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# Seasonal Effects on Accumulation of Microbial Indicator Organisms by *Mercenaria mercenaria*

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**The ability of hard-shelled clams (*Mercenaria mercenaria*) to accumulate fecal coliforms and other microorganisms (*Escherichia coli*, *Clostridium perfringens*, and male-specific bacteriophages) was determined over a 1-year period. Twenty separate trials were conducted during different seasons to encompass a wide range of water temperatures. The greatest accumulation of microorganisms in hard-shelled clams occurred during certain periods in the spring, at temperatures ranging from 11.5 to 21.5°C. These periods of hyperaccumulation did not always coincide for all organisms; the accumulation of bacteriophages was not predicted by the accumulation of either fecal coliforms or *C. perfringens*. Bacteriophages and *C. perfringens* showed significantly higher rates of accumulation than either the fecal coliform group or *E. coli*, especially during the spring. The higher incidence of human viral gastroenteritis associated with the consumption of shellfish during this period may be a result of the extraordinary concentration of certain microorganisms, including enteric viral pathogens.**

Molluscan shellfish are well identified as vectors of bacterial and viral pathogens (21, 23). The reported number of outbreaks and cases of illness associated with the consumption of shellfish in the United States has been steadily increasing since the early 1900s. Coincident with these overall increases has been a steady rise in illnesses attributed to viral pathogens (23). In these case reports a seasonal pattern is evident, with incidents occurring most often in the early spring and least frequently in the late summer (Fig. 1).

Public health problems associated with shellfish consumption have resulted in a program to classify shellfish-growing areas that is based, at least in part, on the sanitary quality of surface waters, as indexed by the level of fecal coliforms (31). One of the shortcomings of this indicator group is that it does not reliably index the presence of enteric viruses in either estuarine waters or shellfish (13, 14, 16, 28). The reason may be in part that fecal coliforms are not as resistant to chlorine disinfection (3, 15, 17, 19, 27) and environmental stresses of salinity (22) and sunlight (5, 26) as some enteroviruses are.

The ability of hard-shelled clams (*Mercenaria mercenaria*) to accumulate a variety of microorganisms (both bacterial and viral) has been previously examined in two general ways. First, environmental studies have examined the densities of particular microorganisms in overlying waters before and during the shellfish harvest and in the harvested shellfish (6, 8, 14). Such studies have not demonstrated relationships between the concentration of any particular microorganism (fecal coliforms, *Escherichia coli*, enterococci, *Clostridium perfringens*, and male-specific bacteriophages) in the water column at any given time and the concentration found in the shellfish, regardless of the season or water temperature. Second, accumulation studies have been conducted under controlled conditions in laboratories. Shellfish were exposed to suspensions of pure strains of bacteria and viruses for various periods. Hard-shelled clams accumulated *E. coli* and *Salmonella typhimurium* at concentrations 6.5 to 8.5 times greater than those found in the surrounding water (7, 29). However, accumulations of certain viruses by hard-shelled clams under similar conditions were generally reported to be greater than those found for the bacterial species. An attenuated strain of human poliovirus type 1 was found in hard-shelled clams at densities 10 to 100 times greater than those found in the surrounding water (25), and coliphage S-13 was found in clams at densities 10 to 1,100 times greater than in water (9).

The problem with the laboratory studies is that the accumulation of pure laboratory suspensions of microorganisms is not a realistic approach to reliably assess bioaccumulation rates. Particle-bound microorganisms may show rates of uptake that are substantially different from those of purified suspensions. For example, crude suspensions and purified cultures of poliovirus have been shown to accumulate differently (21). In addition, such studies must take into account the effects of time of exposure (duration) and several other variables that influence the accumulation of microorganisms.

The objective of this study was to examine the effects of season and temperature on the ability of *M. mercenaria* to accumulate both fecal coliforms and other sanitary indicator organisms (*E. coli*, *C. perfringens*, and male-specific bacteriophages) as they would occur in estuarine water. Each of these microorganisms is present in high densities in untreated wastewater (32) and in various degrees in receiving waters (6). Accordingly, unamended human-derived wastewater was used as the source of all indicator organisms for studying uptake rates in shellfish. Replicate trials were conducted throughout a 1-year period to determine the extent to which season (and corresponding temperatures) influences the accumulation of these microorganisms. Also, the stability of each indicator after introduction into the shellfish was assessed to distinguish results attributed to differential indicator accumulation from die-off.

