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Microbiological Studies of Chesapeake Bay Soft-shell Clams (*Mya arenaria*)

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ABSTRACT

A total of 472 samples of soft-shell clams (*Mya arenaria*), collected from three major clam harvest areas in the Chesapeake Bay and dockside check stations, was analyzed for standard plate count (SPC), total coliforms, fecal coliforms, *Escherichia coli*, and coliphages. SPC increased during the summer season. SPC geometric means of 2.6×10^4, 6.9×10^4, and 7.2×10^4/g, respectively, were found in three major harvest areas. Fecal coliforms remained relatively stable with geometric means of 30, 54, and 62/100 g. As seasonal temperatures increased, the total coliform geometric means declined slightly ranging from 1,500 to 6,300/100 g. *E. coli* means were low (< 27/100 g). The occurrence and levels of male-specific coliphages were also low and did not correlate with bacteriological quality. No significant microbiological quality difference was found between soft-shell clams sampled from harvest waters and check stations. Results indicate that the microbiological quality of soft-shell clams either at harvest or check stations was satisfactory.

The soft shell clam (*Mya arenaria*) is one of Maryland's major commercial seafood harvests in the Chesapeake Bay. Clams are harvested by hydraulic dredges which use a conveyer to bring the soft-shell clams to the vessel. Clean, legal size (≥ 2") clams are placed in containers and iced or mechanically refrigerated on board the harvest boats. The cooled soft-shell clams are transported to check stations where the Department of Health and Mental Hygiene monitors product temperature and issues shipping tags. The clams are then trucked to processing plants for shipping, shucking, washing, and packaging for retail market. Maryland soft-shell clams, fresh in the shell or shucked, are commonly shipped to New England and New York.

During the warm summer months, fecal coliform (FC) levels in Maryland soft-shell clams received in out-of-state markets frequently exceed recognized national criteria (*2,5*). These high levels have made the wholesomeness of clams questionable and has resulted in rejection of the clams at the market level. Although scattered data exist suggesting that the elevated FC levels are a natural phenomenon related to the increase by Bay air and water temperatures, no formal documentation has been published.

A similar problem with the summer harvest of oysters was investigated in Louisiana. During summer ambient air temperatures in excess of 26.7°C (80°F), the standard plate count (SPC) and FC levels in Louisiana oysters from approved harvest areas were found to exceed the national bacteriological market standard for shellstock (*4*). Although recognized as having no public health significance, these high levels of indicator organisms have continued to concern regulatory agencies. Adequate refrigeration, below 4.5°C (40°F), was demonstrated to suppress the proliferation of indicator organisms in Louisiana oysters and maintain the SPC and FC levels within the market standard if the oysters met this standard at harvest (*4*).

To improve market quality, the Maryland Departments of Health and Mental Hygiene (DHMH), Environment (MDE), and Natural Resources (DNR) have worked together to establish regulations requiring cold storage of soft-shell clams on harvest boats. The DHMH and MDE conducted a study to examine the microbiological quality of soft-shell clams. Microbial examination included SPC, total coliforms (TC), FC, *Escherichia coli*, and male-specific phages in soft-shell clams. Samples were collected from three different geographical environments during the entire peak harvest season. Ninety percent of Maryland's clam harvest is from these three environments. This paper compares the microbiological quality of soft-shell clams at harvest with the quality of clams at the check stations.

MATERIALS AND METHODS

Harvesting areas and sampling locations

Twenty sampling locations were selected from three geographical areas. These three areas comprised the major Maryland Chesapeake Bay soft-shell clam harvesting areas (Fig. 1). Water depth ranged from 5 to 12 ft. Area 1 included four sampling locations (Stations 1 to 4) in the Chesapeake Bay along the western shore of Kent Island. Area 1 was sparsely populated cropland and wooded area relatively unimpacted by human activities. Area 2 included seven sampling locations (Stations 5 to 11) along the midwestern shore of the Bay contiguous to the dense urban population of Annapolis and vicinity. Area 3 included nine sampling locations (Stations 12 to 20) in the lower Chester River on the eastern shore of Maryland and was surrounded by extensive agricultural cropland.

