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Microscale gradients of planktonic microbial communities above the sediment surface in a mangrove estuary

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Abstract

The microscale (1 and 4 cm sampling resolution) distributions of chemical (O_2, NH_3, NO_3^-, NO_2^-, PO_4^{3-}) and biological (Chl *a*, phytoplankton, bacterioplankton, viruses) parameters were measured in the 16 cm of water immediately overlaying the sediment-water interface (SWI) within a temperate mangrove estuary in South Australia during December 2003 and March 2004. Shear velocities (u_*) during the time of sampling were very low (<0.1 cm s^{-1}), and we consequently predict that resuspension of organisms and materials was negligible. In December 2003, profiles were often characterised by strong gradients in nutrients and organisms, with the highest concentrations often observed within 0.5 cm of the SWI. Microscale patterns in O_2, NH_3, NO_3^- and NO_2^- indicated that a variety of anaerobic and aerobic transformation processes probably occurred at the SWI and within profiles. Strong gradients in PO_4^{3-} were indicative of nutrient flux across the SWI as a consequence of degradation processes in the sediments. Pico- and nanophytoplankton concentrations were strongly correlated ($p < 0.01$) to PO_4^{3-}, and exhibited 12- and 68-fold changes in abundance, respectively, with highest concentrations observed nearest to the SWI. Several bacterial subpopulations were discriminated using flow cytometry and significant shifts in the 'cytometric structure' of the bacterial community were observed within microscale profiles. Two populations of viruses were correlated to the phytoplankton and low DNA (LDNA) bacteria, and each exhibited elevated concentrations within 0.5 cm of the SWI. In March 2004, microscale distributions of O_2 and nutrients were more homogenous than in December 2003, and dissimilar microbial community structure and patterns were observed above the SWI. The patterns observed here support the prediction that benthic processes can strongly influence the ecology of planktonic communities in the overlaying water, and provide further evidence for the existence of microscale variability amongst communities of aquatic microorganisms.

Keywords: sediment; estuary; bacteria; virus; phytoplankton; gradient

Regional terms: Australia; South Australia; St Kilda

1. Introduction

Mangrove estuaries represent important habitats for numerous coastal and pelagic species. The high levels of productivity that are characteristic of most mangrove estuaries are strongly coupled to the activity of the rich and diverse microbial communities inhabiting them (Holguin et al., 2001). Unlike the oceanic realm, where the bulk of organic matter cycling occurs in the water column, in shallow-water estuaries substantial amounts of organic matter settles to the bottom and is subsequently incorporated into the benthos, where it is degraded and modified by dense microbial communities (Köster et al., 2000). Benthic microbial communities are characterised by

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diverse consortia of microorganisms that are often highly stratified as a consequence of steep chemical gradients (Mackin and Swider, 1989; Barbara and Mitchell, 1996; Paerl and Pinckney, 1996; Holguin et al., 2001). These communities conduct a complex suite of aerobic and anaerobic transformations that ultimately control the concentrations of organic and inorganic nutrients within and above the sediments (Köster et al., 2000; Rieling et al., 2000).

Benthic biogeochemical transformation processes can have a considerable influence on the ecology of the planktonic organisms in the overlying water column (Jørgensen, 1983; Hopkinson et al., 1998; Middelboe et al., 1998). Microbial degradation processes occurring within the sediments lead to the liberation and diffusion of remineralisation products across the sediment–water interface (SWI), potentially providing an important proportion of nutrient requirements for phytoplankton in the overlying water column (Boynton et al., 1980; Hopkinson, 1987; Vidal et al., 1997; Asmus et al., 2000; Rieling et al., 2000; Baric et al., 2002; Qu et al., 2003). Similarly, concentration gradients generated by the large quantities of deposited organic matter in the sediments (Burdige et al., 1992; Burdige and Homstead, 1994), and the release of biodegradable dissolved organic carbon (DOC) from benthic microalgae (Middelboe et al., 1998) can lead to the flux of DOC from the sediments into the water column. Consequently, heterotrophic bacterioplankton may gain access to increased DOC concentrations, enhancing bacterial growth and productivity in the overlying water column (Middelboe et al., 1998). Hopkinson et al. (1998) demonstrated that bacterioplankton growth is indeed stimulated by the DOC in estuarine sediments, and suggested that benthic systems represent sites of both inorganic nutrient remineralisation, important for phytoplankton production, and DOC generation, important for bacterioplankton production in the overlaying water column.

Tide- or wind-induced resuspension of particulate material from the sediments into the water column is also likely to influence communities of planktonic microorganisms in estuarine habitats (Wainright and Hopkinson, 1987). Resuspension of particulate material from the sediments stimulates bacterial production and increases biomass in the overlaying water column (Wainright, 1987; Ritzrau and Graf, 1992) and may supply phytoplankton with increased dissolved nutrients (Fanning et al., 1982). Periodic resuspension of benthic microbial communities (Wainright, 1990) will also influence microbial foodweb structure and nutrient cycling rates in the water column (Demers et al., 1987).

As a consequence of diffusion and resuspension processes occurring at the SWI, coherent gradients in organic and inorganic nutrients are likely to provide both heterotrophic and autotrophic planktonic organisms with a unique microhabitat where levels of growth and productivity may be enhanced. Chemical gradients (e.g. O_2, H_2S) at and above the SWI may also influence the composition and activity of these communities. While several studies have demonstrated the existence of microscale variability of nutrients and microbial communities within the interstitial sediments (Hewson et al., 2001; Middelboe et al., 2003) and on the surface of the benthos (Franklin et al., 2002; Seuront and Spilmont, 2002) within estuarine systems, the microscale distributions of the planktonic community in the waters immediately overlaying the sediments remains poorly studied. Fluxes and interactions between the benthos and water column will have profound implications for nutrient cycling processes, and the structure of microbial and higher order foodwebs within estuarine systems. In the current study we have investigated the influence of the benthos on the ecology of estuarine planktonic microbial communities by studying the microscale dynamics of phytoplankton, bacterioplankton and virus populations within the column of water (<20 cm) immediately overlaying the sediment within a temperate mangrove system.

2. Materials and methods

2.1. Sample site description and characterisation of environment

Samples were collected from a shallow (40 cm) lagoon within a temperate mangrove estuary at St Kilda (34°44′S, 138°33′E) South Australia, on two occasions corresponding to the beginning (9/12/03: from here on Dec. 2003) and end (1/3/04: from here on March 2004) of the austral summer of 2003–2004. The St Kilda site is characterised by a mangrove forest, incorporating a system of shallow lagoons and streams, many of which are isolated from significant tidal flushing, leading to hyper-salinisation and stagnation of the water column (Barbara and Mitchell, 1996). The site hosts a complex microbial community including populations of sulphur-oxidising bacteria and dense microbial mats (Barbara and Mitchell, 1996). All sampling during this study was conducted from a timber board-walk that runs through the St Kilda mangrove system at a height of approximately 50 cm above the water surface.

Bulk physical conditions (temperature, salinity) were measured during each sampling date using a Hydrolab DataSonde® 4*a*. A Sontek acoustic Doppler velocimeter (ADV) was employed to characterise the hydrodynamic conditions at the site, and velocity fields in three dimensions (u_x, u_y, u_z) were computed using WinADV software (Tony Wahl, US Bureau of Reclamation). These values were subsequently employed to calculate the three-dimensional root-mean-square turbulent velocity w_{rms} (m s^{-1}) as (Tennekes and Lumley, 1972):

$$w_{rms}^2 = \frac{1}{3}\left(u_x^2 + u_y^2 + u_z^2\right) \quad (1)$$

We then estimated the average turbulent kinetic energy dissipation rate ε (m^2 s^{-3}) in the water column for each sampling date as (Taylor, 1938):

$$\varepsilon = k\left(\frac{w_{rms}^3}{L}\right) \quad (2)$$

where k is a constant ($k = 1$, Wolk et al., 2001) and L the integral length scale of turbulence, i.e. a characteristic length

scale representing the largest turbulent vortexes. Here L was assigned as 0.4 m, which is the approximate size of the largest possible eddies in the sampling system, which in this case is the depth of the lagoon.

Flow velocities obtained with the ADV were also applied to calculate the shear velocity (u_*) above the sediment surface, to allow for an estimation of the extent of resuspension of bottom sediments as a consequence of tidal or wind-induced water movement (Wainright, 1990). ADV measurements, as described above, were made at three depths, corresponding to distances of 10, 20, 30 cm above the sediment surface. The shear velocity u_* was subsequently calculated as (Dyer, 1986):

$$u_* = \frac{\kappa u}{\log(30d/k_b)} \quad (3)$$

where κ is the von Karman constant (0.41), u is the mean streamwise velocity calculated for a distance d from the bottom and k_b is the average diameter of the grains of sediment. Here k_b was assigned as 75 μm, from previous measurements made in the St Kilda site (Environmental Protection Authority of South Australia Report, 2000).

