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**PROCEEDINGS  
TENTH NATIONAL SHELLFISH  
SANITATION WORKSHOP**

*JUNE 29 AND 30, 1977  
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**DEBORAH S. WILT**  
EDITOR

UNITED STATES DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE  
PUBLIC HEALTH SERVICE  
FOOD AND DRUG ADMINISTRATION  
SHELLFISH SANITATION BRANCH

# COLIFORM DEPURATION OF CHESAPEAKE BAY OYSTERS<sup>1</sup>

by

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## ABSTRACT

Oysters contaminated in nature depurated fecal coliforms to levels below 50/100 g in 48 hr over a wide range of environmental conditions typical of the lower Chesapeake Bay region. Temperature was found to be the most critical environmental factor with conditions below 10-12°C having the potential of inhibiting depuration. Coliform clearance did not appear to be correlated with pumping rate or biodeposition activity of oysters. Oysters infected with the pathogens *Dermocystidium marinum* and *Minchinia nelsoni* (MSX) depurated as rapidly as uninfected ones. Meat quality and size of oysters likewise did not affect depuration.

Four commercial-size tanks of different designs were found to yield satisfactory results in 48 hr. Water flow rates over the ranges studied and location of trays within the tanks did not influence depuration.

Biodeposits contained high levels of total and fecal coliforms, but their accumulation in the tanks did not have a detrimental effect under the conditions studied.

Pooling oysters during monitoring of depuration samples was necessary due to the variation of coliform levels in individual oysters. Samples of 6-8 pooled oysters appeared to be adequate for estimating coliform levels.

The Medium A-1 test was superior to the elevated temperature coliform plate (ETCP) procedure of Cabelli and Heffernan for determination of fecal coliforms in oysters.

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## INTRODUCTION

The investigation was completed in two phases (I and II). First, laboratory studies were conducted using small, shallow plastic trays which held 25 to 36 oysters in a single layer. Experiments were designed to determine the effect of different environmental conditions such as oxygen, turbidity, chlorophyll levels, temperature, salinity, pumping rates and oyster diseases on the depuration process. They were especially designed to give an indication of the intrinsic capability of oysters to depurate coliform bacteria under varying environmental conditions. After completion of the tray studies, depuration in commercial-size tanks was evaluated. Accompanying phases I and II were experiments which evaluated or contrasted various techniques for determination of fecal coliform counts in oyster meats.

A full report of this study has been forwarded to the FDA under the title, "Technical studies of the engineering and biological aspects of the controlled purification of the eastern oyster" (Haven, Perkins, Morales-Alamo and Rhodes, 1976). Its scope is beyond the limits of this review; therefore, only the most pertinent aspects are presented here.

## MATERIALS AND METHODS

### WATER SUPPLY

Water was pumped from the York River to the laboratory where depuration studies were conducted. Bacterial quality was monitored daily at the laboratory during the course of each experiment and the water always met the NSSP standards for approved shellfish growing areas. Bacterial analysis of the water flowing from ultraviolet treatment units was monitored daily; with very rare exceptions, coliform MPN levels were indeterminate (<1.8/100 ml).

## SOURCES OF EXPERIMENTAL OYSTERS

Oysters used in the experiments came from several river systems in Virginia and routinely had very low coliform levels. To increase coliform counts prior to our studies, oysters were transferred to Wormley Creek (a polluted tributary of the York River located approximately 2.5 nautical miles downriver from Gloucester Point) and held in wire trays for periods ranging from two days to two weeks. Total coliform and fecal coliform levels in oysters were usually high after exposure.

## PROCEDURES USED DURING DEPURATION

At the end of every 24 hr period of depuration (except where otherwise indicated), oysters were removed and both oysters and tanks or shallow trays were rinsed with fresh water of potable quality. Gapers and dead oysters were removed. Water samples for bacteriological analysis were usually collected at these 24-hr intervals prior to rinsing.

Environmental factors such as temperature, salinity, dissolved oxygen and turbidity in the water supply were monitored on a regular basis every day during a depuration run.

Two types of ultraviolet lamp units were used to satisfactorily treat water flowing through experimental tanks and trays. One was manufactured commercially by AquaNomics, Inc. of California as Model 4L-368-P-50, a four-lamp unit constructed of PVC in which the lamps were 34" long (Westinghouse No. G36T6L). The unit was provided with an audio and visual warning system for monitoring the intensity of the lamps' radiation passing through the water surrounding them. The preceding unit was used in many of the large-tank studies. A second type of ultraviolet lamp unit (of which several sizes were used in tray and large tank studies) was constructed following the Kelly-Purdy design (Furfari, 1966).

Turbidity of natural water was increased to the desired levels in Phase I studies by metering kaolonite clay into troughs containing depurating oysters. Solids were determined as mg/l by filtration. Ambient dissolved oxygen concentrations were lowered to the desired levels by bubbling nitrogen gas into the inflowing water before it flowed into the experimental oyster trays (Silver, Warren and Doudoroff, 1963). Levels of dissolved oxygen were measured with a polarographic probe and meter.

As indices of biological activity, the pumping rates of oysters were measured, and also the volume of feces and pseudofeces (biodeposits) produced. In measuring pumping rates, oysters were held in specially designed aquaria and each oyster was fitted with a cone-shaped plastic apron that directed the water flow into a chamber where it could be measured (Galtsoff, 1964). Biodeposits were collected with a suction pipette and allowed to settle 24 hrs in a graduated cylinder prior to determining volume (Haven and Morales-Alamo, 1966).

Studies on depuration in commercial-size tanks were conducted using four basic designs which will be described later.

## BACTERIAL ANALYSIS

Total and fecal coliform levels in water and oyster samples were determined using the MPN technique (based on 5 tube replicates) according to procedures outlined in APHA (1970). Oyster samples were generally analyzed as a pool consisting of 6-8 oysters but in several instances individual oysters were analyzed. Levels below the limits of the technique were labelled indeterminate (ID) and were equivalent to <18/100 g for oysters and <1.8/100 ml for water.

The standard MPN method using EC broth was compared to two rapid techniques for the determination of fecal coliform content of oysters. The elevated temperature coliform plate (ETCP) method was performed according to Cabelli and Heffernan (1970a and b). When parallel-testing with the ETCP method, the MPN procedure was modified to include a gravimetric step. One MPN procedure employing Medium A-1 as described by Andrews and Presnell (1972) was also investigated. The latter method included a resuscitation step in which oyster samples were incubated at  $35 \pm 0.3^\circ\text{C}$  for 3 hr prior to being transferred to a water bath at  $44.5 \pm 0.2^\circ\text{C}$  for an additional 21 hr.

