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Differential Depuration of Poliovirus, *Escherichia coli*, and a Coliphage by the Common Mussel, *Mytilus edulis*

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The elimination of sewage effluent-associated poliovirus, *Escherichia coli*, and a 22-nm icosahedral coliphage by the common mussel, *Mytilus edulis*, was studied. Both laboratory- and commercial-scale recirculating, UV depuration systems were used in this study. In the laboratory system, the logarithms of the poliovirus, *E. coli*, and coliphage levels were reduced by 1.86, 2.9, and 2.16, respectively, within 52 h of depuration. The relative patterns and rates of elimination of the three organisms suggest that they are eliminated from mussels by different mechanisms during depuration under suitable conditions. Poliovirus was not included in experiments undertaken in the commercial-scale depuration system. The differences in the relative rates and patterns of elimination were maintained for *E. coli* and coliphage in this system, with the logarithm of the *E. coli* levels being reduced by 3.18 and the logarithm of the coliphage levels being reduced by 0.87. The results from both depuration systems suggest that *E. coli* is an inappropriate indicator of the efficiency of virus elimination during depuration. The coliphage used appears to be a more representative indicator. Depuration under stressful conditions appeared to have a negligible affect on poliovirus and coliphage elimination rates from mussels. However, the rate and pattern of *E. coli* elimination were dramatically affected by these conditions. Therefore, monitoring *E. coli* counts might prove useful in ensuring that mussels are functioning well during depuration.

Bivalve shellfish have been implicated as vectors in the transmission of enteric diseases for many decades (15). The advent of depuration in Europe in the 1920s played a major role in preventing the transmission of human enteric bacterial pathogens via shellfish, and depuration technology has remained largely unchanged since then (4). However, it has not proven as successful in curtailing the transmission of human enteric viruses through shellfish, as recent epidemiological and laboratory studies have shown (7, 8). Hepatitis A virus, the Norwalk agent, and small round structured viruses constitute the infectious viruses of major public health concern associated with transmission via shellfish at present (15).

Much of the literature on depuration has focused on oysters (2, 6, 14, 19) and clams (3, 9, 11) for economic reasons and also because these shellfish have frequently been implicated in disease outbreaks (15). Model enteric viruses (necessitated by the inability to cultivate the viruses of concern in vitro), such as poliovirus, and fecal bacteria, such as *Escherichia coli*, have been used frequently in depuration experiments, usually independently (17). Little information is available on comparative viral and bacterial depuration, despite the volume of work reported in this area. Only four such reports (3, 10, 14, 18) could be located, each using different shellfish species and two using nonhuman viruses as models.

Little is known of the virus depuration capabilities of mussels because only one study is reported in the literature (5). No information is available about the comparative abilities of mussels to depurate viruses and bacteria. This information seemed important in Ireland in view of a rapidly expanding mussel culture industry. This paper describes a comparative study of the efficiency of depuration in eliminating poliovirus, *E. coli*, and a 22-nm icosahedral coliphage from mussels. Experiments were performed in laboratory- and commercial-scale UV recirculating depuration systems, since these are the most common types of purification plants used in Ireland and the United Kingdom.

MATERIALS AND METHODS

Mussels and seawater. The common mussel, *Mytilus edulis*, was used throughout this study. Mussels were harvested in Cork Harbour, Ireland, and transported to the laboratory within 1 h. In the laboratory they were scrubbed vigorously to remove detritus and fouling organisms, and subsequently the byssus threads were removed. Seawater was collected from the same location as the mussels to minimize environmentally induced stress during experimentation.

Tissue culture cells and viruses. Buffalo green monkey (BGM) kidney cells (Flow Laboratories, Inc., McLean, Va.) were used to propagate the poliovirus. Cell stocks were maintained in 75-cm^2 culture flasks (Costar, Cambridge, Mass.) by using Eagle minimal essential medium supplemented with 5% newborn calf serum. Titers of virus stocks were determined, and the virus content of contaminated mussels was enumerated on cells grown in 25-cm^2 culture flasks (Costar) with a semisolid tragacanth gum overlay plaque assay technique, as described by Manning and Collins (13). Plaque-purified poliovirus type 1 vaccine strain was chosen as the model enterovirus for experimental purposes. Poliovirus stocks were prepared by inoculating confluent monolayers of BGM cells with virus at a multiplicity of infection of 10 PFU per cell. When greater than 90% cytopathic effect was evident (18 h), cells and medium were subjected to three cycles of freezing (−80°C), thawing, and sonicating (1 min). The lysate was centrifuged at 4,000 × *g* for 10 min. The clarified lysate was used as the virus stock and contained 10^7 to 10^8 PFU/ml. The virus stock was stored at −80°C until required.

