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VIRUSES AND BACTERIA IN COASTAL WATERS AND SHELLFISH

The fate of viruses and pathogens discharged into the ocean in municipal effluent is not known for certain. They probably become inactivated in the same manner that coliform bacteria are—through sedimentation, predation by marine bacteria and filter-feeders, damage by ultraviolet light or abrupt changes from reducing to oxidizing environment, or because of some antiviral agent present in the sea. Past observations and measurements suggested that viruses survive longer in seawater and shellfish than do human enteric bacteria, but only the results of field studies can confirm this with certainty and provide guidance for wastewater discharge and public health policy. Thus the objectives of our research this year were to (1) determine the concentrations and rates at which enteric viruses enter the sea via municipal wastewater effluents, (2) measure concentrations of viruses in the digestive glands of mussels suspended from buoys near outfalls, and (3) determine the relationship between virus and coliform concentrations in mussels and seawater.

We measured coliforms and viruses (and occasionally salmonella) in 34 samples of primary and secondary effluents and 39 mussels from sea buoys and shore stations; the coliforms in 26 samples of the seawater to which the mussels were exposed were also determined. In addition, tests were run to determine the efficiency of our virus recovery methods.

Our most important finding to date is that viruses can be detected in shellfish near outfalls and do appear to survive relatively longer in mussels than do total coliforms. We estimated coliform-to-virus ratios in effluent, seawater, and mussels. Using a formula based on conventional first-order decay rates that was developed for the conditions of this study by Dr. T. Hendricks of the Project, virus inactivation rate was compared to coliform die-off rate: The relative time required for 90 percent of the viruses in seawater to be inactivated was estimated to be three to six times as long as that for total coliform.

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METHODS

Sewage samples (3.8 to 20 liters) were examined for the presence of enteroviruses by use of selective adsorption to and elution from fiberglass disc filters. Viruses in an effluent sample were adsorbed to the filter at a pH of 3.5 and were then eluted from the filter with a small volume of pH 11.5 buffer. The eluates from the filters were reconcentrated on a precipitate formed by ferric chloride at a pH of 3.5. The viruses on the precipitate could then be eluted into a small volume of fetal calf serum, which was frozen until analysis.

Samples of the California mussel, Mytitus calif omianus, were also examined for the presence of enteroviruses. Mussels were collected from stations remote from sewage outfalls (Oil Platform Hilda, near Santa Barbara, and Scotsmans Cove, in the southern part of Orange County), placed in cages, and suspended at various depths below buoys located offshore near outfall diffusers (Table 1). This caged mussel system is a modification of one previously described (Coastal Water Research Project 1975, pp. 127-31). After 1 to 5 weeks, approximately two kilograms of mussels (10 animals) were collected at each station and kept refrigerated; later, the digestive glands were removed and combined in a composite of approximately 26 grams. The composite was homogenized and washed with a pH 5.5 buffer at low-salt concentration to remove substances toxic to tissue culture cells. Following centrifugation, the tissue sediment was adjusted to pH 3.5 with a 0.7 percent saline buffer to elute viruses from the tissue. The sample was again centrifuged, fetal calf serum added to 10 percent, and the concentrate frozen until analysis.

Sewage and seawater samples were examined for total coliform bacteria as described in Standard Methods (1975), using a membrane filter technique. Mussel samples (digestive gland pools) were examined for the presence of total coliform bacteria using a fermentation tube, most-probable-number (MPN) technique following Recommended Procedures (1962).

Tests were carried out on five samples to estimate the efficiency of recovery of natural viruses in the samples. A known number of Poliovirus Type III (attenuated) plaque forming units (PFU's) were added to effluent, mussel, and seawater samples. The samples were processed as before; from the number of virus PFU's obtained in the final concentrate, the efficiency of recovery was determined.

RESULTS

As noted in the table below, our efficiency of virus recovery ranged from 32 to 67 percent, with lower recovery for mssels than seawater or primary effluent.

Sample	Efficiency of Recovery
Primary effluent, one sample	52%
Seawater	
Sample 1	43%
Sample 2	67%
Mussels	
Sample 1	38%
Sample 2	32%

Enteroviruses in effluent samples from the three major treatment plants were detected and quantified (Table 2). The coliform levels were also determined so that we could obtain an average ratio of total coliform bacteria to virus PFU's for each treatment plant. The average ratios varied from 15.2 million coliform per virus plaque in Orange County primary effluent to 1.53 million coliform per virus plaque in Hyperion treatment plant primary effluent. The estimated virus recovery efficiency of 52 percent would reduce the ratios given by one-half (9.6 million and 765,000 coliform per virus, respectively).