The significance of biodeposits on depuration was investigated in commercial-size tanks which received various flushing treatments. Shellfish and biodeposits were collected after various time intervals and were characterized with respect to coliform types and levels. In addition, feces and pseudofeces were collected separately from oysters depurated in small acrylic plastic trays and analyzed for coliform content.

## TREATMENT OF DATA

Data collected were grouped according to different ranges of environmental factors and coliform MPN levels. The grouped data were also ranked according to the MPN values for determination of the plotting positions to be used in application of the graphical method of Velz (1951) for determination of confidence intervals around the mean.

For convenience in presentation of MPN figures, the dimensional units for MPN are omitted. It is to be understood that levels for oysters are MPN/100 g and for water samples they are MPN/100 ml.

In computational analysis, a value of 17 was used when the MPN was ID. When one or more samples was ID in a group for which a mean value was computed, the resulting mean value was categorized as being less than the figure computed. Likewise, if one or more of the samples had an MPN value  $\geq$  a certain figure the resulting computed mean for the group would be preceded by the sample symbol ( $\geq$ ). MPN values preceded by the

symbol < are illustrated in our figures by an arrow pointing down below the plotted value on the graph. If the mean was preceded by the symbol > the plotted MPN value has an arrow pointing upward.

Confidence intervals for the mean of grouped MPN values appear as vertical lines extending above and below the plotted mean in our figures. No confidence intervals are given for any group of data having more than 10% of the values 20 or less or when there was no difference in the slopes of the lines for the plotted experimental data and the line resulting from the intrinsic variation of the MPN technique.

MPN values of 50 for fecal coliforms and 230 for total coliforms were selected as reference points to guide in interpretation of the data in terms of desirable levels of achievement. Therefore, these values are emphasized throughout the presentation and discussion of results.

## RESULTS AND DISCUSSION

### PART I - SHALLOW TRAYS

Forty-four experiments were conducted in shallow trays. Flows of water were relatively high (equivalent to between 1.7 and 8.0 GPM/Bu) to ensure an adequate supply of dissolved oxygen and food to the experimental animals. Thus, the effect of other factors such as disease, turbidity, etc., could be assessed without concern for food and oxygen levels, except when the latter were intentionally lowered.

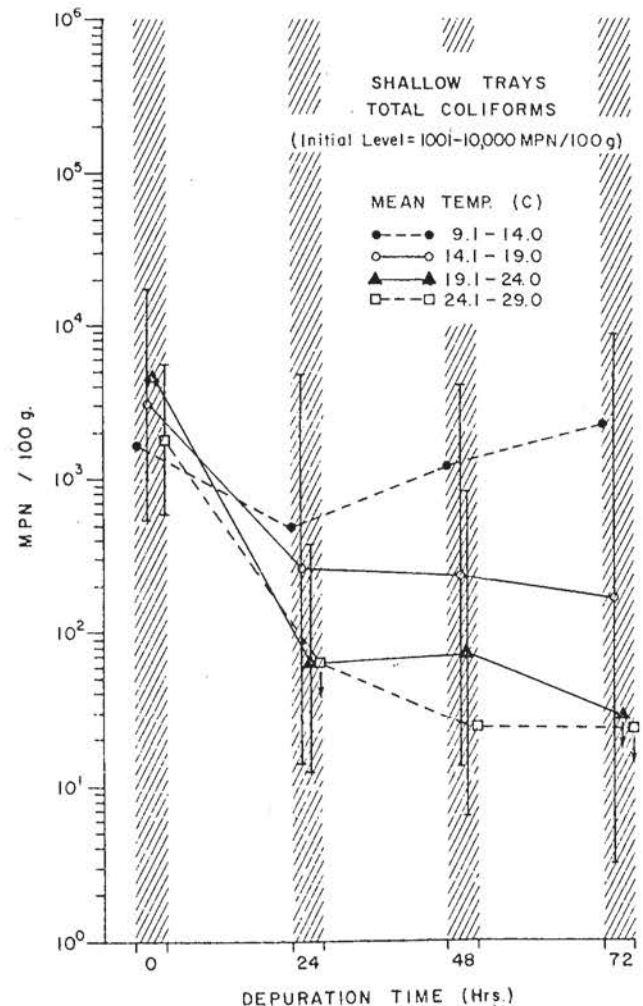
#### Temperature

Most of the experiments on the effect of water temperature on the depuration of oysters were conducted at ambient water temperatures during different seasons in the laboratory at Gloucester Point, but on occasions the water was cooled or heated.

All other environmental factors (except low oxygen conditions, i.e., 0.8 and 0.6 mg/l in shallow trays) were ignored in grouping the data according to temperature because our analyses indicated that their effect was minimal in the ranges studied (see below). The low oxygen levels had a decided adverse effect on depuration and, therefore, experiments conducted under those conditions were omitted in the following analysis.

Results of depuration of total coliforms in relation to temperature levels were erratic. There was wide variation around most of the means and the slopes of corresponding sections of different decay curves often diverged from each other (Figure 1). At initial levels between 1,001-10,000 MPN levels were reduced to less than 100 in 24 hr at temperature ranges 10-24°C (mean temp. = 21.9°C). In the temperature range 9-14°C (mean temp. = 10.4°C) the MPN level was never reduced below 490 and the 48- and 72-hr levels were higher than that at 24 hr. At temperatures between 14-19°C levels were slightly over 230 at 24 and 48 hr and under 200 after 72 hr.