Sample collection

Soft-shell clam samples were collected weekly from May 15 to September 12, 1989. At each harvest area sampling location, 35 to 50 clean, market size (≥ 2 inches) soft-shell clams

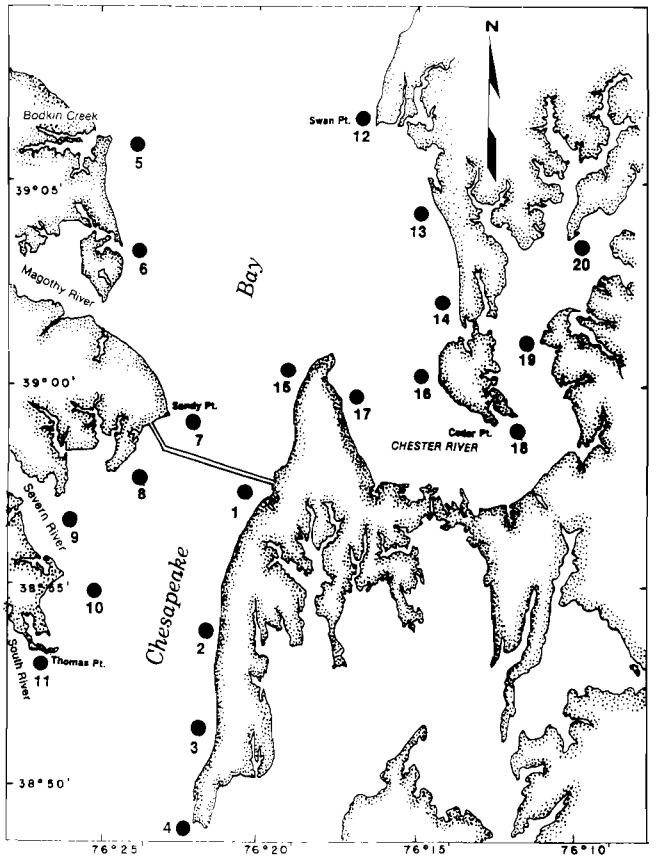

Figure 1. *Soft-shell clam sampling locations.*

were taken directly from the clam dredge conveyor belt. Each clam sample was placed in a double 1-kg sterile polyethylene whirly-pack bag, sealed and iced. Three individual clams were sacrificed to determine the average internal clam temperature at the time of harvest. Air temperature, top (6-12 inches below the surface) and bottom water temperature, salinity, and dissolved oxygen were recorded at each sampling location. Ten soft-shell clam samples of known harvest area origin were collected weekly from check stations following the same procedure used for harvest area clam samples.

Bacteriological analyses

Within 24 h of sample collection, clam samples were subjected to bacteriological examination. Ten to 20 live clams, with a combined weight of over 200 g of meat and liquor, were randomly selected from each clam sample and carefully shucked according to the method described by the American Public Health Association (APHA) (*1*). The shucked clams with liquor were blended with an equal weight of dilution buffer and analyzed for SPC, TC, FC, and *E. coli* counts according to APHA methods (*1*).

Coliphage enumeration

Male-specific coliphages were enumerated by the method developed by Havelaar (*7,8*) using *Salmonella typhimurium* WG49 and WG45 as host strains. WG49 is a F^+ strain containing the plasmid for producing *E. coli* pili and is a good host cell for male-specific coliphages (*8*). WG45, a F^- strain, is the parental strain of WG49 and contains no pili. The same clam homogenates prepared for bacteriological analyses were used for the male-specific coliphage determination. An equal volume of 1:10 sample dilution and agar concentrate containing 2 g of clam sample was mixed and placed on six overlay plates for each host cell. Direct plaque assay using the single agar layer technique (*6*) was compared with the soft agar overlay method. For each coliphage examination, a control coliphage MS2 lysate with known plaque forming unit (PFU) was tested for recovery and quality of the host cells. All plates were incubated at 35°C for 12 h before enumeration of the PFU.

Statistical analyses

Data were analyzed statistically for variance and significant difference (*3,9*). Computer programs of Sigma Plot, LOTUS, and SAS were used to illustrate representations and execute statistical data into conclusive results (*10*).