***E. coli* and coliphage.** A wild-type strain of *E. coli* isolated during routine bacteriological screening of shellfish was used in this study. This isolate was used to avoid using a laboratory strain which may not reflect typical *E. coli* uptake and survival in shellfish in the marine environment. The strain

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was chromosomally marked by isolating spontaneous rifampin-resistant (Rif^r) mutants by spreading ca. 10^{10} cells on violet red bile agar (Oxoid Ltd., London, England) plates containing 100 μg of rifampin (Sigma Chemical Co., St. Louis, Mo.) per ml. This marking facilitated recovery and enumeration of the strain in contaminated mussels. In addition, from this Rif^r isolate, a strain resistant to a broad spectrum of coliphages was selected by a challenge with a collection of environmental coliphage isolates (including the coliphage isolate used in this study) at a high multiplicity of infection. This prevented the possibility of reduced *E. coli* levels and elevated coliphage levels resulting from coliphage replication during depuration. This Rif^r, coliphage-resistant strain was designated *E. coli* 4A.

E. coli A-19 (Hfr) (kindly provided by R. E. Stetler, U.S. Environmental Protection Agency), which exhibits a broad-spectrum phage sensitivity, was used to isolate wild-type coliphages from sewage effluent. These phages were plaque purified by using a double-agar-overlay technique, as described by Maniatis et al. (12), with Luria broth (LB) agar and *E. coli* A-19 (Hfr) as the host bacterium. A number of the plaque-purified isolates were examined under transmission electron microscopy by negative staining with phosphotungstic acid. One isolate displayed a tailless, 22-nm icosahedral morphology which closely mirrored the morphology of enteroviruses and hence was chosen as the model coliphage for experimental purposes. It was designated ϕA1-5a. The double-agar-overlay technique described above (12) was used to prepare phage stocks, determine the phage titers, and enumerate the coliphage content of contaminated mussels. Coliphage stocks were prepared as outlined by Maniatis et al. (12). Briefly, *E. coli* A-19 (Hfr) was inoculated with ϕA1-5a at a multiplicity of infection of 10 PFU per cell. The coliphage was harvested from plates exhibiting confluent lysis after 8 h of incubation at 37°C by flooding the plates with 5 ml of salt-magnesium-gelatin buffer (12), incubating them for 4 h at 4°C with intermittent shaking, and removing the buffer. The plates were flooded with a further 1 ml of buffer and stored tilted at 4°C for 15 min. Again, the buffer was removed and added to the initial 5 ml. This constituted the crude coliphage stock. The stock was treated with 1% chloroform, vortexed briefly, and centrifuged at 4,000 × *g* for 10 min. The clarified stock contained 10^9 to 10^{10} PFU/ml and was stored at −80°C until required.

Preparation of contaminated sewage. To simulate natural conditions of contamination, the model contaminating organisms were thoroughly mixed with sewage effluent obtained from the local municipal sewage works. Prior to use, the sewage effluent was heat treated at 85°C for 1 h to inactivate any indigenous enteric viruses, *E. coli*, or coliphages that may have been present. A 1-ml sample of the poliovirus stock was added to 500 ml of the heat-treated effluent, and the mixture was stirred for 1 h at room temperature. The pH was reduced to 3.5 with hydrochloric acid to actively adsorb the virus to fecal particulates. After stirring at room temperature for 1 h, the pH was readjusted to 7 with sodium hydroxide. Subsequently, 10 ml of an overnight culture of *E. coli* 4A and 1 ml of ϕA1-5a stock were added to the effluent, and the entire mixture was stirred overnight at 4°C. Poliovirus was omitted from experiments undertaken in the commercial depuration plant to prevent the unacceptable risk of cross-contaminating valuable commercial shellfish.

