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Microscale patchiness of virioplankton

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The microscale spatial distributions of viruses were investigated in three contrasting environments including oligotrophic open ocean, eutrophic coastal and estuarine habitats. The abundances of two discrete populations of both viruses and heterotrophic bacteria were measured at spatial resolutions of between 1 and 5 cm using purpose-designed microscale sampling equipment and flow cytometric sample analysis. Within open water samples, virus distributions were characterized by non-normal distributions and by ‘hotspots’ in abundance where concentrations varied by up to 17-fold. In contrast to patterns generally observed at larger spatiotemporal scales, there was no correlation between bacterial and viral abundance or correspondence between bacteria and virus hotspots within these samples. Consequently, strong hotspots and gradients in the virus:bacteria ratio (VBR) were also apparent within samples. Within vertical profiles taken from above the sediment–water interface within a temperate mangrove estuary, distributions of planktonic viruses were characterized by gradients in abundance, with highest concentrations observed within the 1–2 cm immediately above the sediment surface, and virus distributions were correlated to bacterial abundance ($P < 0.01$). The patterns observed in these contrasting habitats indicate that microscale patchiness of virus abundance may be a common feature of the marine environment. This form of heterogeneity may have important implications for virus–host dynamics and subsequently influence microbial trophodynamics and nutrient cycling in the ocean.

INTRODUCTION

Many ecological interactions amongst marine microbial communities are defined on spatial scales that are often orders of magnitude smaller than those typically studied in oceanographic research. Marine microbial communities experience heterogeneity in resources and environmental variables across distances of micrometres to centimetres, and are well adapted to life in a patchy micro-environment (Azam, 1998; Fenchel, 2002). Subsequently, it has been shown that the abundance, activity and composition of marine bacterioplankton and phytoplankton can vary substantially over these small scales (Seymour et al., 2000, 2004, 2005b; Long & Azam, 2001; Waters et al., 2003), and it has been postulated that this type of heterogeneity will have important consequences for microbial ecology and oceanic biogeochemical cycles (Azam, 1998). While the role of viruses has now been integrated into marine microbial food webs (Fuhrman, 1999), little is currently known about how microscale heterogeneity in the distribution of viruses and their host cells can influence viral ecology in the ocean.

In terrestrial systems it has been demonstrated that spatial heterogeneity can strongly influence virus–host population dynamics (Grosholz, 1993; Liu et al., 2000). As viruses are not motile, relying on passive exposure to hosts, the densities and dynamics of planktonic virus and host populations, particularly at spatial scales relevant to

diffusive transport, will be an important deterministic factor in the success and spread of viruses in the sea (Murray & Jackson, 1992). Indeed, in the same way that patchy distributions of organisms can influence predator–prey interactions in the ocean (Rothschild, 1992), microscale spatial heterogeneity is likely to play a critical role in virus–host dynamics.

To date, most investigations into the microscale distributions of marine viruses have focused on sediment communities and these have shown significant shifts in viral abundance across centimetres, which have been related to microbial abundance and diagenetic activity (Hewson et al., 2001; Middelboe et al., 2003). However, significantly less is currently known about the microscale patterns of planktonic viral communities. Shifts in the abundance and composition of pelagic virus populations over scales of a few metres have been demonstrated, but this variability is often likely to be driven by heterogeneities in the physical environment rather than specific virus–host interactions (Riemann & Middelboe, 2002; Frederickson et al., 2003). Concentrations of viruses can be significantly elevated in the few centimetres immediately above the surface of coral colonies (Seymour et al., 2005a), and in temporal experiments it has been demonstrated that numbers of marine viruses can vary by a factor of 2–4 within only 6–10 min (Bratbak et al., 1996). These observations indicate that microscale factors may sometimes influence the ecology and dynamics of marine viruses, but whether they represent ubiquitous phenomena, or

Table 1. Mean microbiological and physico-chemical characteristics of sampling stations.

Site	Latitude	Longitude	Temperature (°C)	Salinity (ppt)	Chlorophyll- <i>a</i> ($\mu\text{g l}^{-1}$)	NO ₂	NO ₃	PO ₄	Virus ($\times 10^7 \text{ ml}^{-1}$)	Bacteria ($\times 10^6 \text{ ml}^{-1}$)	VBR
SO 1	60°45'S	81°27'E	1.39	33.9	—	—	—	—	0.53	0.86	6.2
SO 2	65°39'S	80°20'E	-1.02	32.7	—	—	—	—	0.38	0.87	4.4
SO 3	66°42'S	77°42'E	-1.69	32.7	—	—	—	—	0.35	0.64	5.5
EEC	50°41'N	13°1'E	9.6	34.0	46.6	2.2	0.2	0.4	5.2	3.3	16.7
St Kilda	34°44'S	138°33E	25.1	49.2	16.3	2.0	8.8	69.8	55.0	69.0	8.2

VBR, virus to bacteria ratio; SO, Southern Ocean sites; EEC, eastern English Channel.

