



ABSTRACT

The occurrence of bacteriophages infecting bacterioplankton in the Gulf of Mexico was studied during a research cruise in July of 2005. The initial activity of bacteriophage (FVIC) has also been determined. FVIC was determined by using a modified plaque assay. FVIC has also been determined. Viral abundance significantly decreased with depth in the nutrient-rich Mississippi River Plume from 3.6×10^7 ml⁻¹ at 3 meters to 3.06×10^6 ml⁻¹ at 100 meters (P=0.025). At an oligotrophic station, viral abundance decreased significantly from 3 meters to 100 meters (P=0.0013). The results of the phage induction experiments as well as the negative correlation shows that as bacterial production decreases, lysogeny becomes more prevalent in comparison to phage lysis in the Gulf of Mexico.

INTRODUCTION

- Viral abundances in the marine environment are highest in coastal areas and lowest in the open ocean.
- Viruses are believed to play an important role in carbon cycling in the marine environment.
 - When a bacteriophage infects a bacterium, it can enter either the lysis or lysogeny lifecycle.
 - In the lysis cycle, cells are lysed 10-30 minutes after infection but in the lysogeny cycle, the bacteriophage incorporates itself into the bacterium and the cell is not lysed immediately.
- Carbon from lysed cells enters the DOM pool.
- Using direct measurement methods, Wilhelm et al. found that viral production rates were high in systems that were in a non-steady state such as stratified waters that experienced tidal mixing (Wilhelm et al. 2002, Microb Ecol. 43: 168-173)
- The Mississippi River Plume forms every summer in the Gulf of Mexico as a result of Mississippi River transporting inorganic nutrients into the GOM. (Williamson & Paul. Aquat Microb Ecol. 2004. 36:9-17)

- The MRP is a high productivity, low-salinity zone as opposed to the rest of the GOM which is oligotrophic with low levels of bacterial productivity.
- Thorough studies of the factors influencing the lysic/lysogenic lifestyles in marine bacteriophages is essential for a complete understand of global cycles in the marine environment.
- In this study, viral production rates in the MRP and the Gulf of Mexico were measured directly using the

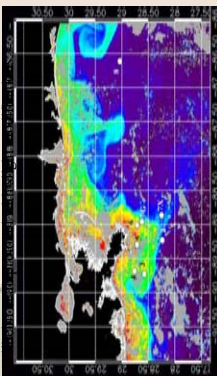


Figure 1. Sea-viewing wide-of-view sensor (SeaWiFS) image showing chlorophyll a concentrations in the Gulf of Mexico in July 2005. Sampling sites are labeled except Station 1, which is off the image.

Phage Lysis and Lysogeny in Bacterial Populations in the Gulf of Mexico

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MATERIALS AND METHODS

- Sample Collection**
 - Samples were collected from 11 different stations at the surface and the sub-surface chlorophyll maximum (SCM).
 - Water was pre-filtered with a 50 µm mesh screen to remove grazers before any experiments were started.
- Viral Production and Phage Induction**
 - Samples were viral-reduced using the Weinbauer & Suttle method with slight modifications.
 - Slides were made in triplicate at each station and every depth for ambient viral and bacterial counts.
 - Viral-reduced samples were split into two treatments, control and mitomycin C (a mutagen). Mitomycin C cultures were treated at a concentration of 1.0 µg ml⁻¹.
 - Samples were taken at T0, T4, T8, and T24.
 - Slides were immediately made using the Noble & Fuhrman method except that SYBR gold nucleic acid stain was used and staining time was 12 minutes. Slides were frozen at -20 °C until counted under epifluorescence microscopy.
- Bacterial Production**
 - Bacterial production was measured via leucine uptake using the Kirchmann protocol with slight modifications.
- Frequency of Visibly Infected Cells (FVIC)**
 - Samples were preserved with buffered Formalin and stored at 4 °C until needed.
 - Samples were ultra centrifuged at 20,000 rpm for 20 minutes.
 - Visibly infected cells were examined using electron microscopy and burst size was calculated.
- Determination of Viral Rates and Statistical Analysis**
 - Viral production and phage induction rates were determined by performing a first order regression on viral counts from each T0, T4, T8, and T24. The slope of the line is the rate of viral production per hour.
 - Statistical analysis was performed using Sigma Stat 3.1 and Minitab 13
 - One-way ANOVA was carried out on each station to determine if viral production was significant.
 - To determine if phage induction was significant, viral counts from the control and mitomycin C treatment at T24 were compared by a 2-sample t-test.

Phage Induction and Viral Production

- Significant viral production was observed at 3 of the 11 stations tested (Figure 3).
- All three were surface stations and none were located inside the MRP.
- The viral production rate at the station located in the MRP was negative.
- Significant phage induction was observed at 3 of 11 stations, none of which were in the MRP (Figure 4).
- The depth of all three stations was at the SCM or deeper.

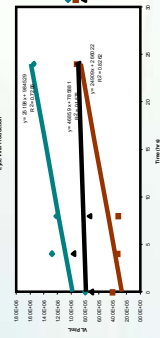
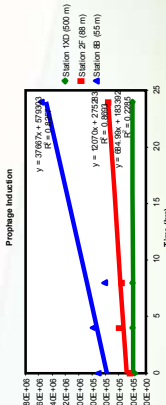


Figure 3. First order regression of stations where viral abundance significantly increased from T0 to T24. Stations 8A and 8B were significant at a 95% CI, and Station 2A was significant at the 90% CI.

Figure 4. First order regression of stations where phage induction was significantly greater than viral production at T24. Stations 2F and 8B were significant at the 95% CI, and station 1XD was significant at the 90% CI.



Lysogenic Fraction & FVIC

- The preliminary average burst size as determined by FVIC was at or greater than 22.06.
- Lysogenic fraction of cells at each station where FVIC was done was calculated by the following equation:

$$\left(\frac{V_{-V_0} B_0}{B_{act}} \right) \times 100$$

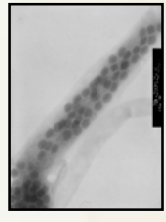


Figure 5. Electron micrograph of a visibly infected cell.

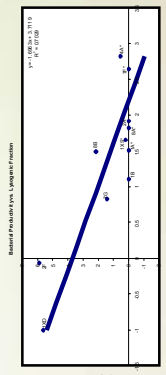


Figure 6. Calculated lysogenic fraction plotted against the log-transformed leucine uptake. * denotes a surface station.

- Lysogenic fraction was greatest in the subsurface samples, and negative or close to zero in the surface samples.
- Based upon the Pearson correlation of lysogenic fraction to leucine uptake, -0.838 ($P=0.001$), leucine uptake increased as the lysogenic fraction decreased.

CONCLUSIONS

- Viral abundance is greatest in the Mississippi River Plume even though neither viral production nor phage induction occurred
 - Viruses are being removed as fast as they are being produced in plume waters.
 - High viral production was seen only in oligotrophic waters in the Gulf of Mexico
- Significant phage induction occurred only at the SCM or deeper.
 - This agrees with previous studies that have found that lysogeny is favored in light-limited ecosystems. As depth increases, bacterial activity decreases due to decreased sunlight and nutrients. Lysogeny becomes the preferred lifestyle, and it is a steady-state system.
- These experiments set the stage for future studies exploring bacteriophage populations in the Gulf of Mexico to gain a deeper understanding of bacteriophage and bacterioplankton interactions and system dynamics.

Acknowledgements

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