Aquarium and tank designs for acclimatization, contamination, and depuration. In the laboratory, a polypropylene aquarium was used to acclimatize the mussels prior to experimentation. It had dimensions of 60 by 40 by 20 cm and could hold ca. 20 liters of seawater. A plastic-coated aluminum grid, placed on the bottom of the aquarium, served to elevate the mussels from the bottom and curtail recontamination with feces and pseudofeces. A flowthrough pump (Camlab, Cambridge, England) was used to recirculate the water. During circulation, water was passed through a charcoal-glass wool filter to remove nitrogenous waste and excess suspended material. The water was continuously aerated by using an air pump (Rena 101) through attached air stones to maintain dissolved oxygen (DO) levels above 60% saturation. The air stones were placed far enough from the mussels that the local turbulence did not resuspend settled biodeposits into the water column.

Mussels were contaminated in the above system with the charcoal-glass wool filter already removed to prevent virus adsorption to the charcoal.

For depuration purposes, the laboratory aquarium was modified by incorporating a UV sterilizing system. The water was diverted onto a Perspex tressel (30 by 4 cm) before being introduced to the filter unit. The tressel provided a very thin film of water, which facilitated sterilization. A Hanovia 30-W UV lamp with dimensions similar to the tressel was placed 2 cm above the water film to effect sterilization.

The commercial depuration unit was a recirculating UV system. It was composed of three tanks in parallel, each 6 by 1.8 by 0.6 m. Each tank had a capacity of 5,000 liters. The water was recirculated by using a Beresford pump at a rate of 15,000 liters/h. The water was pumped to an elevated weir which contained the UV sterilizing system. This consisted of two 30-W low-pressure tubes (TUV 30 W; Philips, Eindhoven, The Netherlands) which were elevated 5 cm above the water. The water was effectively aerated by the fall (3 m) to the tanks after UV irradiation.

Acclimatization, contamination, and depuration. All mussels were acclimatized for at least 4 days prior to contamination. Mussel activity was monitored constantly to ensure viability. Activities monitored included shell opening, the extension of sensory papillae, foot extension and movement, byssus thread production and reattachment, and the rapidity of response to disturbance. Mussels showing reduced activity levels and failure to respond to physical disturbance were removed from the aquarium. Water temperature, DO, salinity, and pH were monitored to ensure that suitable physicochemical parameters prevailed, as suggested by Canzonier (4).

Contamination was carried out over a 24-h period. Laboratory-contaminated sewage suspension (500 ml) was added to 12.5 liters of fresh seawater. The contaminated seawater was recirculated for 30 min to facilitate uniform contaminant distribution in the system. The maximum concentrations of the contaminants in the water during these experiments were as follows: poliovirus, 5×10^2 to 5×10^3 PFU/ml; *E. coli* 4A, 5×10^4 to 5×10^5 CFU/ml; ϕA1-5a, 5×10^4 to 5×10^5 PFU/ml. Acclimatized mussels were placed into the contaminated water for 24 h to accumulate contaminants. During this time, mussel activity and water temperature were regularly monitored.

After 24 h the mussels were removed from the aquarium and washed thoroughly in tap water prior to depuration. At this point, four mussels were retained as an undepurated control zero time sample. The remaining mussels were immersed in either the laboratory-scale or the commercial depuration system. In the commercial system, the mussels were depurated in association with at least 500 other shell-

TABLE 1. Physicochemical parameters under which the depuration experiments were performed

Phase	Temp range (°C)	Salinity range (‰)	pH	DO (% saturation)
Acclimatization	15–21	27–30.6	7.3–8.1	>60
Contamination	15–20	27–29.3	7.4–8.1	>60
Depuration	15.5–19.5	27–29.3	7.4–8.3	>60

fish. Depuration was undertaken over a 52-h period. Mussels were removed at 0, 10, 26, and 52 h for assay. Mussel activity and water temperature were routinely monitored throughout depuration, as were DO, salinity, and pH.

Assay procedures. For the laboratory-depurated samples, 25 g of mussel tissue was used for poliovirus extraction and another 25 g was used for *E. coli* 4A enumeration and ϕA1-5a extraction. For samples depurated in the commercial plant, 25 g of mussel tissue was used for *E. coli* 4A enumeration, and a separate 25 g was used for ϕA1-5a extraction. All mussels were washed thoroughly before being shucked. In all cases, infrabranchial water was discarded during shucking.

