GMR
The Original

Environmental Surveillance Of Viral Communities In South Indian Brackish Water Ecosystems Using Sybr Green Ii Epifluorescencae Microscopy

Chitra Venkatachalam[1*], Satheesh Kumar Sabapathy[2], Chinnadurai Sivasamy[3], Senthilkumar Kalimuthu[4], Sumithra Maniraj[5], Kayalvizhi Rangasamy[6], Sudhakar Cholan[7]

[1*]Assistant Professor Department of Chemistry (PG), Vellalar College for women (Autonomous), Thindal, Erode-638012, Tamil Nadu, India. Email.chitu.sv@gmail.com
[2]Assistant Professor-Research, Saveetha Institute of Basic Medical Sciences (SIBMS), Saveetha Institute of Medical and Technical Sciences. Saveetha University, Chennai-602105, Tamil Nadu, India. Email. Satheeshkumars.sibms@saveetha.com.
[3]Associate Professor, Department of Biochemistry, The Oxford Medical College Hospital and Research Center, Yadavanahalli, Attibele Hobli, Anekal Taluk, Bangalore – 562107, Karnataka, India. Email ID: chinnsaiims@gmail.com
[4]Professor, Department of Biochemistry, Saveetha Institute of Basic Medical Sciences (SIBMS), Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-602105, Tamil Nadu, India. Email: senthilkumarkalimuthu.sibms@saveetha.com
[5]Assistant Professor, Department of Chemistry (PG), Vellalar College for women (Autonomous), Thindal, Erode-638012, Tamil Nadu, India; sumithramaniraj@gmail.com
[6]Associate Professor, Department of Science and Humanities, Sri Eshwar College of Engineering, kinathukadavu, Coimbatore-641202, Tamil Nadu, India. Email.kayalvizhi.r@sece.ac.in
[7]Ph.D Research Scholar, Saveetha Institute of Basic Medical Sciences (SIBMS), Saveetha Institute of Medical and Technical Sciences. Saveetha University, Chennai-602105, Tamil Nadu, India. Email: sumu7000@gmail.com
*Corresponding author address; Chitra Venkatachalam
*Assistant Professor Department of Chemistry (PG), Vellalar College for women (Autonomous), Thindal, Erode-638012, Tamil Nadu, India. Email.chitu.sv@gmail.com

ABSTRACT

The infectious viruses constitute a major component of microbial communities and significantly influence ecological processes through host regulation, nutrient recycling, and maintenance of microbial diversity. Brackish water ecosystems represent dynamic environments that may harbor a wide range of viral populations. Despite their ecological and potential public health importance, information on viral abundance in Indian brackish water habitats remains scanty. Therefore, the present investigation was conducted to evaluate the occurrence and abundance of viral communities in selected brackish water ecosystems of Southern India. Water samples were collected from twelve coastal and brackish water-associated locations across Andhra Pradesh and Tamil Nadu. Viral concentrates were obtained by ultrafiltration and subsequently analyzed by epifluorescence microscopy following SYBR Green II staining. Fixed samples were filtered through 0.02 µm Anodisc membranes, stained with fluorescent dye, and examined under ultraviolet excitation using a Carl Zeiss epifluorescence microscope. Virus-like particles (VLPs) were enumerated using ImageJ image analysis software and expressed as particles per mL of water. Viral particles were observed in all investigated samples, confirming the widespread distribution of viral communities in brackish water environments. The abundance of VLPs exhibited substantial variability among sampling locations, ranging from 6.1×10^2 to 7.3×10^7 viruses mL^{-1}. The greatest viral abundance was recorded in samples collected from Bhimavaram, Andhra Pradesh, whereas the lowest concentration was detected in Velankanni, Tamil Nadu. Sites associated with health and aquaculture industry activities generally displayed elevated viral loads, indicating that environmental productivity and host microbial populations may contribute to viral proliferation.