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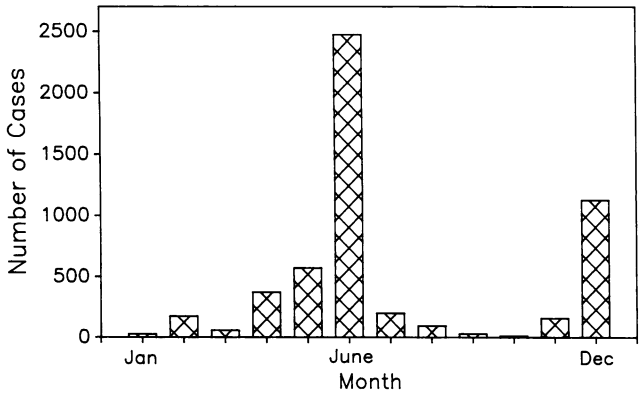


FIG. 1. Number of cases of shellfish-associated diseases by month in the United States for all viral and suspected viral agents for 1902 to 1990. (Adapted from reference 23.)

## MATERIALS AND METHODS

**Shellfish holding and purification.** Hard-shelled clams (*M. mercenaria*) used in this study were harvested from Narragansett Bay, R.I., relayed to a land-based marine laboratory located at Allen Harbor, R.I., and placed into a tank continuously supplied with UV-disinfected seawater. This system consisted of a rectangular, plywood-reinforced resin tank (350 by 30 by 25 cm) with a working volume of 210 liters. Shellfish in this tank were placed in a monolayer on galvanized hardware cloth (0.25-in. [ca. 0.64-cm] mesh) suspended 10 cm above the bottom. Before entering the system, ambient seawater (flowing at approximately 3 liters/min) was disinfected with a four-bulb Kelly-Purdy (18) UV irradiation unit (15-V germicidal lamp; General Electric, Cleveland, Ohio). The effectiveness of disinfection was determined daily by examining exit water (from the UV unit) for the presence of all microbial indicators included in the study. Shellfish were maintained in this system for a minimum of 2 weeks before each accumulation trial to eliminate background levels of the indicator organisms. During this elimination period, both the tank and the shellfish were rinsed daily to remove debris and expelled materials.

**Shellfish contamination.** Bioaccumulation experiments were conducted routinely in the following manner. Ninety-five hard-shelled clams were removed from the depuration system and placed in a circular fiberglass resin tank (90-cm diameter; 127-liter maximum capacity) with a working volume of 40 liters. Immediately before the contamination phase of each experiment, 18 animals were removed to determine the initial densities, if any, of each of the indicator microorganisms within the shellfish. Indicator densities were consistently below the detection limits for each of the assay methods used. Ambient seawater was delivered into this flowthrough uptake system at a rate of 3.0 liters/min. Raw sewage, collected from a local sewage treatment facility in East Greenwich, R.I., was delivered into this contamination tank by a proportioning pump at a rate of 3 ml/min so that a constant concentration of raw wastewater was maintained in the tank. Seawater and sewage were constantly mixed in the uptake tank by a submersible pump circulating 6 liters/min (model 1; Little Giant, Oklahoma City, Okla.). At selected intervals (0, 24, 48, and 168 h) after the initial contamination, tank water and 18 randomly selected clams were collected and analyzed to determine the densities of the indicator microorganisms. Accumulation factors were calculated as the geometric mean indicator density of each microorganism in the shellfish divided by the corresponding geometric mean density of the particular indicator found in the overlying water.

**Survival of microbial indicators.** The stability of indicator organisms within hard-shelled clams was determined by using animals previously exposed to raw sewage, as described above, for a period of 72 h. After this period, a subsample of 18 animals was analyzed to determine indicator organism densities. The remaining shellfish were rinsed with raw seawater to remove debris, banded tightly shut with elastic bands to prevent all filter feeding, and placed in a flowthrough, UV-disinfected seawater system. At 24-h intervals (up to 168 h), 18 animals were removed and analyzed for each indicator organism.

