OCN 626: Marine Microplankton Ecology

Viruses in the Marine Food Web
Lecture 2 of 4
Viral Abundance and Distribution
## Methods for Counting Viruses

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cultivation</strong> <em>(plaque assay or most probable number)</em></td>
<td><em>Count specific <strong>infectious</strong> viruses</em></td>
<td><em>Very specific; No total count possible</em></td>
</tr>
<tr>
<td><strong>Transmission Electron Microscopy (TEM)</strong></td>
<td><em>More confident identification of VLPs</em></td>
<td><em>Laborious; Expensive equipment</em></td>
</tr>
<tr>
<td><strong>Epifluorescence Microscopy</strong></td>
<td><em>Easy, Cheap</em></td>
<td><em>Lower confidence in VLP identification</em></td>
</tr>
<tr>
<td><strong>Flow Cytometry</strong></td>
<td><em>Easy (relatively), Cheap (reagents)</em></td>
<td><em>Expensive equipment; Lower confidence in VLP identification</em></td>
</tr>
</tbody>
</table>
Most Probable Number

Dilutions

http://www.microbiologie.info/images/tellingen/mpnschema1laag.GIF
Most Probable Number

Results

Plaque Assay

Direct Centrifugation for TEM counts of Viruses

Seawater

EM grid

Acrylic holder or Epoxy Plug

Ultracentrifuge
100,000 x Gravity!
• Improved filters
  - track-etched polycarbonate
  - aluminum oxide
  - very flat; precise pore sizes

• Fluorescent DNA Stains
  - detect below limit of resolution
polycarbonate track-etched

aluminum oxide
Bacteria viewed by Epifluorescence Microscopy

0.2 µm Filter
DAPI stain
Bacteria and Viruses viewed by Epifluorescence Microscopy

0.02 µm Filter
SYBR Green I stain

- Bacterium
- Virus
Virus Counts by Flow Cytometry

Comparison Methods

Viral Isolate

FIG. 2. Infection experiment of P. pouchetii. Phaeocystis cells were enumerated by FCM for uninfected (open triangles) and infected (open circles) cultures. Virus counts were performed by three different techniques: EFM (solid triangles), TEM (solid circles), and FCM (solid squares). Virus counts reported for FCM correspond to the average value of counts obtained on 0.5% glutaraldehyde-fixed samples, frozen in liquid nitrogen, at different dilutions and analyzed after incubation at room temperature (Table 1) or after heating at 80°C in the presence of Triton X-100.

FIG. 3. Infection experiment of P. pouchetii. Comparison between FCM, EFM, and TEM virus counts obtained for samples collected every 4 h (Fig. 2; Table 1). FCM versus EFM (solid circles, straight line; EFM = 0.91 × FCM − 8.5 × 10⁵, r = 0.97, n = 14). FCM versus TEM (solid squares, dotted line, FCM = 0.95 × TEM − 3.7 × 10⁵, r = 0.96, n = 8). The dashed line corresponds to a 1:1 relationship.

Comparison Methods
Natural Communities

Fig. 1. Counts of viral particles using Yo-Pro and transmission electron microscopy in natural waters. Data from Hennes & Suttle (1995) and this study. Solid line: relationship of 1:1. Standard deviation (x and y error) is calculated from triplicate samples. Regression equation: $y = 1.78x - 5.57$. Where error bars are not shown the SD was smaller than the width of the symbols.


Abundance and Distribution of Viruses

Examples from various locations
Open Ocean

Coral Sea

North Pacific

Figure 4. Depth profile of prokaryote (Bacteria + Archaea) and viral abundance from the Coral Sea (April 1998), as determined by epifluorescence microscopy of SYBR Green-stained samples; note the log scale. (Method from Noble and Fuhrman 1996b.)


Prokaryotes ($\times 10^9$ per l)

Viruses ($\times 10^{10}$ per l)

- Monterey Bay
- Bering Strait
- Arctic Ocean

Steward unpublished data


Steward unpublished data
Southern CA Bight

Fig. 1. Depth profiles of total virus numbers, bacterial numbers and chlorophyll a from the Southern California Bight, Sep 1990 and 1991. (A) Coastal station 303A (1990): virus abundance covaried with bacterial numbers both according to a Pearson product moment correlation \( p < 0.05, r = 0.88 \) and a non-parametric Spearman’s rank test \( p < 0.05, r_s = 0.89 \); chl a was not correlated \( p > 0.05 \) with virus numbers by either method. (B) Mid-station 304 (1990): neither bacterial numbers nor chl a were correlated with virus numbers according to a Pearson correlation \( p > 0.05 \) or a Spearman’s rank test \( p > 0.05 \). (C) Off-shore station 305 (1990): virus abundance covaried with bacterial numbers (non-normal distribution) according to a Spearman’s rank test \( p < 0.05, r_s = 0.70 \); chl a was not correlated \( p > 0.05 \) with virus numbers by either parametric or non-parametric tests. (D) Off-shore station 305 (1991): virus abundance was correlated with bacterial abundance both according to a Pearson correlation \( p < 0.001, r = 0.88 \) and a Spearman’s rank test \( p = 0.02, r_s = 0.94 \); chl a was correlated with virus numbers only according to a Pearson correlation \( p < 0.05, r = 0.88 \). Shading in (D) represents sea floor.


