Flow cytometric detection of viruses in the Zuari estuary, Goa

Viruses are the most abundant biological entities in global ecosystems and are recognized as a crucial and ubiquitous component of the microbial food web, with abundance in marine waters ranging from 10^6 ml^{-1} in the deep sea to 10^8 ml^{-1} in coastal waters and 10^9 g^{-1} of dry weight in the marine sediments[1,2], which is usually 15-fold greater than bacterial and archael abundance[3]. Viruses are the smallest known organisms (20–200 nm) with simple biological structures consisting of nucleic acid, either DNA or RNA (single- or double-stranded), with a protein coat (capsid).

Viruses are known to infect prokaryotes and microalgae, the most abundant organisms in the ocean and also microzooplankton[2]. They have been implicated in phytoplankton mortality and the decline of phytoplankton blooms[1]. Marine phytoplankton is responsible for up to half of the total primary production on earth and hence plays a critical role in global carbon cycling[4]. Until two decades ago, loss of phytoplankton in the natural environment was attributed to zooplankton grazing and sedimentation below the photic zone. This view was transformed after the identification of marine viruses as agents of mortality of photoautotrophic organisms (both prokaryotic and eukaryotic), thereby associated with the reduction in primary productivity[5,6].

Viral cellular lysis results in the release of cell constituents, thereby contributing to the dissolved organic carbon that becomes available for bacteria and hence forcing the food web towards a more regenerative pathway[7,8]; this highlights the importance of viruses in the microbial loop. Because prokaryotes and autotrophic and heterotrophic protists play pivotal roles in the biogeochemical cycles and global ocean functioning, viral infections of these groups of organism have important ecological consequences[2].

In spite of the importance of viruses in the microbial food web, there are no reports from the Indian waters, except for one where the effect of formaldehyde preservation on the viral abundance from Cochin backwaters was determined by epifluorescence microscopy[9]. A lot of information has been collected on the viral abundance from the world oceans. Amongst the three major approaches specifically adapted for the enumeration of viruses, transmission electron microscopy (TEM), epifluorescence microscopy and flow cytometry, the last one is faster and more accurate for the direct detection and quantification of viruses[10]. Flow cytometry is a high throughput method in which the fluorescent staining of nucleic acids allows virus particles to be counted, even though they are too small to scatter light in a predictable way[8]. In addition, based on the characteristics of fluorescence and light scattering, subpopulations within the viral community can be distinguished from the natural sea-water samples[11], information of which is lacking from the Indian waters. To the best of our knowledge this is the first attempt to record the presence and abundance of viruses in the Indian waters through flow cytometry.

Surface water samples of 1.8 ml were collected in duplicate in cryovials in March 2011 from 10 stations in the Zuari estuary, with salinity ranging from 34‰ to 1.5‰. After fixation with glutaraldehyde (final conc. 0.5%), the samples were kept for 30 min in the dark and then flash-frozen in liquid nitrogen and stored at –80°C until analysis (within a month). Samples were analysed using flow cytometry (BD FACSAria™ II equipped with a blue laser emitting at 488 nm). Before analysis, thawed samples were 100-fold diluted in 0.2 µm filtered, autoclaved TE-buffer (pH 8.0) and stained with SYBR Green I (final conc. 0.5×10^{-4} of the commercial stock) for 10 min at 80°C in the dark, followed by a cooling period of 5 min at room temperature before analysis[10].

Based on their relative green fluorescence and side scatter signatures, two virus subpopulations were distinguished, defined as V1 and V2, corresponding to populations observed previously in sea-water samples[11] (Figure 1). The viral subpopulation V1 had higher green fluorescence and contributed 14–21% to the total abundance. The viral subpopulation V2 which was characterized by the lowest nucleic acid green fluorescence dominated the virus community throughout the estuarine transect, making up 79–86% of the total abundance. Total viral abundance ranged from 1.0×10^7 to 2.6×10^7 cells ml^{-1}, out of which V1 ranged from 0.1×10^7 to 0.5×10^7 cells ml^{-1} and V2 ranged from 0.9×10^7 to 2.2×10^7 cells ml^{-1}. The total viral abundance was close to that reported for the Cochin backwaters (3.9×10^7 cells ml^{-1}) in India[9] and other estuaries of the world[12]. Higher abundance was recorded from the brackish waters (< 30‰), which were two times richer in viral particles compared to saline waters (Figure 2).