RESULTS AND DISCUSSION

Soft-shell clam temperature

Soft-shell clam temperatures increased as harvest water temperatures increased. Ambient clam temperatures ranged from 16 to 20°C in May, gradually increased to 19 to 24°C in June, reached a high temperature of 25 to 26°C in July and August, and then decreased slightly after September (Fig. 2). Clam temperatures during May, June, and early July in Area 3 were higher than clam temperatures in Areas 1 and 2. The ambient soft-shell clam temperatures reflected the bottom water temperature of the sample location (data not shown). Soft-shell clam temperatures at time of harvest were always 0.6 to 2°C lower than the bottom water temperature throughout the entire study period.

The relationship between clam temperature, top water temperature, and bottom water temperature was similar among the three shellfish harvest areas. However, the temperature difference between clams and water sampled in May and June was smaller in Area 3 than the differences in Areas 1 and 2 (data not shown). Area 3 was more responsive to the effects of extremely high rainfall in May and June which caused greater mixing of the water and more uniform temperature.

Bacterial density in soft-shell clams from different geographic regions

A total of 334 soft-shell clam samples was collected from May to September 1989, the peak harvesting season, from three geographical regions. Samples were examined for bacteriological quality including SPC, TC, FC, and *E. coli*. Fig. 3 shows that geometric means of SPC values were

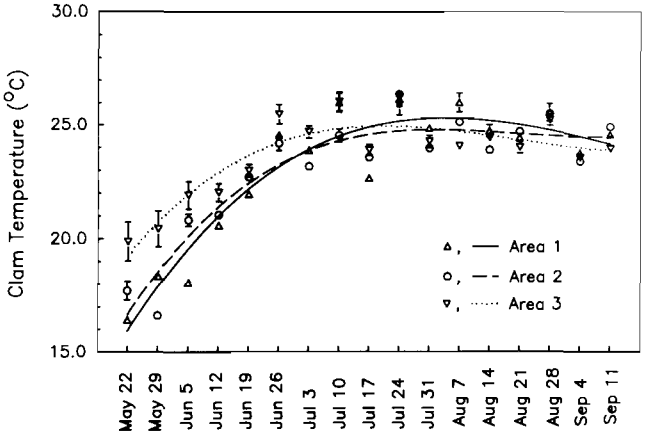

Figure 2. *Changes of soft-shell clam temperature during the harvesting season.*

low, ranging from 3.3 to 4.5 log in May and early June, 3.8 to 5.4 log in late June and July, increasing to 5.1 to 5.6 log during August, and decreasing to less than 5.0 log in September. SPC ranges were broader in late spring and early summer than later summer. Different geographical areas exhibited different bacterial loads. Area 1 had consistently lower values than those of Areas 2 and 3. SPC geometric means per gram of 26,300, 69,200, and 72,400 were found in Areas 1, 2, and 3 clam samples, respectively (Table 1). Results of Duncan's multiple range test for variance indicated that clams from Area 1 had a SPC geometric mean significantly different from Areas 2 and 3 clams ($P < 0.001$). Comparison of Areas 2 and 3 clams showed no significant difference (data not shown).

In contrast to the SPC profile, the numbers of TC found in soft-shell clams did not increase as seasonal temperature rose but decreased slightly during the summer season. Two exceptions were noted on May 29 and August 7 when unusually high counts were observed (Fig. 4) in clams sampled from Areas 1 and 2. These unusual counts were reflected by the FC counts but not by the *E. coli* or coliphage counts. In general, Area 1 soft-shell clams had a lower total coliform MPN geometric mean than Areas 2 and 3 clam samples.

Except for August 7 samples, FC levels remained relatively constant throughout the entire study period, ranging from 1.1 to 2.3 log. FC levels showed less variation than SPC or TC levels among the three geographical regions (Fig. 5). Samples collected from Areas 2 and 3 had a higher MPN FC level than samples from Area 1. *E. coli* counts were relatively low in all three areas and did not exhibit seasonal variation. Some fluctuations occurred among sampling dates (Fig. 6). Area 1 clams exhibited lower *E. coli* counts than clams harvested from Areas 2 and 3. The *E. coli* counts of Area 3 clams exhibited occasional spikes that were 3 to 8 times higher than the average.

