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Review
Viruses in Antarctic ecosystems

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Abstract: This review seeks to highlight the potential importance of viruses in Antarctic ecosystems and describe the limited scope of Antarctic virus studies to date, including studies of marine, terrestrial and freshwater communities. Although much of the existing work focuses on the microbial community, there are also studies of virus infection in Antarctic animal and plant populations. We describe methodologies available for the study of viral ecology in the field and in calling for a more intensive research effort discuss how microbial ecology might benefit from the study of viruses in Antarctic ecosystems.

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Introduction

A virus consists of protein and nucleic acid (DNA or RNA) and can only replicate within a host cell. The viral genome usually encodes structural proteins and the enzymes necessary for viral gene expression, but viruses have no intrinsic metabolism, relying on the host for all necessary precursor molecules (Singleton & Sainsbury 1993). Considerable evidence exists to suggest that viruses are key components of many microbial communities, yet relatively little attention has been paid to their role in either terrestrial or aquatic Antarctic ecosystems. There is only a limited understanding both of the occurrence and distribution of viruses in microbial ecosystems, and of the *in situ* relationships between viral and host communities in the natural environment. This is in contrast to extensive information on the biology and genetics of viruses in the laboratory.

Outside the Antarctic, the abundance, distribution and dynamics of viruses have been investigated in various environments (Cohlan *et al*. 1993, Weinbauer *et al*. 1993, Wommack & Colwell 2000, Wommack *et al*. 2000). However, Antarctic viral ecology is a subject still very much in its infancy, with only a limited number of studies to date addressing virus mediated mortality, viral community diversity and specific virus-host associations – all of which are necessary to describe viral dynamics in Antarctic ecosystems and to determine their ecological importance.

A number of comprehensive reviews have already been written which describe the role of viruses in the marine ecosystem (Wilson & Mann 1997, Fuhrman 1999, Wommack & Colwell 2000). This is because much of the research on interactions between free-living bacteria and bacteriophages (viruses which infect bacteria) has been performed in the marine environment, and these reviews contain detailed information on the ecology and molecular biology of viruses in that ecosystem. However, little information exists to date on viral dynamics in freshwater environments, although high viral abundances have been observed (Mathias *et al*. 1995, Klut & Stockner 1990) and viruses have been shown to be an ecologically significant component of these ecosystems (Hennes *et al*. 1995, Weinbauer & Hofle 1998, Wilhelm & Smith 2000). Terrestrial environments and their associated viral communities have received even less attention.

To date relatively few studies have directly addressed the role of viruses in Antarctic ecosystems, and much of the information we have about the role of viruses in the Antarctic environment comes from studies based elsewhere, which incorporate an Antarctic sample, or a sample from an Antarctic organism as part of a study of global distribution. For example, Muller & Stache (1992) studied the global distribution of virus infection in filamentous marine brown algae, in which they included an isolate from *Leptonematella* in Antarctica.

The ecological importance of viruses

An ever-growing number of studies have described the abundance and ecological importance of viruses, and it is now widely accepted that viruses are a dynamic and ubiquitous component of the planktonic microbial community in many aquatic environments (Bergh *et al*. 1989, Bratbak *et al.* 1994, Suttle 1994, Wilson & Mann 1997, Fuhrman 1999, Wommack & Colwell 2000). Virioplankton abundance has been shown to correlate well with bacterioplankton abundance and is numerically the most abundant component of the plankton (Bergh *et al.* 1989, Børsheim *et al*. 1990, Bratbak *et al*. 1990, Smith *et al*.

Table I. Selected ecological data from marine virus studies

	Value	Source
Bacteria lysed through phage infection per day	10–20%	Suttle 1994
Virus production rates	30–54% h^{-1}	Bratback *et al*. 1992.
Bacteria lysis h^{-1} to sustain this productivity	2–24%	Wommack & Colwell 2000
Maximum bacterial production consumed by viral lysis	6–12%, 30%	Wommack & Colwell 2000
Daily bacterial cell lysis by viral infection	8–26%	Wommack & Colwell 2000
Visibly infected bacteria in aquatic communities	1–4%	Wommack & Colwell 2000
Bacteria at some stage of phage infection	2–40%	Wommack & Colwell 2000
Virus-mediated bacterioplankton mortality	15–20% d^{-1}	Wommack & Colwell 2000
Viral mortality in heterotrophic marine bacteria	3–31%	Bird *et al.* 1993
Bacteria infected on a daily basis	4–13%	Suttle & Chen 1992
Marine bacteria containing mature virions	0.9–4.3%	Proctor & Fuhrman 1990
Marine bacteria containing all virions	3.3–30.5%	Proctor *et al*. 1993
Marine bacteria infected	17%	Suttle 1994
Bacterial mortality attributed to viruses	34%	Suttle 1994
Marine heterotrophic bacteria infected by viruses	20%	Suttle 1994
Bacteria lysed daily	15%	Suttle 1994
Primary production lost to viral lysis	2–3%	Suttle 1994
Number of contacts resulting in infection	2–3%	Garza & Suttle 1998
Number of virus particles in the marine system	10^6–10^9 ml^{-1}	Suttle *et al*. 1990
Host density necessary for viral propagation	10 ml^{-1}	Suttle & Chan 1994
Increase in VBR during an algal bloom	10 x	Yager *et al*. 2001
VBR in marine systems	5–25 x	Suttle *et al*. 1990
Virus induced death rates (bacteria in temperate freshwater)	10.8–43.2%	Mathias *et al*. 1995
Reduction in photosynthesis rate by virus increase	50%	Suttle *et al*. 1990
Total viral bacterial mortality in surface waters	10–50%	Suttle 1992
Total viral bacterial mortality in the absence of protists	50–100%	Suttle 1992
Reduction in eukaryotic phytoplankton productivity	2%	Suttle 1994
Mortality of bacteria (bactivory)	25%	Paul & Kellogg 2000
Mortality of bacteria (viral lysis)	25%	Paul & Kellogg 2000
Average burst size per cell lysed	few – > 500	Borsheim 1993
Proportion of dissolved DNA (viral)	17–30%	Jiang & Paul 1994