The results highlight the extensive presence and heterogeneous distribution of viral communities within Indian brackish water ecosystems. These findings provide valuable baseline data on environmental viral abundance and emphasize the importance of incorporating viral monitoring into brackish water ecosystem management programs. Furthermore, the study contributes to a better understanding of viral ecology in coastal environments and may support future biosecurity, aquaculture industry, health industry, and environmental surveillance initiatives.

Key words: Brackish Water; SYBR Green; Epifluorescence Microscopy; Virus Abundance; Environmental Virology.

INTRODUCTION

Brackish water ecosystems form transitional zones between freshwater and marine environments and support complex biological communities comprising bacteria, phytoplankton, zooplankton, shellfish, and viruses. Among these organisms, viruses are considered the most numerous biological entities in aquatic habitats and are recognized as key regulators of microbial activity, nutrient recycling, and ecosystem processes (Suttle, 2007; Rohwer and Thurber, 2009). Besides naturally occurring aquatic viruses, these ecosystems may also harbour a variety of human pathogenic viruses, including rotaviruses, noroviruses, enteroviruses, adenoviruses, and hepatitis viruses. The ability of these pathogens to persist under favourable environmental conditions raises concerns regarding their ecological and public health impacts (Bosch et al., 2008; Haramoto et al., 2018).

The occurrence and concentration of viruses in brackish water systems are governed by a combination of biological and environmental factors such as host availability, salinity, temperature, nutrient status, and sediment composition (Wommack and Colwell, 2000;Weinbauer, 2004). Brackish water habitats are especially susceptible to viral inputs due to their close association with aquaculture activities, freshwater runoff, wastewater discharge, and naturally occurring microbial communities. As a result, these environments may facilitate the circulation and transmission of viruses affecting both aquatic organisms and humans (Sankar et al., 2026), thereby posing challenges for environmental management and public health protection (Farkas et al., 2020; Yadalam et al., 2023).

Shellfish species, particularly clams, constitute an important economic resource in many Indian brackish water regions. Owing to their filter-feeding nature, these organisms can concentrate viral particles from surrounding water and sediments, making them useful indicators of environmental contamination as well as potential vectors for viral transmission through the food chain (Lees, 2000). Numerous outbreaks of gastroenteritis have been linked to the consumption of shellfish contaminated with enteric viruses such as noroviruses and rotaviruses, highlighting the necessity for routine monitoring of aquatic viral contaminants. Phytoplankton communities are another essential component of brackish water ecosystems and contribute significantly to primary production and nutrient turnover. In shrimp aquaculture systems, these microalgae influence water quality and interact closely with microbial populations. Nevertheless, the ecological interactions between phytoplankton and viral communities remain insufficiently understood. Viral infections can influence algal bloom dynamics, alter microbial food webs, and affect the persistence and spread of pathogens within aquaculture environments (Brussaard, 2004; Short, 2012).

Both water and sediment serve as important reservoirs for viral survival and dissemination. Previous studies have demonstrated that sediments may contain substantially higher concentrations of viruses than the overlying water column and can provide conditions that support prolonged viral persistence (Danovaro et al., 2008). Despite increasing recognition of the ecological importance of aquatic viruses, comprehensive information regarding their abundance, distribution, and diversity in Indian brackish water environments remains limited. In particular, data describing the occurrence of both aquatic and human-associated viruses in coastal water and sediment systems are scarce.

A detailed assessment of viral abundance and spatial distribution in brackish water ecosystems is therefore essential for improving our understanding of aquatic viral ecology, identifying potential environmental reservoirs, and evaluating associated health risks. Such information is critical for the development of effective surveillance programs and biosecurity measures aimed at minimizing disease transmission in aquaculture systems and protecting public health. Furthermore, environmental monitoring of viral populations may contribute to the early detection of emerging viral threats and support sustainable management of coastal ecosystems (Haramoto et al., 2018; Farkas et al., 2020). The objective of this study was to investigate the abundance, distribution, and ecological relevance of viral communities in brackish water ecosystems and to evaluate their potential contribution to environmental persistence and disease transmission. Particular emphasis was placed on establishing baseline information regarding viral abundance in Indian brackish water environments to support future environmental surveillance and aquaculture biosecurity initiatives.