From the shear velocity u_* it was possible to estimate the diffusive sublayer δ_d and viscous sublayer δ_v thickness as (Jumars, 1993):

$$\delta_v = \frac{11.6\mu}{\rho u_*} \quad (4)$$

$$\delta_d = \frac{2\mu}{\rho u_*} \quad (5)$$

where μ is the dynamic viscosity (approx. 0.97×10^{-3} kg m^{-1} s^{-1} in seawater at 25 °C) and ρ is the density of seawater (approx. 1.024×10^3 kg m^{-3} at 25 °C).

2.2. Microscale profiles of chemical and biological parameters

We measured microscale vertical patterns in dissolved O_2, inorganic nutrients (NH_3, NO_3^-, NO_2^-, PO_4^{3-}), chlorophyll *a* and suspended particulate material in the 16 cm of water immediately overlaying the sediment–water interface. Dissolved O_2 vertical profiles were obtained at a spatial resolution of 4 cm using a CellOx 325-3 oxygen electrode (Wissenschaftlich-Technische Werkstatten).

Vertical profiles for inorganic nutrients, chlorophyll *a* and suspended particulate material were obtained using a pneumatically operated syringe sampler, consisting of a linear array of 50 ml syringes, each separated by a distance of 4 cm and connected along a hollow copper pipe (Waters et al., 2003). This device was employed to obtain vertical depth profiles from a distance of 0.5 cm to 16.5 cm above the surface of the muddy sediment.

Samples for inorganic nutrient analysis (20 ml) were put into sterile 50 ml centrifuge tubes and frozen prior to analysis. All nutrient analysis for ammonia (NH_3), nitrite (NO_2^-), nitrate (NO_3^-) and filterable reactive phosphorous (PO_4^{3-}) was conducted using an Alpkem segmented autoanalyser at the Australian Centre for Tropical Freshwater Research, James Cook University. Chlorophyll *a* levels were determined in profile samples by filtering 20 ml samples onto 0.45 μm HA (Millipore) filters. Chlorophyll was later extracted in 90% acetone and assayed using a Turner 10AU fluorometer.

Concentrations of suspended particulate material were determined by filtering 45 ml samples through pre-weighed 0.45 μm HA (Millipore) filters. Filters were rinsed with MilliQ water, dried at 60 °C for 24 h, and reweighed to determine the mass of suspended solids (>0.45 μm) retained on the filter (Hewson et al., 2001).

2.3. Microscale profiles of phytoplankton, bacterioplankton and viruses

Prior to the analysis of nutrient, chlorophyll *a* and suspended solid profiles, 1 ml aliquots were taken from each sub-sample to allow for the enumeration of planktonic microorganisms within the samples. Aliquots were added to sterile 2 ml cryovials and fixed with paraformaldehyde (1% final concentration) for 20 min before being quick frozen in liquid nitrogen and stored at −80 °C.

During the sampling conducted in March 2004, another specifically designed microsampler, closely based on the design of the syringe sampler described above, but capable of obtaining sub-samples at a spatial resolution of 1 cm, was also employed to investigate the microscale distributions of the planktonic microbial community. This pneumatically operated device consisted of a linear array of 1 ml syringes, allowing for the collection of 600 μl sub-samples, and was used to obtain five microscale vertical profiles from a depth of 0.5 to 15.5 cm above the sediment surface. Samples collected with this device were fixed and frozen as described above.

2.4. Sediment samples

Virus and bacterial abundance within the compact muddy sediments was measured in triplicate benthic samples obtained using cut-off 50 ml sterile syringe sediment corers. Cores were taken from the sediment surface to a depth of 2 cm and placed into sterile 50 ml centrifuge tubes containing 35 ml of 0.02 μm filtered TE buffer (10 mM Tris, 1 mM EDTA pH 7.5). Samples were agitated on a flask shaker for 15 min before being centrifuged at $1000 \times g$ for 10 min (Hewson et al., 2001), and 1 ml aliquots were then taken from the supernatant and fixed and frozen as described above.

2.5. Flow cytometry

All flow cytometric analysis was conducted using a Becton Dickinson FACScan flow cytometer, equipped with an air-cooled argon laser (15 mW, 488 nm), and phosphate buffered saline (PBS) solution employed as a sheath fluid. Prior to analysis, frozen samples were quick thawed, and divided into separate aliquots for enumeration of autotrophic phytoplankton, and heterotrophic bacteria and viruses. Samples for bacteria and virus enumeration were diluted 50-fold in TE buffer

(10 mM Tris, 1 mM EDTA pH 7.5) before being stained with SYBR-I Green solution (5:100,000 dilution; Molecular Probes, Eugene, OR), and incubated in the dark for 15 min (Marie et al., 1999a,b). We tested the virus abundance in samples of different dilution factors (1:5, 1:10, 1:50, 1:100) and found good correspondence between the calculated abundances in each instance. However, we found that the populations were easiest to differentiate, while also keeping the flow cytometry event rate below 800 (to avoid coincidence of particle counts, Marie et al 1999), using the 1:50 dilution. Fluorescent beads of 1 μm diameter (Molecular Probes, Eugene, OR), were added to all samples in a final concentration of approximately 10^5 beads ml^{-1} (Gasol and del Giorgio, 2000), and all measured cytometry parameters were normalised to bead concentration and fluorescence.

For each sample, forward scatter (FSC), side scatter (SSC), green (SYBR-I) fluorescence, red fluorescence, and orange fluorescence were acquired, and instrumentation set-up and methodology closely followed the protocols described by Marie et al. (1999b). For phytoplankton analysis, sample acquisition was run until at least 100–200 μl of unstained sample was analysed at a rate of approximately 40 μl min^{-1}. Populations of autotrophic nano- and picophytoplankton were discriminated according to differences in red (chlorophyll) fluorescence, orange (phycoerythrin) fluorescence and side scatter (Marie et al., 1999b) (Fig. 1E,F). For enumeration of bacteria and viruses, sample acquisition was run until at least 50–100 μl of sample was analysed at an event rate of $<800\ s^{-1}$. Individual virus and bacterial populations were separated according to variations in SYBR-green fluorescence and light side scatter (Marie et al., 1997, 1999a,b) (Fig. 1C). Data for individual sub-samples was collected in list-mode files, and populations were identified and enumerated using Win Midi 2.8 (© Joseph Trotter) flow cytometry analysis software.

2.6. Statistical analysis

All data was tested for normality using the Kolmogorov–Smirnov test for normality, and in the cases where normality could not be assumed, data was log transformed to allow for the use of parametric analysis. Distributions of variables within microscale profiles were compared by calculating the Pearson correlation coefficient. All data analysis was performed using SPPSS version 11.0 statistical software.

3. Results

3.1. Environmental parameters

Environmental conditions at the St Kilda mangrove estuary differed slightly between the two sampling dates. Mean temperature and salinity levels were relatively high during both sampling periods, although other parameters including bulk concentrations of suspended particulate material and inorganic nutrients varied significantly between the two sampling dates (Table 1). A sharp O_2 gradient above the sediment–water interface was observed in Dec. 2003 (Fig. 2A) but was not apparent in March 2004. While turbulent kinetic energy levels were relatively low on both sampling dates, levels were slightly higher in Dec. 2003 (Table 1). Shear velocity rates (u_*) were also slightly higher in Dec. 2003 (Table 1). By applying the shear velocities calculated here, we estimated the thickness of the diffusive (δ_d) and viscous (δ_v) sublayers in the St Kilda site during the sampling periods to be 2.2 mm and 1.3 cm respectively in Dec. 2003, and 1.1 cm and 6.1 cm in March 2000.

Field observations at the sampling site also indicated dissimilarities in the characteristics of the sediment surface between the two sampling occasions. In Dec. 2003 the sediment was characterised by a dark muddy surface with no algal cover evident. In March 2004 however, the sediment was partially covered in a fine layer of micro- and macroalgae. On both occasions, white and purple plumes indicative of sulphur bacteria (Bernard and Fenchel, 1995) were also intermittently present on the sediment surface.

3.2. Differentiation of microbial sub-populations

Using flow cytometry, multiple populations of microorganisms were identified within water samples. Within unstained samples, two discrete populations of phytoplankton were discriminated, which according to relative size and autofluorescence parameters were defined here as a group of picoeukaryotic phytoplankton and a group of small nanophytoplankton (Fig. 1E,F). Although populations of *Synechococcus*-type cyanobacteria can readily be identified using flow cytometry, according to orange phycoerythrin fluorescence (Olson et al., 1990), and have been found in coastal and estuarine habitats previously (Moreira-Turcq et al., 2001), there was no evidence of a population of cells high in orange fluorescence, indicative of *Synechococcus*, in the St Kilda mangrove estuary during the sampling conducted here.