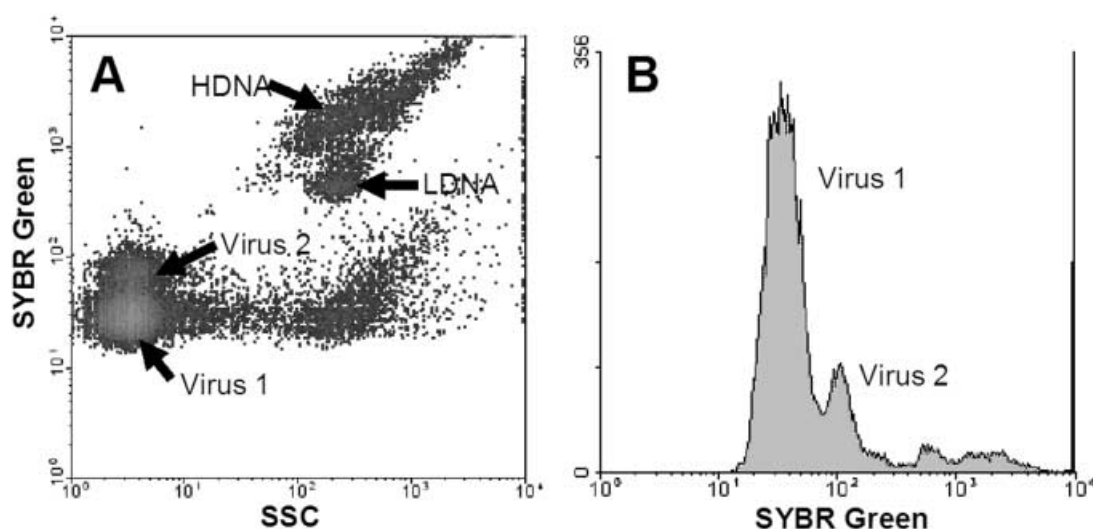


Figure 1. (A) Density plot of Side Scatter vs SYBR Green fluorescence showing discrimination of discrete virus and bacterial populations; and (B) histogram of SYBR Green fluorescence. Virus sub-populations are defined as discrete peaks on histogram plot.

rare occurrences in specific environments, is as yet not known.

As viral lysis can be a major cause of mortality of marine bacteria and phytoplankton (Proctor & Fuhrman, 1990; Suttle, 1994; Fuhrman & Noble, 1996), viruses play a definitive role in the function of the microbial loop and subsequently influence the cycling of organic matter in the ocean (Fuhrman, 1999; Wilhelm & Suttle, 1999; Riemann & Middelboe, 2002). More specifically, viral lysis of cells reduces the rate at which carbon is removed from the upper ocean via the 'biological pump', leading to a greater accumulation of carbon in the ocean's surface waters and an increased flux of CO₂ to the atmosphere (Suttle, 2005). Therefore, in light of the need for accurate global climate change predictions (Longhurst, 1991), it is important that an accurate perception of the factors controlling virus–host dynamics in the ocean is obtained. To fully comprehend any ecological process or interaction, measurements at spatial scales corresponding to the aggregation and interactions of populations are required (Wiens, 1989). It has been predicted that short-lived microscale patches of viruses should occur in the ocean (Bratbak et al., 1996), but to our knowledge *in situ* measurements of viral distributions at these scales have

not been attempted. The objective of this research is to gain a first insight into how marine virus populations are distributed near to the scales of microbial interaction, and how these distributions are related to, or controlled by, patchiness of microbial host populations.

MATERIALS AND METHODS

Study sites and sampling strategy

Microscale distributions of planktonic viruses were studied in dissimilar marine habitats, incorporating polar and temperate systems, and including oligotrophic open ocean, eutrophic coastal and estuarine environments. At each site, different microscale sampling devices, each designed according to the specific physical and biological characteristics of the environment, were employed to measure the microscale distributions of planktonic viruses.

Open ocean samples were obtained from the Southern Ocean during the CLIVAR expedition in January 2005. Southern Ocean (SO) samples were collected from three locations along a transect extending from the Kerguelen Plateau to the Antarctic ice-shelf (60°45'S 81°40'E–66°42'S 77°42'E). At each location samples were collected

Table 2. Mean microscale distribution characteristics of virus community.

Site	Mean ($\times 10^7 \text{ ml}^{-1}$)	CV	Maximum/ minimum	Skew	Kurtosis	Correlation bacteria	Correlation Chl- <i>a</i>	V1-V2 correlation
SO 1	0.53	28.0	4.9	2.6	3.7	No	–	0.79**
SO 2	0.38	27.1	4.2	1.9	5.4	No	–	0.79**
SO 3	0.35	25.2	5.5	2.3	11.0	No	–	0.85**
EEC	5.2	23.1	3.5	4.4	28.7	No	No	No
St Kilda	55.0	26.2	2.1	1.5	1.6	0.83**	No	0.99**

*, $P < 0.05$; **, $P < 0.01$; SO, Southern Ocean sites; EEC, eastern English Channel.

using a purpose-designed sampling device, which has previously been employed for the examination of 2-dimensional microscale distributions of marine bacteria (Seymour et al., 2004). This spring-loaded system consists of a 10×10 matrix of 1 ml syringes, each separated by a distance of 1.2 cm and set to sample volumes of $100 \mu\text{l}$. A messenger weight is employed to trigger the sampling mechanism, allowing for the simultaneous collection of 100 sub-samples across 116 cm^2 . While this device can be used to take samples anywhere from the surface to 25 m depth, during this study samples were taken from a depth of approximately 1 m. Concurrent conductivity–temperature–depth (CTD) and chlorophyll measurements were made at each site.

