6	33.4	11.4	22.6	31.0	27.5
$[NO_2^- + NO_3^-]$ (µmol L^{-1})	13.3	13.1	14.0	15.0	12.4	13.1	17.9	13.0	17.0
$[PO_4^{3-}]$ (µmol L^{-1})	3.0	5.6	7.1	5.3	2.9	2.0	22.0	10.7	28.0
DOC (µmol L^{-1})	82.1	77.1	72.5	78.3	78.8	75.8	75.0	77.5	81.3
DON (µmol L^{-1})	6.4	4.3	2.9	×	7.1	6.4	5.0	4.3	2.9
C: N DOM	12.8	18.0	25.4	×	11.0	11.8	15.0	18.1	28.4
SPM (mg L^{-1})	16.5	16.1	15.5	30.4	16.4	15.2	32.0	30.9	30.1
PON (µmol L^{-1})	1.9	1.1	1.0	1.5	0.7	1.0	1.6	0.8	0.7
POC (µmol L^{-1})	9.5	14.0	11.0	14.1	6.9	7.5	23.8	7.0	7.1
C: N POM	5.1	12.6	11.6	9.4	9.8	7.8	14.8	8.6	10.4

Table 2. Average (±SE) free-living (FL) and particle-associated (PA) prokaryotes abundances (cell ml^{-1}) observed (i) inshore, (ii) within the kelp and (iii) offshore of the kelp forest at the 3 sampling sites: Archway Bay, Macaroni Bay 1 and Macaroni Bay 2.

Sampling sites	Stations	Total abundances (cell mL^{-1})	
		FL	PA
Archway Bay	Inshore	2.54×10^5 ($\pm 3.50 \times 10^3$)	4.26×10^3 ($\pm 1.50 \times 10^3$)
	Kelp	3.66×10^5 ($\pm 3.34 \times 10^3$)	7.14×10^3 ($\pm 3.60 \times 10^3$)
	Offshore	3.63×10^5 ($\pm 1.11 \times 10^4$)	5.45×10^3 ($\pm 1.31 \times 10^3$)
Macaroni Bay 1	Inshore	6.47×10^4 ($\pm 4.22 \times 10^3$)	3.87×10^3 ($\pm 7.47 \times 10^2$)
	Kelp	5.50×10^4 ($\pm 2.07 \times 10^3$)	7.08×10^3 ($\pm 2.84 \times 10^3$)
	Offshore	7.48×10^4 ($\pm 9.51 \times 10^3$)	5.36×10^3 ($\pm 6.67 \times 10^2$)
Macaroni Bay 2	Inshore	3.51×10^5 ($\pm 1.90 \times 10^4$)	6.71×10^3 ($\pm 2.10 \times 10^3$)
	Kelp	2.97×10^5 ($\pm 8.02 \times 10^4$)	1.53×10^3 ($\pm 4.80 \times 10^2$)
	Offshore	4.44×10^5 ($\pm 3.19 \times 10^4$)	9.71×10^3 ($\pm 1.52 \times 10^3$)

SE: Standard Error.

Table 3. Prokaryotic heterotrophic production (PHP; µmol C m^{-3} d^{-1}) and potential respiration estimated via ETS measurements (R-ETS; µmol C m^{-3} d^{-1}) of free-living (FL) and particle-associated prokaryotes (PA) observed (i) inshore, (ii) within the kelp and (iii) offshore of the kelp forest at the 3 sampling sites: Archway Bay, Macaroni Bay 1 and Macaroni Bay 2.

Sampling sites	Stations	PHP (µmol C m^{-3} d^{-1}) Average (±SE)		R-ETS (µmol C m^{-3} d^{-1})	
		FL	PA	FL	PA
Archway Bay	Inshore	2.9 (±0.2)	26.7 (±8.1)	38.9	106.7
	Kelp	2.9 (±0.5)	7.2 (±1.1)	18.6	×
	Offshore	4.2 (±0.3)	15.9 (±0.7)	86.3	59.3
Macaroni Bay 1	Inshore	0.5 (±0.0)	43.1 (±5.9)	21.0	1.1
	Kelp	4.0 (±1.9)	49.1 (±2.9)	1.1	74.3
	Offshore	12.0 (±1.5)	22.4 (±5.7)	74.3	81.9
Macaroni Bay 2	Inshore	11.3 (±0.0)	11.5 (±1.6)	38.9	15.2
	Kelp	9.5 (±4.3)	18.1 (±11.3)	32.2	43.8
	Offshore	20.8 (±5.3)	47.2 (±13.0)	19.3	8.8

SE: Standard Error × : no data available.