When samples were stained with the nucleic acid stain SYBR Green, it was possible to discriminate several populations of heterotrophic bacteria and virus-like particles. Two populations of virus-like particles, defined here as V1 and V2, and corresponding to populations observed previously in seawater samples (Marie et al., 1999), were identified as discrete peaks on histogram plots of SYBR Green fluorescence (Fig. 1A) and clusters in biparametric cytograms (Fig. 1B).

The bacterioplankton community could also be divided into separate subpopulations according to differences in SYBR Green fluorescence (DNA content) and cell side scatter. In the Dec. 2003 samples, the bacterial community could be divided into three discrete groups, differing on the basis of increasing SYBR Green fluorescence. The low DNA (LDNA) and high DNA (HDNA) populations generally evident in most aquatic systems (Gasol et al., 1999) were clearly present in all samples (Fig. 1C). The HDNA population also exhibited two well-defined peaks on histogram plots of SYBR Green fluorescence and could subsequently be divided further into HDNA I and HDNA II categories (Fig. 1B). In March 2004 however, the bacterial community was characterised by a substantially different 'cytometric structure'. While the LDNA, HDNA I and HDNA II populations were all still present, at least two more subpopulations were evident within all samples. The B4

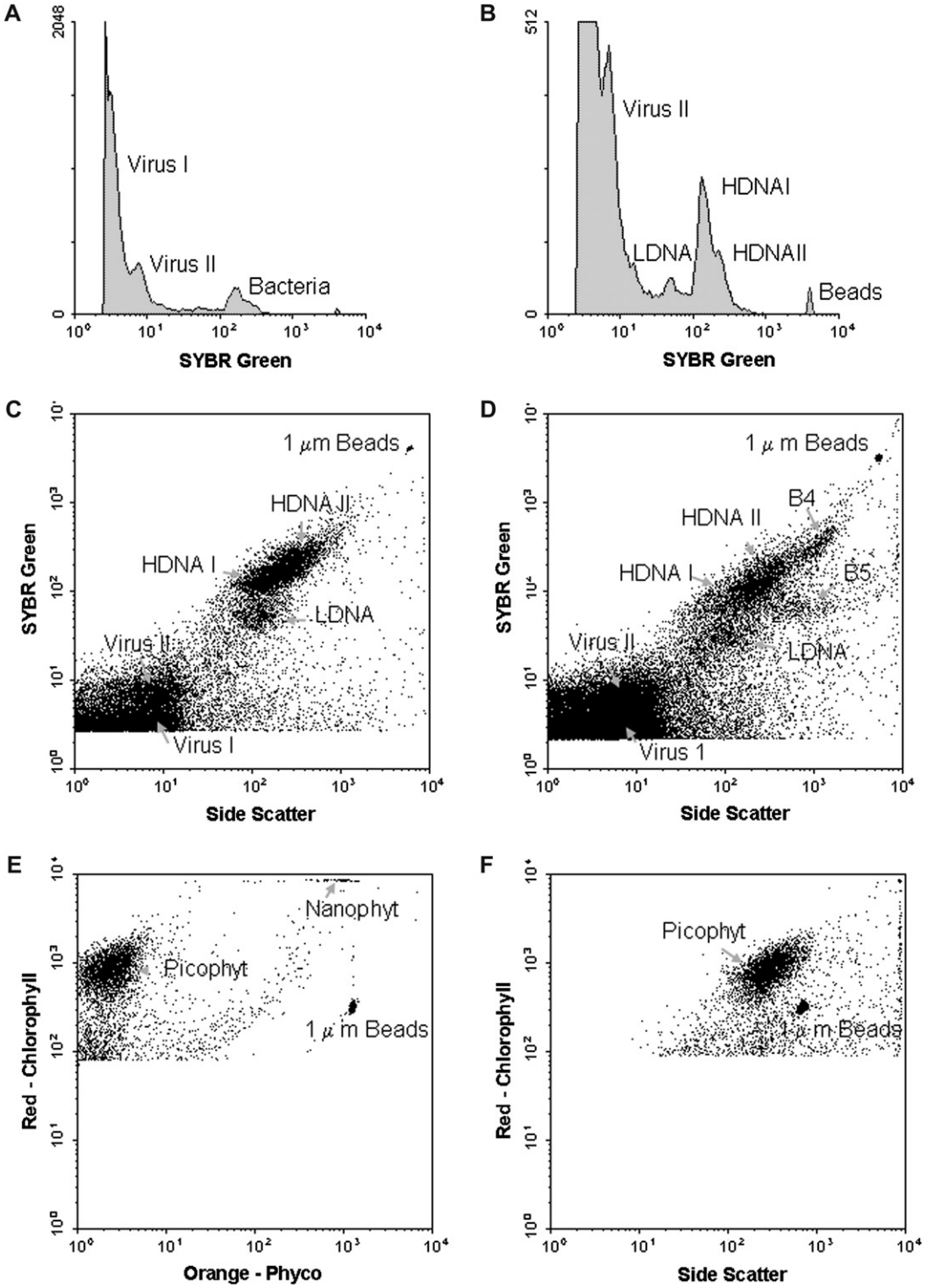

Fig. 1. Flow cytometric characterisation of microbial sub-populations. (A) Discrimination of virus sub-populations and heterotrophic bacteria. (B) Discrimination of heterotrophic bacteria subpopulations as discrete peaks on SYBR Green fluorescence histogram plot. (C) Scatter plot of cell side scatter vs SYBR Green fluorescence illustrating separation of virus and bacterial subpopulations in water samples from 9/12/03. (D) Scatter plot of side scatter vs SYBR Green fluorescence illustrating separation of discrete virus and bacterial subpopulations in water samples from 1/3/04. (E,F) Discrimination of pico and nano- phytoplankton populations according to side scatter, and red and orange autofluorescence.

population exhibited much higher levels of SYBR Green fluorescence than the other bacterial subpopulations, and the B5 population was characterised by higher levels of side scatter than the other bacterial populations (Fig. 1D). Neither the B4 nor B5 populations exhibited significant levels of orange or red fluorescence that would be indicative of autotrophic cyanobacteria such as *Synechococcus* (Jacquet et al., 1998).

In addition to the dissimilarities in the cytometric structure of the bacterial community, we observed differences in the total abundance of the virus, bacterial and phytoplankton

Table 1
Bulk physical characteristics of the St Kilda mangrove estuary sampling site on 9/12/03 and 1/3/04

Sample date	Temperature (°C)	Salinity (‰)	Dissolved O_2 (mg l^{-1})	Chl *a* (μg l^{-1})	SPM[a] (mg l^{-1})	NH_3 (μg N l^{-1})	NO_3^- (μg N l^{-1})	NO_2^- (μg N l^{-1})	PO_4^{3-} (μg P l^{-1})	ε (cm^2 s^{-3})	u_* (cm s^{-1})
9/12/03	25.0	43.0	1.9	n/a	20.4	24.4	13.8	3.1	205.8	1.3×10^{-3}	0.086
1/3/04	25.1	49.2	4.1	16.3	99.2	1.6	8.8	2.0	69.8	2.4×10^{-4}	0.018

[a] SPM, suspended particulate material.

populations between the two sampling dates (Table 2). Within sediment samples assessed for microbial abundance, it was often not possible to clearly identify the specific sub-populations that were evident in the water samples. Therefore only total bacterial and viral abundance could be enumerated in these samples. Concentrations of total viruses and bacteria in the sediments during the March 2004 sampling were 1.9×10^9 cm^{-3} and 4.2×10^8 cm^{-3} respectively.