1992, Wommack *et al*. 1992, Suttle & Chan 1994, Mathias *et al*. 1995, Wommack *et al.* 1999). Indeed, it has even been demonstrated that bacterial abundance can be used to predict viral abundance (Fuhrman 1999). A range of studies have documented that viruses are typically about ten times more abundant than the bacteria, the next most abundant plankton class (Fuhrman & Suttle 1993, Wommack & Colwell 2000), and virus like particles have been reported at up to 72 times the concentration of bacteria in Arctic sea ice (Maranger *et al*. 1994).

In addition, it has been established that viruses play critical roles in the structure and function of aquatic food webs and nutrient cycles in the sea (Murray & Jackson 1993, Wilhelm & Suttle 1999). Indeed, up to 20% of marine heterotrophic bacteria have been shown to be infected by viruses (Noble & Fuhrman 1997), and up to 10–25% of the bacterial community can be lysed per day (Suttle 1994, Steward *et al.* 1996). A summary of these data is given in Table I.

Studies from outside the Antarctic show the importance of viruses in microbial ecology. It has been demonstrated for the marine ecosystem, for example, that a threshold abundance of *Synechococcus* (a cyanobacterium), occurs beyond which concentrations of cyanophages (viruses which infect Cyanobacteria) increase dramatically (Suttle & Chan 1994). It is, therefore, probable that largely species-specific virus-induced mortality could be responsible for maintaining biodiversity among the microbial community, keeping population densities of particularly successful species below critical levels. Natural selective events, such as nutrient input, can stimulate rapid growth of a specific bacterial strain, and once a critical host density has been reached, a viral 'epidemic' may occur (Wommack & Colwell 2000).

Mortality attributable to viruses may equal that due to protozoan grazing (Noble & Fuhrman 1997). Viral concentrations and frequencies of infected cells are highly significantly correlated with grazing rates, suggesting that protistan grazing may stimulate viral activity (Simek *et al*. 2001) or vice versa through grazers detecting dissolved organic matter from viral lysis. Increased viral and flagellate abundances suggest that both lysis and grazing could play a role in the phylotype-specific mortality (Riemann *et al*. 2000).

Virus populations may influence food web interactions. In one of the few Antarctic marine studies undertaken to date, prokaryotic mortality was shown to result mostly from viral infection rather than bacteriovory (Guixa-Boixereu *et al*. 2002). It has also been suggested that they may influence community productivity as virus enrichment experiments

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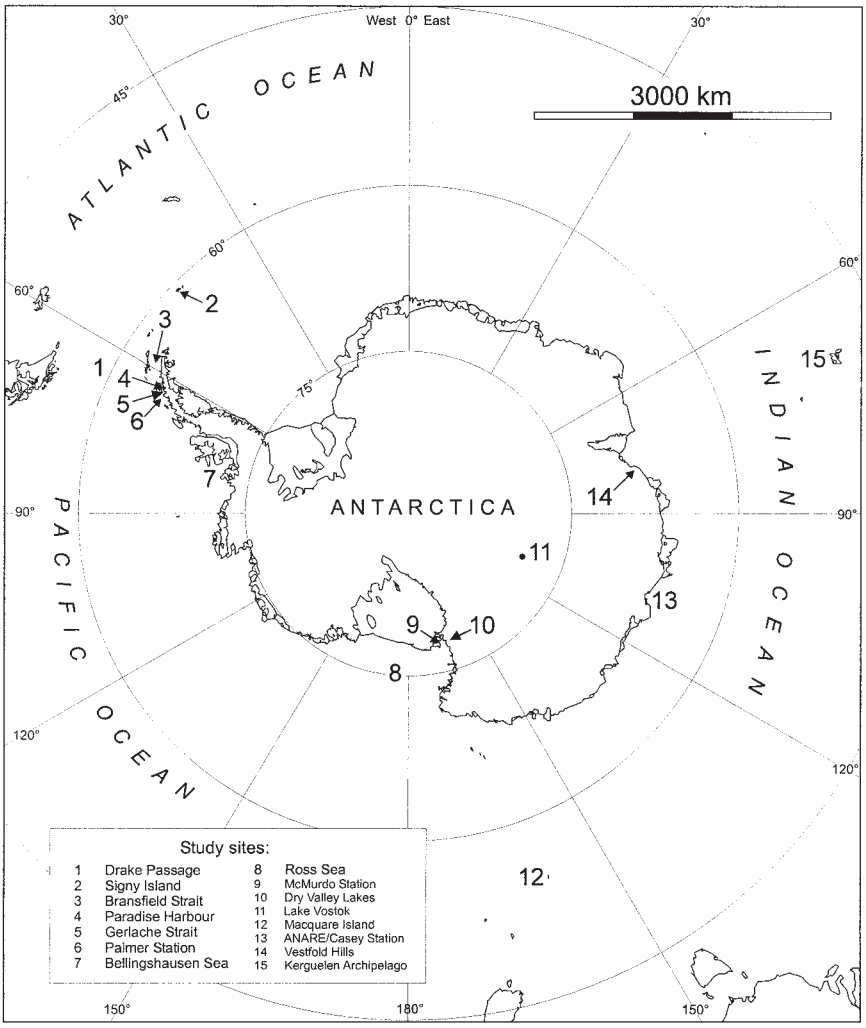

Fig. 1. Location of study sites.

show that the productivity of eukaryotic phytoplankton could be ~2% higher in the absence of viruses (Suttle *et al.* 1990, Suttle 1994). In addition, viruses may also indirectly affect the primary productivity by modifying community structure (Suttle *et al.* 1991).