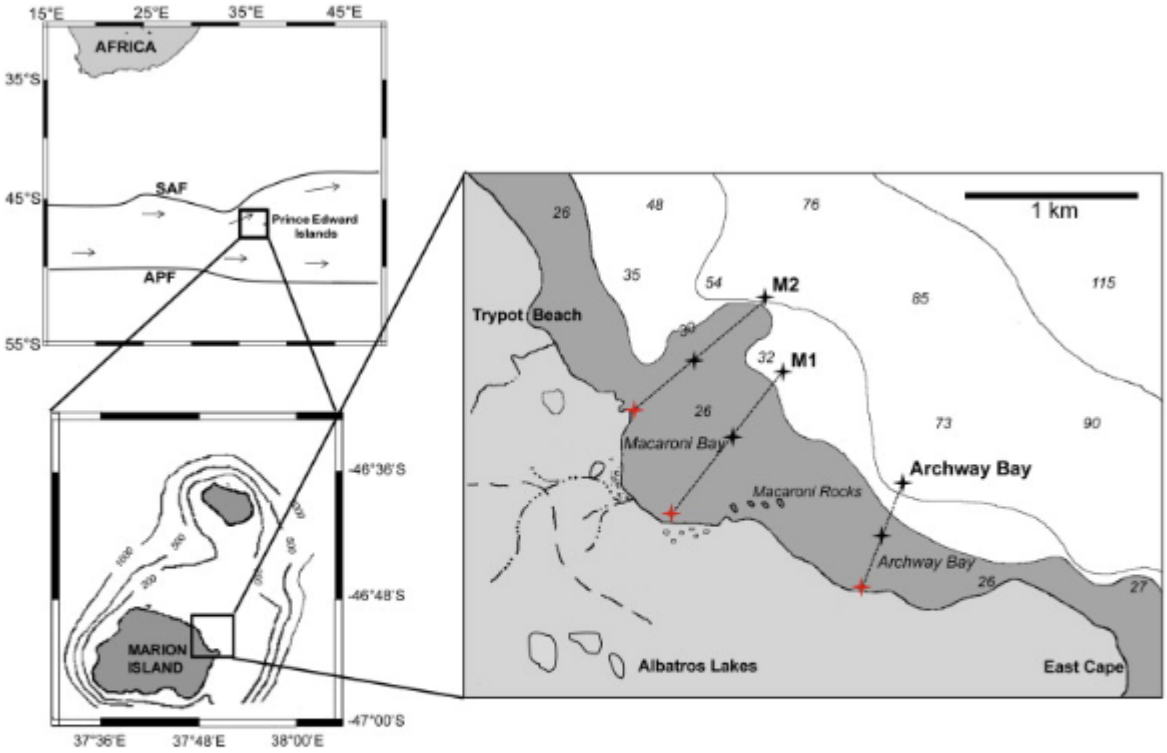

Fig. 1. Location of sampling sites in the vicinity of Prince Edward Islands. Transects were conducted in 3 kelp forests (*Macrocystis pyrifera*) located along the east coast of Marion Island: Archway Bay, Macaroni Bay 1 (M1) and Macaroni Bay 2 (M2). Dark grey area between Marion Island coastline and the 30 m isobaths shows the kelp forest. At each site, samples were collected at 3 stations (black stars) perpendicular to the coast: in the near-shore kelp-free waters (i.e. depth <5 m), within the forest and offshore. SAF: sub-Antarctic Front. APF: Antarctic Polar Front. ACC: easterly-flowing Antarctic Circumpolar Current.