Samples from within a eutrophic coastal environment were collected from the eastern English Channel (EEC) ($50^\circ 40' 75'' \text{N}$ $1^\circ 31' 17'' \text{E}$) in April 2004. This sampling site was situated approximately 1.8 km from the north-eastern coast of France, and characterized by high levels of tidal mixing, eutrophic conditions, and during the time of sampling was strongly influenced by the annual bloom of the Prymnesiophyceae phytoplankton *Phaeocystis globosa*. Samples were collected using an up-scaled version of the 2-dimensional sampling device described above, which consisted of a 10×10 array of 50 ml syringes, separated at a spatial resolution of 5 cm. This pneumatically operated device was attached to a series of electric pumps allowing for the simultaneous collection of 100 samples across an area of 0.2 m^2 . Samples were taken from a depth of 1 m at each of the sampling sites. The larger sample volume applied here was employed to allow for concurrent measurements of chlorophyll-*a* levels, associated with the *P. globosa* bloom, within each sub-sample. Bulk physical conditions (temperature and salinity) were measured from the surface to the bottom with a Sea-Bird SBE 25 Sealogger CTD probe.

Samples from an estuarine habitat were collected from a shallow (<1 m) lagoon within a temperate mangrove estuary at St Kilda ($34^\circ 44' \text{S}$ $138^\circ 33' \text{E}$) in South Australia, during March 2004. The St Kilda mangrove forest is characterized by a system of hypersaline lagoons and streams, and hosts a complex microbial community including populations of sulphur-oxidizing bacteria and dense microbial mats (Barbara & Mitchell, 1996). Within this habitat the vertical microscale distributions of viruses and other microbial parameters were measured in the few centimetres immediately overlaying the muddy sediment surface. 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., 2002). 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. To analyse viral distributions at a finer resolution, another specifically designed micro-sampler, capable of obtaining sub-samples at a spatial resolution of 1 cm, was also employed. This pneumatically operated device consisted of a linear array of 1 ml syringes, allowing for the collection of $600 \mu\text{l}$ sub-samples, and was used to obtain micro-scale vertical profiles from a depth of 0.5 to 14.5 cm above the sediment surface.

At the St Kilda site, bulk physical conditions (temperature, salinity) were measured using a Hydrolab Data-Sonde[®] 4a. A Sontek Acoustic Doppler Velociometer (ADV) was also employed to characterize the hydrodynamic conditions at the site, and flow velocities obtained with the ADV were applied to calculate the shear velocity (u_*) above the sediment surface, to allow for an estimation of the extent of resuspension of bottom sediments and organisms, as a consequence of tidal or wind induced water movement (Wainright, 1990). The ADV measurements 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):

$$u_* = \frac{\kappa u}{\log(30d/k_b)} \quad (1)$$

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 $75 \mu\text{m}$, from previous measurements made in the St Kilda site (South Australian Environmental Protection Authority, 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):

$$\delta_v = \frac{11.6 \mu}{\rho u_*} \quad (2)$$

$$\delta_d = \frac{2 \mu}{\rho u_*} \quad (3)$$

where μ is the dynamic viscosity (approximately $0.97 \times 10^{-3} \text{ Kg m}^{-1} \text{ s}^{-1}$ in seawater at 25°C) and ρ is the

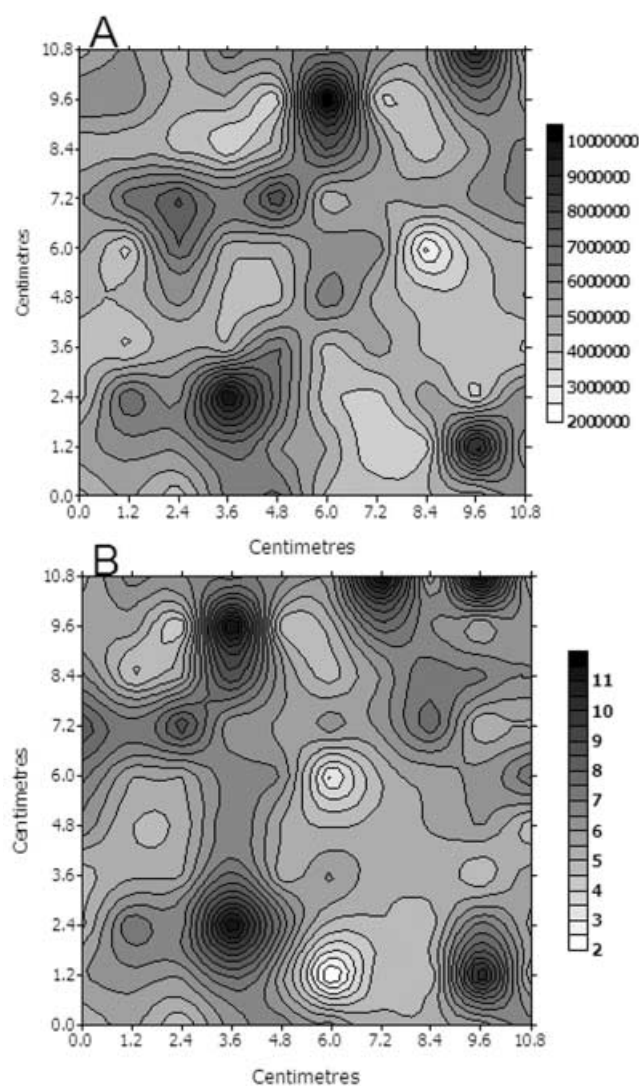


Figure 2. Microscale distributions in Southern Ocean sample ($60^{\circ}45'S$ $81^{\circ}40'E$). (A) Total viruses. Scale bar represents number of viruses ml^{-1} ; and (B) virus:bacteria ratio.

density of seawater (approximately $1.024 \times 10^3 \text{ Kg m}^{-3}$ at $25^{\circ}C$).