Abruptly high levels of TC and FC occurred on August 7 only in Areas 1 and 2 clams (Fig. 4 and 5). Climatic and environmental factors were suspected and investigated. Rainfall data for these areas were not unusual. Water column nutrients were examined and found to be normal (MDE, unpublished data). The possible discharge of inadequately treated sewage from waste water treatment plants was ruled out since the bacteriological quality of the harvest water was normal. Corresponding *E. coli* counts were also normal. A high concentration of pleasure boats in Areas 1 and 2 was suspected and eliminated, since boating patterns remained the same over the entire study period. The August 7 results probably represent the occasional outliers normally found in extensive environmental sampling.

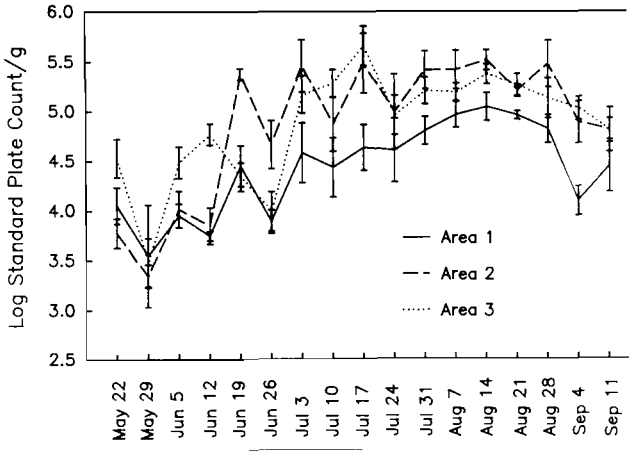

Figure 3. *Standard plate count of soft-shell clams. The vertical bar stands for the SPC geometric mean ± SD. The lines were connected at the geometric means of each week's samples.*

Clam samples meeting the FDA recommended 5×10^5/g SPC limits (2,5) were 98.5% in Area 1, 86% in Area 2, and 88.2% in Area 3 (Table 1). As shown in Table 2, 89.7, 87.6, and 86.3% of the clam samples from Areas 1, 2, and 3, respectively, met the criterion for the recommended FC limit of 230 MPN/100 g. Variance analysis showed significantly different TC and *E. coli* counts among clams from each of the three areas ($P < 0.001$). TC geometric means were 1,585, 6,310, and 2,692/100 g in Areas 1, 2, and 3, respectively (Table 2). Geometric means of *E. coli* found in Areas 1, 2, and 3 clams were 14.1, 20.9, and 27.5/100 g, respectively. Area 2 clams had a narrower range of *E. coli* counts than Areas 1 and 3. However, FC levels in Areas 2 and 3 clams were not significantly different. FC levels in

TABLE 1. *Standard plate count of soft-shell clams harvested from different growing waters.*

SPC range/g	Area 1		Area 2		Area 3	
	Units	RCF[a]	Units	RCF	Units	RCF
≤900	0	0	0	0	1	0.7
1,000 - 5,000	2	2.9	9	7.9	3	2.6
5,100 - 10,000	13	22.1	8	14.9	8	7.9
11,000 - 50,000	26	60.3	20	32.5	39	33.6
51,000 - 100,000	11	76.5	16	46.5	32	54.6
110,000 - 500,000	15	98.5	45	86.0	51	88.2
510,000 - 1,500,000	1	100.0	11	95.6	15	98.0
1,600,000 - 5,000,000			2	97.4	2	99.3
5,100,000 - 10,000,000			2	99.	1	100.0
11,000,000 - 50,000,000			1	100.0		
Total number samples	68		114		152	
Geometric mean/g	26,303		69,183		72,444	
Range of values/g	4,400 - 630,000		1,250 - 12,020,000		700 - 5,330,000	

[a]RCF, relative cumulative frequency.

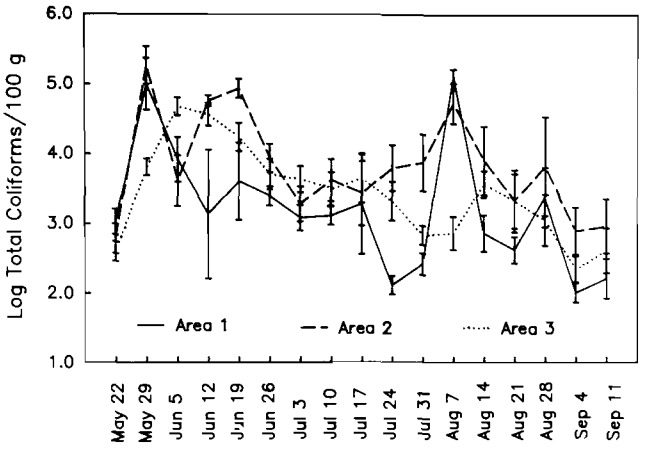

Figure 4. *Coliforms in soft-shell clams. The vertical bar stands for the TC geometric mean ± SD. The lines were connected at the geometric means of each week's samples.*