Viruses are being increasingly recognized and accepted as important contributors to element cycling within the microbial loop (Wilson & Mann 1997). It has even been suggested that viruses may influence pathways of energy transfer in the system (Fuhrman 1999). Planktonic bacteria are regulated by the availability of inorganic and organic nutrients ("bottom up control"), by bacteriovorous protists ("top-down control"), and by viral lysis (Jurgens *et al.* 1999). Antarctic lakes have very low rates of phytoplankton and heterotrophic bacterioplankton production, and the role of "top-down" control of these assemblages is limited (see references in Butler 1999). This suggests that the relative ecological role of viruses in these systems might be greater than in temperate and tropical systems (Kepner *et al.* 1998).

Viruses are likely to be selective in the species or strains they infect and may, therefore, shape the species composition of bacterio- and phytoplankton host communities (see references in Yager *et al.* 2001). It is therefore possible, that virus populations could have a marked effect on host community structure. Hennes *et al.* (1995) suggested that autochthonous viruses are capable of selectively lysing abundant bacterial strains within the bacterioplankton. Conversely, the production and distribution of viruses in aquatic environments could be determined by factors which affect the productivity and density of host populations, especially the bacterioplankton (Wommack & Colwell 2000). There is also evidence that factors affecting algal distribution can affect those of the virioplankton (Wommack & Colwell 2000), and that even viruses which are present at low abundances in natural aquatic viral communities, can control microbial community structure (Hennes *et al.* 1995). Even if viruses cause only a small proportion of the mortality of a group of organisms, they can still have a profound effect on the relative proportions of different species or strains in the community (Fuhrman 1999). Indeed, in some cases the abundances of viruses and hosts appears to be positively correlated, while in other instances viruses appear to cause the apparent lysis of entire populations within the microbial

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community (Hennes *et al.* 1995). There may even be a disproportionate effect - the greatest impact of viruses on rates of photosynthesis probably occurs in specific situations, such as the demise of large monospecific phytoplankton blooms. Viral lysis will cause the release of cellular contents into the dissolved organic matter pool, which can lead to changes in both bacterial and eukaryotic community structures (van Hannen *et al*. 1999, Yager *et al*. 2001).

Many investigators have speculated that another important impact of virus infection on microbial communities may be its influence on the diversity and clonal composition of bacterioplankton and phytoplankton populations (see references in Wommack & Colwell 2000). So virus infection may be an important mechanism for maintaining clonal and genetic diversity of host populations (Fuhrman & Suttle 1993, Thingstad *et al*. 1993) as viruses control the population sizes of specific planktonic hosts (Wommack *et al*. 1999).

Weibe & Liston (1968) proposed that viral lysis and infection could both influence the growth and diversity of bacterioplankton and mediate genetic exchange. Probably the most uncertain aspect of viruses is their role in genetic exchange amongst microorganisms and its effect on short-term adaptation, population genetics and evolution (Fuhrman 1999). Also viruses may influence diversity at the population genetic level, since it has been demonstrated that natural virioplankton consortia can mediate genetic exchange among bacterial strains via transduction (virus-mediated transfer of DNA from a donor to a recipient).

What is certain, is that viral impact on host populations can be very rapid. Steward *et al*. (2000), in their study of genome size distributions in marine systems, showed marked short-term spatial and temporal variability in marine viral assemblages, with some significant shifts occurring within a period of two days or less. Extreme variability in numbers is distinctive for this component of the plankton. Such large variation in numbers suggests that the viruses are indeed active components of aquatic microbial communities (Wommack & Colwell 2000). Significant changes in abundance have also been noted over time intervals of as little as twenty minutes, suggesting that viral production may occur through a series of synchronous lysis events (Bratbak *et al*. 1996).

Studies of virus communities in aquatic microbial ecosystems across the globe, have demonstrated that viruses can influence community structure, productivity and function, they may also influence nutrient cycling and food web interactions. It has also been suggested that viruses have a role in the maintenance of biodiversity, the control of population size, the clonal composition of populations and genetic exchange. It is, therefore, likely that viruses also play a significant role in Antarctic ecosystems.

The Antarctic

Viruses in Antarctic marine microbial communities

Much of the work to date concerning virology in the Antarctic region has arisen from work in the marine system, or through the study of the global distribution of pathogenic organisms (for study locations see Fig. 1). The question of whether viruses were present in the Southern Ocean was first addressed in 1991, when Smith *et al*. (1992) investigated viral abundance with depth and latitude, and its relationship to the distribution of bacteria. It was not until 1993 that Bird *et al*. reported the first study of viral ecology in the Antarctic, on a cruise around the coastal regions of East Antarctica on the RV *Polar Duke* in 1992. In this study, they counted free-living viruses in the water column and classified them into different size classes by head capsid diameter. They then monitored population dynamics with time. They were also able to determine the virus to bacteria ratios[†] for the Southern Ocean (at between 0.7 and 6:1), when typical virus to bacteria ratios for the marine system have been quoted at between 1:1 and 50:1 (see review by Wommack & Colwell 2000). Following this work, Antarctic viruses were observed in the Drake Passage by Smith *et al*. (1992), and in the coastal waters of Paradise Harbour by Bird *et al*. (1993). The importance of the virioplankton is illustrated by its inclusion in the Palmer Long Term Ecological Research Grid (Bird *et al*. 1993). The virus concentrations and virus to bacteria ratios indicate that viruses are no less important in the Southern Ocean ecosystems than elsewhere in the world's oceans (Marchant *et al*. 2000).