Sample preparation and analysis

In all environments, samples for viral enumeration were fixed with 2% (final concentration) paraformaldehyde and quick frozen in liquid nitrogen. Viruses were enumerated in all samples using flow cytometric (FCM) analysis. Prior to flow cytometry, samples were quick thawed, diluted (1:10 or 1:50) in $0.02 \mu\text{m}$ filtered TE buffer (10 mM Tris, 1 mM EDTA [pH 7.5]), stained with SYBR-I Green (5:100,000) (Molecular Probes) and incubated in the dark for 15 min (Marie et al., 1999a,b). Samples obtained from the Southern Ocean were also heated to $80^{\circ}C$ for 10 min during SYBR-I Green incubation, as heating has recently been shown to optimize FCM viral counts (Brussaard, 2004). Fluorescent beads of $1 \mu\text{m}$ diameter (Molecular Probes, Eugene, Oregon), were added to all samples in a final concentration of approximately 10^5 beads ml^{-1} (Gasol & del Giorgio, 2000), and

all measured cytometry parameters were normalized to bead concentration and fluorescence.

The EEC samples were analysed using an Epics Elite flow cytometer (Beckman Coulter Inc., Fullerton, CA, USA), while the SO and St Kilda estuary samples were analysed using a Becton Dickinson Facsanto flow cytometer. For all samples, forward scatter (FSC), side scatter (SSC), and green (SYBR-I) fluorescence were acquired, and sample acquisition was run until at least $50\text{--}100 \mu\text{l}$ of sample was analysed at an event rate of $<800 \text{ s}^{-1}$. Individual virus and heterotrophic bacterial populations were separated according to variations in SYBR-Green fluorescence and SSC (Marie et al., 1997, 1999a,b; Brussaard, 2004). 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.

RESULTS

Samples were obtained from three habitats of widely divergent physical and chemical characteristics (Table 1). The habitats studied were also characterized by dissimilar abundances of microbial populations, with bulk concentrations of chlorophyll-*a*, bacteria and viruses all varying by over two orders of magnitude between sampling environments (Table 1).

Flow cytometric discrimination of viruses

In all SYBR Green stained samples, it was possible to discriminate multiple populations of viruses and heterotrophic bacteria. 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 (Figure 1A) and clusters in biparametric cytograms (Figure 1B) in all samples from each of the habitats. These populations differed in relative abundance according to sample location, with the V1 population representing 81, 27 and 76% of the total virus community in the SO, EEC and St Kilda environments respectively.

The heterotrophic bacterioplankton community could also be divided into separate subpopulations according to differences in SYBR Green fluorescence (DNA content) and cell side scatter (Figure 1B). In all samples the community could be divided into at least two clear clusters of cells, corresponding to the high DNA (HDNA) and low DNA (LDNA) populations typically observed in aquatic samples (Gasol et al., 1999). Here the proportion of HDNA bacteria was applied as a proxy measure of bacterial activity according to previous experimental observations (Lebaron et al., 2001, 2002), and represented 65, 92 and 61% of the total bacterial community in the SO, EEC and St Kilda sites respectively.

Microscale virus distributions in the Southern Ocean

Mean concentrations of viruses and bacteria differed slightly between the three SO sample stations employed (Table 1). However, at each site the virus population exhibited marked microscale patchiness, with coefficients