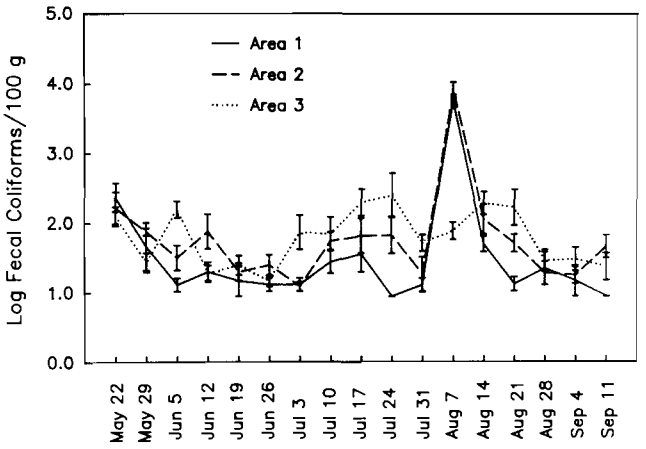

Figure 5. *Fecal coliforms in soft-shell clams. The vertical bar stands for the FC geometric mean ± SD. The lines were connected at the geometric means of each week's samples.*

Area 2 and 3 clams were significantly different from Area 1 clams ($P < 0.001$, data not shown).

Comparison of bacteriological quality of soft-shell clams collected from harvest waters and check stations

To determine the microbial growth, if any, in soft-shell clams between time of harvest and transport to the check stations, 138 clam samples with known harvesting locations were collected from the check stations. Data comparisons of SPC, TC, FC, and *E. coli* are shown in Fig. 7. SPC levels found in check station clams had a similar level and pattern of increasing counts during the summer season as the clams at harvest. The SPC geometric mean and range were 57,500/g and 700-12,020,000/g, respectively, for harvest area clam samples; and 87,100 and 850-11,240,000/g, respectively, for check station samples. Variance analyses of SPC showed no significant difference between clams sampled at harvest area locations and those sampled at check stations ($P < 0.001$). FC levels in check station samples had a mean of 75/100 g and a range from < 18 to 160,000/g compared to a mean of 51/g and a range from < 18 to 17,000/100 g in harvest area clams. *E. coli* levels were low with a geometric mean of 23/100 g and a range from < 18 to 2,200/100 g in both harvest area and check station clams (data not shown).

FC and *E. coli* counts were relatively constant throughout the entire study period for clams of both sources. A slight increase in FC level occurred during July and August. Variance analyses revealed that check station clams contained slightly higher levels of TC and FC than clams sampled at harvest ($P < 0.001$). No significant difference in *E. coli* levels was found between clams taken from harvest areas and check stations.

Male-specific phages

Two host strains, *S. typhimurium* WG49 and WG45, were used to determine the presence of phages. Coliphage PFU was determined at a sensitivity level of 0.5 PFU/g. The soft agar overlay method was found to give a 30% higher count than the single-agar-layer plaque assay and was therefore the method used in this study. PFUs of coliphages for WG45 were much lower than that for WG49 (data not shown). The results indicated that few somatic phages were detected using host strain WG45. Havelaar and Hogeboom (*7*) have reported that coliphage counts on F^- salmonella host cells were usually 1-3 orders less than phages detected on F^+-salmonellae produced by male-specific phages (*8*). The PFUs of male-specific phage active against