Poliovirus was extracted from shellfish meat by the elution-adsorption technique described by Richards et al. (17). The final homogenate and appropriate dilutions were assayed in triplicate as described above.

The mussel tissue used for *E. coli* 4A enumeration and ϕA1-5a extraction was finely chopped with sterile scissors and placed in a Stomacher 400 (Coleworth) for 2 min. The homogenate obtained was diluted 1:2 (wt/vol) in 0.25× Ringer buffer (Oxoid). Triplicates containing 1 ml of the final homogenate and appropriate dilutions were overlaid with 5 ml of tryptone soy agar (Oxoid) containing 100 μg of rifampin per ml. The plates were allowed to stand for 1 h at room temperature and were then overlaid with violet red bile agar. When set, the plates were incubated at 44.5°C for 24 h. Typical *E. coli* 4A colonies were enumerated.

For ϕA1-5a extraction, the remaining homogenate from laboratory experiments and the entire homogenate from commercial plant experiments were diluted with sterile double-distilled H_2O to give a final dilution of 1:5 (wt/vol). This homogenate was decanted into 250-ml centrifuge bottles and shaken for 5 min at maximum speed on a multitube shaker (The Vortex Manufacturing Co., Cleveland, Ohio). It was then centrifuged at 10,000 × *g* for 15 min, and the supernatant was retained as the coliphage extract. Triplicates of the extract and appropriate dilutions were assayed as described above.

RESULTS

Physicochemical parameters were similar for all experiments (Table 1).

The results of comparative depuration under suitable physicochemical conditions and mussel activity are plotted in Fig. 1. *E. coli* 4A was eliminated very rapidly over the first 10 h, showing a 2.65-log reduction. Thereafter, this organism was eliminated slowly, and a final 2.8-log reduction resulted within 52 h. Poliovirus elimination, on the other hand, appeared to be relatively constant throughout the 52-h depuration period, and a final 1.86-log reduction was obtained. The ϕA1-5a elimination rate appeared to be intermediate between those of *E. coli* 4A and poliovirus. During the initial 10 h of depuration, the coliphage level was reduced by the logarithm of 1.5, and a final 2.16-log reduction was obtained within 52 h.

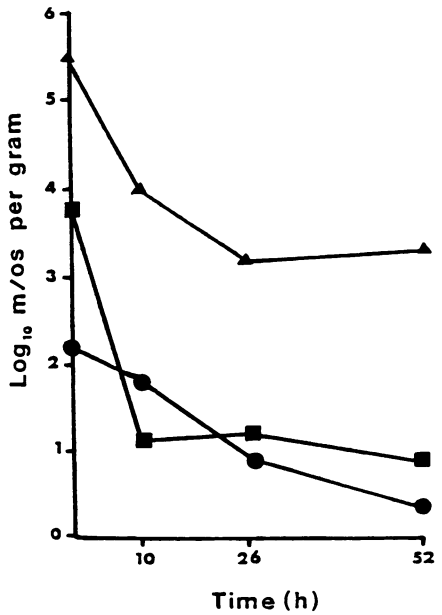

FIG. 1. Elimination of poliovirus (PFU per gram) (●), *E. coli* 4A (CFU per gram) (■), and ϕA1-5a (PFU per gram) (▲) from mussels during depuration in the laboratory-scale system. Environmental conditions during depuration were as follows: temperature, 15.5 to 19.5°C; salinity, 27 to 29.3 ‰; DO, >60% saturation; pH, 7.4 to 8.3.

The results of a comparative elimination study undertaken in the commercial depuration plant are shown in Fig. 2. The pattern and extent of *E. coli* 4A elimination closely resembled the results obtained in the laboratory-scale system. A 2.61-log reduction was obtained within 10 h, followed by a slower elimination that resulted in a 3.18-log reduction after 52 h. In contrast, ϕA1-5a was not efficiently eliminated.

