These observations regarding the survival of E. coli and S. schottmuelleri in soft clams and shell oysters which were artificially polluted and stored under natural conditions will be helpful in the rational control of one of the more turbulent fields of sanitation.

Survival of Enteric Organisms In Shellfish

By C. B. KELLY and WILLIAM ARCISZ

SANITARY control of the shellfish industry has relied largely on assurance that shellfish are harvested from waters beyond the influence of dangerous pollution. Among the stipulations for an approved shellfish area, the Public Health Service Manual of Recommended Practice for the Sanitary Control of the Shellfish Industry (*1*) includes a limiting coliform most probable number (MPN) of the overlying water.

Recognizing the desirability of bacteriological criteria for shellfish as well as for waters from the growing areas, the Public Health Service has included in the manual certain tentative coliform MPN values for oysters and other shellfish as taken from the growing area. Interpretation of bacterial findings in shellfish at the market, particularly in species of shellfish other than oysters, is quite difficult in the absence of specific data on the reliability of the indicator organisms as representative of the presence or absence of enteric pathogens. A criterion of the value of any indicator organism is obviously the relative survival as compared to the pathogens, not only in water, but also in the shellfish after the shellfish have been removed from the water.

There is no question concerning the persistence of enteric pathogens in shellfish after they have been harvested. Epidemiological data, such as that summarized by Fisher (*2*) and more recent reports (*3–5*), contain incontrovertible evidence of the transmission of enteric diseases by shellfish. Furthermore, there is sufficient experimental evidence, at least with *Salmonella typhosa*, to show that this organism will survive for a long period of time in both shell and shucked oysters. This is borne out by Hunter's (*6*) conclusions, based on a review he made of the literature as of 1928, that *S. typhosa* will remain viable within the bodies or shell liquor of oysters long enough to cause illness when oysters are eaten within the usual period elaps-

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ing between the time they are removed from the infected water and the time they are consumed.

Experimental data on species of shellfish other than oysters, however, are quite limited. Except for a few experiments conducted by Klein (*7*) with mussels and cockles, no studies have been made with other commercially important species of shellfish.

Therefore, during 1952 and 1953, in Woods Hole, Mass., at the Shellfish Sanitation Laboratory of the Public Health Service, we conducted a number of experiments to determine the relative survival of *Escherichia coli* and *Salmonella schottmuelleri* in oysters and soft clams—two of the more commercially important shellfish in the North Atlantic coast area. This investigation was considered desirable, in view of the limited data on survival of either coliforms or enteric pathogens in these species of shellfish: *Crassostrea virginica*, the eastern oyster of the Atlantic and Gulf coasts, and *Mya arenaria*, the soft clam of the New England and Middle Atlantic coast. Furthermore, improvements in bacteriological techniques and development of methods since the earlier studies have made possible more quantitative estimations of enteric pathogens.

The Plan of Study

The basic principles in this reinvestigation of survival of enteric organisms in shellfish were considered to be as follows:

1. Experimental animals should be allowed to become polluted by natural physiological processes—by exposure to sea water containing predetermined suspensions of the polluting organisms.

2. Polluting organisms should be present in the experimental animals in concentrations within the range found under naturally occurring circumstances. The sensitivity of the available methods for enumerating the test organisms would be a limiting factor in establishing the level of concentration, particularly of enteric pathogens.

3. In order to make a direct comparison of the survival of the selected indicator organism, *E. coli*, with the selected pathogen, *S. schottmuelleri*, mixtures of the two organisms, in appropriate ratio, should be used as the polluting agents.

4. Storage of the polluted shellfish should be conducted at controlled temperatures.

5. Survival of test organisms should be determined by quantitative methods of enumeration.

6. Storage should be conducted for a length of time equivalent to the longest period the shellfish might be expected to be in transit from harvesting to consumption, except that the experiment should terminate when inspection and other tests indicate that the shellfish have deteriorated to the extent that they are unsuitable for human consumption.