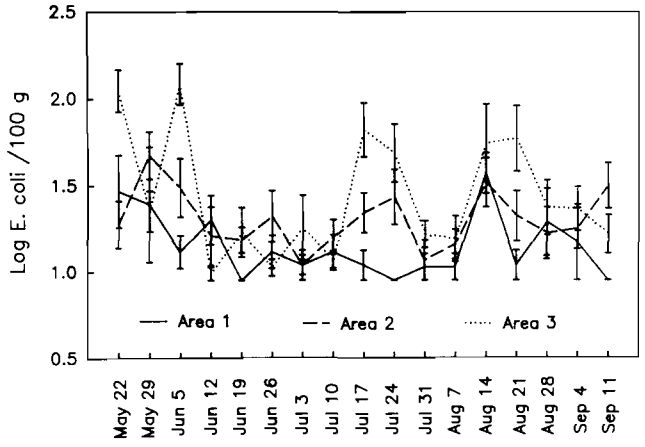

Figure 6. *The level of* E. coli *in soft-shell clams. The vertical bar stands for the* E. coli *geometric mean ± SD. The lines were connected at the geometric means of each week's samples.*

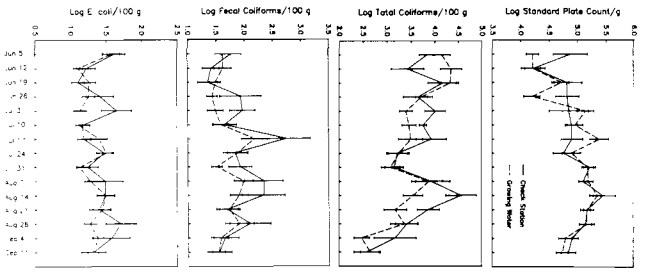

Figure 7. *Bacteriological quality of soft-shell clams sampled from harvesting waters and check stations. All clam samples from both check stations and growing water were collected from Area 2 sampling location. The vertical bar stands for bacterial count geometric mean ± SD. The lines were connected at the geometric means from each week's samples.*

WG49 in soft-shell clams from the three harvest areas and at check stations are shown in Fig. 8 and 9. The incidence of male-specific coliphage found in soft-shell clams harvested from Area 3 was higher than Areas 1 and 2.

Contrary to initial speculation, the clams harvested from Area 2, the high density residential area, contained few coliphages. Results indicated that there was no correlation between densities of TC, FC, or *E. coli* and coliphages. The high incidence of male-specific coliphages found in Area 3 clams may be related to the wildlife present in this heavily agricultural region. Feces from raccoons and seagulls, active in this area, contain 2×10^3 to 2×10^5/g male-specific phages (Olivieri, personal communication). The occurrence of these wild animal and bird wastes may explain the higher prevalence of coliphage in soft-shell clams from Area 3. The occurrence of coliphage observed in the harvest waters at the time of clam sample collection was consistent with levels observed in clams (data not shown).

Coliphage levels in check station clams were lower than those in clams at harvest. Coliphage may accumulate in the siphon and hepatopancreas of the soft-shell clam; however, the rate and the mechanism of the phage binding to the soft-shell clam tissue are not known. The large amount of liquor lost during storage and handling of soft-shell clams after harvest may be one of the causes for lower coliphage levels in the check station clams.

The relatively low level of male-specific phage found in this study was surprising. At least three possible explanations for this result exist. First, there may be a low incidence of coliphage in environments not affected by direct sewage discharges. Second, coliphage may not persist any length of time under general environmental conditions. Third, the accumulation and retention of coliphage by

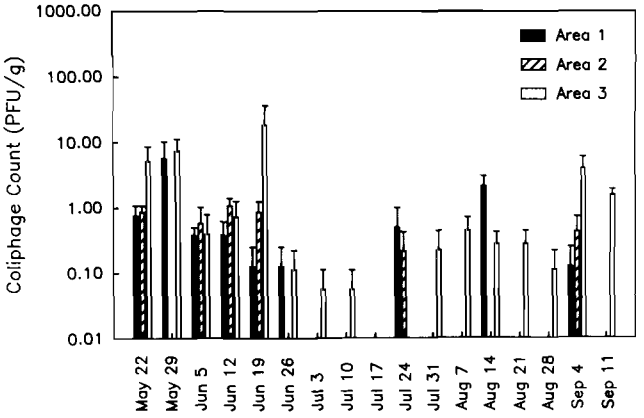

Figure 8. *Levels of male-specific coliphages active against* Salmonella typhimurium *GW49 in soft-shell clams. Phage results of July 24 samples from Area 3 were not included.*