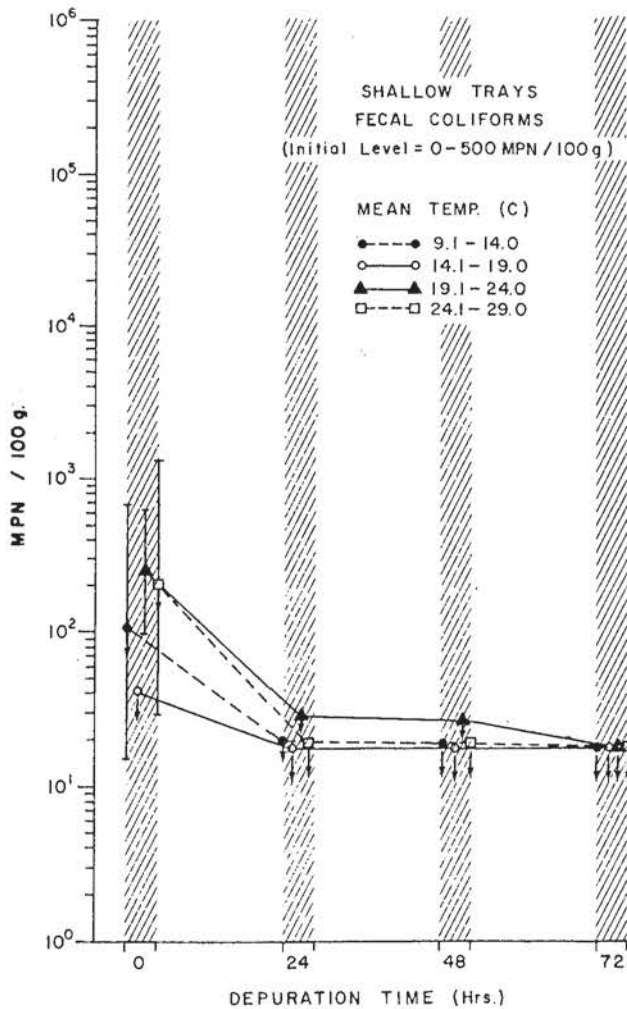
**FIGURE 1** Mean total coliform levels in oysters depurated in shallow trays with respect to initial MPN level and mean temperature. Vertical lines represent the 90% confidence intervals for the mean. Oysters contaminated in nature.



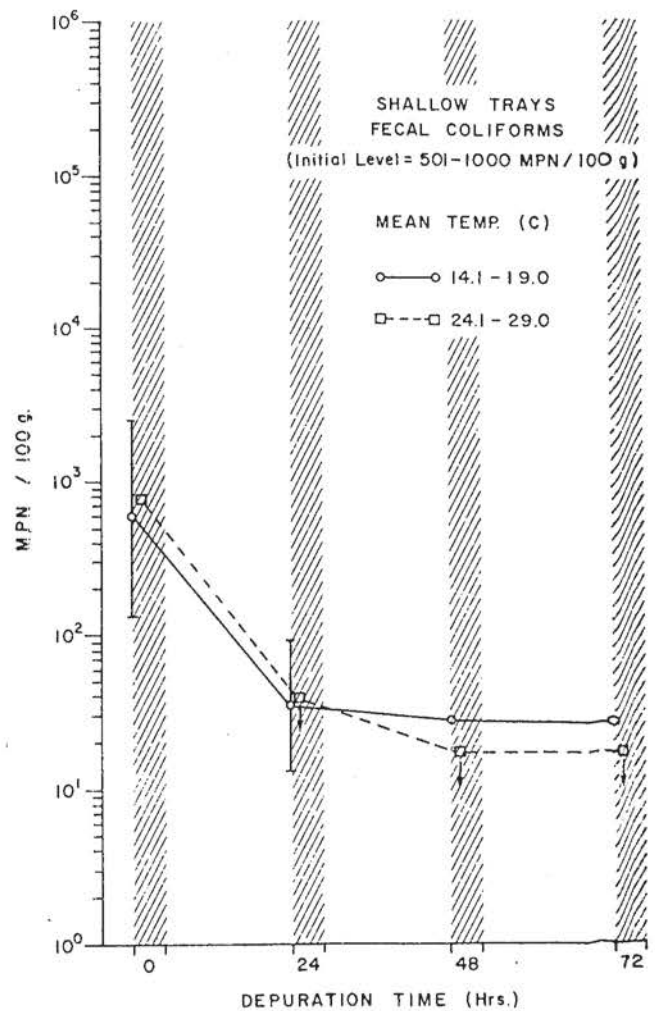
The erratic nature of the total coliforms MPN in shallow tray experiments and the lack of consistency of most of the data for the various temperature ranges at the different initial level categories did not permit establishment of end point levels at any given combination of initial MPN level and mean temperature. A similar variation was noted in almost all other reduction curves in tray and tank studies. Because of this variability, results of total coliform tests are not given in the following pages, unless otherwise specifically stated.

Fecal coliform MPN at different temperature and initial level groupings at 24, 48 and 72 hr followed more regular patterns and were more consistent than those for total coliforms. With a single exception, at all combinations of temperature and initial MPN level, MPN values were under 50 after 24 hr of depuration and remained below that level through the subsequent 48 hr of depuration (Figures 2, 3 and 4).

**FIGURE 2** Mean fecal coliform levels in oysters depurated in shallow trays with respect to initial MPN level and mean temperature. Vertical lines represent the 90% confidence intervals for the mean. Oysters contaminated in nature.



**FIGURE 3** Mean fecal coliform levels in oysters depurated in shallow trays with respect to initial MPN level and mean temperature. Vertical lines represent the 90% confidence intervals for the mean. Oysters contaminated in nature.



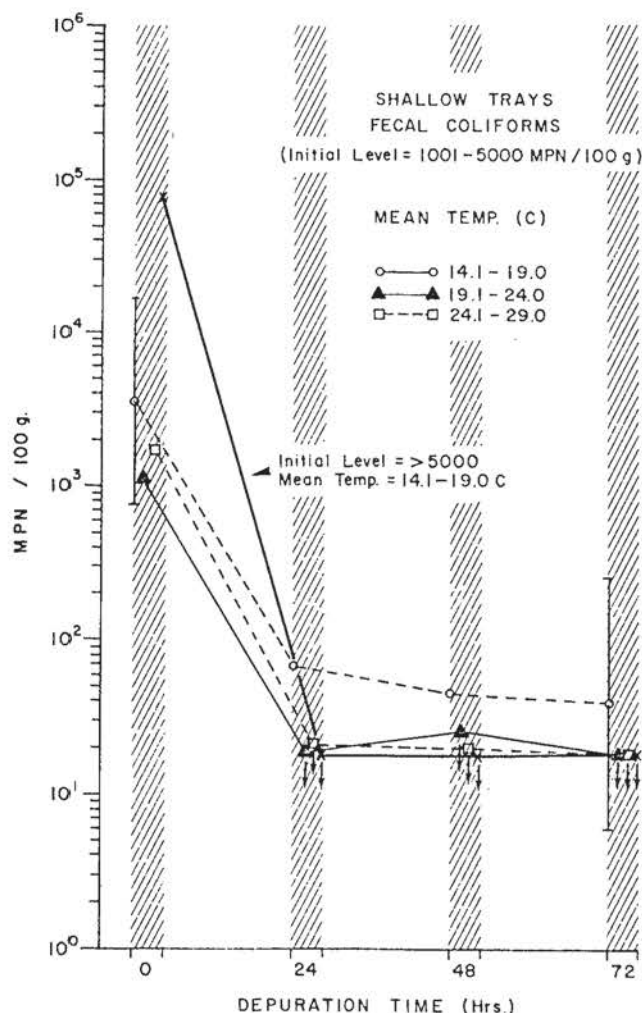
The only exception was the initial MPN level of 1,001-5,000 at temperatures between 14-19°C (Figure 4). In that case, the 24-hr MPN was 68 but was reduced to 45 by 48 hr; however, these two values were based on single samples. The mean MPN, based on five samples, after 72 hr was <40 with the 90% confidence interval estimates ranging from 11 to 135. Four of these five samples (80%) showed MPN values under 50 and three were under 20. The fifth sample had an MPN value of 330.