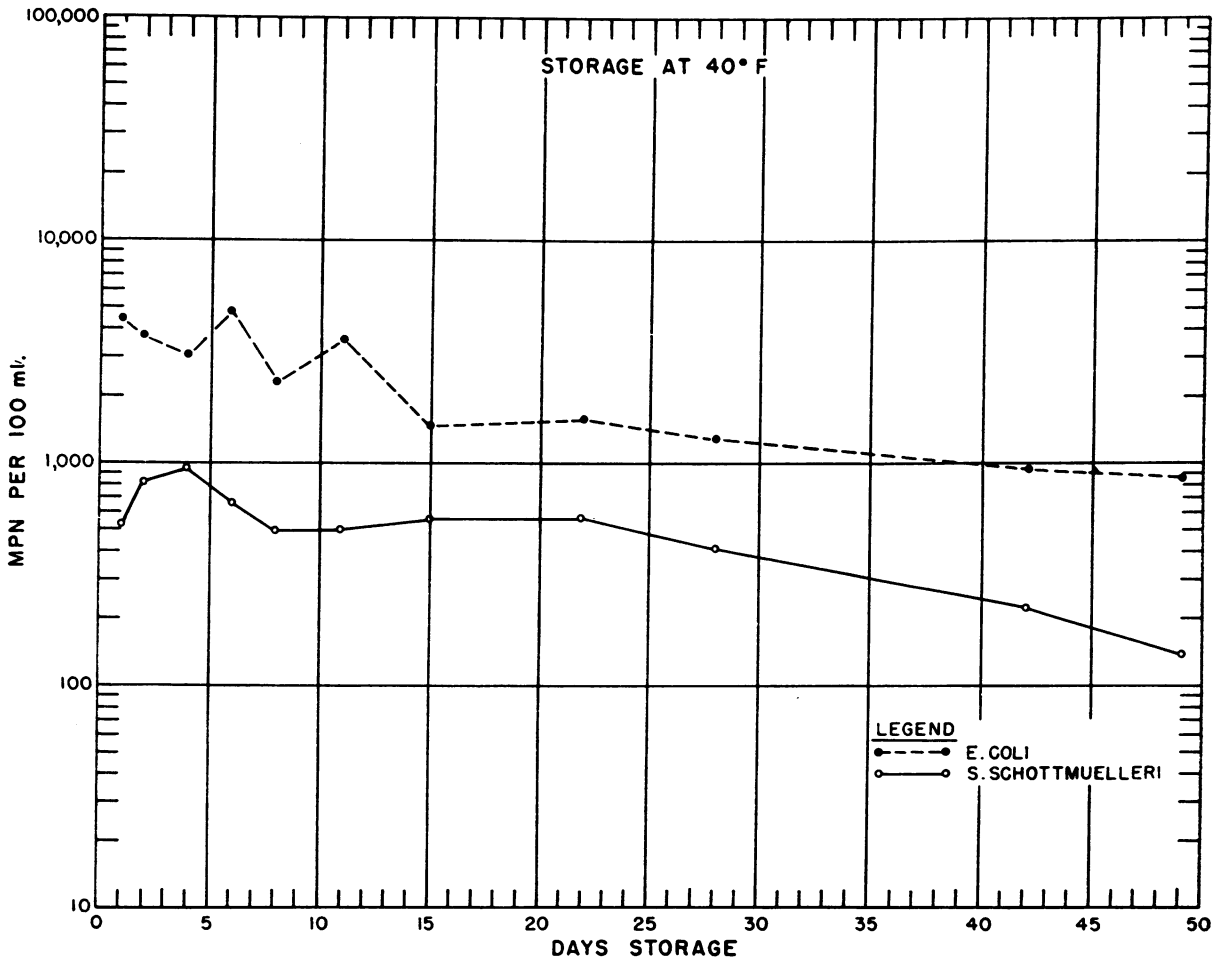
Pollution

Pollution of the shellfish was accomplished by immersing the shellfish in a wooden aquarium containing sea water to which had been added a sufficient quantity of suspensions of *E. coli* and *S. schottmuelleri* to produce a bacterial density in the water of approximately 10,000 and 1,000 per 100 milliliters, respectively. The shellfish were allowed to remain in this water for a period of 6½ to 8 hours in order to effect the accumulation of the maximum