Recently, Guixa-Boixereu *et al*. (2002) studied variability in the abundance of virus-like particles, virus-like particle decay rates and prokaryotic mortality caused by viral infection in three Antarctic seas: the Bellingshausen Sea, the Bransfield Strait and the Gerlache Strait. They found only small spatial variability in the three areas, but that virus-like particle abundance decreased by one order of magnitude from the surface to the bottom, in addition to low diel and seasonal variation. Comparatively recently, the concentration of virus particles was determined in the surface waters of the Southern Ocean during the spring, where no significant relationship was found between the viral abundance and either the cyanobacterial population density or the chlorophyll *a* concentrations (Marchant *et al*. 2000). In addition, a study of large viruses and infected microeukaryotes in Ross Sea summer pack ice habitats, was published earlier this year (Gowing, 2003). Significantly, the technology is now available to enable us to study viruses in much more detail (see methods section below). However, it is important to note that the viruses are not a new group.

[†]Although often quoted in studies of virus ecology, the ecological relevance of the virus to bacteria ratio has yet to be determined.

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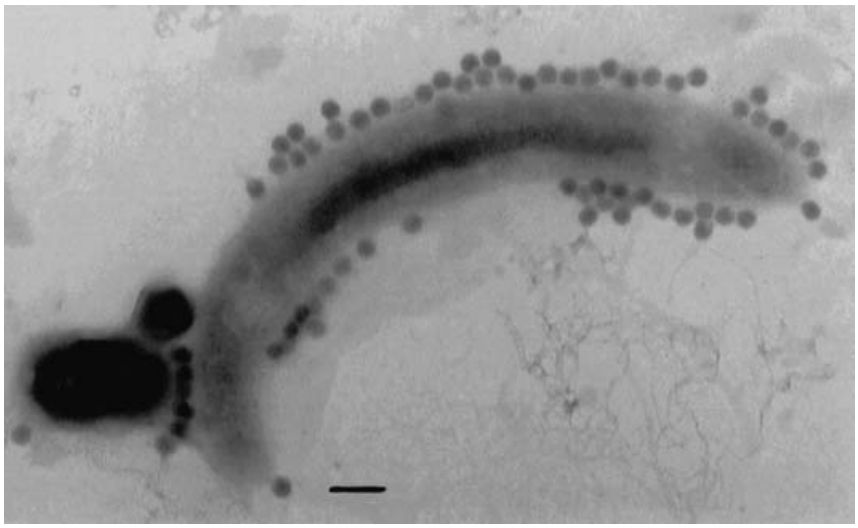

Fig. 2. A transmission electron micrograph of a bacterium or cyanobacterium and several virus particles. The sample was collected from Moss Lake on Signy Island in the South Orkney Islands, during the 1998/99 field season. Scale bar *c.* 100 nm.

Cyanobacteria are an ancient branch of evolution (Woese 1987), thought to have been well established 3.5 billion years ago (Schopf & Packer 1987). This fact alone implies that the origin of phage might even be earlier than this (Suttle 2000). Yet data for this important component of the Antarctic ecosystem is limited.

Viruses in Antarctic terrestrial and freshwater microbial communities

To our knowledge no studies of Antarctic viruses in terrestrial microbial communities have been reported in the literature, and it was not until 1998 that the first observations of viruses were made in perennially ice-covered polar lakes, when Kepner *et al*. (1998) found abundant large virus particles in Lakes Fryxell, Hoare, Joyce & Bonney in Taylor Valley, Southern Victoria Land (77º00'S, 162º52'E). Yet Maranger & Bird (1995), working on freshwater lakes in Quebec, have shown that virus to bacteria ratios are often significantly higher in freshwater than in marine systems. Antarctic lake systems are of particular interest, as perennially ice-covered Antarctic lakes are ecosystems that are dominated by microorganisms and exist at environmental extremes. It is, perhaps, surprising that such limited observations exist, given the heightened interest in the possibility of the discovery of novel microorganisms in subglacial Lake Vostok (Karl *et al*. 1999). Antarctic lakes in general may be a reservoir of previously undescribed viruses that possess novel biological and biochemical characteristics.

More recently, the number of studies of viruses in Antarctic ecosystems has increased. Villeneuve & Mueller (2000) studied the distribution of viruses in microbial mats in meltwater ponds and streams on the McMurdo Ice Shelf, and in cryoconite communities. In the Dry Valley Lakes, as mentioned above, Kepner *et al*. (1997, 1998), found that the ratio of virus-like particles to heterotrophic bacteria was higher than those reported for many other aquatic systems. They went on speculate that viruses constitute an important food web component in these lakes. The abundance of planktonic virus-like particles was also determined for other Antarctic lakes (Lyons *et al*. 1997). Further north on Signy Island, Wilson *et al*. (2000a) examined water samples from a range of freshwater lakes of different trophic status, using transmission electron microscopy (Fig. 2). It was found that virus-like particles were ubiquitous, morphologically diverse and abundant, and occurred at high concentrations. Laybourn-Parry *et al*. (2001) determined virus like particle abundances in nine freshwater to saline lakes in the Vestfold Hills, East Antarctica. They revealed a range of morphologies, but no correlation was found between virus-like particles and either bacterial numbers or chlorophyll *a* concentrations. They went on to speculate that as the plankton in Antarctic lakes is almost entirely made up of bacteria and protists, and that virus-like particle abundances are high, it is likely that viruses play a pivotal role in cycling carbon in these extreme ecosystems.

In spite of the observation that there are abundant viruses in Antarctic lakes, and that viruses may contribute significantly to bacterial mortality in planktonic systems (Noble & Fuhrman 1997), little information exists on the role of viruses in structuring microbial assemblages in such natural environments. Such studies would be of particular interest in the Antarctic, as many of the freshwater ecosystems in the Antarctic are dominated by the microorganisms. It is likely, therefore, that viruses may have a larger effect on bacterial mortality in these unique systems than in lower latitude systems with a higher trophic complexity. In addition, the relative ecological role of extreme environmental conditions, such as UV on virus communities, might be greater than in temperate or tropical latitudes.