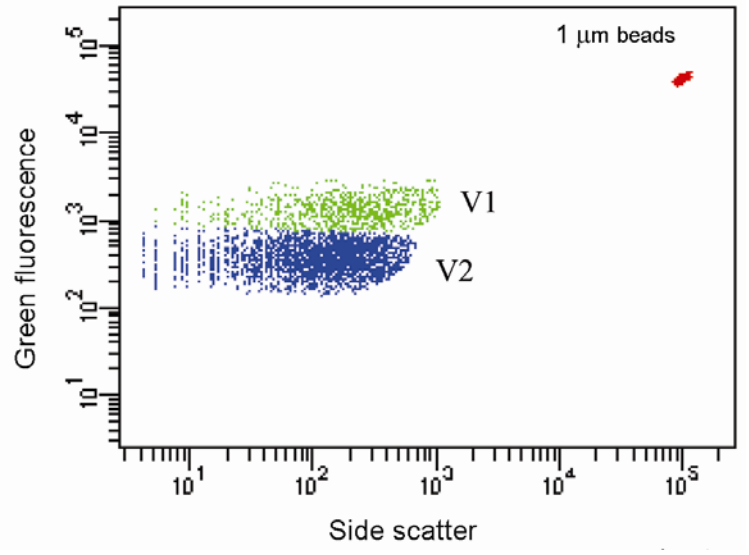

Figure 1. Flow cytometric analysis of samples collected from the surface waters of Zuari estuary on 21 March 2011. Scatter plot of side light scatter (proxy for cell size) versus green fluorescence (SYBR Green I) shows two viral populations (V1 and V2).

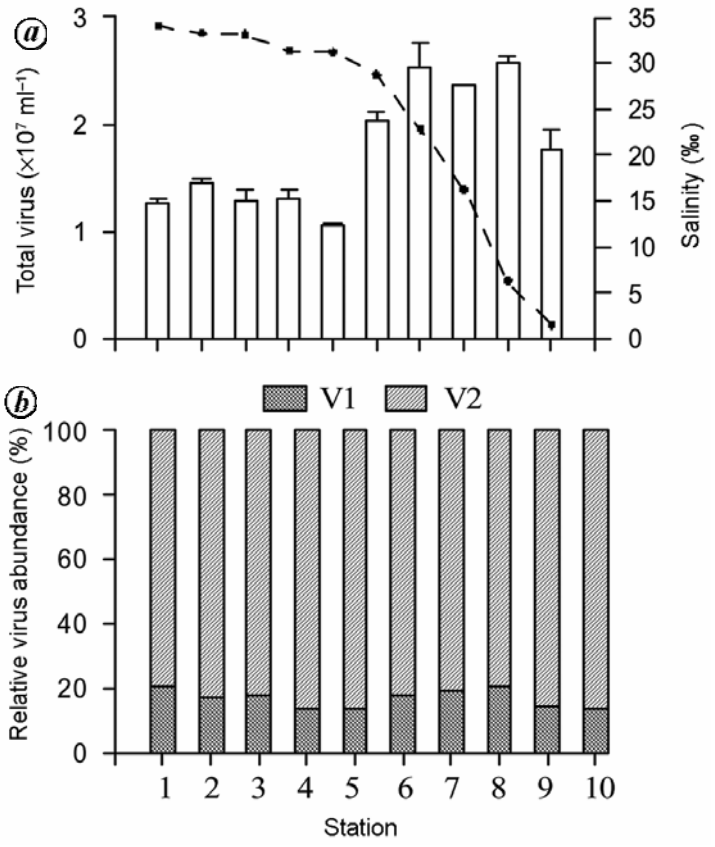

Figure 2. (***a***) Total virus abundance ($\times 10^7$ ml^{-1}) and salinity (‰) and (***b***) percentage contribution of V1 and V2 populations to the total viral abundance along the Zuari estuary. (Vertical lines in (***a***) indicate standard deviation.)

Although little is known about the composition of the marine virus community, research has shown that 20% of the V1 group, which displays the highest SYBR Green-I fluorescence intensity and 100% of the V2 viruses could pass through 0.2 μm pore-size filters, suggesting that V1 viruses are larger[9]. Also, the position of the V1 and V2 groups in the cytograms is the same as that of cultures of several marine phytoplankton phages and bacteriophages[13]. This observation is supported by the pulsed-field gel electrophoresis results of natural virioplankton samples where the most abundant genome size ranges from 23 to 97 kb, which matches that of bacteriophages[14]. This suggests that V1 viruses could be infectious to eukaryotic phytoplankton, and V2 viruses could be related to the more numerous heterotrophic bacteria and usually make up the majority of viruses within the virioplankton. In some observations additional subpopulations, V3 and V4, have been observed which represent the phytoplankton viruses[10].

Studies have shown that viruses can cause up to 30% of the bacterial mortality and 2–10% of the phytoplankton mortality in aquatic systems and also play a role in algal bloom termination[15]. Similar bacterial mortality rates have been reported from protistan grazing, thus showing that viruses cannot be ignored in studies of the microbial loop.

These observations show that viruses which are abundant in the Indian marine environment can be a major source of mortality of marine microorganisms, consequently affecting the nutrient and energy cycles as well as the microbial community structure. Unravelling the viral diversity through molecular methods will further help in understanding the complex relationships between viruses and their hosts. Hence, future studies in this region should incorporate viruses and virus-mediated processes for better understanding of the microbial food web and the biogeochemistry.

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