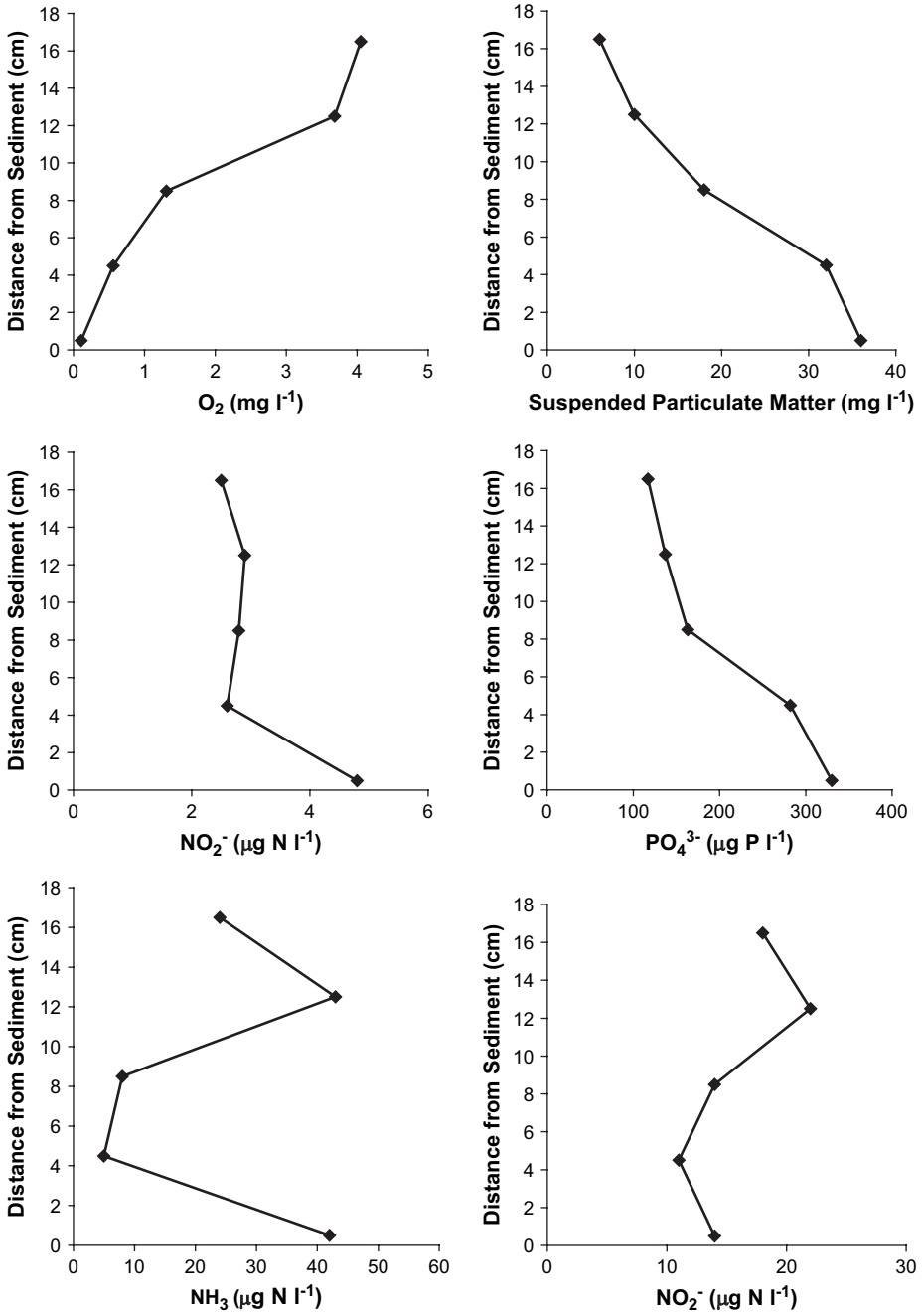

Fig. 2. Microscale vertical profiles of physical parameters immediately above the sediment–water interface within the St Kilda mangrove estuary on 9/12/03. (A) Dissolved oxygen, (B) suspended particulate material, (C) nitrite, (D) filterable reactive phosphorous, (E) ammonia, (F) nitrate.

Table 2
Microbial community characteristics at the St Kilda mangrove estuary sampling site on 9/12/03 and 1/3/04

Sample date	Total bacteria ($\times 10^7$ ml^{-1})	%HDNA	Total viruses ($\times 10^8$ ml^{-1})	VBR	Picophytoplankton ($\times 10^4$ ml^{-1})	Nanophytoplankton ($\times 10^3$ ml^{-1})
9/12/03	9.5	75.6	3.4	4.7	6.9	1.5
1/3/04	6.9	70.9	5.5	8.2	1.9	1.0

3.3. Microscale profiles

On each of the sampling dates, concentration gradients were observed in the microscale distributions of physical and biological variables within the 16.5 cm immediately above the sediment–water interface. As the nature of these microscale patterns and the community characteristics sometimes differed significantly between the two sampling dates, the patterns observed will be described separately.

3.3.1. December 2003

Microscale vertical profiles of suspended particulate matter, NO_2^- and PO_4^{3-} all exhibited increasing trends towards the sediment, with maximum concentrations observed within the 5 mm closest to the sediment surface (Fig. 2). Alternatively, concentrations of NO_3^- were highest 16.5 cm from the sediment surface (Fig. 2E), while NH_3 exhibited discrete concentration peaks at 0.5 cm and 12.5 cm above the sediment surface (Fig. 2F). Dissolved O_2 exhibited a decreasing trend toward the sediment with concentrations within the 5 mm closest to the sediment surface over 30 times lower than in the overlaying water column (Fig. 2A).

The picophytoplankton and nanophytoplankton populations also exhibited marked gradients in abundance across the 16 cm profile, with up to 12- and 68-fold changes in abundance observed respectively (Fig. 3E,F). Highest concentrations were observed within the 0.5 cm closest to the sediment surface, although profiles were characterised by coherent gradients in abundance across 8–12 cm. The picophytoplankton and nanophytoplankton populations were strongly correlated to each other ($r > 0.963$, $p < 0.01$) and to the concentration of PO_4^{3-} ($r > 0.925$, $p < 0.01$).

In contrast to the phytoplankton populations, total heterotrophic bacterial abundance exhibited slight decreasing trends towards the sediment surface, with lowest concentrations always observed within the 5 mm closest to the sediment surface. Total bacterial abundance was not significantly correlated ($p > 0.05$) to any other measured parameter in any profiles. The three bacterioplankton subpopulations generally exhibited dissimilar microscale patterns to one another (Fig. 3B). In all profiles the HDNA I population exhibited a decreasing trend towards the sediment surface, with lowest concentrations observed within the 5 mm closest to the sediment surface. Conversely, the LDNA and HDNA II populations were significantly ($r > 0.883$, $p < 0.05$) correlated to one another, and both exhibited a slight increasing trend towards the sediment surface, with highest concentrations observed within the 5 mm closest to the sediment surface (Fig. 3B). These patterns generated microscale shifts in the dominant population within the bacterial community. Within most sub-samples the HDNA I population represented the numerically dominant group, but within the samples closest to the sediment surface both the LDNA and HDNA II populations generally occurred in higher concentrations. These microscale changes in the bacterial community structure were also apparent when histogram plots of SYBR Green fluorescence were compared between depth intervals, with marked shifts in the relative size and shape of the three peaks corresponding to the different bacterial subpopulations (Fig. 4). Both the LDNA and HDNA II populations were significantly correlated ($r > 0.891$, $p < 0.05$) to the phytoplankton populations and to PO_4^{3-} concentrations, while the HDNA I population was not significantly correlated ($p > 0.05$) to any other measured parameter. Microscale shifts in the relative proportions of the LDNA and HDNA populations generated decreasing trends in the proportion of HDNA cells with proximity to the sediment surface (Fig. 3C).

The virus populations exhibited slight increasing trends towards the sediment surface (Fig. 3A), with the highest concentrations of both the V1 and V2 populations observed within the 5 mm closest to the sediment. Unlike the bacterial subpopulations, the virus subpopulations exhibited very similar microscale dynamics, with strong correlations between the V1 and V2 populations observed ($r > 0.994$, $p < 0.01$), and the relative proportions of V1 and V2 virus populations remained stable within and between profiles. Both the V1 and V2 populations were correlated ($r > 0.888$, $p < 0.01$) to the nanophytoplankton, picophytoplankton and LDNA populations, and the V2 population was correlated ($r > 0.936$, $p < 0.05$) to the HDNA II population. The total virus population and both the V1 and V2 populations were also correlated ($r > 0.932$, $p < 0.05$) to the concentration of suspended particulate material. The mean virus:bacteria ratio (VBR) was 4.7 and exhibited an increasing trend with proximity to the sediment surface in all profiles (Fig. 3D).

3.3.2. March 2004

Strong vertical gradients in suspended particulate matter and chlorophyll *a* were apparent in vertical profiles, with highest concentrations observed within the 5 mm closest to the sediment surface (Fig. 5B,C). However, with the exception of a slight gradient in the concentration of PO_4^{3-} (Fig. 5D), the concentrations of inorganic nutrients remained homogenous in vertical profiles (data not shown). In comparison to the strong gradient observed in Dec. 2003, only a slight shift in dissolved O_2 levels was observed, although concentrations close to the sediment surface were again lowest (Fig. 5A).