**Microbiological analyses. (i) Shellfish.** Eighteen animals were collected, placed in polypropylene bags, stored on ice, and examined (within 2 h) according to recommended procedures (2). Earlier results, obtained from 12 to 18 animals, revealed substantial variability in densities of indicator organisms, possibly caused by the differences in filtering activity between animals. To determine whether this was the actual cause of the variability, we subdivided the clams into three equal subsamples of six animals each. Each subsample was scrubbed, and the shell contents (including meats and liquors) were collected in a sterile blender jar (Waring Corp., Corning, N.Y.) and homogenized for 2 min at high speed. The homogenates were held on ice until analysis (within 60 min). Concentrations of fecal coliforms, *E. coli*, and *C. perfringens* were determined for each subsample with a five-tube, multiple-dilution most-probable-number (MPN) procedure. Fecal coliform and *E. coli* concentrations were determined with lauryl tryptose broth (Difco Laboratories, Detroit, Mich.) as the presumptive test medium (2) and EC-MUG (Difco) as the confirmatory medium (24). Enterococcal concentrations were determined with azide dextrose broth (Difco) as the presumptive test medium. Tubes that showed turbid growth at 24 and 48 h were confirmed by streaking a portion from each tube onto membrane filters (HC filters; Millipore Corp., Bedford, Mass.) that had been placed on mE agar plates (20) modified (11) by the addition of indoxyl-β-D-glucoside (750 μg/ml) (Sigma Chemical Co., St. Louis, Mo.). Each membrane accommodated up to five linear streaks. Inoculated plates were inverted and incubated for 24 h at 41°C. Streaks (and corresponding tubes) were scored positive if blue growth developed. *C. perfringens* concentrations were determined by the iron milk method procedure (1). Male-specific bacteriophage densities were determined by a modified double-agar-overlay method described by Cabelli (6). The concentration of each indicator organism reported for each subsample is the geometric mean number of organisms per 100 g, calculated from the densities determined for each of the three subsamples.

**(ii) Water.** Samples were collected in sterile, 1-liter, screw-cap, polypropylene sample bottles (Nalgene Laboratories Inc., Rochester, N.Y.) and stored on ice until analysis. Fecal coliform and *E. coli* densities were determined by the mTEC procedure (12), enterococcal densities were determined by the modified mE procedure (11), and *C. perfringens* densities were determined by the mCP procedure (4). Densities of male-specific bacteriophages were determined by a modified double-agar-overlay method (10). This method uses an *E. coli* strain (HS[pFamp]R) that is highly selective for the enumeration of these bacteriophages from municipal wastewaters and environmental waters.

**Other parameters.** Certain ambient physical and chemical

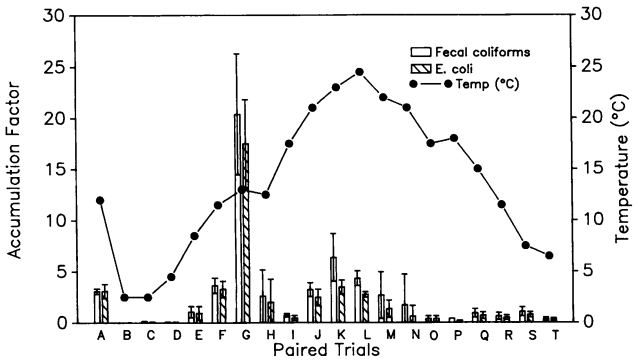


FIG. 2. Bioaccumulation of fecal coliforms and *E. coli* by *M. mercenaria*. Results shown are for paired trials in relation to seawater temperatures. Accumulation factors (and their 95% confidence limits, indicated by error bars) are calculated as the geometric mean indicator MPN values determined for the shellfish divided by the respective density of the particular indicator found in the overlying contaminated water.

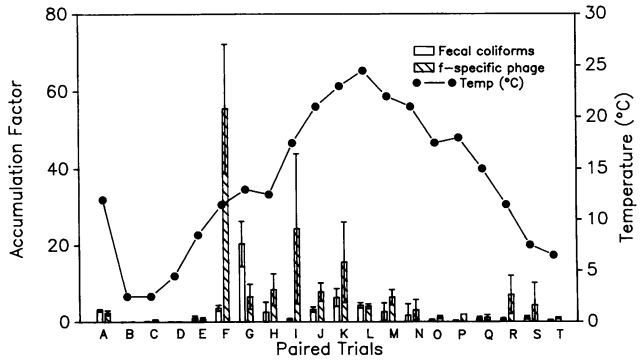


FIG. 3. Bioaccumulation of fecal coliforms and male-specific bacteriophages by *M. mercenaria*. Results shown are for paired trials in relation to seawater temperatures. Accumulation factors (and their 95% confidence limits, indicated by error bars) are calculated as the geometric mean indicator MPN values (for fecal coliforms) and PFU (for male-specific bacteriophages) for the shellfish divided by the respective density of the particular indicator found in the overlying contaminated water.