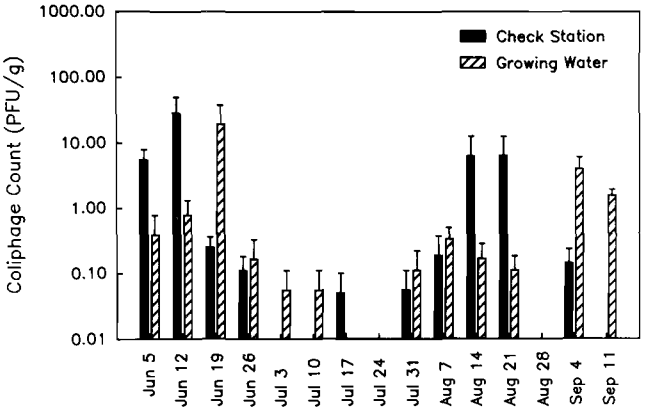

Figure 9. *Comparison of male-specific phage counts between soft-shell clams sampled from harvesting waters and check stations. Phage results of July 24 samples from Area 3 were not included.*

TABLE 2. *Total coliforms, fecal coliforms, and* E. coli *in soft-shell clams from different harvest areas.*

MPN[a]/100 g	Total coliforms						Fecal coliforms						*E. coli*					
	Area 1		Area 2		Area 3		Area 1		Area 2		Area 3		Area 1		Area 2		Area 3	
	Units	RCF[b]	Units	RCF	Units	RCF	Units	RCF	Units	RCF	Units	RCF	Units	RCF	Units	RCF	Units	RCF
≤18	1	1.7	1	0.8	0	0	3	48.5	29	25.7	29	20.0	48	70.6	51	45.1	67	43.8
19 - 90	6	10.3	2	2.7	5	3.3	25	85.3	52	71.7	72	66.0	19	98.5	56	94.7	58	81.7
91 - 140	4	16.2	5	7.1	8	8.5	1	86.7	10	80.5	16	76.5			6	100.0	13	90.2
150 - 230	7	26.5	4	10.6	10	15.0	2	89.7	8	87.6	15	86.3	1	100.0			7	94.8
240 - 330	5	33.8	2	14.1	11	22.2	2	92.6	4	91.2	8	91.5					4	97.4
340 - 700	6	42.6	11	22.1	17	33.3	1	94.1	2	92.9	3	93.5					2	98.7
790 - 1,700	15	64.7	18	38.1	29	52.3			3	95.6	4	96.1					2	100.0
1,800 - 7,000	12	82.4	27	61.9	21	66.0	2	97.1	3	98.2	4	98.7						
7,900 - 54,000	7	92.6	21	80.5	37	90.2	2	100.0	2	100.0	2	100.0						
55,000 - 230,000	3	97.1	16	94.7	11	97.4												
240,000 - 700,000	2	100.0	6	100.0	4	100.0												
Total number samples	68		113		153		68		113		153		68		113		153	
Geometric mean/ 100 g	1,585		6,310		2,692		29.5		53.7		61.6		14.1		20.9		27.5	
Range of values/ 100 g	18 ~ 240,000		18 ~ 240,000		20 ~ 240,000		<18 ~ 7,900		<18 ~ 17,000		<18 ~ 14,000		<18 ~ 230		<18 ~ 130		<18 ~ 1,300	

[a]MPN, most probable number.
[b]RCF, relative cumulative frequency.

soft shell clams have not been studied; the clams may not retain coliphage. Since the coliphage levels in the harvest waters were very low, accumulation may not be a factor.

Male-specific phages have been suggested as potential indications of human sewage. Harvest areas where clams were collected in this study were well removed from known treated sewage discharges. No untreated sewage discharges were known to exist. This study demonstrates the potential for some level of male-specific coliphage to be identified in the absence of large sewage discharges. Whether these coliphages are associated with sewage from wildlife is not known.

The microbiological quality of soft-shell clams harvested from the three different geographic regions in Maryland's Chesapeake Bay proved to be generally acceptable, meeting FDA recommended market quidelines. The higher bacterial counts in Area 3 were most likely associated with nonpoint pollution from nutrient rich farmland. Higher summer temperatures resulted in higher SPCs. With sound refrigeration practices and proper handling techniques, clams arriving at check stations prior to the wholesale market were of satisfactory quality.

ACKNOWLEDGMENTS

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