Complications of depuration during the principal months of mussel harvesting include spawning and accompanying poor mussel activity. Experiments were undertaken to determine the effect of these conditions on depuration efficiency. The results of a representative experiment are illus-

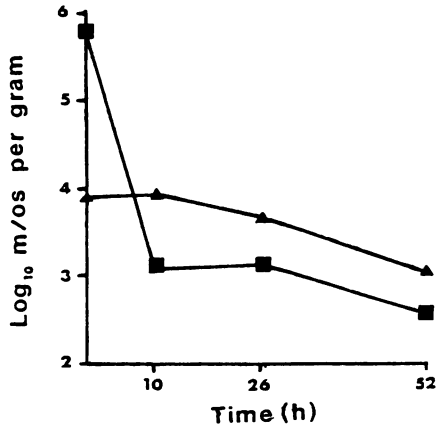

FIG. 2. Elimination of *E. coli* 4A (CFU per gram) (■) and ϕA1-5a (PFU per gram) (▲) from mussels during depuration in the commercial-scale system. Environmental conditions during depuration were as follows: temperature, 15.5 to 19.5°C; salinity, 27 to 29.3‰; DO, >60% saturation; pH, 7.4 to 8.3.

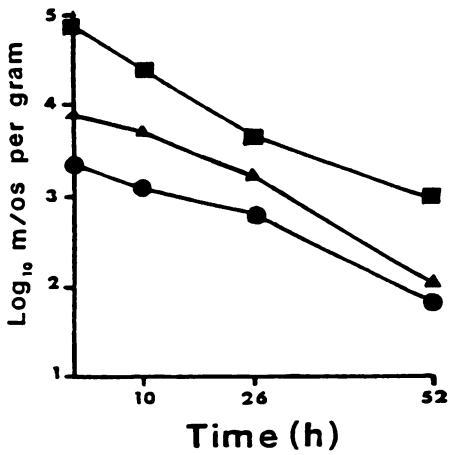

FIG. 3. Elimination of poliovirus (PFU per gram) (●), *E. coli* 4A (CFU per gram) (■), and ϕA1-5a (PFU per gram) (▲) from mussels during depuration in the laboratory-scale system under conditions of spawning-associated poor mussel activity. Environmental conditions during depuration were as follows: temperature, 15.5 to 19.5°C; salinity, 27 to 29.3‰; DO, >60% saturation; pH, 7.4 to 8.3.

trated in Fig. 3. The effect of spawning on poliovirus elimination appeared to be negligible, since the characteristic pattern of gradual reduction over the 52-h period was retained, and the logarithm of the poliovirus level was reduced by 1.54 within this time. Like poliovirus, the extent of ϕA1-5a elimination appeared to be virtually unaffected by these adverse conditions, with a log reduction of 1.88 occurring within 52 h. However, the pattern of elimination changed and became similar to the pattern observed for poliovirus. In contrast to the viruses, the effect of these stressful conditions on *E. coli* 4A elimination was dramatic. First, the rapid elimination normally seen over the first 10 h was not evident. The elimination pattern was gradual over 52 h, resembling that of poliovirus. Second, the final 1.93-log reduction obtained was quite inefficient when compared with the 2.8-log reduction that occurred under suitable conditions.

For all experiments undertaken, the relative rates of elimination during depuration were as follows: *E. coli* 4A > ϕA1-5a > poliovirus type 1, irrespective of experimental conditions.

DISCUSSION

This study was undertaken to determine the efficiency with which poliovirus is eliminated from the common mussel during depuration and to compare the relative rates and patterns of its elimination with those of *E. coli*, the internationally accepted fecal indicator, and a 22-nm icosahedral coliphage. Coliphages are currently generating considerable interest as alternatives to *E. coli* as environmental indicators of enteric viruses. The experiments were performed in a laboratory-scale recirculating UV system and a commercial UV recirculating depuration plant.

Depuration, under the conditions outlined for the laboratory-scale system (Fig. 1), resulted in significant reductions in the levels of poliovirus type 1, *E. coli* 4A, and ϕA1-5a in contaminated mussels. Both the laboratory- and commercial-scale depuration systems produced similar rates of *E. coli* 4A elimination, and although the rate of ϕA1-5a elimination was lower in the commercial-scale system, a substantial reduction in the level of this coliphage was obtained nonetheless. Thus, the process of depuration, as reported in this communication, considerably reduces the risk of virus or bacterial infection from contaminated mussels, if it can be assumed that the behavior of the model contaminating organisms we used reflects that of pathogens commonly associated with mussels, such as hepatitis A virus.