parameters of the uptake water (salinity, temperature, dissolved oxygen, and turbidity) were determined daily. Salinities and temperatures were determined with an electrodeless induction salinometer (model RS 5-3; Beckman, Cedar Grove, N.J.). Concentrations of dissolved oxygen were measured with a Yellow Springs Instruments (Yellow Springs, Ohio) model 57 oxygen meter. Turbidities were measured with a nephelometer (model 21PE; Monitek Inc., Hayward, Calif.).

## RESULTS

The bioaccumulation of microbial indicator organisms from seawater by hard-shelled clams was investigated from November 1989 through December 1990. Typical seasonal variations for temperate climates were observed for water temperatures (2.5 to 24.5°C). Salinities throughout these trials remained relatively constant (29 to 31 ‰). Dissolved oxygen levels always exceeded 90% saturation. Turbidities were consistently <5.0 nephelometric turbidity units.

Fecal coliforms and *E. coli* were concentrated by the shellfish to various degrees over the 13-month period. Figure 2 summarizes data collected from 20 trials during this study. There was a seasonal influence on the rates of accumulation of these vegetative indicators. The mean accumulation factors for fecal coliforms and *E. coli* in hard-shelled clams for the 20 trials were 2.7 (range, 0.02 to 20.4) and 2.0 (range, 0.02 to 17.5), respectively. The greatest accumulation for both organisms occurred during May, when the water temperature was 13.0°C (trial G). May was considered a period of hyperaccumulation, defined as the level of uptake at which the mean accumulation factor of an indicator is at least two standard deviations above the mean overall accumulation factor for all trials. This period of hyperaccumulation was preceded by steadily increasing water temperatures during the seasonal transition from winter (low water temperature, 2.5°C) to spring. Uptake rates (accumulation factors) just before and just after the period of hyperaccumulation were significantly lower (3.6 and 2.6 for fecal coliforms and *E. coli*, respectively). Spring and summer accumulation rates for fecal coliforms and *E. coli* (excluding the period of hyperaccumulation) were 0.7 to 6.4 and 0.5 to 3.5, respectively. As temperatures declined through the fall and winter months, the accumulations of these indicator organisms were generally lower than for any other time of the year. When autumn water temperatures decreased from 12.0 to 2.5°C, the accumulation factors for fecal coliforms and *E. coli* ranged from <0.1 to 3.1 (generally <1.0). In each of the three determinations made when water temperatures were below 7.0°C, the accumulation factors for both of these indicators were <0.2. With the exception of the springtime phenomenon, accumulations of the fecal coliforms and *E. coli* were very similar, regardless of the season or the water temperature.

Accumulation factors for fecal coliforms and male-specific (f-specific) bacteriophages in hard-shelled clams are shown in Fig. 3. The pattern of uptake was somewhat similar to those shown for fecal coliforms and *E. coli*. However, one distinct period of hyperaccumulation that did not correspond to the hyperaccumulation period for fecal coliforms and *E. coli* was found for male-specific bacteriophages (trial F). Hyperaccumulation of all three indicators did occur within a relatively short time (2 weeks). This period of extraordinary uptake (when the water temperature was 11 to 12°C) was reflected by an accumulation factor of 55.5 for male-specific bacteriophages. A second difference between the bacteriophage and fecal coliform indicator groups was their overall mean accumulation factors. The mean accumulation factor for male-specific bacteriophages was 7.6 (range, <0.1 to 55.5), which is more than twice that found for fecal coliforms. When water temperatures were below 6.5°C, bacteriophage accumulation factors dropped to ≤1.0, which is similar to that for fecal coliforms, probably because hard-shelled clams appear to stop filter feeding at temperatures below 4°C (data not shown).