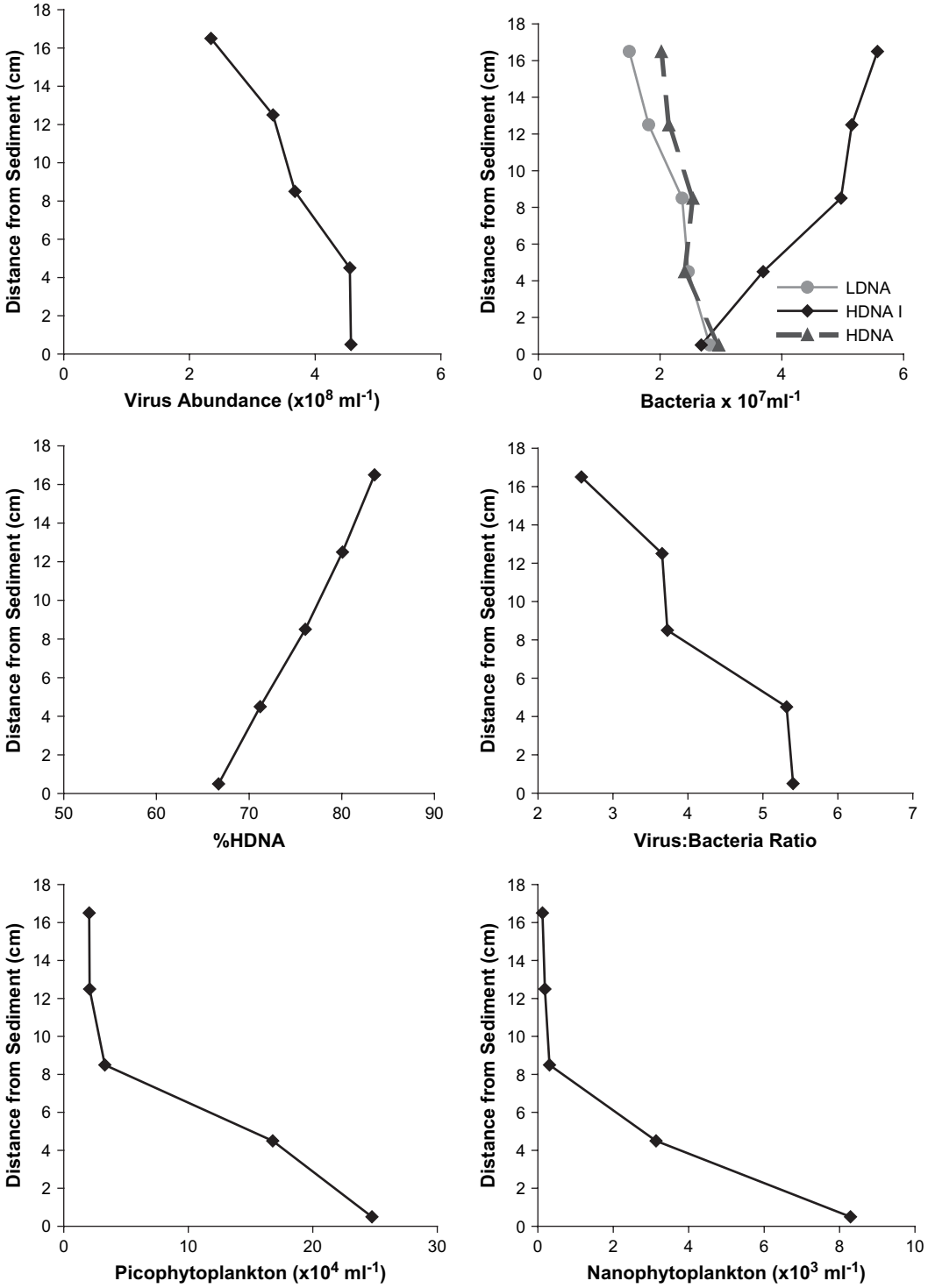

Fig. 3. Microscale vertical profiles of microbial populations immediately above the sediment–water interface within the St Kilda mangrove estuary on 9/12/03. (A) Total virus abundance, (B) LDNA, HDNA I and HDNA II bacterial subpopulations, (C) %HDNA (employed here as an index of bacterial activity), (D) virus:bacteria ratio, (E) picophytoplankton, (F) nanophytoplankton. Error bars represent the 95% confidence intervals obtained from replicate ($n = 10$) flow cytometric analysis of a single bulk sample (1 ml) collected from the sample site at the time of sampling.

Microbial populations were again compared to physical parameters at a spatial resolution of 4 cm, but were also investigated using a high resolution microsampler that measured distributions at a resolution of 1 cm (Fig. 6). Each of the phytoplankton and virus populations exhibited similar trends to those observed in Dec. 2003, with highest concentrations observed closest to the sediment surface (Fig. 6). Within 16 cm vertical profiles the picophytoplankton and nanophytoplanton populations exhibited up to 7- and 35-fold changes in abundance, respectively, and both populations were significantly

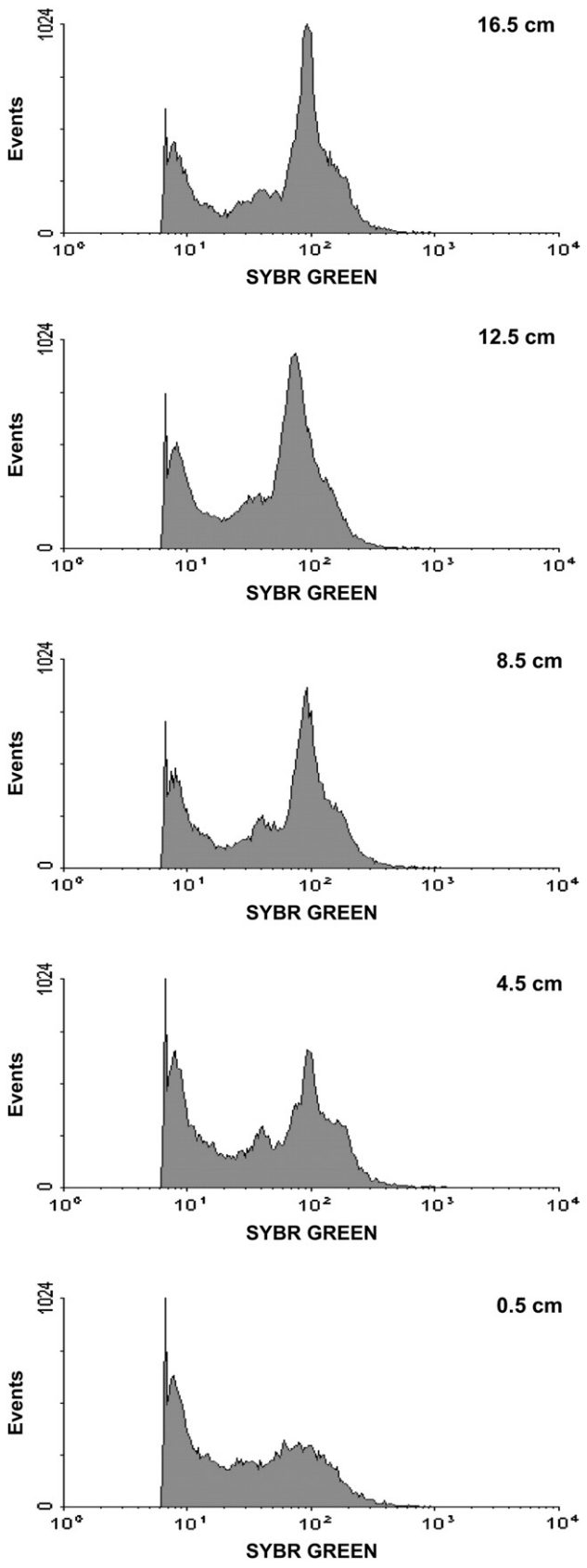

Fig. 4. Shifts in the flow cytometric population structure of the bacterioplankton community in a microscale vertical profile within the St Kilda mangrove estuary on 9/12/04.

correlated to each other ($r > 0.791$, $p < 0.01$), chlorophyll *a* ($r > 0.990$, $p < 0.01$), NH_3 ($r > 0.883$, $p < 0.05$) and PO_4^{3-} ($r > 0.885$, $p < 0.05$).

Virus concentrations varied only slightly within microscale profiles, with less than two-fold changes in abundance observed in most instances. However, highest concentrations of total viruses (Fig. 6A) and V1 and V2 populations again occurred within the 5 mm closest to the sediment surface, and correlations ($r > 0.733$, $p < 0.01$) between the V1 and V2 populations were again observed in all samples. However, unlike the Dec. 2003 samples, neither of the virus populations were significantly correlated ($p > 0.05$) to the concentration of suspended particulate matter. Both virus populations were correlated to the nanophytoplankton in all samples ($r > 0.567$, $p < 0.05$), but to the picophytoplankton in only 1 profile ($r > 0.768$, $p < 0.01$). In 3 out of 5 profiles both the V1 and V2 populations were significantly correlated ($r > 0.537$, $p < 0.05$) to all of the bacterial subpopulations. While the VBR varied within microscale profiles, no coherent trends or gradients, like those observed in Dec. 2003, were apparent in any of the profiles (Fig. 6D).