Table 1. Survival of Escherichia coli and Salmonella schottmuelleri in shell oysters

Days of storage	*E. coli* (MPN per 100 ml.)		*S. schottmuelleri* (MPN per 100 ml.)	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2
1	6,000	4,400	160	520
2	3,500	3,800	130	820
3	1,500		160	
4	1,400	3,100	270	960
5	1,400		220	
6	2,400	4,900	190	660
7	1,300		170	
8		2,300		500
10	1,300		240	
11		3,600		500
12	600		210	
14			210	
15		1,500		570
16	600		130	
20	200		140	
22		1,600		570
28		1,300		420
42		960		230
49		890		140

Figure 1. Survival of Escherichia coli and Salmonella schottmuelleri in shell oysters.



amounts of the polluting bacteria. At the end of that time, the shellfish were transferred to watertight metal containers for storage.

Storage

Refrigerated storage of the shellfish was conducted in a household type refrigerator, which had been adjusted to maintain a temperature of approximately 40° F. (5° C.). The temperature of the refrigerator during the storage period ranged between 33.6° F. and 48° F. (1°-9° C.).

The soft clams were also stored at a warmer temperature, in an attempt to simulate conditions normally present in commercial handling. Storage at a temperature of 66° F. (19° C.) was considered to be most representative of conditions prevailing in the marketing areas where clams are handled. Storage at this temperature was conducted in a bacteriological incubator adjusted to maintain the desired temperature.

Bacteriological Methods

Quantitative estimation of bacterial densities was conducted by the dilution method in which at least 4 decimal dilutions—5 tubes for each dilution—were used.

The method for enumerating *S. schottmuelleri* involved enrichment in tetrathionate broth or selenite F broth, with isolation of typical colonies from brilliant green agar or bismuth sulfite agar. The test was completed by confirmation on Russell's double sugar agar, with occasional confirmation serologically using salmonella group B antiserum.

The test for enumerating *E. coli* consisted of preliminary plantings of decimal dilutions in lactose broth, with confirmation in brilliant green lactose bile broth.

Results of tests for both organisms were expressed as most probable numbers per 100 milliliters according to Hoskins tables (*8*).

Results

The results obtained in two experiments on storage of shell oysters are shown in table 1. In order to absorb variations in results inherent in the MPN method of enumeration, the data are presented as 3-day logarithmic moving averages of the observed values.

In experiment 1, where the shell oysters were stored for a period of 22 days, it was found that the numbers of *E. coli* reduced throughout the entire period of storage, but little, if any, reduction in *S. schottmuelleri* was observed.

In experiment 2, which is illustrated in figure 1, storage was continued for a longer period—49 days. The reduction in the numbers of the two test organisms was more nearly parallel, particularly after the fifth day of storage. Both *Salmonella* organisms and *E. coli* were recovered in significant numbers at the end of the period of storage.

We observed little, if any, deterioration of the oysters. Some dehydration of the meats did occur, owing to storage under dry conditions, but the oysters were still in salable condition at the end of the storage periods of 22 and 54 days.

Two series of experiments, two experiments each, were conducted on soft clams; one series was conducted during cold weather, in January and February, and the other in April and May during more moderate weather. The results of these experiments are shown in table 2.