The total estimated mass emission rates of enteroviruses for the three treatment plants was 2.7×10^{11} PFU per day, not considering an estimated efficiency of recovery of 50 percent (Table 3).

Mussels and other shellfish are efficient concentrators of bacteria and viruses, and we used the mussels collected from buoys and shore-station mussels to intercept and concentrate these microorganisms from dilute solution in the field. Enteric viruses were detected in 18 of the 39 mussel samples. We detected viruses in mussels from only two of the nine shore stations—Ballona Creek and the Marina del Rey Yacht Channel in Santa Monica Bay. One of the virus-positive Ballona Creek samples was found to contain 2.5×10^6 MPN total coliforms per kilogram of digestive gland. The number of enteroviruses in the digestive glands of mussels from the 30 buoy stations ranged from none detected to 1,475 PFU/kg. Table 4 lists the 16 buoyed mussel samples in which virus plaques were detected and quantified. Concentrations and frequencies of recovery were lower in mussels exposed near the surface (0 to 1 meter) than in those at greater depths (8 to 45 m); however, during the winter and early spring months, when the thermocline was not present, there was a more even distribution of virus concentration in the water column. The number of coliform bacteria per virus plaque ranged from 11,400 to 10.1 million; if a virus-recovery-efficiency factor of 35 percent is appliedto the results, the ratios would be reduced by about two-thirds.

As shown in Table 4, the majority of the mussel samples had total-coliform-to-virus ratios approximately 100 times lower than the average ratio for primary effluent, indicating that viruses were more concentrated with respect to coliform bacteria in the mussels than in sewage. The coliform levels in the water near the buoys ranged from 10^2 to 7.8×10^3 cells per liter

and were typically three orders of magnitude lower than those found in a roughly equivalent amount of mussel digestive gland (4.9×10^5 to 9.8×10^8 cells per kg), Indicating the degree to which these shellfish concentrate bacteria and, presumably, viruses from the water. Additional calculation indicates that the buoyed mussels positive for viruses contained from 0.1 to 8 viruses per individual mussel.

To obtain some idea of the relative die-off rate of viruses in the field, we estimated relative survival rates of bacteria (t_b) and viruses (t_v.) at the Palos Verdes buoys using the following formula:

$$t_v/t_b = \frac{1n(n_b/n_b^0 + 1nD}{1n(RFn_b/n_v^0) + 1nD}$$

where

D = the dilution of sewage in seawater,

n_b^0 = the initial bacteria concentration in sewage,

n_v^0 = the initial virus concentration in sewage,

n_b = the concentration of bacteria in seawater near the mussels,

R = the measured ratio of virus to bacteria in mussels, and

$$F = (t_b/t_v)\frac{1 - e^{-4}}{1 - e^{-4}(t_b/t_v)}$$

The assumptions entering our calculations were that both types of microorganisms followed a single exponential "decay" law, that the number of bacteria in the seawater was representative of the seawater filtered by the mussels, that the bacteria-to-virus ratio in seawater was the same as in mussels, and that the dilution of the effluent near the buoys ranged from 400:1 to 2,000:1.

Applying these conditions to the data from the 10 paired samples of Palos Verdes buoyed mussels and water (given in Table 4), we found t_v to range from 3 to 6 times t_b. Thus we estimate that it takes three to six times as long for the viruses to undergo the same mortality as the bacteria during their concurrent residence in the sea and mussels.

Additional sampling is required to confirm the trends seen in these analyses. Large-volume sampling of seawater near southern California outfalls for viruses would be useful in confirming the levels of viruses present in the water column. It would also be useful to compare coliform and virus levels in the seawater to determine if the coliform index is satisfactory for assessing virus contamination.

Details of this work will appear in a technical memorandum to be published later this year.

REFERENCES

Coastal Water Research Project. 1975. Annual report. El Segundo, Calif.
Recommended Procedures for bacteriological examination of seawater and shellfish, 1972. Washington, D.C. Standard Methods for examination of water and wastewater, 14th ed. 1975. Washington, D.C.

Table 1. Location of mussel buoys used in study of uptake viruses.