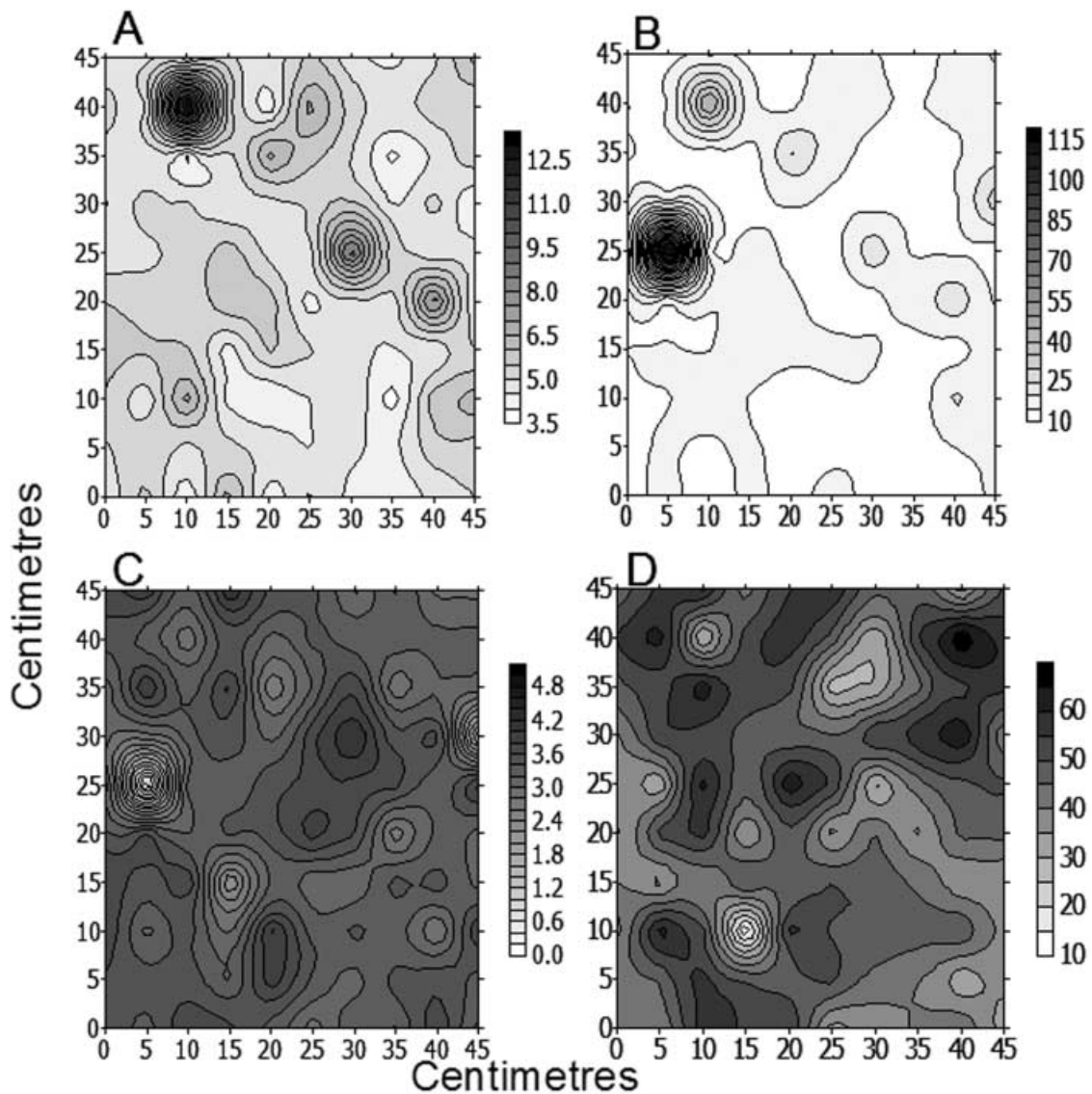


Figure 3. Microscale distributions in eastern English Channel sample. (A) Total viruses. Scale bar represents total viruses $\times 10^7 \text{ ml}^{-1}$; (B) virus:bacteria ratio; (C) heterotrophic bacteria. Scale bar represents total bacteria $\times 10^6 \text{ ml}^{-1}$; and (D) chlorophyll-*a*. Scale bar represents $\mu\text{g l}^{-1}$.

of variation $>25\%$ and changes in total viral abundance of between 4.2 to 5.5-fold observed in all 2-dimensional sample-sets (Table 2). Significant skew and kurtosis values (Table 2), indicative of non-normal distributions, dominated by the presence of a few dense patches (or hotspots) over a wide range of lower concentrations, were also observed. Higher levels of spatial heterogeneity were apparent when the virus community was treated as two separate sub-populations, with the V1 and V2 populations varying by up to 5 and 17-fold respectively. In each of the sample sets, the distributions of the V1 and V2 populations were significantly correlated to one another (Table 2). The heterotrophic bacterial community and the proportion of HDNA bacteria also varied within microscale profiles, but always to a lesser degree than viral abundance, with bacterial abundance changing by between 1.6 and 2.6-fold within microscale profiles. However, microscale virus distributions were not significantly correlated ($P > 0.05$) to heterotrophic bacteria distributions in any of the profiles.

The virus:bacteria ratio (VBR) also exhibited patchy distributions across centimetre scales, and varied by up to 7.5-fold within microscale profiles (e.g. Figure 2B).

Microscale distributions in the eastern English Channel

In the eutrophic coastal EEC, high mean chlorophyll-*a* levels during the time of sampling were associated with a dense *Phaeocystis globosa* bloom. Unlike the other environments sampled during this study, the virus community at this site was dominated by the V2 population, which occurred in concentrations of $3.8 \times 10^7 \text{ ml}^{-1}$ and comprised 73% of the total virus community. Like the SO samples, the microscale spatial patterns of the virus community were characterized by the presence of single point hotspots in abundance, where concentrations were sometimes more than three times higher than background levels (Figure 3A). Significant skew and kurtosis values were also observed (Table 2). However, unlike the SO samples, the