In general, *C. perfringens* accumulation factors were significantly higher than those observed for fecal coliforms (Fig. 4) and male-specific bacteriophages (Fig. 5). Of the 20 trials, three (G, H, and J) qualify as periods of hyperaccumulation for *C. perfringens*. The accumulation factors determined for these trials were >130 and occurred during the spring when water temperatures ranged from 13.0 to 21.0°C. The mean accumulation factor for *C. perfringens* for all 20 trials was 61.8, with a range from 0.4 to 229.6. Again, the seasonality of hyperaccumulation of this species was similar

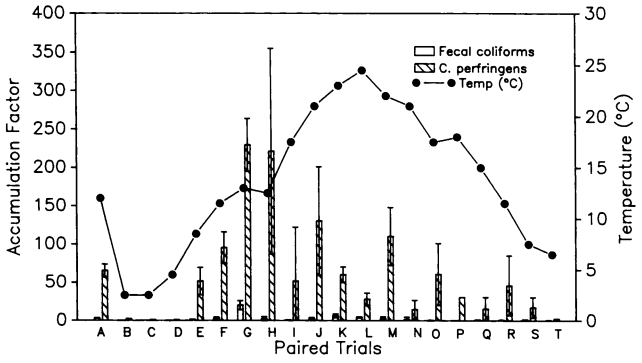


FIG. 4. Bioaccumulation of fecal coliforms and *C. perfringens* by *M. mercenaria*. Results shown are for paired trials in relation to seawater temperatures. Accumulation factors (and their 95% confidence limits, indicated by error bars) are calculated as the geometric mean indicator MPN values determined for the shellfish divided by the respective density of the particular indicator found in the overlying contaminated water.

to that of fecal coliforms and bacteriophages. In fact, hyperaccumulation of *C. perfringens* occurred simultaneously with hyperaccumulation of fecal coliforms in trial G but not with hyperaccumulation of male-specific bacteriophages.

The rates of uptake of the fecal coliforms, *C. perfringens*, and male-specific bacteriophages by *M. mercenaria* were significantly correlated ($P < 0.05$) during the year when hyperaccumulation values were not included in the data set (Tables 1 and 2). As water temperatures increased, substantial changes in accumulation rates occurred between 4.5 and 11.5°C, particularly with *C. perfringens* and male-specific bacteriophages. Accumulations of each indicator had been relatively low just before this period. During the fall, as water temperatures dropped below 11.5°C, the ability of the shellfish to accumulate all microorganisms declined. A sharp and dramatic decrease in uptake was seen when temperatures reached 6.5°C, although shellfish siphon extension activity was still observed. Accumulation factors at this time

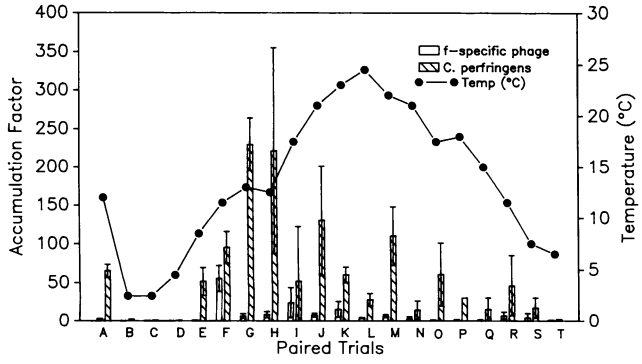


FIG. 5. Bioaccumulation of *C. perfringens* and male-specific bacteriophages by *M. mercenaria*. Results shown are for paired trials in relation to seawater temperatures. Accumulation factors (and their 95% confidence limits, indicated by error bars) are calculated as the geometric mean indicator MPN values (for fecal coliforms) and PFU (for male-specific bacteriophages) for the shellfish divided by the respective density of the particular indicator found in the overlying contaminated water.

TABLE 1. Shellfish accumulation of microbial indicators by season

| Season | Temp (°C) | Accumulation factor[a] for: | | |
|---|---|---|---|---|
| | | Fecal coliforms | *C. perfringens* | Male-specific phages |
| Spring | 4.5 | 0.1 | 0.4 | 0.1 |
| Spring | 8.5 | 1.1 | 51.7 | 0.6 |
| Spring | 11.5 | 3.6 | 95.5 | 55.5 |
| Fall | 11.5 | 0.6 | 46.2 | 7.1 |
| Fall | 7.5 | 1.1 | 17.4 | 4.1 |
| Fall | 6.5 | 0.4 | 2.0 | 1.0 |

[a] Ratio of the density of the indicator organism in shellfish (number of organisms per 100 g) to the density of the indicator organism in the uptake seawater (number of organisms per 100 ml).

were comparable to those found during the winter when water temperatures were 3.0°C.