In a single experiment in the mean temperature range 14-19°C (mean temp. = 14.7°C) and initial level of 79,000, the MPN value was reduced to <18 in 24 hr and stayed at that level for the next 48 hr of depuration (Figure 4).

Initial mean MPN levels in the mean temperature range of 9-14°C were all under 500. MPN values for fecal coliforms ranged from <18 to 390 in samples of oysters from four locations at water temperatures under 15°C. It appears, therefore, that at temperatures under 14°C fecal coliform MPN levels greater than 500 occur infrequently in tributaries of southern Chesapeake Bay.

In summary, at a temperature range of 14 to 29°C, oysters were depurated of fecal coliforms from MPN levels as high as 10<sup>4</sup> to less than 50 in 24 hr. The same was found at temperatures as low as 12°C when initial MPN levels were 230 or less.

**FIGURE 4 Mean fecal coliform levels in oysters depurated in shallow trays with respect to initial MPN level and mean temperature. Vertical lines represent the 90% confidence intervals for the mean. Oysters contaminated in nature.**



### Dissolved Oxygen

Oysters depurated fecal coliform bacteria over a wide range of dissolved oxygen values. Fecal coliform levels in oysters were reduced to less than 50 in 24 hr at D.O. concentrations of 1.8 mg/l or higher in experiments conducted at water temperatures of 26°C with mean initial levels as high as 2100. At oxygen concentrations higher than 0.8 mg/l fecal coliforms were reduced to less than 50 in 48 hr. When oxygen concentration was reduced to 0.6 mg/l the MPN was reduced from a mean of 2100 to a mean less than 50 in 72 hr.

### Turbidity

Turbidity levels in Chesapeake Bay over productive oyster beds may range from about 5 to 65 mg/l and average about 31 to 36 mg/l (Brehmer and Haltwanger, 1966; Nichols, 1972; Haven and Morales-Alamo, 1972). In a series of experiments, turbidity was increased by adding kaolinite clay to flowing water in experimental troughs to levels as high as 77 mg/l over a temperature range of from 12 to 27°C. These levels did not inhibit depuration of fecal coliform bacteria, and in all instances fecal coliform levels were less than 50 MPN in 24 to 48 hr.

### Salinity

Oysters live and reproduce successfully over a wide range of salinities, ca. 5-32‰ in the Chesapeake Bay. Because of this wide tolerance, it was expected that they would depurate over a wide range of salinities.

Oyster depurated fecal coliforms in our study over a salinity range of 14.0‰ to 21.4‰ to an MPN less than 50 in 24 to 48 hr. In all probability, depuration above 21.4‰ would be equally practical since there is no evidence in the literature that salinities from 25‰ to 32‰ inhibit oyster activity (Galtsoff, 1964). The lower limit for depurating oysters has not been determined since ambient salinities at Gloucester Point were never below 14‰ during the study period. However, 10‰ is the suggested lower limit for depuration of oysters in the Gulf of Mexico region (Huntley and Hammerstrom, 1971).

### Effect of the Oyster Pathogens *Dermocystidium marinum* and *Minchinia nelsoni* (MSX) on Depuration Rates

Since one would expect that physiologically depressed or diseased oysters would depurate more slowly than healthy ones, depuration rates were examined in diseased oysters with known intensities of infection by the pathogens *Dermocystidium marinum* and *Minchinia nelsoni* (MSX).

In a series of studies, two populations of oysters, infected and uninfected, were selected, placed in the shallow trays, and processed individually for total and fecal coliform analyses at 0, 24, 48 and 72 hr after placement in the depuration system.

*D. marinum* causes necrosis of connective and epithelial tissue in heavily infected oysters, thus, one would expect that depuration rates would not be as high or depuration would not occur under those conditions. However, our studies showed that infected oysters (including heavily infected ones) depurated as rapidly as uninfected ones (Figures 5 and 6).

FIGURE 5 Mean fecal coliform levels in infected oysters depurated in shallow trays. Oysters were infected with *Dermocystidium marinum* and *Minchinia nelsoni* (MSX). Oysters contaminated in nature.