Viruses in Antarctic animal populations

Viral diseases spread over a wide geographical range (Harder *et al.* 1991), and as such Antarctic organisms, particularly birds, mammals and fish are often included in epidemiological studies.

Penguin populations. Antarctic penguins appear to be relatively free of infectious diseases, although there is serological evidence of infection with a number of avian diseases found elsewhere (Morgan & Westbury 1981, Morgan & Westbury 1988, Austin & Webster 1993, Clarke & Kerry 1993). Gardner *et al*. (1997) reported evidence for the occurrence of a specific avian pathogen, infectious bursal disease virus (IBDV), in wild Antarctic penguins. This raises concern for the conservation of avian wildlife in Antarctica. The presence of seroreactors has been observed near centres of human activity, and raises the possibility that the virus may have been introduced. IBDV is a pathogen of domestic chickens (*Gallus domesticus*), but antibodies have been detected in a variety of wild aquatic bird species (Wilcox *et al*. 1983). It affects lymphoid organs, primarily the bursa of fabricius in chicks, which is the main site for the development of antibody producing B-lymphocyte populations. IBDV was found in both emperor (*Aptenodytes forsteri* Gray) and Adélie (*Pygoscelis adeliae* (Hombron & Jacquinot)) penguins. Serum antibodies to influenza A viruses and paramyxoviruses have also been detected in Adélie penguin (*Pysoscelis adeliae*) and Antarctic skua (*Stercorarius skua maccormicki* (Saunders)) sera in the Ross Sea Dependency. In addition, virus infection has been detected in sub-Antarctic penguins on Macquarie Island in the Southern Ocean (Morgan *et al*. 1981) and serological evidence for the king penguin, Aptenodytes patagonicus, in the Crozet Archipelago, has been found for antibodies to infectious bursal disease, influenza A and Newcastle disease virus (Gauthier-Clerc 2002).

Seal populations. Phocine distemper virus (PDV) of the genus *Morbillivirus*, is believed to have been responsible for the deaths of over 18 000 harbour seals (*Phoca vitulina* (L.)) and a small number of grey seals (*Halichoerus grypus* Fabr.) in Northern Europe (Heidejorgensen *et al*. 1992). The origin of PDV remains unknown, but evidence of prior infection has been found in both Arctic and Antarctic seal populations (Have *et al*. 1991, Heidejorgensen *et al*. 1992). Antibodies against European phocine herpesvirus isolates have also been detected in the sera of Antarctic seals (Harder *et al*. 1991). The detection of neutralizing antibodies to phocine, feline and canine herpesviruses in sera of two Antarctic pinniped species, Weddell (*Leptonychotes weddelli* Lesson) and crabeater seals (*Lobodon carcinophagus* (Hombron & Jacquinot)) was reported by Harder *et al*. (1991) and Stenvers *et al*. (1992). In addition, antibodies to canine distemper virus were found in Antarctic seals by Bengtson *et al*. (1991). Harder *et al*. believe that the nomadic movements of crabeater seals rather than the sedentary life of Weddell seals could assist in spreading viruses over a wide geographical range. Further details of the molecular biology of these viruses are given by Lebich *et al*. (1994), Harder & Osterhaus (1997) and Harder *et al*. (1998).

Other examples of viruses found in Antarctic animal populations cited in the literature include evidence of orthomyxoviruses and paramyxoviruses (Austin & Webster 1993), the discovery of new strains of murine cytomegalovirus isolated from wild mice (Booth *et al*. 1993) and the isolation of arboviruses including a new flavivirus and a new bunyavirus from *Ixodes uriae* collected at Macquarie Island (Doherty *et al*. 1975, St. George *et al*. 1985).

Viruses in Antarctic human populations

The isolation of research staff for long periods of time in close proximity to their colleagues, and a reduction in the number of potential challenges to the immune system, has led to a number of immunity and viral studies among Antarctic researchers, since stressful environmental conditions are a major determinant of immune reactivity. Much work was conducted in this area in the late 1970s and early 1980s and is reviewed elsewhere.

Recent contributions to this area include a study of Australian National Antarctic Research Expedition populations exposed to prolonged periods of isolation in the Antarctic. Tingate *et al*. (1997), documented alterations of T cell function, including depression of cutaneous delayed-type hypersensitivity responses and a peak 48.9% reduction of T cell proliferation to the mitogen phytohaemagglutinin, during a nine-month period of isolation. These findings have important long-term health implications.

Prolonged Antarctic isolation was also associated with altered latent herpesvirus homeostasis, including increased herpesvirus shedding and expansion of the polyclonal latent Epstein-Barr virus-infected B cell population. In one such study, it was found that Epstein-Barr virus reactivation could be associated with diminished cell-mediated immunity in Antarctic expeditioners (Mehta *et al*. 2000). Epstein-Barr virus reactivation and cell-mediated immune (CMI) responses were followed in sixteen Antarctic expeditioners during winter-over isolation at two Australian National Antarctic Research Expedition stations. Delayed-type hypersensitivity (DTH) skin testing was used as an indicator of the CMI response, which was evaluated twice before winter isolation and three times during isolation. A polymerase chain reaction (PCR) assay was used to detect Epstein-Barr virus DNA in saliva specimens before, during and after winter isolation. The probability of Epstein-Barr virus shedding increased from 6% before or after winter isolation to 13% during the winter period. Epstein-Barr

virus appeared in saliva during winter isolation more frequently when DTH response was diminished than when DTH was normal. The findings indicated that the psychological, physical, and other stresses associated with working and living in physical isolation during the Antarctic winter result in diminished cell mediated immunity and to an accompanying increased reactivation and shedding of latent viruses (Mehta *et al*. 2000, Lugg & Shepanek 1999, Tingate *et al*. 1997).