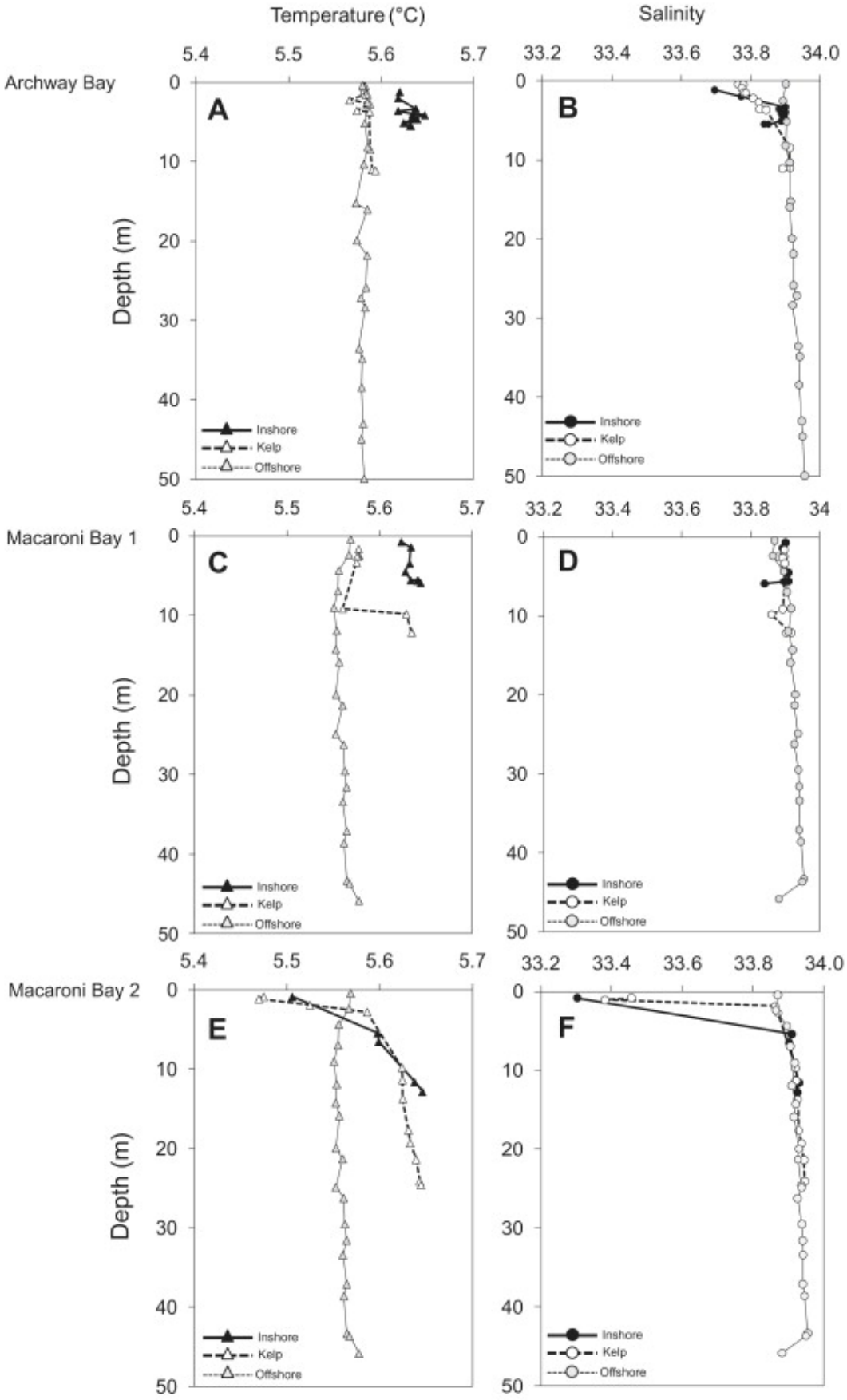

Fig. 2. Temperature (°C) and salinity profiles recorded along transects at Archway Bay (A and B), Macaroni Bay 1 (C and D) and Macaroni Bay 2 (E and F). Inshore (black marks), inside the kelp forest (white marks) and offshore (grey marks).