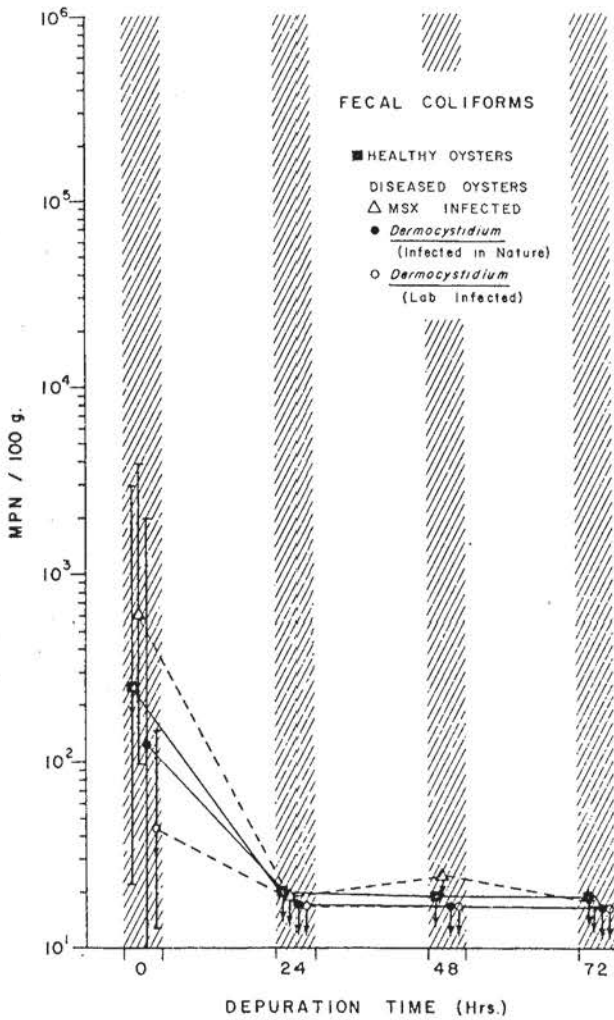
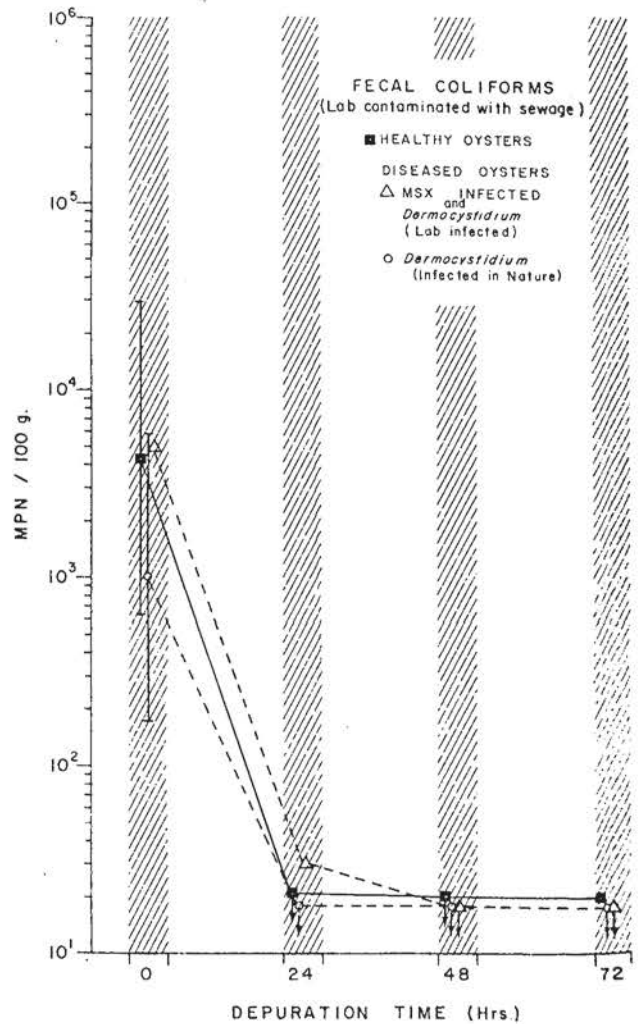


FIGURE 6 Mean fecal coliform levels in oysters depurated in shallow trays with respect to infection with *Dermocystidium marinum* and *Minchinia nelsoni* (MSX). Oysters contaminated with raw sewage.



### Pumping Rate and Biodeposition Activity

There was no observed relation between pumping rates of oysters, or rates of biodeposition and depuration. In a series of studies involving 19 oysters, average pumping rates ranging from 1.4 to 10.5 l/hr were observed. Oysters with lower rates depurated as rapidly as those with higher rates; all depurated to satisfactory levels in 48 hr or less. In other studies oysters with low or zero production of biodeposits depurated as rapidly as those producing high levels.

### Other Parameters

The effects on depuration rates of oyster size, meat quality, source of oysters and amount of food were investigated. None had an adverse influence on depuration. When data were grouped according to these four parameters, fecal coliforms were depurated to mean levels of less than 50 by 48 hr.



## PART II - COMMERCIAL TANKS

Experiments in shallow trays were designed to gather data on the optimal ranges of physical and chemical environmental factors for successful depuration of oysters. They were conducted under conditions which eliminated crowding and gave oysters access to an abundant supply of water in a laminar flow with very little water recirculation. Having established those optimal ranges in shallow trays, experiments were conducted in large commercial-size tanks: 1) to determine if the results from shallow trays applied to the more crowded conditions and different hydraulic circulations found in the large tanks; and 2) to test the relative efficiency for depuration of large tanks of different design.

### Tank Studies

Forty-one experiments were conducted using commercial-size depuration tanks of various types. Their design, size, and capacity in terms of bushels of oysters differed widely (Table 1). Moreover, their design incorporated diverse techniques for aeration, systems of water flow and methods of drainage. In all these tanks and under a diversity of environmental conditions oysters depurated fecal coliform bacteria to acceptable levels within 24 to 48 hr.

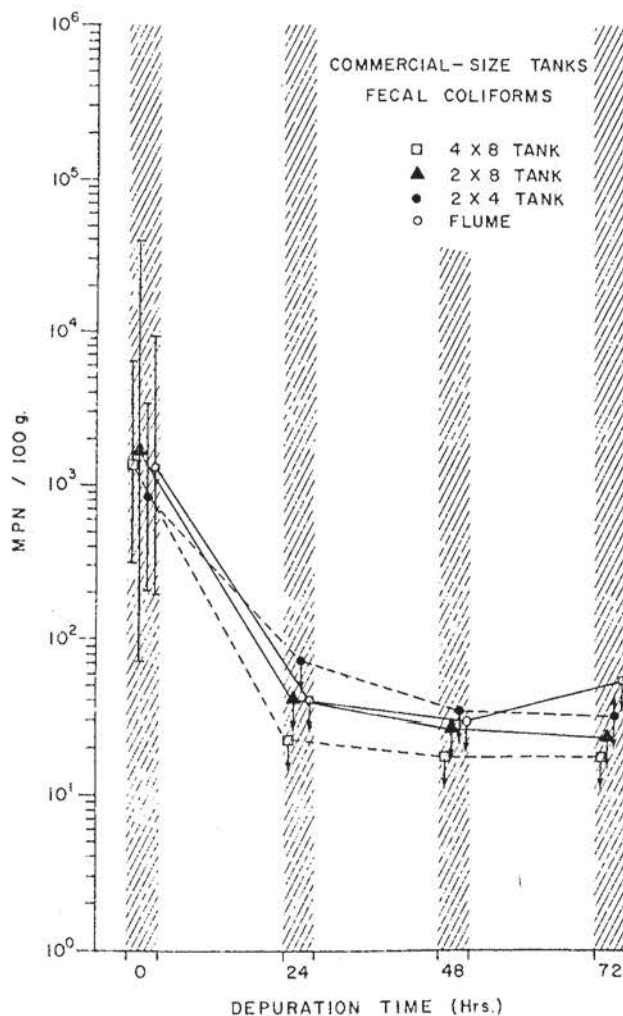
Decay curves for mean MPN levels of fecal coliforms in tank studies show that oysters depurated to low levels (<40) in 48 hr in all tanks (Figure 7). The fact that mean levels recorded for samples from 4' x 8' tank are significantly lower than those in the other tanks may be associated with a difference in the temperatures at which the experiments were conducted. Mean temperatures in experiments in the 2' x 8' tank ranged from 10.1 to 26.5°C. In the 2' x 4' tank they ranged from 10.1 to 18.4°C. Those in the 4' x 8' tank only ranged from 20.2 to 28.8°C. Mean temperatures in flumes ranged between 24.4 and 29.0°C, but results obtained were not as consistent as those in the 4' x 8' tank. Temperature cannot be considered the factor associated with the difference.