The bacterioplankton community exhibited marked differences to the patterns observed in Dec. 2003, although the patterns observed also varied between individual microscale depth profiles. In two out of five vertical profiles the highest concentrations of bacteria were observed closest to the sediment surface, with gradients in abundance apparent over a distance of 2–4 cm (e.g. Fig. 6B). In the other three profiles, the bacterial community remained comparatively homogenous, not exhibiting any clear trend in abundance, with no evidence for the decreases in abundance and %HDNA near to the sediment surface that was apparent in Dec. 2003. In each of the high resolution microscale profiles, each bacterial sub-population exhibited very similar distributions, and all populations were significantly correlated ($r > 0.590$, $p < 0.05$) to one another. There was no evidence for the shifts in bacterial community structure in histogram plots of SYBR Green fluorescence between depth intervals that were observed in the Dec. 2003 samples, and no clear trends in the %HDNA were observed in any of the profiles (Fig. 6C). None of the bacterial subpopulations nor the %HDNA were significantly correlated ($p > 0.05$) to chlorophyll *a*, suspended particulate material or inorganic nutrients.

4. Discussion

4.1. Environmental variability above the sediment–water interface

Planktonic communities inhabiting the few centimetres immediately overlaying the SWI are likely to experience a number of biotic and abiotic forces unique to that specific microenvironment. Our results suggest that physical and chemical parameters within the 10–20 cm above the sediment surface are highly heterogeneous, with marked gradients in the concentrations of dissolved and particulate material.

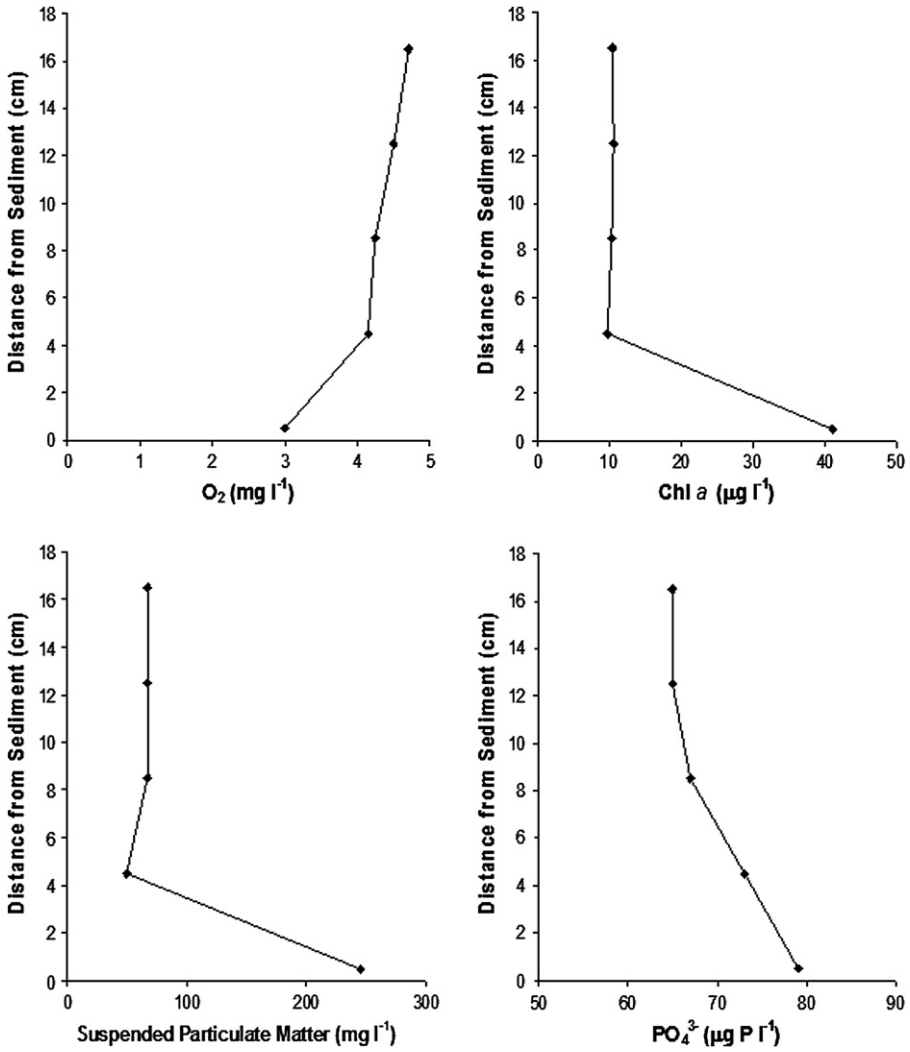

Fig. 5. Microscale vertical profiles of physical parameters immediately above the sediment–water interface within the St Kilda mangrove estuary on 1/3/04. (A) Dissolved oxygen, (B) chlorophyll *a*, (C) suspended particulate material, (D) filterable reactive phosphorous.

Gradients in the concentrations of dissolved oxygen and inorganic nutrients above the SWI, like those observed here, have been observed over equivalent spatial scales previously (Jørgensen, 1980; Yamamuro and Koike, 1994). The gradient in O_2 concentrations observed in Dec. 2003, characterised by very low O_2 levels (<1 mg l^{-1}) in the 4–8 cm immediately above the sediment surface, presumably occurred as a consequence of microbial respiration of organic matter within the sediments and relatively stagnant conditions in the overlaying water. The remineralisation of organic matter in the sediments and subsequent flux of inorganic nutrients across the SWI is probably also responsible for the strong gradient in the concentration of PO_4^{3-} observed above the sediment surface (Hopkinson, 1987; Köster et al., 2000; Baric et al., 2002).

We also observed significant variability and complex patterns in the microscale vertical distributions of the nitrogen species measured. Small-scale oxygen gradients, like those observed here, form redox gradients, which can subsequently generate microscale variability in the types and rates of nitrogen cycling in and above the sediments (Rysgaard et al., 1994; Paerl and Pinckney, 1996; Kemp and Dodds, 2001). The NO_2^- maximum, and low concentrations of NO_3^-, observed immediately above the SWI here are indicative of denitrification occurring at the sediment surface, where low O_2 concentrations and large amounts of organic matter facilitated the respiration of NO_3^- by denitrifying bacteria (Randall and Ingraham, 1981; Seitzinger, 1988, 1990). The increases in NO_3^- 12–16 cm away from the SWI are alternatively indicative of increased nitrification rates occurring when O_2 levels were higher (Henriksen and Kemp, 1988; Kemp and Dodds, 2001). A peak in NH_3 at the sediment surface (Fig. 2F), where anoxic conditions prevailed, is likely to have resulted from release during denitrification processes (Paerl and Pinckney, 1996). The second increase in NH_3 levels, 12.5 cm from the sediment surface (Fig. 2F), probably occurred as a consequence of heterotrophic remineralisation occurring at higher rates as oxygen levels increased, and as oxygen levels increased further, NH_3 decreased slightly, probably as a consequence of increased rates of nitrification (Libes, 1992).