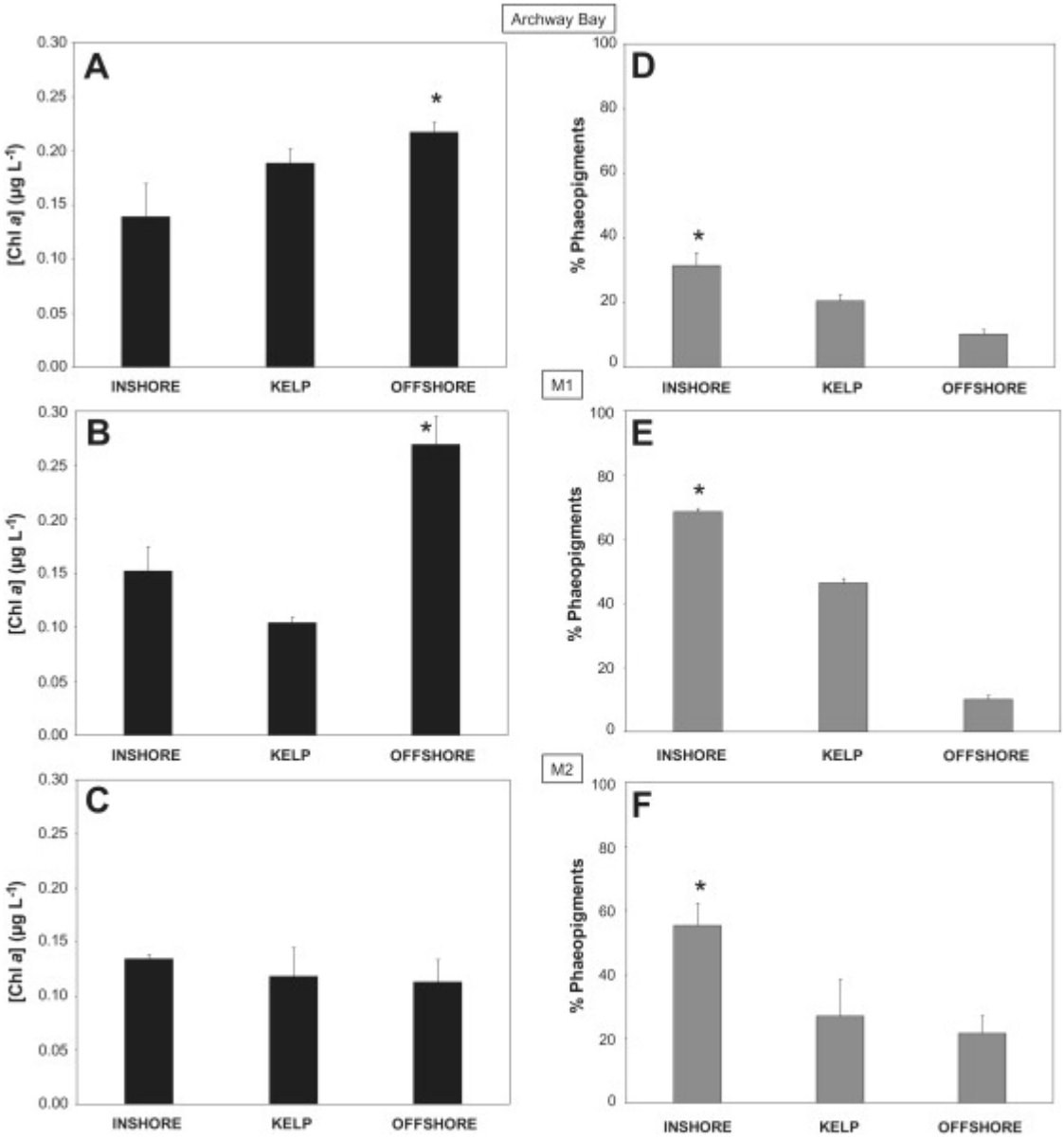

Fig. 3. Average chlorophyll *a* concentrations ([Chl *a*] µg L^{-1}) in black bars (A, B and C) and relative concentration of phaeopigments to total chlorophyll *a* (% phaeopigments) in grey bars (D, E and F) along transects (inshore, kelp and offshore) at Archway Bay (A and D), Macaroni Bay 1 (M1; B and E) and Macaroni Bay 2 (M2; C and F). The error bars are standard errors. * Significant differences (Tukey test; $p < 0.05$).