**TABLE 1** Details of Depuration Tank Design Flow Characteristics, Tray Capacity<sup>a</sup> and Number of Studies Conducted in Each

Number of Studies	Tank Dimensions	Volume Capacity (Gal)	Tray Capacity	Ratio Gal. Water/Bu Oysters
6	4' x 8' x 18"	253	8	46.5
12	2' x 8' x 42"	457	8	88.7
10	2' x 4' x 33"	155	5	51.5
9	26" x 13' x 6" (Flume)	97	3	37.3

<sup>a</sup>Each tray held 0.6 bushels of 3.5" oysters.

**FIGURE 7** Mean fecal coliform levels in oysters depurated in commercial-size tanks - all experiments combined for each tank type. Oysters contaminated in nature.



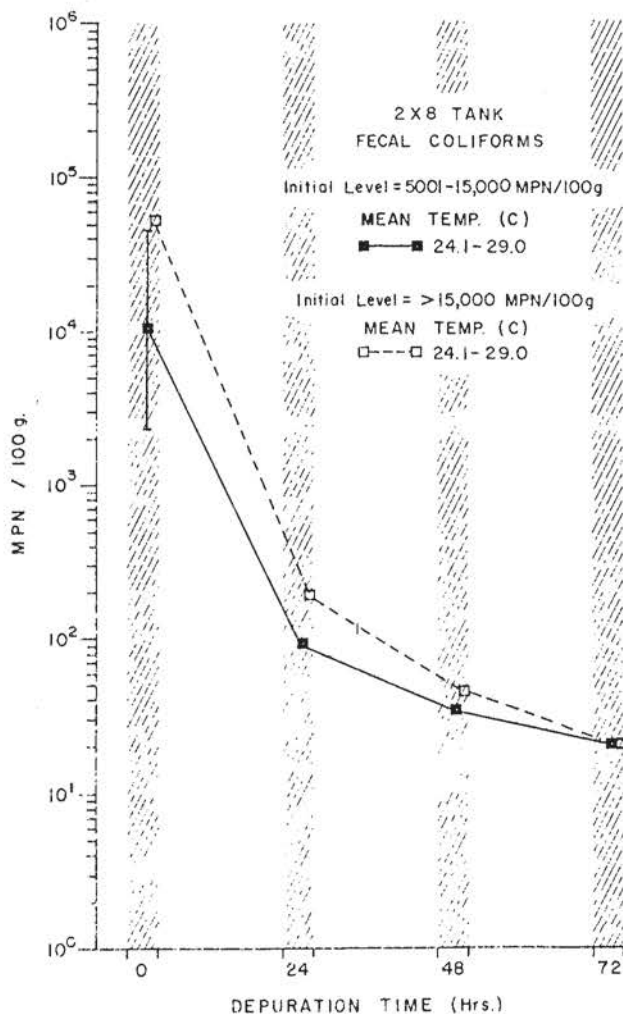
Mean MPN's remained below 40 after 72 hr of depuration in three of the tanks (4' x 8', 2' x 8' and 2' x 4'). An unexplainable increase in the MPN level between the 48- and 72- hr periods in some of these experiments resulted in a 72-hr mean of <56. Ignoring the 72-hr data, the flume is satisfactory for depuration of oysters in 48 hr if a fecal coliform level of <40 is considered acceptable.

The mean MPN of fecal coliforms after 48 hr for the 153 pooled samples analyzed from the 41 depuration runs made in commercial-size tanks was <26. Ninety-nine percent of the samples had an MPN under 790 and 98% were 230 or less.

### Anticipated Coliform Levels During Depuration

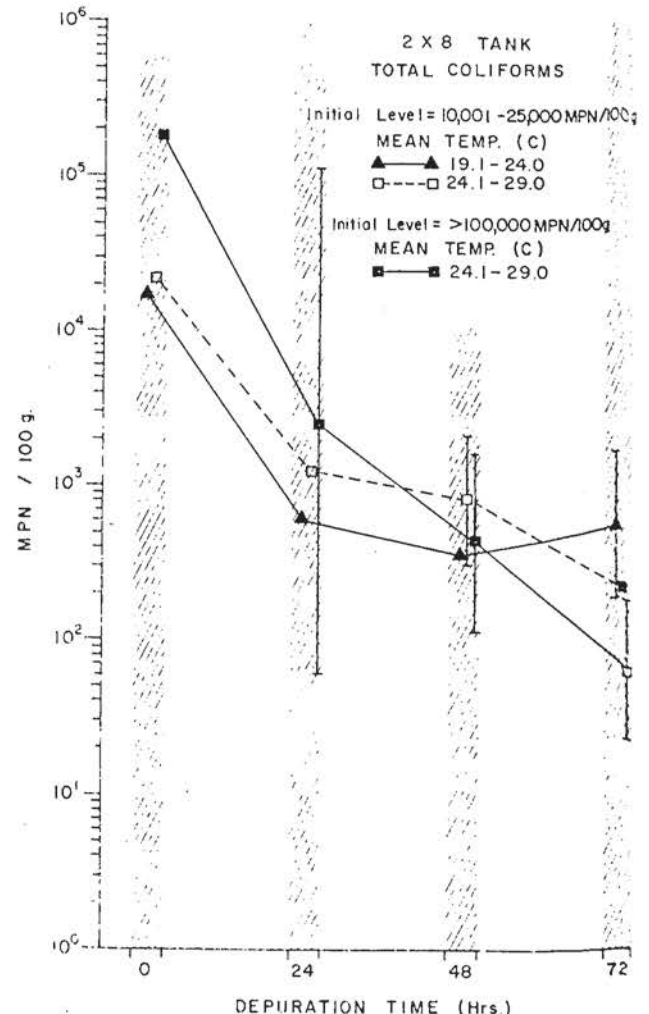
Initial densities of total and fecal coliforms were determining factors in the time required to reduce coliform populations to specified levels. Fecal coliform densities dropped below 50 within 48 hr when mean levels were as high as 52,000 and water temperature was 26°C (Figure 8). Fecal coliform levels dropped below 50 within 24 hr when initial levels were 300-3,300 and temperatures ranged between 15 to 29°C. Forty-eight hr were required to reduce the fecal coliform content of oysters containing 180-190 to 50 when depurated at 10.1-11.5°C. How oysters with higher initial levels would respond to cold water depuration is not known. Oysters collected when water temperatures were low, 8-14°C, routinely had fecal coliform levels below 400.