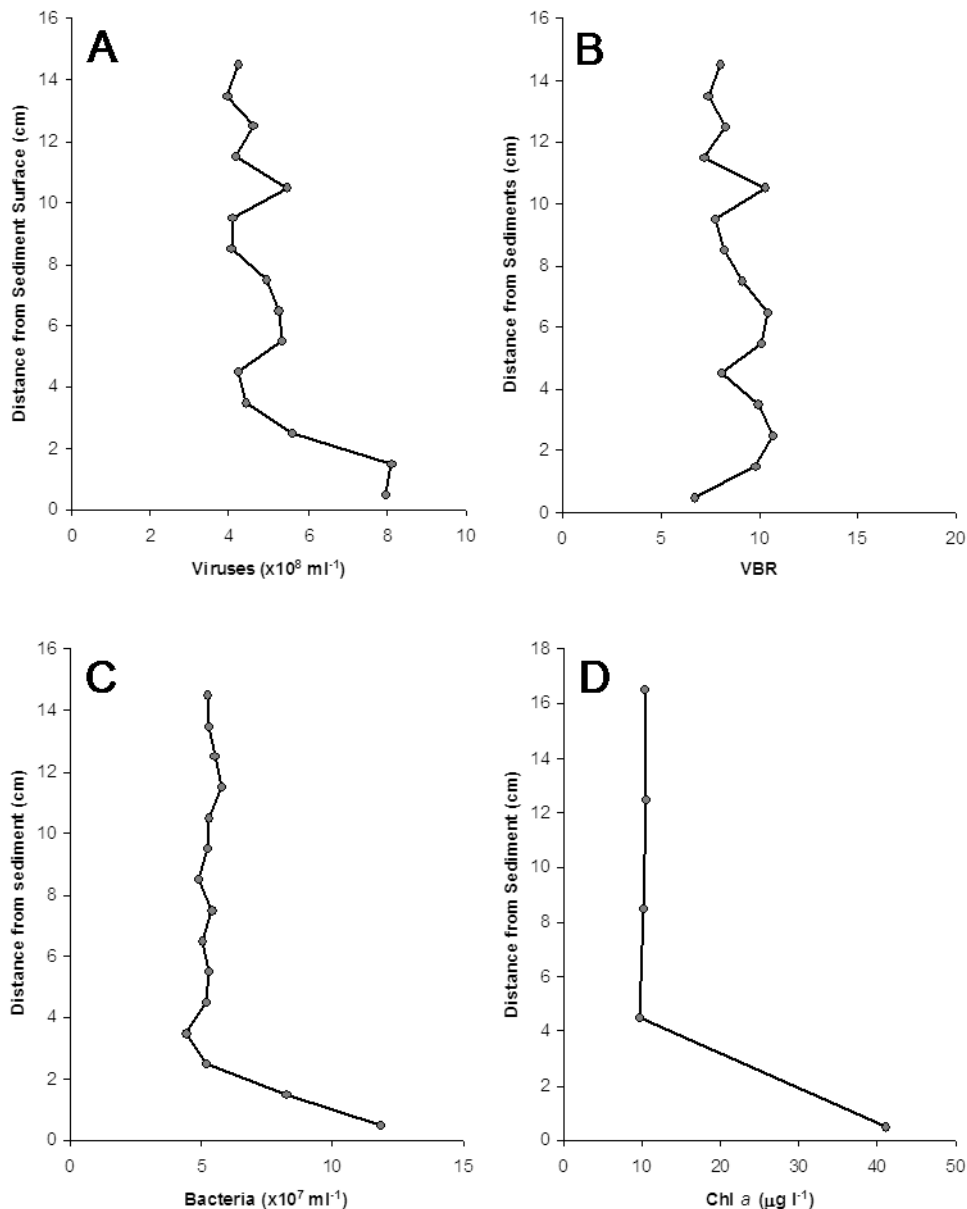


Figure 4. Microscale vertical profiles above the sediment surface in the St Kilda estuary. (A) Total viruses; (B) virus:bacteria ratio; (C) heterotrophic bacteria; and (D) chlorophyll-*a*.

two virus sub-populations exhibited significantly different spatial distributions to one another (Table 2). Heterotrophic bacterial abundance and activity (%HDNA), and chlorophyll-*a* also exhibited marked variability within the microscale profile (Figure 3C,D). Chlorophyll-*a* concentration varied by 8-fold and exhibited gradients in abundance extending over several centimetres, while the total concentration of heterotrophic bacteria varied by over 11-fold, and the proportion of HDNA bacteria varied between 78.5 and 97% within the profile. However, the microscale distributions of neither virus population were significantly correlated to any of these parameters ($P > 0.05$), and hotspots in virus abundance did not correspond to hotspots in bacteria or chlorophyll-*a*. The VBR also exhibited marked microscale variability, ranging from 9.9 to 128, and the distribution of this parameter was again dominated by the presence of hotspots that

occurred as a consequence of both peaks in virus abundance and points of low bacterial abundance (Figure 3).

Microscale distributions in the St Kilda mangrove estuary

In the St Kilda estuary, 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 (Figure 4). However, concentrations of inorganic nutrients remained homogenous in the vertical profiles (data not shown). The shear velocity (μ_*) above the sediment surface during the time of sampling was calculated to be 0.018 cm s^{-1} .

Both the virus and bacterial populations exhibited highest concentrations closest to the sediment surface (Figure 4). Virus concentrations varied by over two-fold,

with highest concentrations observed 1.5 cm from the sediment surface. The vertical distributions of the V1 and V2 populations were correlated with one another (Table 2) and to the heterotrophic bacteria ($r > 0.780$, $P < 0.05$). Neither virus population, nor total virus abundance, was correlated to chlorophyll-*a*, suspended particulate matter or the proportion of HDNA bacteria ($P > 0.05$). The VBR varied between 6.8 and 10.5 within the profiles above the sediment surface, although no clear trend in relation to the sediment surface was apparent (Figure 4).

DISCUSSION

It is now well recognized that important ecological interactions amongst microbial assemblages in the ocean are often played out across spatial scales of centimetres or less (Azam, 1998; Fenchel, 2002), and it is at these scales that many biogeochemical transformations occur within specific microzones, where conditions are markedly different to bulk phase conditions (Paerl & Pinckney, 1996; Bidle & Azam, 1999). As virus–host interactions are intrinsically controlled by host-densities, small-scale transport dynamics and organism motion (Murray & Jackson, 1992), it is likely that biological and environmental variability at the ocean's microscale could also influence the ecology of viruses in the ocean (Weinbauer, 2004). Here we have shown that the distributions of pelagic viruses are indeed often heterogenous at centimetre scales within a variety of marine habitats.