Elsewhere, (Shult *et al*. 1991) studied the transmission and susceptibility of the population to Adenovirus-21 (Ad-21) infection during a 1977 outbreak at McMurdo station. The unique circumstances at McMurdo allowed 125 men from the US to join and intermingle with 75 men who had wintered for six months in complete isolation. For an additional five week isolation period, respiratory illness etiology and transmission were monitored in the combined population. A total of 89% of the population were susceptible to Ad-21 but only 15% were infected. Despite a harsh environment and frequent prolonged gatherings of susceptible personnel, a respiratory virus type with known epidemic potential was surprisingly difficult to transmit. Such investigations are important for understanding the physiological and psychological disturbances that occur during long periods of confinement.

Viruses in Antarctic plant populations

Very little work has been published on Antarctic higher plant virus communities and this may reflect the limited extent to which higher plants have colonised the Antarctic continent. To our knowledge there is only one direct study of viruses in higher plants in an Antarctic ecosystem, and that was on a sub-Antarctic island. Skotnicki *et al*. (2003) describe the first sub-Antarctic plant virus from Macquarie Island, a *Stilbocarpa* mosaic bacilliform badnavirus (SMBV), as the first vascular plant virus reported from any of the remote sub-Antarctic islands, and it represents the southernmost plant virus found anywhere.

Antarctic viruses in ecological studies

Economic interest in the potential presence of novel biochemicals with pharmaceutical or industrial application, has led to increased bioprospecting, and the screening of many Antarctic compounds for useful properties. These searches have led to the discovery of new compounds, for example: sulphated polysaccharides from the giant kelp *Durvillaea antarctica* (Chamisso) Hariot (Matsuhiro *et al*. 1996), and cytotoxic and viricidal trisulphated triterpene glycosides from the Antarctic sea cucumber (*Staurocucumis liouvillei* (Vaney)), which have been shown to be active against herpes simplex virus type I (HSV-I) at concentrations below 10 µg ml^{-1} (Maier *et al*. 2001). In addition, the efficient and direct resolution of cis-1-amino-2-indanol using *Candida antarctica* lipase B catalysing the alcoholysis of its N, O-diacetyl derivative was also reported (Anilkumar *et al*. 1999). A study of the immune response of the Anatarctic teleost *Notothenia rossi* to the bacteriophage MS2 was reported by O'Neill (1981), illustrating the potential significance of viruses to the Southern Ocean fisheries and bacterial ice nucleation activity after T4 bacteriophage infection was investigated by Kozloff *et al*. (1992).

Although strictly outside the area covered by the Antarctic Treaty, another potential economic interest arose through the use of viruses in the biological control of introduced rabbits. Sobey *et al*. (1973) reported the release of the European rabbit flea, *Spilopsyllus cuniculi* (Dale), as a possible vector for myxomatosis, on Macquarie Island in 1968. More recently, an experiment was conducted on an island of the Kerguelen archipelago in January and December 1987. Despite a small founding population, the species succeeded in establishing itself. Three years after introduction, the rate of spread was approximately 600 m between 1990 and 1993. Despite the sub-Antarctic climate of Kerguelen, the burdens were similar to those noted in habitats favourable to this ectoparasite. The circulation of myxoma virus, present on this island for several decades, has been favoured by the presence of the flea. As a result, the proportion of rabbits with antibodies rose from 34% before the introduction of the flea to 85% in 1998 (Chekchak *et al*. 2000). In addition, the success of an attempt to control of the feral cat *Felis catus* through the introduction of a viral disease on Marion Island was evaluated by Aarde (1984) and Howell (1984). Sub-Antarctic islands with their low biodiversity and high rates of endemism, provide ideal models for the assessment of natural biological control methods.

High latitude / high altitude studies elsewhere

Some indication of the potential importance of virus communities in Antarctic ecosystems can be determined from virus studies in high latitude/high altitude communities elsewhere, particularly the Arctic and alpine systems. However, it should be stressed that there is a fundamental difference as the Antarctic is surrounded by the Southern Ocean and is isolated, whereas the Arctic and alpine regions are surrounded by land, which permits the free movement of organisms and consequent gene flow.

The potential exists in high latitude systems for the discovery of novel viruses such as the arctic squirrel hepatitis virus (ASHV) - a member of the hepadnavirus family, which is endemic in wild arctic ground squirrels (*Spermophylus parryi kennicotti*) in Alaska (Testut *et al*. 1996). Of particular concern recently, was the discovery of 15 strains of Tomato Mosaic Tobamovirus (ToMV) in Arctic ice cores from three sites in Greenland, as glacial ice is an enormous repository of airborne microorganisms

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deposited from lower latitudes over long time periods (Abyzov 1993). The plant pathogenic tobamoviruses are extraordinarily stable viruses with a wide host range. Castello *et al*. (1999) detected tomato mosaic tobamovirus RNA by reverse-transcription polymerase chain reaction amplification in glacial ice sub-cores < 500 to *c*. 140 000 years old from drill sites in Greenland. They observed that sub-cores contained multiple tomato mosaic tobamovirus sequences nearly identical to contemporary ones. Detection of tomato mosaic tobamovirus in ice raises the possibilities that stable viruses of humans and other hosts might be preserved there, and that entrapped ancient viable viruses may be continually or intermittently released into the modern environment or lead to lethal epidemics (Baker 1999, Davies 1999, Walker 1999). These releases, if extensive, could affect gene flow, RNA evolution, measurements of mutation rates, and disease outbreaks via the release of virulent virus strains. Depending upon the degree of genotype recycling, calculations of evolutionary distances and mutation rates based on contemporary genotypes may be inaccurate and misleading. They suggest a recently developed immunocapture RT-PCR assay for ToMV (Jacobi *et al*. 1998) would allow the detection of intact virions in ice, to distinguish it from potential fragmented virons or naked viral RNA.