The pattern of poliovirus elimination from mussels was very similar to the pattern of poliovirus elimination from Olympia oysters depurated in a free-flowing system as reported by DiGirolamo et al. (6). *E. coli* 4A and ϕA1-5a elimination patterns also reflected those reported elsewhere for other shellfish species (3, 9, 15). Hence, mussels appear to be depurated in a similar fashion to other shellfish under conditions suitable for good activity.

The relative rates and patterns of removal of poliovirus, *E. coli* 4A, and ϕA1-5a from mussels during depuration in the laboratory-scale systems under suitable conditions (Fig. 1) indicate that the contaminating microorganisms were differentially eliminated. This differential elimination was emphasized by the depuration results from the commercial plant (Fig. 2). Canzonier (3) reported similar results for clams. If depuration were a function of defecation alone, similar rates of removal would be expected. The differential elimination found indicates that another factor(s) must influence the removal of the contaminants from mussels during depuration. Such factors may include adsorption to gastrointestinal tract cells, interstitial sequestering, and spontaneous and/or hemocyte-induced inactivation.

The differences seen in the rates of depuration of *E. coli*, coliphage, and poliovirus also suggest that the mechanism(s) effecting elimination may vary from one microorganism to another. This coincides with the work of Canzonier (3) and Scotti et al. (18). Since poliovirus and ϕA1-5a have similar sizes and shapes, the discrepancies in the elimination rates and patterns of these viruses suggest that virus elimination from mussels is not a product of particle size and shape alone. Sobsey et al. (19) recently reported similar discrepancies in the rates of poliovirus and hepatitis A virus elimination from oysters.

When the rate of *E. coli* 4A elimination is compared with the rate of elimination of coliphage or poliovirus under suitable conditions of depuration, it is evident that *E. coli* is not a reliable indicator of the efficiency of depuration in eliminating these viruses from mussels. Canzonier (3) and Scotti et al. (18) arrived at similar conclusions when reporting findings with hard clams and Pacific oysters, respectively. Comparative analysis of the results suggests that ϕA1-5a is more reliable than *E. coli* as an indicator of the efficiency with which enteric viruses are eliminated from mussels. The appropriateness of this coliphage as an experimental indicator is greatly enhanced by the ease and rapidity of assaying and its innocuous nature. These properties facilitate safe and rapid elucidation of information about virus depuration mechanisms and the factors affecting them under commercial conditions. However, the differences in the rates of elimination of poliovirus and ϕA1-5a reported here and of poliovirus and hepatitis A virus as reported by Sobsey et al. (19) suggest that caution is required in applying conclusions from such coliphage-based experiments to depuration characteristics of enteric viruses in general.

None of the depuration experiments were successful in eliminating either of the viruses or *E. coli* 4A to undetectable levels within 52 h. It is likely that the high initial levels of contamination contributed to this failure. There are reports in the literature of successful virus depuration from shellfish

contaminated to levels similar to those used in the present study and within a similar period (5, 11, 16). However, these studies invariably used monodispersed viruses and were undertaken at a time when procedures for extracting viruses from shellfish were less reliable than that of Richards et al. (17). The inability to eliminate *E. coli* 4A to undetectable levels corresponds to the work of Buisson et al. (2), who reported consistently unsuccessful fecal-coliform depuration from oysters contaminated with $>10^3$ MPN/g. Hence, it is evident that depuration cannot be relied on to render mussels safe for human consumption after they have become contaminated with high levels of viruses and bacteria. This coincides with other reports (2, 4, 9), which suggest that successful cleansing of shellfish depends to a large extent on the initial contamination levels.

The results presented in Fig. 3 suggest that conditions affecting mussel activity during depuration dramatically affect the rate and pattern of *E. coli* 4A elimination. In contrast, the extent of mussel activity appeared to have little or no effect on the rates at which poliovirus and ϕA1-5a were removed. Canzonier (3) observed similar relationships in hard clams. During depuration under stressful conditions associated with spawning, the rate and pattern of *E. coli* 4A elimination reflected those of poliovirus (Fig. 3). The poor activity was probably related to the spawning, since spawning is physiologically stressful (1). In experiments on *E. coli* and poliovirus elimination from Eastern oysters, Mitchell et al. (14) showed that both microorganisms exhibited very similar elimination rates and patterns. They concluded that the mechanisms of elimination of both organisms by shellfish are very similar, if not identical. However, a salinity range of 8.5 to 23‰ was reported for one of the experiments; this was likely to have been stressful for the oysters. Hence, the results obtained by Mitchell et al. (14) would appear to coincide with those illustrated in Fig. 3.