The ability of *M. mercenaria* to concentrate each microbial indicator was analyzed in relation to water temperature by Pearson's correlation coefficient analysis (Table 2). Data from all 20 trials showed no significant relationships between water temperature and the accumulation factors for any of the indicators. However, when hyperaccumulation trials for each microorganism were omitted, significant relationships emerged. Treated in this manner, accumulations for each of these microorganisms by *M. mercenaria* had a significant correlation to water temperature.

Linear regression analyses (Table 3) revealed that the accumulations of the bacterial indicators (fecal coliforms, *E. coli*, and *C. perfringens*) had relatively strong correlations to one another throughout all seasons and temperatures. In contrast, the accumulation factors found for each of the bacterial species showed no significant relationships to those for the viruses (male-specific bacteriophages).

As a means of determining the die-off rates of each indicator group, contaminated hard-shelled clams were banded shut to prevent filter feeding and placed in seawater maintained at one of two temperature ranges. Indicator organism densities were determined 48 and 168 h after banding. At least 94% of the initial density of each indicator group was recovered after 48 h (Table 4). After 7 days, the greatest decline among the indicator organisms occurred for fecal coliforms, with reductions of 24% in 10 to 12°C waters. In hard-shelled clams, the densities of *C. perfringens* and male-specific bacteriophages were extremely stable, with 92

TABLE 2. Pearson correlation coefficient analyses of accumulation factors and temperatures

| Microbial indicator | Results for: | | | |
|---|---|---|---|---|
| | All data ($n = 20$) | | Data with hyperaccumulation omitted | |
| | $r$ | Probability | $r$ | Probability[a] |
| Fecal coliforms | 0.219 | 0.356 | 0.626[b] | 0.004* |
| *E. coli* | 0.127 | 0.595 | 0.478[b] | 0.039* |
| *C. perfringens* | 0.260 | 0.268 | 0.495[c] | 0.044* |
| f-specific bacteriophages | 0.147 | 0.536 | 0.554[d] | 0.017* |

[a] *, significant ($P < 0.05$) correlation between indicator uptake by hard-shelled clams and water temperature.
[b] Trial G omitted; $n = 19$.
[c] Trials G, H, and J omitted; $n = 17$.
[d] Trial F omitted; $n = 19$.

TABLE 3. Correlation of linear regression analyses of indicator organism accumulations by *M. mercenaria*

| Microbial indicators | Pearson correlation ($n$ = 20) | |
|---|---|---|
| | $r$ | $P^a$ |
| Fecal coliforms vs *E. coli* | 0.99 | <0.001 |
| Fecal coliforms vs *C. perfringens* | 0.68 | 0.001 |
| Fecal coliforms vs f-specific bacteriophages | 0.14 | 0.560* |
| *C. perfringens* vs f-specific bacteriophages | 0.25 | 0.280* |

[a] *, no significant correlation (Pearson analysis) at $P < 0.05$.

and 90% of their initial densities, respectively, remaining after 7 days. These results suggest that die-off did not affect the accumulation rates determined by this study.

## DISCUSSION

The experimental design of this study incorporated the more positive aspects of earlier investigations to determine the ability of shellfish to concentrate a variety of microorganisms. These previous investigations used two separate approaches: environmental monitoring and laboratory experiments with pure cultures. In this study, we used shellfish exposed to ambient seawater to which a constant amount of raw wastewater was added in an effort to keep the indicator levels in overlying water relatively constant. This type of exposure alleviated two problems inherent in earlier studies that used pure cultures. First, our method using raw wastewater more closely replicates indicator densities in estuaries, which are unpredictable and constantly changing; exposures can never be known with certainty. Second, by not using pure cultures, we alleviated the problem of uptake of individual organisms, which may accumulate at rates appreciably different from those of particle-bound microorganisms (21).

Shellfish are continually subjected to changing environmental conditions that influence their physiological state and thus strongly affect their ability to accumulate particulate materials. This study examined the effects of two parameters, season and water temperature, on the ability of hard-shelled clams to filter and retain several different indicator microorganisms. The ability of shellfish to concentrate contaminants was considerably reduced when ambient seawater temperatures were below 7°C, partly as a result of their diminished physiological activity. When water temperatures fell below 4.5°C, bioaccumulation was essentially halted. Water quality during such periods of relative dormancy had virtually no effect on the sanitary quality of *M. mercenaria*. However, water temperature alone did not appear to explain shellfish accumulation rates. During the spring, when water temperatures were increasing, there were threshold periods in which animal activity was significantly influenced. Temperatures between 4.5 to 11.5°C correlated with a marked increase in the accumulation of all indicators. However, each of the indicator groups displayed this phenomenon during different trials. These findings suggest that accumulation of microbial species by shellfish is differentially selective and may be based on biochemical changes in shellfish tissues, particular characteristics such as size, shape, and surface change, and possibly other factors as well. The hyperaccumulation phenomenon has been observed during the fall (6), although it was not observed during this study year.