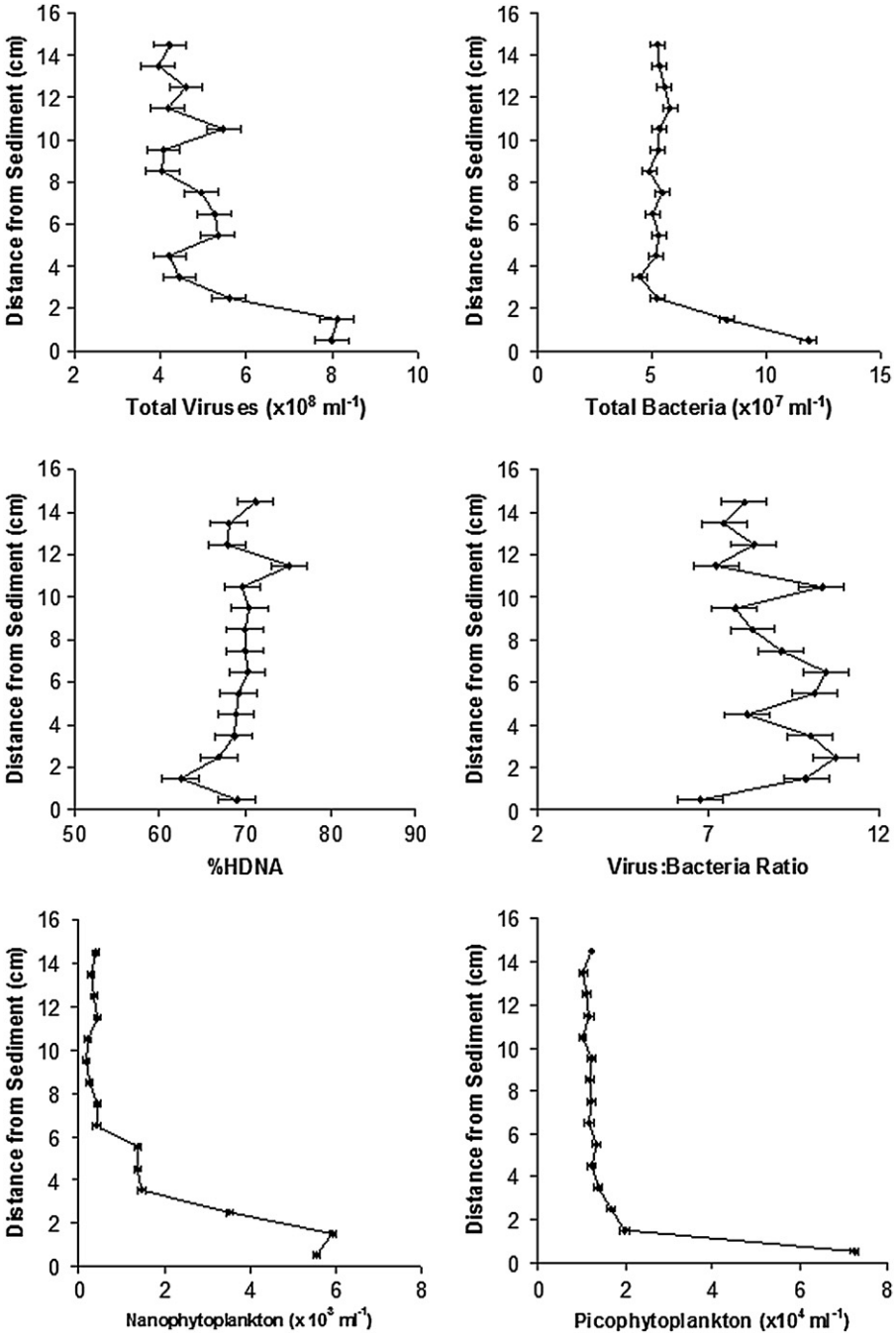

Fig. 6. Microscale vertical profiles of microbial populations, at a sampling resolution of 1 cm, within the 15.5 cm immediately above the sediment–water interface within the St Kilda mangrove estuary on 1/3/04. (A) Total virus abundance, (B) Total bacterial abundance, (C) %HDNA (employed here as a measure of bacterial activity), (D) virus:bacteria ratio, (E) Small nanophytoplankton, (F) picophytoplankton. Error bars represent the 95% confidence intervals obtained from replicate ($n = 10$) flow cytometric analysis of a single bulk sample (1 ml) collected from the sample site at the time of sampling.

In March 2004 the microscale patterns in nutrients were not as complex as in Dec. 2003. While a slight gradient in PO_4^{3-} was again observed, all other inorganic nutrients remained relatively homogenous. This pattern is likely to be a direct consequence of the relatively uniform O_2 concentrations and the absence of a strong anoxic zone. The reason for the homogenous and higher concentrations of O_2 may be decreased levels of deposited organic matter and subsequently reduced microbial respiratory activity at the sediment surface. The lower abundance and activity levels (%HDNA) of the bacterial community observed in March 2003 are consistent with this hypothesis. Alternatively, the higher concentrations of O_2 observed near the sediment surface may have been associated with the communities of macro and microalgae present on the sediment surface in March 2004 (Qu et al., 2003). The assimilation of inorganic nutrients by these communities may also explain the lower nutrient concentrations observed during this period (Kuwae et al., 1998; Cabrita and Brotas, 2000; Qu

et al., 2003). These dissimilar patterns indicate that, as well as being highly variable across small spatial scales, the processes influencing the chemical environment above the SWI can also vary markedly with time.

Physical mixing processes, generated by tidal movement or wind influence, can also alter the physical and biological status of the water overlaying the SWI, by resuspending organisms and nutrients (Fanning et al., 1982; Demers et al., 1987; Wainright, 1987, 1990). Several studies have illustrated high levels of resuspension of microbial communities from the benthos, and the critical shear velocities (u_{*c}) required to resuspend microorganisms and detritus have been calculated for different environments (Wainright, 1990; Blanchard et al., 1997; Beaulieu, 2003). In the present study we calculated shear velocity (u_*) rates of approximately 0.09 and 0.02 cm s^{-1} during the Dec. 2003 and March 2004 sampling dates respectively. These values are relatively low in comparison to previously published critical shear velocities (e.g. 0.95–1.35 cm s^{-1} for bacteria (Wainright, 1990), and 0.4–1 cm s^{-1} for phytoplankton (Blanchard et al., 1997; Beaulieu, 2003), and we subsequently predict that the influence of resuspension and mixing processes at the St Kilda site, at least during the time of sampling, would be low.

Using the shear velocity rates calculated here it was possible to estimate the thickness of the viscous (δ_v) and diffusive (δ_d) sublayers above the sediment surface in the St Kilda site. The viscous sublayer corresponds to the region above the SWI where surface friction retards flow and viscous forces damp out turbulent mixing. Viscous sublayers are most prominent in muddy bottomed, low-energy environments and are generally ~1 cm thick (Crawford and Sanford, 2001). The diffusive sublayer represents the thin layer of water immediately adjacent to the sediment surface where molecular diffusion becomes the dominant form of mass transfer. The size of the diffusive sublayer controls the extent of exchange of nutrients and gases across the SWI, and depending upon the environment is generally a few millimetres thick (Jørgensen and Revsbech, 1985; Jørgensen and Des Marais, 1990; Dade, 1993; Crawford and Sanford, 2001). As a consequence of the very low shear velocities occurring at the St Kilda site, the viscous and diffusive sublayers observed here were relatively thick. By controlling mass transfer and limiting mixing, thick diffusive and viscous sublayers will maintain larger and more persistent gradients of substrates above the SWI, and this probably explains the strong O_2 and nutrient gradients observed here. As a consequence, the planktonic community inhabiting the water immediately above the SWI within this specific environment is likely to experience a highly stratified microhabitat.

4.2. Microscale shifts amongst planktonic microbial communities

Both of the phytoplankton populations identified here exhibited high levels of centimetre-scale spatial variability, with highest concentrations always occurring within 0.5 cm of the SWI. Resuspension of phytoplankton cells from the sediment into the overlaying water column has been predicted to significantly increase phytoplankton biomass and primary production levels in the overlaying water column (Fanning et al., 1982). However, as is discussed above, due to the low u_* levels observed here, we predict that rates of resuspension of phytoplankton cells from the sediment surface would be low to negligible, particularly due to the small cell size (<10 μm) of the populations investigated here.

Both populations studied here were strongly correlated to PO_4^{3-} gradients in all samples, and phytoplankton abundance peaks also corresponded with peaks in NH_3 concentration near to the SWI. A number of studies have previously discussed the potential importance of benthic nutrient flux for phytoplankton communities in the overlaying water column (Rowe et al., 1975; Boynton et al., 1980; Hopkinson, 1987; Rieling et al., 2000), and the relevance of small-scale nutrient gradients in the ecology of phytoplankton has also been theorised for decades (Goldman et al., 1979; Lehman and Scavia, 1982). The patterns observed here imply that the microscale distributions of the phytoplankton community above the SWI are closely coupled to gradients in nutrient concentration. The observation that changes in phytoplankton abundance were almost twice as great in Dec. 2003, when nutrient gradients were also most pronounced, adds further support to this prediction. As a consequence of the patterns observed here, primary production levels in the few centimetres above the SWI may be significantly higher than in the overlaying water column.

Total changes in bacterial abundance were less intense than those observed for the phytoplankton populations, but the bacterial community generally exhibited more complex patterns and trends within the waters overlaying the SWI. Using flow cytometry, multiple sub-populations of bacteria were discriminated, and as has recently been observed in open water and lake samples (Seymour et al., 2004; Andreatta et al., 2004), these populations sometimes exhibited dissimilar microscale patterns to one another. The subpopulations discriminated in Dec. 2003 appear to closely correspond to the low-DNA (LDNA) and high-HDNA (HDNA) groups previously observed in seawater samples (Li et al., 1995; Jellett et al., 1996; Gasol et al., 1999). The LDNA and HDNA groups are widely expected to represent dormant and active cells respectively (Gasol et al., 1999; Lebaron et al., 2001, 2002), and the proportion of HDNA cells (%HDNA) has therefore been applied as an indicator of bacterial activity (Gasol et al., 1999; Vaqué et al., 2001; Gasol et al., 2002; Seymour et al., 2004). Due to the significantly higher side scatter and green fluorescence of the HDNA II population identified here, this population is predicted to represent the most active cells in the community (Lebaron et al., 2002).