Although *E. coli* appears to be an unreliable indicator of virus elimination from mussels, the rate and pattern with which it is removed appear to reflect the physiological status of the mussels. Thus, monitoring *E. coli* elimination may provide a useful guide to the level of mussel activity during depuration.

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LITERATURE CITED

1. **Bayne, B. L., R. J. Thompson, and J. Widdows.** 1976. Physiology, p. 121–206. *In* B. L. Bayne (ed.), Marine mussels: their ecology and physiology. Cambridge University Press, Cambridge.
2. **Buisson, D. H., G. C. Fletcher, and C. W. Begg.** 1981. Bacterial depuration of the Pacific oyster (*Crassostrea gigas*) in New Zealand. N. Z. J. Sci. **24**:253–262.
3. **Canzonier, W. J.** 1971. Accumulation and elimination of coliphage S-13 by the hard clam, *Mercenaria mercenaria*. Appl. Microbiol. **26**:1024–1031.
4. **Canzonier, W. J.** 1982. Depuration of bivalve molluscs—what it can and cannot accomplish and some practical aspects of plant design and operation. Proceedings of the International Seminar on Management of Shellfish resources. Irish Marine Farmers Association, Tralee, Ireland.
5. **Crovari, P.** 1958. Some observations on the depuration of mussels infected with poliomyelitis virus. Ig. Mod. **5**:22–32.
6. **DiGirolamo, R., J. Liston, and J. Matches.** 1975. Uptake and elimination of poliovirus by West Coast oysters. Appl. Microbiol. **29**:260–264.
7. **Gill, O. N., W. D. Cubitt, D. A. McSwiggan, B. M. Watney, and C. L. R. Barlett.** 1983. Epidemic gastroenteritis caused by SRSV contaminated oysters. Br. Med. J. **287**:1532–1534.
8. **Grohmann, G. S., A. M. Murphy, P. J. Christopher, E. Anty, and H. B. Greenberg.** 1981. Norwalk virus gastroenteritis in volunteers consuming depurated oysters. Aust. J. Exp. Biol. Med. **59**:219–228.
9. **Heffernan, W. P., and V. J. Cabelli.** 1970. Elimination of bacteria by the Northern quahaug (*Mercenaria mercenaria*): environmental parameters significant to the process. J. Fish. Res. Board Can. **27**:1569–1577.
10. **Hoff, J. C., and R. C. Becker.** 1969. The accumulation and elimination of crude and clarified poliovirus suspensions by shellfish. Am. J. Epidemiol. **90**:53–61.
11. **Liu, O. C., H. R. Seraichekas, and B. L. Murphy.** 1967. Viral depuration in the Northern quahaug. Appl. Microbiol. **15**: 307–315.
12. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
13. **Manning, J. S., and J. K. Collins.** 1980. Effects of cell culture and laboratory conditions on type 2 Dengue virus infectivity. J. Clin. Microbiol. **10**:235–239.
14. **Mitchell, J. R., M. W. Presnell, E. W. Akin, J. M. Cummins, and O. C. Liu.** 1966. Accumulation and elimination of poliovirus by the Eastern oyster. Am. J. Epidemiol. **84**:40–50.
15. **Richards, G. P.** 1985. Outbreaks of shellfish-associated enteric virus illness in the United States: requisite for development of viral guidelines. J. Food Prot. **48**:815–823.
16. **Richards, G. P.** 1988. Microbial purification of shellfish: a review of depuration and relaying. J. Food Prot. **51**:218–251.
17. **Richards, G. P., D. Goldmintz, D. L. Green, and J. A. Babinchak.** 1982. Rapid methods for extraction and concentration of poliovirus from oyster tissue. J. Virol. Methods **5**:285–291.
18. **Scotti, P. D., G. C. Fletcher, D. H. Buisson, and S. Fredericksen.** 1983. Viral depuration of the Pacific oyster (*Crassostrea gigas*) in New Zealand. N. Z. J. Sci. **26**:9–13.
19. **Sobsey, M. D., A. L. Davis, and V. A. Rullman.** 1987. Persistance of hepatitis A virus and other viruses in depurated Eastern oysters. Proc. Oceans 87 **5**:1740–1745.