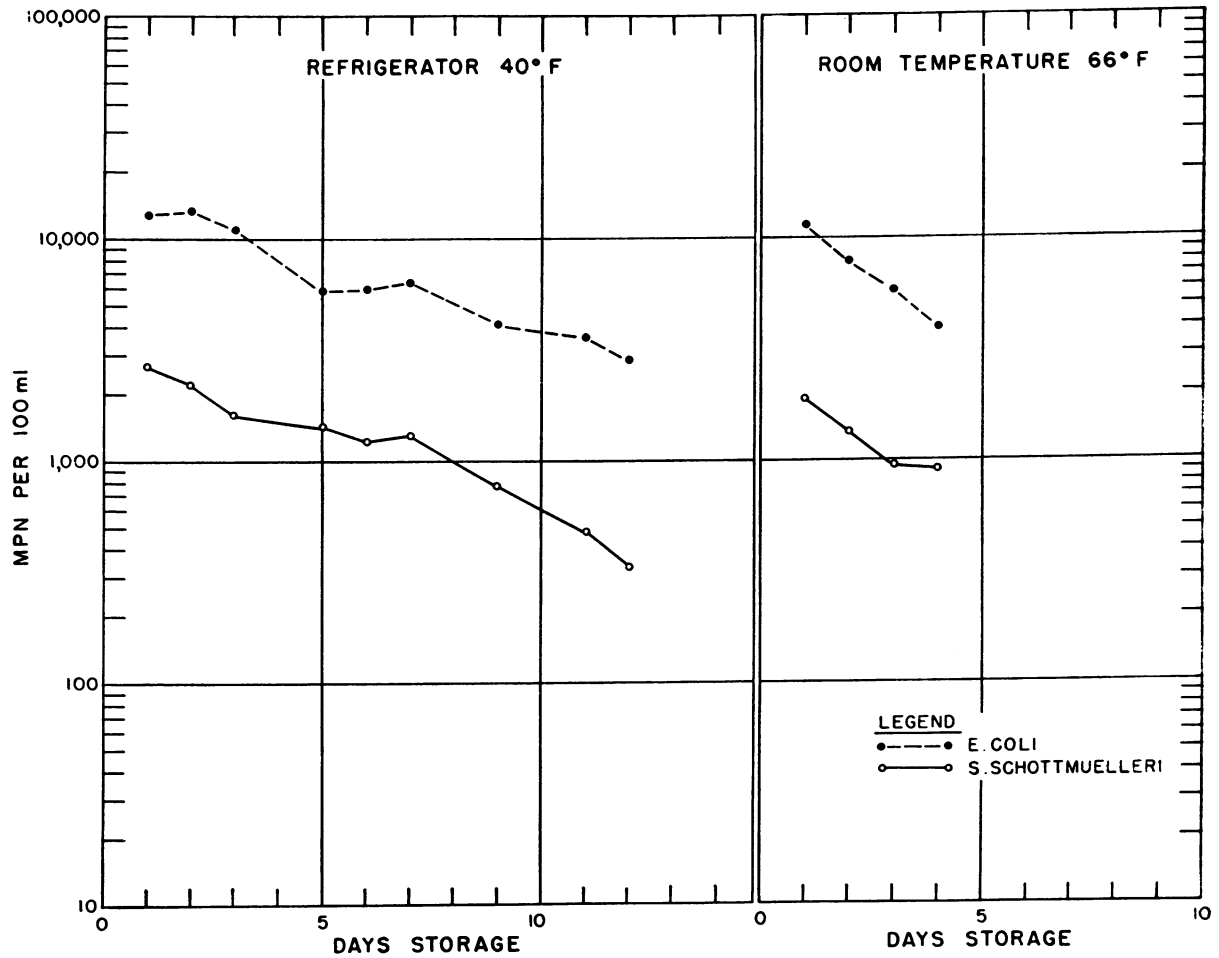
Our comparison of results obtained on refrigerated clams in experiments 3 and 4 indicates a similar rate of decline in numbers of both *E. coli* and *S. schottmuelleri*. The reduction of *E. coli* in the two experiments was not influenced by a more than tenfold difference in initial concentrations.

In experiments 5 and 6, similar results were obtained during the first 10 days of storage. However, at the end of that period, particularly in experiment 5, the clams had deteriorated to a point where they could not be considered suitable for human consumption. In experiment 5, samples which were examined for 2 days after that time showed an increase in numbers of *S. schottmuelleri* but a slight decrease in *E. coli*.