METHODOLOGY

Determination of Total Viral Abundance by Epifluorescence Microscopy

Total viral abundance in brackish water samples was determined using SYBR Green II staining followed by epifluorescence microscopy. Immediately after collection (Water samples have been collected during December 2025 to March 2026), water samples were concentrated using a 0.2 µm hollow-fibre membrane filter. The filtrate was subsequently concentrated using a 100 kDa hollow-fibre cartridge with a peristaltic pump, and the resulting viral concentrate was collected (Alavandi et al., 2015). The concentrate was immediately fixed with 0.02 µm-filtered formalin (37–39% w/v formaldehyde solution) to a final concentration of 2% (v/v) and stored on ice until further processing.

A control slide was prepared using 1 mL of 0.02 µm filter-sterilized Milli-Q water, which was also used for the preparation of the SYBR Green II working solution. Prior to filtration, the filtration tower and associated components

were thoroughly cleaned with filter-sterilized distilled water followed by 70% ethanol. An anodisc membrane filter (0.02 µm pore size, 25 mm diameter) was mounted onto the filter holder. A pre-wetted mixed cellulose ester membrane filter (0.8 µm pore size, 25 mm diameter) was placed centrally on the filter support and moistened with sterile distilled water to ensure a uniform translucent appearance without air bubbles. Excess water was removed by applying gentle vacuum. Subsequently, the anodisc filter was carefully positioned on top of the support filter.

A measured volume of fixed water sample was transferred into the filtration funnel and filtered under a vacuum pressure of approximately 20 kPa. Upon completion of filtration, the Anodisc filter was carefully removed by holding only its plastic rim and separated from the support membrane. The filter was then dried completely by gentle blotting. For staining, 100 µL of SYBR Green II working solution was dispensed onto the center of a Petri dish. The dried Anodisc filter was placed face-down onto the staining solution, allowing microorganisms retained on the filter surface to be stained. Following incubation, excess stain was removed by blotting the reverse side of the filter using a lint free tissue (Kimwipe).

The stained filter was mounted onto a 25 × 25 mm glass coverslip using 27–30 µL of 0.1% (v/v) p-phenylenediamine antifade mounting medium. The prepared slides were examined using a Carl Zeiss epifluorescence microscope under ultraviolet (UV) excitation in dark-field mode. Total viral abundance was determined by SYBR Green II staining and epifluorescence microscopy. Virus like particles were enumerated from 20 random microscopic fields using imageJ software and expressed as VLPs mL^{-1} (Noble et al., 1998).

RESULTS

Total Virus Abundance in Brackish Water Environment

Total viral abundance in concentrated brackish water samples was determined by epifluorescence microscopy following SYBR Green II staining. Viral particles were detected in all sampling locations, demonstrating the widespread occurrence of viruses in the investigated brackish water environments.

The abundance of virus-like particles (VLPs) varied considerably among the sampling sites, ranging from 6.1×10^2 to 7.3×10^7 viruses mL^{-1}. The highest viral abundance was recorded in the Bhimavaram shrimp farming region of Andhra Pradesh (7.3×10^7 viruses mL^{-1}), whereas the lowest abundance was observed in the Velankanni coastal ecosystem of Tamil Nadu (6.1×10^2 viruses mL^{-1}). Intermediate viral concentrations were detected in samples collected from Ongole (5.2×10^5 viruses mL^{-1}), Nellore (3.9×10^5 viruses mL^{-1}), Marakanam (5.5×10^6 viruses mL^{-1}), and various locations in Sadras, Tamil Nadu, where viral abundance ranged from 4.3×10^2 to 4.9×10^5 viruses mL^{-1}.

Marked spatial variation in viral abundance was observed among the brackish water environments studied. Samples collected from intensive aquaculture regions, particularly Bhimavaram and Marakanam, exhibited substantially higher viral concentrations compared with other locations. These findings indicate that viral populations are highly heterogeneous within brackish water ecosystems and may be influenced by local environmental conditions, host abundance, aquaculture practices, and microbial community dynamics.