TABLE 4. Survival of indicators inside banded hard-shelled clams (*M. mercenaria*)

| Microbial indicator | Organism concn at indicated temp and time/initial concn[a] | | | |
|---|---|---|---|---|
| | 10–12°C[b] | | 18.0–21.8°C[b] | |
| | 48 h | 168 h | 48 h | 168 h |
| Fecal coliforms | 0.94 | 0.76 | 0.95 | 0.92 |
| *C. perfringens* | 1.00 | 1.00 | 0.98 | 0.92 |
| f-specific bacteriophages | 0.98 | 0.92 | 0.97 | 0.90 |

[a] The mean indicator organism concentrations in banded clams at 2 and 7 days were divided by the mean initial indicator organism concentrations. Mean concentrations were calculated from results of duplicate trials at each temperature range.

[b] Water temperatures were maintained within this range during each of two trials.

The spring period of hyperaccumulation corresponds to epidemiological reports of outbreaks of illness attributed to the consumption of raw molluscan shellfish (primarily oysters and clams). The times of hyperaccumulation of biological organisms and the increased incidence of human gastrointestinal illness (Fig. 1 to 5) appear to be correlated. The increase in illness rates seen during the spring may be caused by increased accumulation of sewage-derived enteric viral pathogens. Although the fecal coliform data for overlying water may indicate that a growing area is safe for harvest, the animals may present consumers with an unacceptably high degree of viral exposure because of the hyperaccumulation phenomenon. Except during hyperaccumulations (which appear as unpredictable anomalies), water temperature appears to be a good predictor of microorganism accumulation in *M. mercenaria*. However, the quality of molluscan shellfish can never be reliably determined on the basis of overlying water quality, especially as water temperatures begin to rise in mid- to late spring.

The rates of accumulation of fecal coliforms and *E. coli* by hard-shelled clams were virtually identical throughout the year. These results were not unexpected, because *E. coli* generally makes up the majority of the fecal coliform group associated with human fecal waste. Densities of enterococci, although currently used as health effect indicators for recreational waters (30), were not reported in this study because previous investigations have demonstrated that they behave almost identically to other vegetative bacterial indicators.

A strong correlation between the accumulations of fecal coliforms and *C. perfringens* was seen throughout the year. Therefore, monitoring *C. perfringens* concentrations may better reflect seasonal accumulation activity because these organisms are concentrated to a much greater extent than fecal coliforms. As such, *C. perfringens* may be useful for predicting the increased uptake of bacterial pathogens. However, accumulation of the bacterial viruses cannot be predicted by monitoring any of the bacterial groups examined.

Accumulation, inactivation (die-off), and elimination of microbial indicator organisms are processes that occur simultaneously in all physiologically active shellfish. Determining the rates of each of these phenomena is difficult. This study found that dribbling (the elimination of foreign material in the absence of water transmission) and the inactivation of indicators by and within the shellfish had a minimal role in decreasing microbial densities after their accumulation. Accumulation factors derived in this study are, therefore, the results of true accumulations and not those of

apparent accumulations after a significant inactivation of the indicators.

The results of this study suggest that current management practices for shellfish harvesting may not be consistent with public health protection. These inconsistencies are attributable, in part, to the shortcomings of the bacterial indicators used for assessing shellfish quality and to the wide range of biological activity observed for shellfish over a calendar year. These shortcomings include the facts that (i) *M. mercenaria* accumulates male-specific bacteriophages, which are enteric virus simulants, at rates and concentrations different from those for the fecal coliform group or *C. perfringens*; (ii) both season and temperature strongly influence the ability of shellfish to concentrate biological contaminants; and (iii) surface and bottom water quality do not necessarily reflect the sanitary quality of shellfish harvested from those waters. Reliable assessment of that quality requires the examination of the meats and liquors and may be the single most important means for minimizing public health risks.

## ACKNOWLEDGMENTS

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