Identification of viruses

We employed flow cytometry (FCM) for the identification and enumeration of viruses in all samples, as this technique allows for very rapid and accurate analysis of viral abundance in aquatic samples (Marie et al., 1999a,b; Brussaard, 2004; Weinbauer, 2004). The FCM can also often be used to identify multiple populations of viruses, according to differences in fluorescence and light scatter (Chen et al., 2001; Larsen et al., 2004), which is not possible using epifluorescence microscopy (Hennes & Suttle, 1995; Noble & Fuhrman, 1998). In all samples analysed, we could identify two populations of viruses, corresponding to the V1 and V2 groups commonly observed in marine samples (Marie et al., 1999a). Some authors have defined these groups as low and high DNA viruses according to the differing SYBR Green fluorescence (Larsen et al., 2004), although it has been demonstrated that fluorescence intensity does not equal the viral genome size, and the separation of viral groups may instead be related to differing binding efficiencies of SYBR Green to nucleic acids (Brussaard et al., 2000). It has also been speculated that these groups may represent bacteriophages and phytoplankton infecting viruses respectively (Marie et al., 1999a). However, more recent work has indicated that most phytoplankton viruses are generally characterized by higher levels of side scatter and green fluorescence (Brussaard et al., 1999, 2005), and it has been suggested that the V1 and V2 populations probably both represent groups of bacteriophages (Brussaard et al., 2005). This is consistent with assumptions that bacteriophages represent the bulk of the virus community

in most marine habitats (Fuhrman, 1999; Wommack & Colwell, 2000).

In the SO and St Kilda samples, the V1 and V2 populations were highly correlated to one another and exhibited almost identical spatial distributions, perhaps suggesting little ecological separation between the two groups. However, within the EEC samples the two groups were not correlated and dissimilar microscale spatial patterns were apparent. While potentially ecologically important, the reasons for these differences are not clear, and further research is required to fully understand the composition and ecological role of the discrete clusters of viruses that can be discriminated using FCM.

Microscale patchiness of viruses

Both populations of viruses exhibited significant levels of microscale variability, generally characterized by non-normal distributions and the presence of hotspots in abundance. Within the St Kilda habitat, viruses exhibited relatively coherent gradients in abundance apparently associated with the sediment surface. The most immediate explanation for the heterogeneity observed at these small spatial scales is perhaps viral association with patches of host organisms. Heterotrophic bacteria are known to exhibit patchy distributions at millimetre and centimetre scales (Mitchell & Fuhrman, 1989; Duarte & Vaque, 1992; Seymour et al., 2000, 2004; Long & Azam, 2001), and in the samples obtained here bacterial abundance varied by up to 11-fold across distances of a few centimetres. At larger scales, virus abundance is generally strongly correlated to bacterial abundance and activity (Heldal & Bratbak, 1991; Boehme et al., 1993; Cochlan et al., 1993; Drake et al., 1998; Wommack & Colwell, 2000). As virus–host contact is essentially controlled by host density (Wiggins & Alexander, 1985), it may be assumed that microscale hotspots in bacterial abundance should represent micro-environments favouring bacteriophage infection and replication. However, in most samples, the spatial distribution of viruses did not correspond to bacterial abundance or activity (%HDNA), with hotspots in virus abundance not related to bacterial hotspots.

The reason for this apparent uncoupling of microbial and virus microscale spatial patterns may be related to the dynamic and often ephemeral nature of microscale heterogeneity in the ocean. Microscale patchiness of bacteria in the ocean may often be characterized by short-lived events; where chemotactic bacteria respond to a nutrient patch, the patch is depleted or diffused, and the bacteria swim away, all within the course of a few minutes to hours (Blackburn et al., 1998). In this scenario, if it is assumed that viral production is increased within the nutrient patch micro-environment, due to the increased abundance and activity of bacterial hosts, then the creation of intense patches of viral abundance may follow. As diffusion coefficients of viruses are only 1% of simple solutes, with diffusivity rates of between 1.6×10^{-8} and $3 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ (Murray & Jackson, 1992), the spread of viruses after cell lysis will be relatively low, potentially leading to strong localization of viral abundance (Bratbak et al., 1996). This level of viral diffusivity is also low in comparison to the potential movement of bacteria away from a depleted patch (e.g. up to $400 \mu\text{m s}^{-1}$; Mitchell et

al., 1995), and in this sense virus patches may represent historical remnants of bacterial patches, with viral abundances remaining high within the region of the original patch, even after the clustering bacteria have departed.

Alternatively, strong microscale patches of viruses that do not correspond to patches of bacteria, may represent regions where bacterial numbers have been depleted by increased levels of viral lysis. Where densities of bacteria are significantly enhanced within stable micro-environments, such as those attached to organic particulate material (Alldredge & Silver, 1988; Grossart et al., 2003), viruses are likely to play an important role in controlling bacterial abundance. In this instance, in the same way that viruses control blooms of dominant bacterial species at larger temporal and spatial scales (Thingstad & Lignell, 1997), viruses might control peaks in microbial abundance within specific microhabitats. More specifically, at larger scales viral abundances generally peak following a bacterial bloom (Bratbak et al., 1990; Weinbauer et al., 1995; Larsen et al., 2004). Similar microscale control of bacterial abundance by viral lysis, may explain the presence of localized cold-spots that are sometimes found in microscale distributions of bacteria (Seymour et al., 2000, 2004, 2005). However, it is notable that only some virus hotspots observed in this study corresponded to coldspots in bacterial abundance (e.g. Figure 3).