Overall, the results demonstrate that brackish water ecosystems harbour abundant viral communities and may serve as important reservoirs for aquatic and potentially human associated viruses. The wide variation in viral abundance observed across sampling locations highlights the need for continuous environmental monitoring and viral surveillance in aquaculture associated coastal ecosystems.

The SYBR Green II stained virus like particles captured by epifluorescence microscopy from various sampling location are presented below

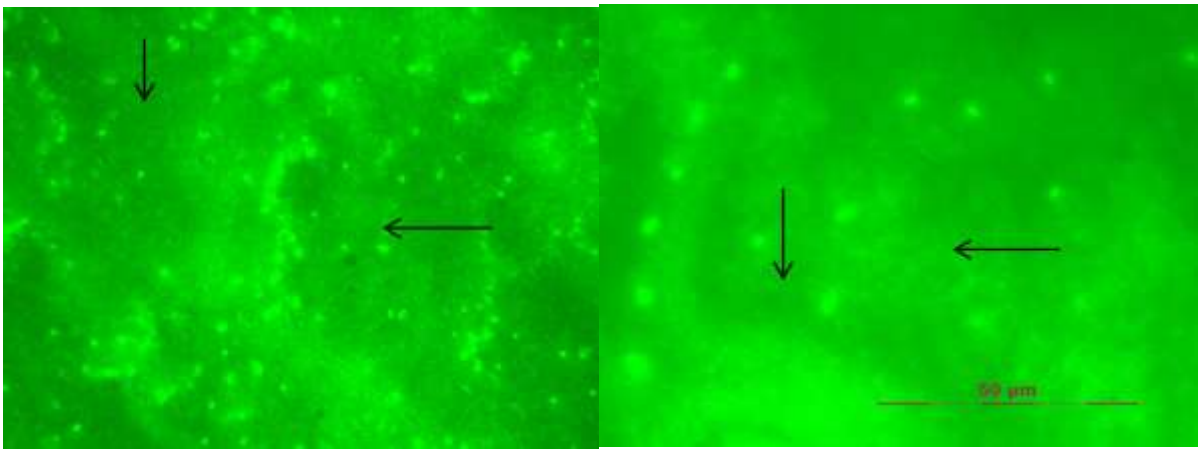

Figure A **Figure B**

Figure A and B show the total viral abundance in Bhimavaram and Ongole water samples, respectively, determined using SYBR Gren II staining followed by Epifluorescence microscopy. The viral like particle are indicated by arrow marks.