A number of epidemiological studies have been conducted on viruses of both aquatic and terrestrial Arctic animals, such as infectious hematopoietic necrosis virus (IHNV) and infectious pancreatic necrosis virus (IPNV) in the Arctic char (*Salvelinus alpinus* L.) (McAllister *et al*. 2000) and morbillivirus infections of polar bear populations (*Ursus maritimus* Phipps) (Garner *et al*. 2000). Of particular concern in this context is the potential for cross species transfer, for example, morbillovirus infection from dogs to seals.

Bio-monitoring programmes have been established in Pacific walrus (*Odobenus rosmarus divergens* (Illeger)) populations to facilitate understanding and detecting trends in marine mammal populations (Calle *et al*. 2002). Of particular importance to the Arctic (with an indigenous human population) is the transfer of rabies infections by the Arctic fox (*Alopex lagopus*) (Arai *et al*. 1997, Ballard *et al*. 2001, Nadin-Davis 1998, Follmann *et al*. 2002).

Human populations in the Arctic have also been used for the study of rabies transmission (Kuzmin 1999), the human polyomavirus JC virus (Sugimoto *et al*. 2002) and influenza infection of the Inuit (Nielsen *et al*. 2001), in addition to specific studies such as the introduction of organisms to the Antarctic on tourist boots (Curry *et al*. 2002) and virus surveillance within a semi-isolated Arctic military community (Fulton *et al*. 1984). Predictions of the changes in tick-borne encephalitis virus (TBEv) distribution under the influence of climate change suggest that TBEv may be driven into increasingly high latitude and high altitude regions, until by the 2080s it may become confined to parts of Scandinavia (Randolph 2002). In terms of human exploitation, the transformation (uptake of exogenous DNA) of Arctic bramble (*Rubus arcticus* L.) by *Agrobacterium tumefaciens* has illustrated the potential for biotechnological applications (Kokko & Karenlampi 1998).

The role of viruses in ecosystem function has also received some attention in Arctic ecosystems, where viruses have been found to be a ubiquitous and dynamic feature and a significant source of bacterial mortality in Arctic marine microbial communities (Steward *et al*. 1996). Viral and bacterial dynamics in Arctic sea ice were studied by Maranger *et al*. (1994) during the spring algal bloom near Resolute, North Western Territories, Canada. High virus counts were found in Arctic sea ice samples taken during the bloom. They also found disequilibrium between phage and bacterial growth and abundance at the bloom maximum, and attributed this to a change in the bacterial community structure or to an increase in viral lytic activity. In addition, they found that virus to bacteria ratios decreased as bacterial abundance increased, but perhaps the most astounding finding was of extremely high VBRs (72:1) in this environment. A similar study of an Alaskan Arctic algal bloom by Yager *et al.* (2001) showed that peak virus to bacteria ratios coincided with observed shifts in microbial activity and community structure. They suggest that substrate, host and virioplankton interactions may control the microbial carbon cycling of the coastal Alaskan Arctic. The occurrence of a rapid increase in viral abundance during the decline of planktonic and bacterial and algal blooms indicates that virus infection might be an important source of bacterial and algal mortality in the sea (Bratbak *et al*. 1990).

However, Pina *et al.* (1998) suggested that considering the low activity and bactivory rates in alpine lakes, even a small mortality rate caused by viruses may have a considerable impact on the bacterioplankton community structure. Even the study of subsurface viruses and bacteria can provide clues to the likely behaviour and activity of viruses in Antarctic systems, as has been shown in the demonstration of the presence of viruses in sediment cores (Bird *et al*. 2001). Of interest from studies of these systems which may be of relevance to the Antarctic, is that at all levels of viral-induced mortality, the greatest impact of viral infection occurred under oligotrophic conditions (Murray & Eldridge 1994), exactly the type of systems typical of the Antarctic. Conversely, a higher virus to bacteria ratio has been demonstrated for eutrophic waters than oligotrophic waters (Bratbak *et al*. 1990). Whichever way we might extrapolate these findings, however, there is no real alternative to direct *in situ* studies involving the environments in question.

So why study Antarctic viruses?

As demonstrated in the studies outlined so far, viruses have

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been shown to influence many biogeochemical and ecological processes, including nutrient cycling, particle size distributions, bacterial and algal biodiversity and species distributions, algal bloom control and genetic transfer. Long periods of geographic isolation may have led to isolated communities with low levels of biodiversity and potentially high rates of endemism. As a result, Antarctic viruses may have evolved with a restricted potential host range. The Antarctic may, therefore, provide a good source of novel organisms and model virus-host associations (which could act as reservoirs of disease), particularly the potential seed bank trapped in glacial ice and within sub-glacial lakes. Existing studies of Antarctic virus populations, particularly as outliers in epidemiological investigations should be extended to address the theory of the ubiquity of microorganisms. This could also be applied through extending the use of latitudinal transects.

Viruses in Antarctic ecosystems are subject to intense and specific selection pressures, including isolation, temperature, nutrient limitation, desiccation and non-ionizing radiation. Combinations of specific environmental stresses on microbial community structures of relatively low complexity can be used in studies of global climate change. In the physical environment, solar UV radiation is known to have a strong impact on the loss of infectivity of free viruses (see review by Jeffrey *et al*. 2000). Infectivity can be sensitive to solar radiation (Suttle & Chen 1992, Wommack *et al*. 1996), which can be high under the Antarctic ozone hole. For comprehensive reviews of the early literature on viral inactivation, see Akin *et al*. (1971) and Kapuscinski & Mitchell (1980). It has also been shown (Murray & Jackson 1993) that increased UV-B irradiance associated with stratospheric ozone depletion could significantly change viral mortality in polar environments.