Table 2. Survival of Escherichia coli and Salmonella schottmuelleri in soft clams

Storage temperature	Days of storage	*E. coli* (MPN per 100 ml.)				*S. schottmuelleri* (MPN per 100 ml.)			
		Experiment 3	Experiment 4	Experiment 5	Experiment 6	Experiment 3	Experiment 4	Experiment 5	Experiment 6
40° F. (5° C.)	1	36,000	2,700	17,000	16,000	2,400	1,500	4,800	3,300
	2	40,000	2,700	15,000	19,000	2,200	1,400	3,600	2,400
	3	32,000	1,100	15,000	26,000	1,600	800	3,000	1,900
	4	--------	510	15,000	20,000	--------	500	2,700	1,700
	5	19,000	370	13,000	12,000	1,400	500	3,000	2,000
	6	16,000	900	11,000	7,700	900	570	3,500	1,300
	7	12,000	1,600	12,000	7,700	850	760	3,800	1,100
	8	12,000	1,200	7,000	--------	560	510	2,600	--------
	9	13,000	530	6,000	7,700	640	450	2,000	600
	10	14,000	450	4,200	--------	380	260	1,300	--------
	11	10,000	760	4,900	4,500	380	270	1,900	260
	12	7,200	860	3,800	2,700	330	210	2,500	82
66° F. (19° C.)	1	20,000	3,000	16,000	20,000	1,500	1,100	2,900	2,700
	2	13,000	1,400	9,900	21,000	1,000	690	2,800	1,600
	3	8,900	710	9,900	17,000	730	320	2,800	1,100
	4	--------	330	6,200	17,000	--------	240	2,800	2,200
	5	5,500	330	4,600	--------	310	240	2,100	--------
	6	9,700	520	3,900	--------	130	440	1,900	--------

Figure 2. Survival of Escherichia coli and Salmonella schottmuelleri in soft clams.



A summarization of results of all soft clam storage tests is shown in figure 2. The data are presented as a series of 3-day logarithmic moving averages of all observations on similar days of storage. The results show a reduction continuing at a rate throughout the entire storage period which is quite similar for the two test organisms. *S. schottmuelleri* were recovered in significant numbers after the clams had been stored for 12 days.

The influence of storage at a warmer temperature (66° F.) is also presented in table 2 and figure 2. Reduction of both *E. coli* and *S. schottmuelleri* was similar to that during the same period of storage in the refrigerator. However, after 5 days of storage at the warmer temperatures, the clams deteriorated to an unsalable condition. Many of the animals were gaping and had lost their shell liquor, and in some instances, definite spoilage had started. It was therefore considered that observations made at the end of 5 days could not be interpreted the same as those under conditions which might normally occur in marketable shellfish. In some instances, a sharp de line in the numbers of both organisms occurred; and in others, we observed multiplication of either or both. Reduction of *S. schottmuelleri* and *E. coli* during the significant portion of the storage period was generally parallel.

Summary and Conclusions

The observed survival of *Salmonella schottmuelleri* in shell oysters was similar to that of *Salmonella typhosa* reported by other investigators. The work of Kinyoun (*9*), Krumweide and associates (*10*), and Tonney and White

(*11*) indicates survival of *S. typhosa* for 15 to 60 days in shell stock. The results obtained in the experiments conducted by us at the Public Health Service Shellfish Sanitation Laboratory at Woods Hole, Mass., indicate that *S. schottmuelleri* persists at least as long as *Escherichia coli*, but the rate of reduction of *S. schottmuelleri* during the usual period of storage is not as great as that of *E. coli*.

The reduction of both organisms in stored soft clams proceeded much in a parallel fashion. The rate of reduction at a moderate temperature (66° F.) was generally similar to that at refrigeration temperature (40° F.) during the same period of storage.

Both *E. coli* and *S. schottmuelleri* survived in shell oysters and soft clams which were stored for a period of time at least as long as the shellfish might be in transit from the point of harvesting to consumption.

There was little evidence of multiplication of either *E. coli* or *S. schottmuelleri* in animals that could be considered in marketable condition. Increase in both organisms, on some occasions in the same lot of shellfish, occurred on prolonged storage.

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