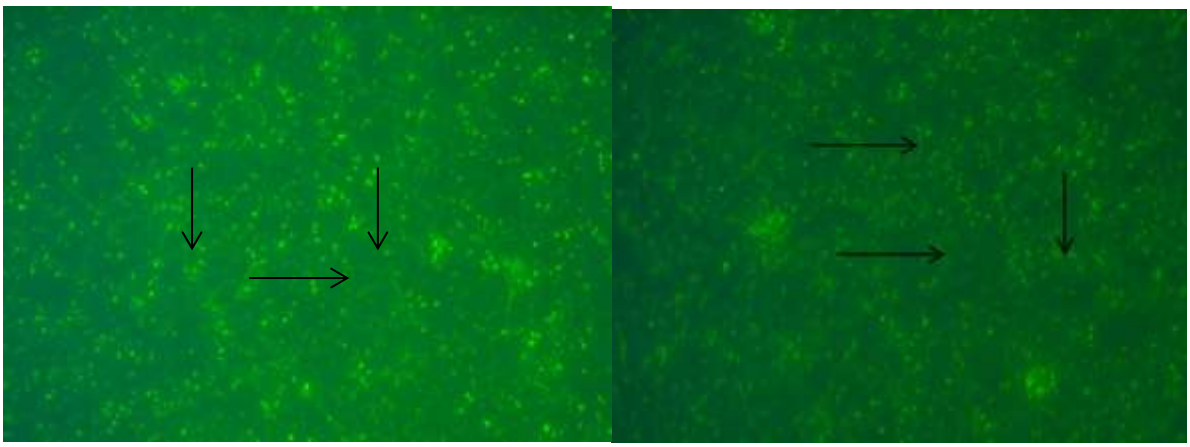

Figure C Figure D

Figure C and D show the total viral abundance in Sadras and Nagapattinam water samples, respectively, determined using SYBR Gren II staining followed by Epifluorescence microscopy. The viral like particle are indicated by arrow marks.

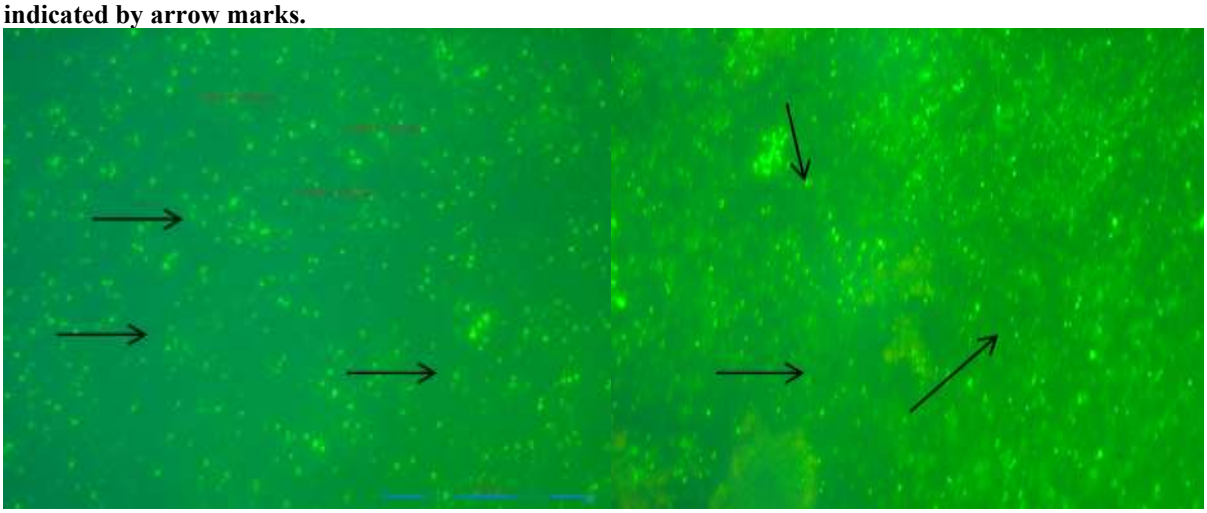

Figure E **Figure F**

Figure E and F show the total viral abundance in Velankanni and Marakkanam water samples, respectively, determined using SYBR Gren II staining followed by Epifluorescence microscopy. The viral like particle are indicated by arrow marks.

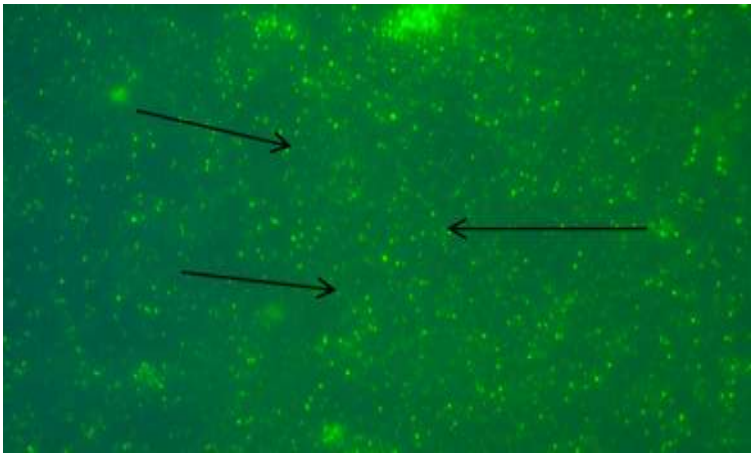

Figure G

Figure G show the total viral abundance in Nellore water samples, determined using SYBR Gren II staining followed by Epifluorescence microscopy. The viral like particle are indicated by arrow marks.