Antarctic microbial communities are of relatively low complexity with short food chains and limited biodiversity. Such communities are particularly amenable to the study of ecosystem function. The Antarctic, in addition, has a historical record preserved in ice cores, which can be used to study the impacts of local versus global pollution and of other human activities.

Methods to study Antarctic viruses

As viral methodology has developed, an opportunity has arisen for a variety of novel studies of Antarctic virus ecology and community interactions in the field. Whilst techniques such as the DNA specific stains SYBR Green I (Noble & Fuhrman 1998) and SYBR Gold (Chen *et al*. 2001), epifluorescence of DAPI stained cells, plaque assays and transmission electron microscopy (TEM) are being used increasingly, new molecular biological techniques are now starting to be applied to virus ecology, though have yet to find extensive use in the Antarctic. A variety of approaches including enumeration of visibly infected microbes, removal of viral particles, decay of viral infectivity, and measurements of viral production rates have been used to infer the impact of viruses on microbial mortality (Suttle 1994). Transmission electron microscopy has evolved into the benchmark for virus enumeration, often in combination with ultrafiltration. The combination of extensive observations of viruses within bacterioplankton cells and incorporation of a radiolabelled tracer into virioplankton (Steward *et al*. 1992) provides direct evidence that viral infection and lysis occur. The most sensitive assays, however, involve titers of infectious viruses, but require isolation and culture of specific host strains (Wommack *et al*. 1999) or ELISA with monoclonal antibodies (Kulonen *et al*. 1991).

Pulsed-Field Gel Electrophoresis (PFGE) has already been used in a marine study, including samples from the Arctic Ocean and the Ross Sea in the Antarctic (Steward *et al*. 2000), to determine genome size distributions. They found that the vast majority of detected virus-like genome sizes were typical of bacteriophages, and that only a few percent were in the size ranges of known algal viruses. Using PFGE it is possible to analyse the community structure of virioplankton populations. Individual virus strains within a virioplankton concentrate are electrophoretically separated according to genome size. After PFGE, virioplankton DNA within the agarose gel is stained with a fluorescent dye, resulting in a banding pattern in which each band represents a sub-population of viruses with a particular genome size. Using a radiolabelled probe, it has been possible to measure the detection limit with PFGE at 10^5 virus particles (Wommack *et al*. 1999). Another strategy for the detection of viruses in environmental samples is through reverse transcriptase PCR (RT-PCR) (Abbaszadegan *et al*. 1999), restriction fragment length polymorphism (RFLP) and hybridization analysis (Kellogg *et al*. 1995). Both the polymerase chain reaction (PCR) and RT-PCR (reverse transcription PCR) have been used to detect and identify DNA and RNA plant viruses (see references in Skotnicki *et al*. 2003).

As recently as 1999, methodological developments have allowed the use of PCR to examine the diversity of natural communities of viruses without the need for culture (Chen & Suttle 1995, Chen *et al*. 1996, Wilson *et al* 1999, 2000b). Algal virus specific primers have now been developed (Short & Suttle 1999), and are used to amplify polymerase gene fragments from natural virus samples which infect microalgae, before separation of the amplification products by denaturing gradient gel electrophoresis (DGGE) (Short & Suttle 1999, 2002). Group specific primers were designed to allow amplificaton of DNA-polymerase gene fragments from viruses PBCV-1 and NY-2A that infect an endosymbiotic *Chlorella* like alga (Chlorophyceae), and a virus MpV-SP1 which infects the photosynthetic flagellate *Micromonas pusilla* (Prasinophyceae) (Chen & Suttle 1995). A further primer set has now been developed for

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Cyanophages - CPS1 and CPS2, which are specific for marine cyanomyoviruses (Fuller *et al*. 1998). Such techniques are now powerful enough to allow the study of genetic variation within Antarctic virus populations. In the not too distant future, the application of microarrays to study viral gene expression will also be possible, as an understanding of the genetic diversity in phages in wider environments such as the soil, freshwater and the ocean is only just beginning (Paul & Kellogg 2000).

So it is now possible with the use of a good epifluorescence microscope with the appropriate filter sets and minimal molecular biology kit (such as a thermal cycler, a freezer, basic electrophoresis equipment and a transilluminator) to undertake many of these protocols directly in the field, particularly on scientific research stations. However, primarily because of logistic constraints and to the increased costs associated with fieldwork in the Antarctic, the Antarctic region has often been slow to benefit from the rapid advances being made in microbial ecology and in molecular biology elsewhere.

Conclusions

Very few studies of viruses in Antarctic ecosystems have been conducted to date, yet the subject has enormous potential for answering some of the very fundamental questions of microbial ecology. However, even some of the basic questions such as whether the Antarctic is especially favourable to viruses, how many viral species are present, what proportion of these viruses are infective, are viruses important for regulating host communities and can they affect genetic diversity, have yet to be answered. Only then might we be able to tackle some of the more complex ecological questions such as: are there threshold abundances for infection, what are the relative importances of lysogeny and pseudolysogeny in maintaining phage populations, do viruses have a role in the maintenance of species diversity and genetic exchange, and what is the role of the virioplankton in microbial community structure and carbon cycling?

The Antarctic, with microbial community structures of relatively low complexity, can be considered an end point of an environmental continuum. The potential exists to address themes such as genetic diversity, the worldwide occurrence and potential ubiquity of viruses, the importance of key environmental factors such as UV radiation and temperature, mortality of specific populations and modifications in community structure, along with studies of evolution and extinction, biodiversity and the effects of climate change. The next decade promises to be extremely exciting as the central role of viruses in aquatic ecosystems continues to emerge (Suttle 2000). The field as a whole is poised to make significant advances and the Antarctic offers a unique environment in which to undertake these studies.

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