Finally, viral adsorption to particulate matter may also be a mechanism for the creation of hotspots of virus abundance. Viruses readily adsorb to colloids and suspended particles in seawater (Bitton & Mitchell, 1974; Bitton, 1975), and this may lead to the passive aggregation of viruses. This mechanism could be particularly relevant in the case of the EEC samples, as during *Phaeocystis globosa* blooms large quantities of transparent exopolymeric particles (TEP) are produced, to which up to 27% of the virus community may become attached (Brussaard et al., 2005b). Similarly, Bratbak et al. (1990) demonstrated that up to 23% of viruses were attached to the mucous layers surrounding dead or senescent diatoms during the decline of diatom blooms. Consequently, microscale aggregation of viruses may sometimes occur independently of specific virus–host spatial dynamics. Accumulation of viruses onto particulate material may, importantly, reduce viral mediated mortality amongst planktonic microbial communities, by removing free viruses from the water column (Brussaard et al., 2005). It is not clear to what extent viruses attached to particulate material can be enumerated using FCM, although Brussaard et al. (2005) predicted that a significant proportion of attached viruses may be counted using this technique following the disintegration of aggregates during the fixation process. Therefore, it is plausible that at least some of the virus hotspots observed here were derived from viral adsorption to particles.

Unlike the open-water SO and EEC samples, we observed significant correlations between bacteria and viruses, and microscale gradients rather than hotspots in viral abundance, in the few centimetres overlaying the sediment surface in the St Kilda estuary. In a previous study, similar correlations between bacteria and viruses occurred immediately above the surface of coral colonies (Seymour et al., 2005). In each of these specific cases, the

micro-environments studied represented relatively stable systems, potentially supporting gradients in microbial and chemical parameters. The shear velocity at the St Kilda site during the time of sampling (0.018 cm s^{-1}) was much lower than the critical shear velocity required for the resuspension of bacteria ($0.95\text{--}1.35 \text{ cm s}^{-1}$) and phytoplankton ($0.4\text{--}1 \text{ cm s}^{-1}$) (Wainright, 1990; Blanchard et al., 1997; Beaulieu, 2003), and we subsequently suggest that resuspension of viruses from the sediments during the sampling period was not likely. Therefore, the gradients in viral abundance observed probably developed in association with gradients in microbial abundance related to nutrient and chemical variability above the sediment–water interface (SWI). By applying the shear velocities calculated here, we estimated the thickness of the diffusive (δ_d) and viscous (δ_v) sublayers to be 1.1 cm and 6.1 cm respectively. The viscous sublayer corresponds to the region above the SWI where surface friction retards flow and viscous forces damp out turbulent mixing and is generally ~ 1 cm thick (Crawford & 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, and depending upon the environment is generally a few millimetres thick (Jørgensen & Revsbech, 1985; Jørgensen & Des Marais, 1990; Dade, 1993; Crawford & 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 materials and organisms above the SWI, and may explain the occurrence of gradients in the abundance of bacteria and viruses overlaying the SWI in the St Kilda estuary.

The dissimilar microscale spatial patterns and virus–host relationships observed between the different environments studied here suggests that microscale variability in the abundance of pelagic viruses can be generated by differing mechanisms related to specific environmental controls and ecological interactions. Nevertheless, the re-occurrence of variability at these spatial scales, within environments differing markedly in physical conditions, nutrient status and microbial abundance, indicates that microscale patchiness of planktonic viruses may be a ubiquitous feature of the marine environment.

Consequences of microscale patchiness

A major consequence of the patterns observed here, is that microbial exposure to virus infection is often not a uniform force at the ocean's microscale. This is highlighted by the microscale variability of the VBR observed here, and in contrast to the patterns observed at larger scales (Heldal & Bratbak, 1991; Boehme et al., 1993; Cochlan et al., 1993; Drake et al., 1998; Wommack & Colwell, 2000), indicates the apparent un-coupling of virus and bacterial distributions at these scales. Patchiness of viruses and host populations at scales relevant to diffusive transport and microbial motility will influence the contact rate of viruses and host particles. Virus–host dynamics have been compared and related to planktonic predator–prey systems, with bacterial and virus abundance fluctuating

in a predator–prey type of oscillation (Weinbauer et al., 1995). However, when patchy, non-uniform distributions of planktonic organisms have been considered in predator–prey models, encounter rates can be substantially modified (Rothschild, 1992). Therefore, future calculations of virus–host dynamics in the pelagic realm should consider that virus and host abundances are not necessarily uniform at the scales of interaction, and that this patchiness may influence contact rates.

Finally, in the same way that microscale variability of all physical, chemical and biological parameters, can lead to misestimates of primary and secondary production and biogeochemical transformation rates (Knauer et al., 1982; Azam, 1998; Karl et al., 2003), patchiness of viruses will potentially confound bulk analysis of viral production and virus induced mortality rates. Bratbak et al. (1996) suggested that microscale temporal variability in viral abundance may be responsible for significant differences between sample bottles. The patterns here further indicate that caution is required in relating ‘average’ conditions to the description of specific microbial interactions, as general rules and relationships derived from large-scale patterns may often not be applicable to microscale processes and dynamics (e.g. correlations between bacteria and virus abundance).

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