S.No	Water sample location	Total virus abundance (viruses mL^{-1})
1	Bhimavaram –AP	7.3×10^7
2	Ongole - AP	5.2×10^5
3	Sadras- TN	4.3×10^2
4	Sadras- TN	4.9×10^5
5	Bhimavaram -AP	3.6×10^3
6	Sadras- TN	4.5×10^4
7	Velankanni - TN	6.1×10^2
8	Bhimavaram -AP	5.5×10^3
9	Nellore- AP	3.9×10^5
10	Nagapatinam - TN	4.1×10^3
11	Marakanam - TN	5.5×10^6
12	Bhimavaram - AP	3.3×10^4

TN: Tamil Nadu; AP: Andra Pradesh

Table 1: Total viral abundance in brackish water samples collected from different coastal and brackish water environments determined by epifluorescence microscopy following SYBR Green II staining.

DISCUSSION

Total Virus Abundance in Environmental Samples

Viruses constitute a major component of aquatic microbial communities and are regarded as the most numerous biological agents in marine and estuarine ecosystems. Their ecological functions extend beyond host infection and include regulation of microbial populations, facilitation of genetic exchange, and participation in nutrient turnover processes that sustain ecosystem productivity (Rohwer and Thurber, 2009; Selvavinayagam et al., 2023). Despite growing recognition of their environmental significance, quantitative information on viral abundance in brackish water aquaculture systems remains relatively scarce, particularly in tropical regions (Williamson et al., 2005).

In the present investigation, viral abundance was estimated using SYBR Green II staining coupled with epifluorescence microscopy. This technique has become a standard approach for the enumeration of virus-like particles in brackish water samples because of its rapid processing time, sensitivity, and reproducibility (Noble and Fuhrman, 1998; Patel et al., 2007). The fluorescent dye binds efficiently to viral nucleic acids retained on ultrafine membrane filters, enabling accurate visualization and quantification of viral particles. Previous studies have demonstrated that SYBR Green I-based counts may exceed those obtained through transmission electron microscopy, reflecting its enhanced detection capability (Noble and Fuhrman, 1998).

The viral concentrations recorded in this study varied markedly among sampling sites, ranging from 6.1×10^2 to 7.3×10^7 viruses mL^{-1}. Such variability reflects the heterogeneous nature of brackish water ecosystems and is broadly consistent with observations reported from other coastal and estuarine environments worldwide (Wommack and Colwell, 2000; Weinbauer, 2004). Viral populations are known to increase with ecosystem productivity and host availability, resulting in elevated abundances in nutrient-rich coastal habitats compared with offshore and deep-sea environments (Weinbauer, 2004; Danovaro et al., 2008).

Several environmental factors may have contributed to the differences observed among the sampling locations. Parameters such as nutrient concentrations, salinity, temperature, dissolved oxygen, organic matter availability, bacterial density, phytoplankton productivity, and aquaculture management practices are known to influence viral replication and persistence in aquatic ecosystems (Fuhrman, 1999; Brussaard, 2004; Wigington et al., 2016). Shrimp ponds characterized by high biological productivity often support large microbial populations, thereby providing suitable hosts for viral propagation and maintenance (Thingstad, 2000).

A substantial proportion of brackish water viral communities is believed to consist of bacteriophages that infect prokaryotic hosts. Through infection and lysis of bacterial cells, these viruses contribute significantly to microbial mortality and influence the structure of aquatic microbial communities (Weinbauer, 2004; Suttle, 2005). Viral lysis releases cellular constituents into the surrounding environment, enhancing the availability of dissolved organic matter and nutrients. This process, commonly described as the viral shunt, redirects energy and carbon flow through microbial pathways and plays a vital role in aquatic biogeochemical cycles (Fuhrman, 1999; Suttle, 2007).