Centimetre-scale shifts, characterised by changes in the "cytometric structure" of the bacterial community (Fig. 4) potentially occurred as a consequence of heterogeneity in the chemical and physical microenvironment above the SWI. We observed lower bacterial activity (%HDNA) and total abundance near to the SWI in Dec. 2003, despite the elevated concentrations of organic and inorganic nutrients expected to occur within this region (Hopkinson et al., 1998). However,

it is pertinent to note that the decreases in cell numbers and %HDNA near to the SWI were primarily driven by decreases in the HDNA I population. As the decreases in HDNA I did not correspond to equal magnitude increases in the LDNA and HDNA II populations, changes in %HDNA were apparently not associated with cells simply shifting between different activity levels. This indicates that either the HDNA I bacteria were actively removed from the environment near to the SWI, or that growth of this population was inhibited within this region. A possible explanation for the reduction in HDNA I growth and numbers is sensitivity to the anoxic conditions above the SWI. This is supported by the observations that increases in HDNA I numbers corresponded to an increase in O_2 concentration in the Dec. 2003 profiles, and that in March 2004, when O_2 concentrations did not decrease below 3 mg l^{-1}, the HDNA I population did not exhibit a significant decrease near to the SWI. Other alternative mechanisms include preferential grazing or infection of this population. Indeed, the HDNA I population corresponds to the bacterial subpopulation previously shown to be most susceptible to grazing pressure (Bouvy et al., 2004), and concentrations of heterotrophic nanoflagellates are often elevated in the few centimetres immediately above the SWI (Shimeta and Sisson, 1999). Numbers of viruses and the VBR were also higher near to the SWI, indicating that the potential for virus infection within this region may also be greater. Increased grazing or infection rates within the few centimetres immediately above the SWI may also explain the concomitant increases in numbers of LDNA bacteria, which have in some cases been predicted to represent dead cells (Gasol et al., 1999).

Alternatively, rather than being indicative of changes in bacterial metabolic state, changes in the relative abundances of the bacterial subpopulations may be a manifestation of microscale shifts in the composition of the bacterial community. Considering the heterogeneous nature of the environment studied here, we predict that the bacterial community will be highly diverse and perhaps characterised by microscale shifts in community composition, probably between communities dominated by aerobic and anaerobic populations (Köster et al., 2000). Microscale shifts in bacterioplankton species richness have been observed previously (Long and Azam, 2001), and Zubkov et al. (2001) demonstrated that different flow cytometric subpopulations of bacteria can represent discrete phylogenetic groups. However, other authors have alternatively shown that cytometric subpopulations can be comprised of multiple phylogenetic groups (Fuchs et al., 2000) and that cells from a single species of bacteria can co-occur in different flow cytometric populations (Servais et al., 2003). Without molecular analysis of sorted flow cytometric groups it is not possible to determine how the shifts in cytometric population structure observed here relate to taxonomic (Zubkov et al., 2001) or physiological (Gasol et al., 1999) variability. Nevertheless, our results indicate that shifts in the structure of bacterioplankton communities can occur over spatial sales of centimetres apparently in response to microscale heterogeneity in the physical and/or chemical environment immediately above the SWI.

In March 2004, both the bacterial community structure and microscale dynamics above the SWI were dissimilar to the patterns observed in Dec. 2003. Two additional populations of bacteria (B4 and B5), characterised by high side scatter and fluorescence levels were apparent in March 2004. It is not clear why these populations were present in March 2004 and not Dec 2003. However, the B4 and B5 populations were always correlated to the other bacterial subpopulations, and did not exhibit patterns, such as association with oxygen gradients, that would be suggestive of specialised populations of bacteria such as sulphur-oxidising bacteria. Indeed, all bacterial subpopulations were generally correlated to one another in March 2004. As shifts in abundance remained consistent between bacterial subpopulations, there was little change in %HDNA observed, indicating that bacterial activity was apparently homogenous within profiles in March 2004. Furthermore, the changes in the 'cytometric structure' of the bacterial population that were evident in Dec. 2003 did not occur in March 2004. This may be a consequence of the greater homogeneity in conditions (e.g. O_2 and nutrients) observed in the water overlaying the SWI in March 2004.

Unlike Dec. 2003, when all profiles were characterised by identical spatial patterns and trends, in March 2004 the distributions observed sometimes differed from one profile to another. In three profiles all of the bacterial populations exhibited homogenous trends and low levels of variability over the 16 cm profile. In two profiles however, each of the populations exhibited maximum bacterial abundance within the 1–3 cm immediately above the SWI. The source of the variability between the profiles is itself an expression of the microscale heterogeneity observed within this environment. Vertical gradients above the sediment surface are ultimately a corollary of processes occurring within or on the sediment. If for instance we consider a dense patch of benthic microalgae covering only a few centimetres of the sediment surface (Seuront and Spilmont, 2002), then the flux of DOC from this patch (Middelboe et al., 1998) will be higher than in the surrounding area, and gradients in bacterial abundance and activity may be more pronounced here than above other points on the sediment surface. As profiles were not all taken from above exactly the same point on the sediment surface, these circumstances may explain the different patterns observed between profiles.

Viruses represent abundant and dynamic populations within the water column and sediments of estuarine systems (Almeida et al., 2001; Hewson et al., 2001; Middelboe et al., 2003; Weinbauer et al., 2003), but to our knowledge few studies have investigated the distributions of virioplankton immediately above the SWI. Like the bacterial populations, we observed dissimilarities in the microscale dynamics of the virus populations between the two sampling dates. In Dec. 2003 both virus populations were significantly correlated to the suspended particulate material in all profiles. Correlations between virioplankton abundance and suspended particulate matter have been observed previously within estuarine systems and may be indicative of viral adsorption to particles (Hewson et al., 2001) or resuspension processes. Within

a variety of marine environments virus abundance is correlated to total bacterial abundance (Cochlan et al., 1993; Boehme et al., 1993; Drake et al., 1998; Middelboe et al., 2003; Seymour et al., 2005), but no such coupling between total viral and bacterial abundance was observed here. However, both virus populations were correlated to the LDNA bacteria subpopulation, and the V2 population was correlated with the HDNA II population, indicating a more complex association between the bacterial and viral communities than is indicated by comparisons of total viral and bacterial abundance. Microscale changes in the VBR observed within profiles indicate a possible de-coupling of bacterial and viral communities and shifts in virus production and loss rates above the SWI. In all profiles the highest VBR was observed close to the SWI, implying that within this microenvironment bacterial exposure to viral infection may be increased. The temporal heterogeneity in viral abundance and dynamics observed here suggests that the virus community represents a dynamic element within the St Kilda mangrove habitat, which along with environmental factors, may play a large role in structuring the population and trophic dynamics of the microbial populations inhabiting the water column within the few centimetres above the SWI.

4.3. Conclusion

Previously, we have observed centimetre scale spatial heterogeneity in the abundance and activity of microbial communities in open water samples (Seymour et al., 2004; Waters et al., 2003) and associated with the surfaces of coral colonies (Seymour et al., 2005). The patterns observed here indicate further that microscale heterogeneity amongst aquatic microbial communities is probably a ubiquitous feature of the marine environment and is likely to occur in response to a variety of generating mechanisms.

Our results also support the prediction that communities of planktonic microorganisms within shallow estuarine habitats can be strongly influenced by benthic processes, such as resuspension of materials and organisms, and diffusion of nutrients across the SWI. The gradients and patterns evident in microscale profiles here suggest that, at least in this specific environment, the physical, chemical and biological nature of the water column immediately above the benthic–water interface can be markedly stratified at scales of centimetres or less. We subsequently argue that estuarine and coastal habitats will often be characterised by a more complex setting than a simple dichotomy of benthic and pelagic realms. Instead, a continuum of micro-niches, influenced by benthic and pelagic processes to different degrees, and characterised by discrete microbial consortia, is likely to exist at and above the SWI. Shifts in the levels of primary and secondary production and trophic interactions within the few centimetres immediately above the SWI may ultimately modify the productivity and function of protected estuarine systems. Consequently, the dynamics and distributions of the communities inhabiting this specific microenvironment warrant further focussed consideration.

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