**FIGURE 8** Mean fecal coliform levels in oysters depurated in the 2 x 8 tank with respect to initial MPN level and mean temperature. Vertical lines represent the 90% confidence intervals for the mean. Oysters contaminated in nature.

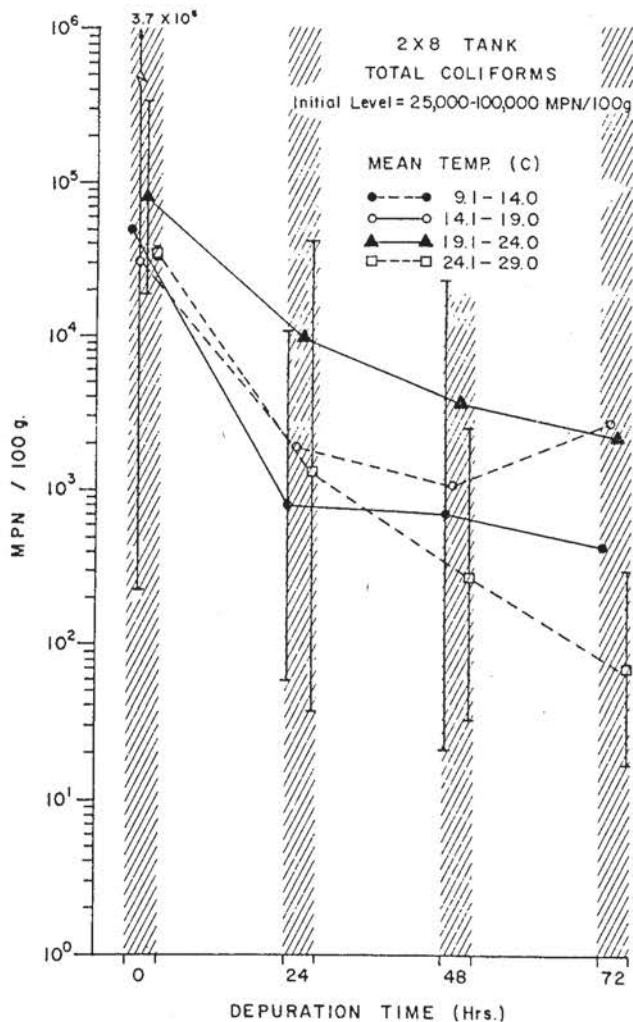


Overall reduction of total coliforms to our reference levels was not as predictable as for fecal coliforms. It was found that mean total coliform levels above 75,000 approached 230 in 72 hr (15-27°C). However, initial levels of approximately 30,000 stabilized at 1,400-1,500 during the three day period. At an initial level of 19,000 in one series of experiments total coliforms were not reduced below 350 in 72 hr (20-27°C). Overall the upper limits were 12,000-15,000 total coliforms if attainment of approximately 230 is desired in 24 hr or 20,000-200,000 if 72 hr is permitted (Figure 9). Success in reducing total coliforms below 230 or in approaching that figure was not necessarily related to temperature as can be seen in Figure 10.

**FIGURE 9** Mean total coliform levels in oysters depurated in the 2 x 8 tank with respect to initial MPN level and mean temperature. Vertical lines represent the 90% confidence intervals of the mean. Oysters contaminated in nature.



**FIGURE 10** Mean total coliform levels in oysters depurated in the 2 x 8 tank with respect to initial MPN level and mean temperature. Vertical lines represent the 90% confidence intervals for the mean. Oysters contaminated in nature.



### Bacteriological Significance of Biodeposits

It has been recorded by other workers prior to this study that oyster biodeposits in a depuration tank contain high levels of coliform bacteria. Examination of oysters containing  $10^4$  total coliform and  $10^3$  fecal coliform showed that the corresponding levels in their feces were  $10^6/100g$  and  $10^5/100g$ , respectively.

Experiments were performed to determine the significance of biodeposits containing elevated coliform levels on depuration. Oyster shell and tank surfaces were flushed free of biodeposits at 6, 12, and 24 hr intervals. Studies were also included in which tanks were not flushed and oyster wastes allowed to accumulate. The results showed that flushing was not necessary in the

tanks studied. However, due to the small number of experiments conducted, elimination of flushing cannot be recommended. The potential for recontamination of oysters by biodeposits exists although coliform levels in tank waters examined were very low, indicating that the bacteria were well aggregated in feces and pseudofeces.

Biodeposits collected during the first few hours of depuration showed that approximately  $10^2$  more coliforms were present in feces than in pseudofeces. Coliform densities declined approximately by  $10^2$  over the three day span.

### Analysis of Rapid Bacteriological Methods

The sanitary quality of commercial shellfish has been routinely monitored using the APHA fecal coliform procedure, which utilizes enrichment in lactose broth followed by observation of gas production in EC medium at elevated temperature (APHA, 1970). This technique is not only laborious but requires 72 hr for completion and necessitates holding depurated shellfish under refrigeration for several days before release to the market. Therefore, two alternate 24-hr methods were investigated.

The first rapid technique examined was the elevated temperature coliform plate (ETCP) method developed by Cabelli and Heffernan (1970a, b) for determination of fecal coliform densities in hard and soft shell clams. Parallel testing using the standard MPN and ETCP methods revealed that plated oyster brei had an inhibitory effect on fecal coliform growth. Dilution of brei yielded higher counts as did longer incubation times thus suggesting that the inhibitory agents were labile. The ETCP method was rejected because at concentrations of brei which yielded reasonable numbers of colonies, the incubation time had to be extended beyond 24 hr.

Comparison of the Medium A-1 test with the standard fecal coliform procedure through the parallel examination of 143 oyster samples showed that the two methods were not significantly different (calculated  $t$  value = 0.63;  $t_{0.05} = 1.98$ ; null hypothesis accepted). A detailed analysis of over 250 positive tubes from Medium A-1 and EC medium showed that Medium A-1 was superior, if any difference was present, to EC medium in terms of its specificity for *Escherichia coli*. Percentages of *E. coli* detection for the standard and Medium A-1 tests were 99.2 and 100% respectively. Only 1% of positive tubes contained additional coliform types which could produce gas at  $44.5^\circ C$  in contrast to 6% of the positive EC tubes. Prolonging the incubation of Medium A-1 by 24 hr increased the number of gassing tubes by 4.9% but only half of the isolates were *E. coli* whereas the remainder were identified as *Enterobacter* sp. and *Klebsiella* sp.