Beyond nutrient regeneration, viruses exert strong selective pressures on microbial populations and contribute to the maintenance of biodiversity through host-specific interactions. Viral-mediated horizontal gene transfer may further promote microbial adaptation and evolution, thereby influencing community composition and ecosystem resilience (Rohwer and Thurber, 2009; Breitbart, 2012). Consequently, viral communities represent an essential ecological component of shrimp pond and brackish water ecosystems.

The widespread occurrence of virus-like particles observed in this study suggests that brackish water habitats may function as reservoirs of diverse viral populations. Continuous surveillance of these environments is therefore important for understanding ecosystem health, strengthening aquaculture biosecurity, and identifying potential environmental risks associated with viral transmission (Bibby and Peccia, 2013; Farkas et al., 2020). Future investigations incorporating molecular tools, including metagenomic sequencing and viral community profiling, will provide deeper insights into viral diversity, host associations, and ecological functions within these ecosystems.

CONCLUSION

The present study demonstrated the widespread presence of virus-like particles in brackish water environments of Southern India. Viral abundance exhibited substantial spatial variability, indicating that local environmental conditions and microbial host populations strongly influence viral distribution patterns. These findings establish important baseline data on viral abundance in Indian brackish water ecosystems and contribute to the growing understanding of present viral ecology.

Brackish water environments are continually influenced by aquaculture and health care sector industry activities, land runoff, wastewater inputs, and natural ecological processes, creating conditions that may support the persistence of diverse viral populations. Although the methodology employed in this study quantified total virus-like particles and did not permit identification of specific viral pathogens, previous investigations have shown that coastal and estuarine

waters can harbour human-associated enteric viruses, including noroviruses, rotaviruses, adenoviruses, enteroviruses, hepatitis A virus, and hepatitis E virus. Filter-feeding shellfish such as clams, oysters, and mussels are capable of concentrating viral particles from surrounding waters and sediments. As a result, contaminated shellfish may serve as vehicles for the transmission of viral diseases to humans when consumed raw or inadequately cooked. Consequently, brackish water ecosystems may represent important environmental interfaces linking aquatic viral reservoirs and public health.

The elevated viral abundance observed in certain aquaculture and coastal sites may indicate environmental conditions favourable for viral survival and circulation. While many of the detected particles are likely bacteriophages and other environmentally important viruses, the potential occurrence of human-associated viral pathogens cannot be disregarded. Therefore, routine monitoring of viral abundance may provide valuable information for water quality assessment, environmental risk evaluation, and early detection of potential public health threats.

Increasing anthropogenic pressure on coastal ecosystems further underscores the need for comprehensive viral surveillance programs. The integration of conventional viral enumeration methods with molecular detection approaches such as quantitative PCR, metagenomics, and next-generation sequencing will facilitate the identification of pathogenic viruses and improve risk assessment strategies. Such efforts will support sustainable aquaculture development, environmental management, and the protection of public health.

Acknowledgments

The authors thank the ICAR–Central Institute of Brackish water Aquaculture (CIBA), Government of India, for providing access to the tangential flow filtration facility used for viral concentration from the collected water samples. The authors also gratefully acknowledge their technical support and assistance throughout the study.

Conflict of Interest

The authors declare no conflict of interest.

Ethical Approval

Not Applicable

Funding Disclosure

None

References:

1. Alavandi, S.V., Bharathi, R.A., Kumar, S.S., Dineshkumar, N., Saravanakumar, C. and Rajan, J.J.S. (2015). Tangential flow ultrafiltration for detection of white spot syndrome virus (WSSV) in shrimp pond water. Journal of Virological Methods, 218, 7–13.
2. Bibby, K. and Peccia, J. (2013). Identification of viral pathogen diversity in sewage sludge. Environmental Science & Technology, 47, 1945–1951.
3. Bosch, A., et al. (2008). Waterborne viruses associated with gastroenteritis. Food and Environmental Virology, 1, 4–12.
4. Breitbart, M. (2012). Marine viruses: truth or dare. Annual Review of Marine Science, 4, 425–448.
5. Brussaard, C.P.D. (2004). Viral control of phytoplankton populations. Journal of Eukaryotic Microbiology, 51, 125–138.
6. Danovaro, R., Dell'Anno, A., Corinaldesi, C., et al. (2008). Major viral impact on the functioning of benthic deep-sea ecosystems. Nature, 454, 1084–1087.
7. Farkas, K., Hillary, L.S., Thorpe, J., et al. (2020). Wastewater and environmental surveillance for viral pathogens. Current Opinion in Environmental Science & Health, 16, 11–17.
8. Fuhrman, J.A. (1999). Marine viruses and their biogeochemical and ecological effects. Nature, 399, 541–548.
9. Haramoto, E., et al. (2018). Occurrence of viruses in water environments. Food and Environmental Virology, 10, 1–17.
10. Lees, D. (2000). Viruses and bivalve shellfish. International Journal of Food Microbiology, 59, 81–116.
11. Noble, R.T. and Fuhrman, J.A. (1998). Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. Aquatic Microbial Ecology, 14, 113–118.
12. Patel, A., Noble, R.T., Steele, J.A., Schwalbach, M.S., Hewson, I. and Fuhrman, J.A. (2007). Virus and prokaryote enumeration from planktonic aquatic environments by epifluorescence microscopy with SYBR Green I. Nature Protocols, 2, 269–276.
13. Rohwer, F. and Thurber, R.V. (2009). Viruses manipulate the marine environment. Nature, 459, 207–212.

14. Sankar, S., Anandharaman, K., Selvam, P., Jayaraman, A., Jayakumar, D., Balakrishnan, P. and Shankar, E.M. (2026). Genomic evolution of SARS-CoV-2 delta variants pre- and post-omicron emergence using alignment-free machine learning models. PLoS ONE, 21(3), e0345259.
15. Selvavinayagam, S.T., Sankar, S., Yong, Y.K., Anshad, A.R., Chandramathi, S., Somasundaram, A., et al. (2024). Serosurveillance of dengue infection and correlation with mosquito pools for dengue virus positivity during the COVID-19 pandemic in Tamil Nadu, India: A state-wide cross-sectional cluster randomized community-based study. medRxiv.
16. Short, S. M. (2012). The ecology of viruses that infect eukaryotic algae. Environmental Microbiology, 14(9), 2253-2271.
17. Suttle, C.A. (2005). Viruses in the sea. Nature, 437, 356–361.
18. Suttle, C.A. (2007). Marine viruses—major players in the global ecosystem. Nature Reviews Microbiology, 5, 801–812.
19. Thingstad, T.F. (2000). Elements of a theory for the mechanisms controlling abundance, diversity and biogeochemical role of lytic bacterial viruses. Limnology and Oceanography, 45, 1320–1328.
20. Weinbauer, M.G. (2004). Ecology of prokaryotic viruses. FEMS Microbiology Reviews, 28, 127–181.
21. Wigington, C.H., Sonderegger, D., Brussaard, C.P.D., et al. (2016). Re-examination of the relationship between marine virus and microbial cell abundances. Nature Microbiology, 1, 15024.
22. Williamson, K. E., Radosevich, M., & Wommack, K. E. (2005). Abundance and diversity of viruses in six Delaware soils. Applied and environmental microbiology, 71(6), 3119-3125.
23. Wommack, K.E. and Colwell, R.R. (2000). Virioplankton: viruses in aquatic ecosystems. Microbiology and Molecular Biology Reviews, 64, 69–114.
24. Yadalam, P.K., Anegundi, R.V., Ramadoss, R., Saravanan, M., Veeramuthu, A. and Heboyan, A. (2023). Indigenous oral and gut phages defeat the deadly NDM-1 superbug. Bioinformatics and Biology Insights, 17, 11779322231182767.