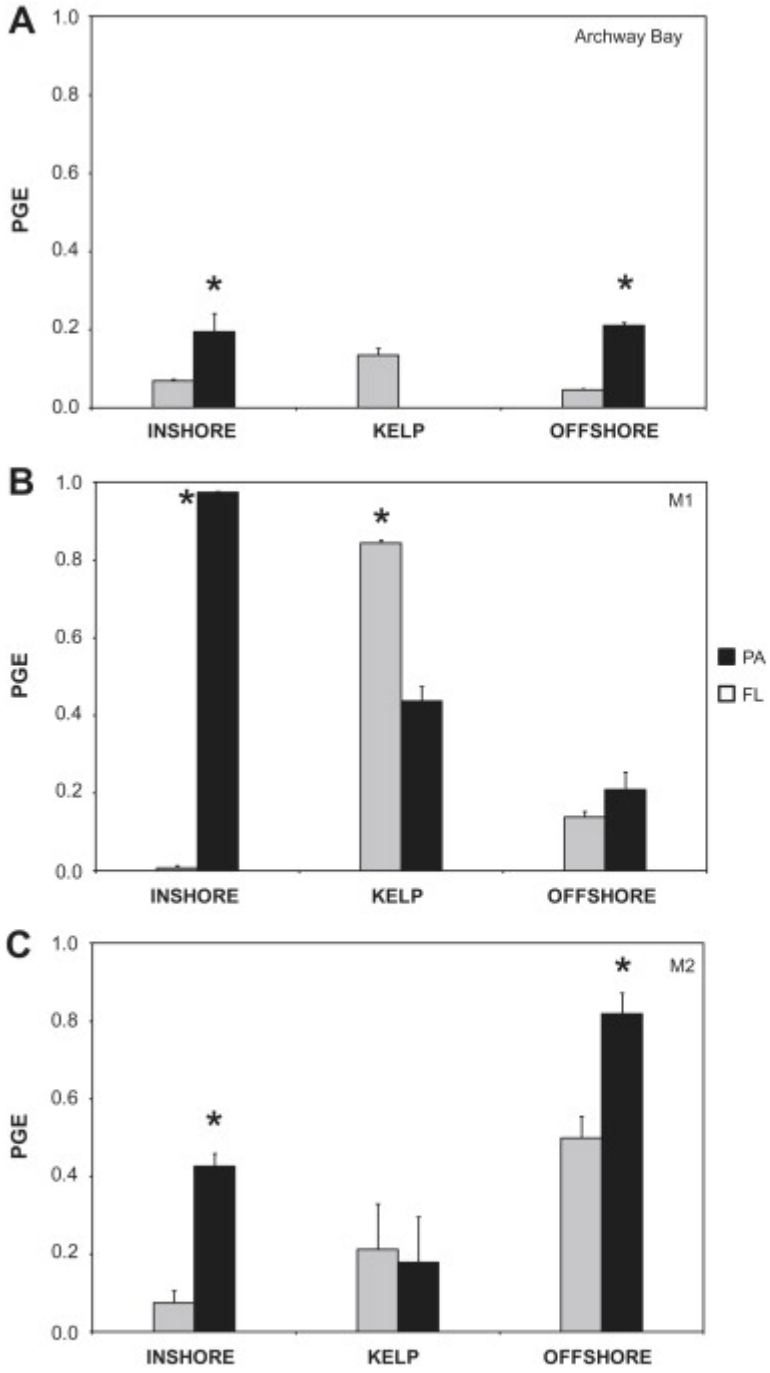

Fig. 4. Average prokaryotic growth efficiency PGE of free-living (FL: in grey) and of particle-associated prokaryotes (PA: in black) along transects (inshore, kelp and offshore) at Archway Bay (A), Macaroni Bay 1 (M1; B) and Macaroni Bay 2 (M2; C). Particle-associated PGE within the kelp at Archway Bay was not calculated as the respirations rates were not available at this station. The error bars are standard errors. * Significant differences between FL-PGE and PA-PGE (Wilconxon-Mann-Whitney *U*-test; $p < 0.05$).