Fig. 3. Virus numbers in the upper 50 m of the water column in transects from coast (<10 km from shore) to off-shore (>45 km from shore). Samples are from (●) the Southern California Bight, (●) the San Diego Trough, and (○) the Gulf of Bothnia. Error bars are 95% confidence intervals, when applicable. A statistically significant negative trend was demonstrated for the combined Pacific Ocean stations by least-squares linear regression \( p = 0.03 \).
Virus Abundance Correlated with Prokaryotes and Chl a

Fig. 2. Relationship between viral and bacterial abundance in fresh- and salt water habitats, including the corrected values for the concentrated marine virus samples. The fitted line represents the regression equation we derived from the overall relationship.

Fig. 4. Scatter diagram of the points obtained from the literature and this freshwater study used to derive regressions of viral abundance and chlorophyll a concentration. Solid line represents the overall regression equation we derived.

Viruses vs. Bacteria

Literature Summary

Table 1. Typical counts of viruses from various marine planktonic environments; see also Figure 4

<table>
<thead>
<tr>
<th>Location*</th>
<th>Viruses (10^8 L^-1)</th>
<th>Method*</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>North Atlantic, spring</td>
<td>150</td>
<td>TEM</td>
<td>Bergh et al. (1989)</td>
</tr>
<tr>
<td>Raunefjord (Norway)</td>
<td>100</td>
<td>TEM</td>
<td>Bergh et al. (1989)</td>
</tr>
<tr>
<td>Raunefjord, late winter</td>
<td>5</td>
<td>TEM</td>
<td>Bratbak et al. (1990)</td>
</tr>
<tr>
<td>Raunefjord, spring</td>
<td>20–100</td>
<td>TEM</td>
<td>Bratbak et al. (1990)</td>
</tr>
<tr>
<td>Southern California, nearshore</td>
<td>111–282</td>
<td>TEM</td>
<td>Cochlan et al. (1993)</td>
</tr>
<tr>
<td>Southern California, offshore, 50 m</td>
<td>13–124</td>
<td>TEM</td>
<td>Cochlan et al. (1993)</td>
</tr>
<tr>
<td>Southern California, offshore, 50 m</td>
<td>4–57</td>
<td>TEM</td>
<td>Cochlan et al. (1993)</td>
</tr>
<tr>
<td>Southern California, offshore, 900 m</td>
<td>25</td>
<td>TEM</td>
<td>Cochlan et al. (1993)</td>
</tr>
<tr>
<td>Bering and Chukchi Seas</td>
<td>20–360</td>
<td>TEM</td>
<td>Steward et al. (1996)</td>
</tr>
<tr>
<td>Northern Adriatic Sea</td>
<td>10–600</td>
<td>TEM</td>
<td>Weinbauer et al. (1995)</td>
</tr>
<tr>
<td>Gulf of Mexico, University of Texas pier</td>
<td>104</td>
<td>TEM</td>
<td>Weinbauer and Suttle (1997)</td>
</tr>
<tr>
<td>Gulf of Mexico, offshore</td>
<td>3–57</td>
<td>TEM</td>
<td>Weinbauer and Suttle (1997)</td>
</tr>
<tr>
<td>Gulf of Mexico, offshore</td>
<td>3–82</td>
<td>Yo-Pro</td>
<td>Weinbauer and Suttle (1997)</td>
</tr>
<tr>
<td>Southern California, 190 km offshore</td>
<td>125</td>
<td>TEM</td>
<td>Noble and Fuhrman (1998b)</td>
</tr>
<tr>
<td>Southern California, 190 km offshore</td>
<td>170</td>
<td>SYBR</td>
<td>Noble and Fuhrman (1998b)</td>
</tr>
<tr>
<td>Equatorial Pacific</td>
<td>53</td>
<td>FCM</td>
<td>Marie et al. (1999)</td>
</tr>
<tr>
<td>Mediterranean Sea</td>
<td>23</td>
<td>FCM</td>
<td>Marie et al. (1999)</td>
</tr>
</tbody>
</table>

*Near-surface and summer unless otherwise indicated.

*TEM is ultracentrifugation directly onto TEM grids for counting, without prior concentration steps. Yo-Pro and SYBR are stains used in epifluorescence direct counts of Anodisc-filtered samples. FCM is flow cytometry with SYBR Green stain.

Summary
Viral Abundance

- Viruses are ubiquitous in marine environment
- Abundance greater in surface than deep waters and in nearshore vs open ocean
- Virus concentrations generally correlated with prokaryotes and Chl a
- Viruses about an order of magnitude more abundant than bacteria