Location	Water Depth (m)	Proximity to Outfall: Distance (km)	Proximity to Outfall: Direction
Santa Monica Bay	105	0.01*	West
Palos Verdes			
Buoy 1	33	1.5	East northeast
Buoy 2	33	2.0	Northwest
Buoy 3	33	3.5	Northwest
Orange County	60	2.5	West

*7-mile outfall.

Table 2. Concentrations of total coliforms and enteroviruses in four southern California wastewater effluents, 1975-76. Averages are geometric means.*

Effluent	Total Coliforms (MPN/liter)	Viruses (PFU/liter)	Ratio, Coliform to Virus
PRIMARY			
Los Angeles City			
Average	1.67×10^8	125.6	1.53×10^6
Range	$5.2 \times 10^7 - 8.5 \times 10^8$	42–238	$2.26 \times 10^5 - 2.23 \times 10^7$
No. of samples	9	13	9
Los Angeles County			
Average	3.14×10^8	88.5	4.93×10^6
Range	$1.4 \times 10^8 - 8.0 \times 10^8$	13–447	$1.06 \times 10^6 - 5.41 \times 10^7$
No. of samples	7	7	7
Orange County			
Average	8.88×10^8	58.3	1.52×10^7
Range	$4.3 \times 10^8 - 2.7 \times 10^9$	46–72	$8.11 \times 10^6 - 3.75 \times 10^7$
No. of samples	5	5	5
SECONDARY			
Los Angeles City			
Average	9.54×10^5	0.621	3.75×10^6
Range	$2.4 \times 10^5 - 4.0 \times 10^6$	0.05–12.7	$6.5 \times 10^4 - 1.6 \times 10^8$
No. of samples	7	10	7

*Values given do not reflect estimated 50 percent recovery efficiency for viruses.

Table 3. Estimated mass emission rates of enteroviruses from three southern California municipal wastewater treatment plants.

Effluent	Flow (cu m/day)	Viruses* Concentration** (PFU/liter)	Viruses* Mass Emission Rate (PFU/day)
Los Angeles City			
Primary	9.27×10^5	125.6	1.16×10^{11}
Secondary	3.97×10^5	0.621	2.47×10^8
Los Angeles County	1.29×10^6	88.5	1.14×10^{11}
Orange County	6.62×10^5	58.3	3.86×10^{10}

*Values do not reflect a 50% efficiency of recovery.
**Geometric means of values.

Table 4. Concentrations of total coliforms and enteroviruses in seawater and in digestive glands of *Mytilus californianus* suspended from buoys.

Table 4. Concentrations of total coliforms and enteroviruses in seawater and in digestive glands of *Mytilus californianus* suspended from buoys.

Location and Water Depth	Date Sampled	Total Coliform Seawater (No./liter)	Mussels (No./kg)	Viruses in Mussels (PFU/kg)*	Ratio, Coliforms to Viruses in Mussels
Santa Monica Bay					
15 m	6 Mar 76	4×10^3	7.9×10^5	263	3×10^4
45 m	6 Mar 76	8×10^3	3.5×10^7	140	2.5×10^5
45 m	11 Feb 76	ND**	7×10^7	30	2.33×10^6
Palos Verdes					
Buoy 1					
30 m	1 Mar 76	7×10^3	1.1×10^7	114	9.65×10^4
Buoy 2					
16 m	3 May 76	1×10^2	3.48×10^7	620	5.61×10^4
16 m	17 May 76	1.2×10^3	1.2×10^8	552	2.17×10^5
32 m	3 May 76	5.9×10^4	3.48×10^7	1,475	2.36×10^4
32 m	17 May 76	7×10^2	7.8×10^6	210	3.71×10^4
32 m	1 Jun 76	8.4×10^3	8.6×10^6	321	2.68×10^4
Buoy 3					
17 m	3 Oct 75	5×10^2	4.9×10^5	41.7	1.18×10^4
25 m	30 Oct 75	3.7×10^2	1.2×10^6	105.3	1.14×10^4
32 m	3 Oct 75	1.1×10^3	8×10^6	315.8	2.53×10^4
32 m	30 Oct 75	1.7×10^3	4×10^6	263.2	1.52×10^4
Orange County					
Surface	6 Feb 76	ND	9.2×10^8	91	1.01×10^7
15 m	6 Feb 76	ND	9.2×10^8	476	1.93×10^6
45 m	6 Feb 76	ND	3.1×10^7	25	1.24×10^6

*Values given do not reflect an estimated efficiency of recovery of 35%.
**ND, no data.