### Monitoring

In this project a rigorous statistical examination of sample size was not conducted. The number of oysters within each sample was less than recommended by

APHA (1970), but this condition was logistically unavoidable due to the experimental need to process numerous samples from various tank locations during the course of each experiment. Examination of the replicate pools of oysters showed that variations were generally factors of 2 to 3-fold for fecal coliforms, but infrequently were as high as 10-fold. Variations of total coliform densities were more widely spread. Examination of individual oysters showed that discrepancies between individual oyster samples within a given population were greater than variations inherent in the MPN technique.

The minimum number of samples required to assay the number of coliform bacteria present in an oyster population during depuration was not determined. However, six samples of 6-8 pooled oysters per sample per tank were adequate for estimating coliform levels. This is evidenced by the predictability with which fecal coliform levels were reduced to less than 50 when physicochemical parameters were acceptable. Total coliform densities were generally predictable but were somewhat more erratic. There was no evidence that position in the tank influenced depuration of total and fecal coliforms in oyster populations from various trays.

## CONCLUSIONS

Depuration of oysters to levels below 50 fecal coliforms and 230 total coliforms is possible in the southern Chesapeake Bay. Levels of 50 fecal coliforms or less can consistently be attained in 48 hr even when the initial levels are very high (i.e., ca. 52,000). It was not determined how high those values could be before oysters will not depurate to 50 fecal coliforms in 48 hr. The mean MPN for 153 pooled samples analyzed from 41 depuration runs in commercial-size tanks after 48 hr was <26 fecal coliforms. Ninety-nine percent of the samples had a fecal coliform MPN under 790 and 98% were under 230 when the initial levels are as high as  $10^4$  to  $10^5$ . The upper limit is not precisely known but probably lies in that range. We do not recommend use of total coliforms as indicators of depuration success or failure.

Reasonable environmental conditions must be in effect for depuration to occur, but the ranges are sufficiently broad to make depuration feasible in southern Chesapeake Bay. The temperature should be above 10-12°C but below 29°C; turbidity can be as high as 77 mg/l mean over 3 days (the upper limit is not known); dissolved oxygen should be above 2 mg/l; water rates should be at least 1 GPM/Bu. If a red tide is present near the depuration plant, caution should be observed in monitoring the final product, but the dinoflagellates will probably not prevent continuation of operations unless concentrations become extremely high. Chlorophyll levels in the water up to 23.6 mg/l and mean salinities of 14 to 21.4‰ have no effect on depuration.

As long as gaping oysters are removed from the system, none of the following factors need to be considered

in undertaking depuration of oysters: size, meat quality, infection by *Dermocystidium marinum* or *Minchinia nelsoni*.

Three tank designs, each holding 2-5 bushels of oysters, performed adequately. We recommend one of those on the basis of design simplicity. It is constructed of 3/4" plywood and is 8' long x 4' wide x 1-1/2' deep.

## DISCUSSION

UNIDENTIFIED SPEAKER: As you were examining your individual shellfish, did you notice whether a significant percentage of them showed little or no loss in fecal coliform?

DR. PERKINS: I don't have the figures at my disposal right now, but I could produce that data very easily from the individual oyster samples I examined.

UNIDENTIFIED SPEAKER: Frank, would the stress effects of commercial harvesting on oysters have to be examined?

MR. HAVEN: I would say that the way we collected these oysters for our study would create no greater or no less stress than what they'd undergo during commercial harvesting conditions.

We dredged up the oysters ourselves from the Rappahannock River and put them in a truck; they'd be out of water for 24 hours, so to speak. Then we'd put them in Wormley Creek, leave them there for three days to three weeks, bring them up and put them in a truck. They'd be out of the water for half a day while they were being scrubbed and washed. It might be longer. They might stay out of water all day; then we'd put them in the tank and start zero hour. So, they were subject to more or less the same conditions that you would have—maybe not quite as rigorous, but they weren't treated gently.

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## REFERENCES

- (APHA) American Public Health Association. 1970. Recommended procedures for the examination of sea water and shellfish. Fourth Edition. American Public Health Association, New York.
- Andrews, W. H. and M. W. Presnell. 1972. Rapid recovery of *Escherichia coli* from estuarine water. *Appl. Microbiol.* 23: 521-523.
- Brehmer, M. L. and S. O. Haltwanger. 1966. A biological and chemical study of the tidal James River. Va. Inst. Mar. Sci. Rept. to the Federal Water Pollution Control Administration under contract PH 86-65-86.

- Cabelli, V. J. and W. P. Heffernan. 1970a. Accumulation of *Escherichia coli* by the northern quahaug. *Appl. Microbiol.* 19: 239-244.
- Cabelli, V. J. and W. P. Heffernan. 1970b. Elimination of bacteria by the soft shell clam, *Mya arenaria*. *J. Fish. Res. Bd. Canada* 27: 1579-1587.
- Furfari, S. A. 1966. Depuration Plant Design. U. S. Dept. Health Educ. & Welfare, Publ. Health Serv., Washington. 119 p.
- Galtsoff, P. S. 1964. The American Oyster *Crassostrea virginica* Gmelin. U. S. Fish. Wildlife Serv., Fish. Bull. 64: 1-480.
- Haven, D. S. and R. Morales-Alamo. 1966. Aspects of biodeposition by oysters and other invertebrate filter feeders. *Limnology and Oceanography* 11(4): 487-498.
- Haven, D. S. and R. Morales-Alamo. 1972. Biodeposits as a factor in sedimentation of fine suspended solids in estuaries. *The Geological Society of America, Memoir* 133: 121-130.
- Haven, D. S., F. Perkins, R. Morales-Alamo and M. Rhodes. 1976. Technical studies on the engineering and biological aspects of controlled purification of the eastern oyster. Volumes I, II and III. Final Contract Report, 15 June 1973 to 15 November 1975, Contract No. FDA 73-183, Dept. of Health, Education and Welfare, Public Health Serv., FDA.
- Huntley, B. E. and R. J. Hammerstrom. 1971. An Experimental Depuration Plant: Operation and Evaluation. *Ches. Sci.* 12: 231-239.
- Nichols, M. M. 1972. Sediments of the James River Estuary, Virginia. *The Geological Society of America, Memoir* 133: 139-212.
- Silver, S. J., C. E. Warren and P. Doudoroff. 1963. Dissolved oxygen requirements of developing steelhead trout and chinook salmon embryos at different water velocities. *Trans. Am. Fish. Soc.* 92: 327-355.
- Velz, C. J. 1951. Graphical approach to statistics. IV. Evaluation of bacterial density. *Water and Sewage Works* 98: 66-73.